INSCOPER

User Guide
Imaging Software

v. 9.3

www.inscoper. com

Table of contents

1. IMAGING

From core solution to custom techniques

An Inscoper-certified installer assembled a hardware configuration for your system using the Configurator software. Now, your system is ready to perform your acquisition workflow.

If you want to perform minor hardware modifications (switch or add objectives, cubes), you can proceed with caution following this tutorial (part 3). For any other changes, do not modify the hardware configuration on your own at the risk of breaking image acquisition functions. Please contact us if you need advice.

The Imaging documentation is structured as follows:

- 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.

1.1. 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.

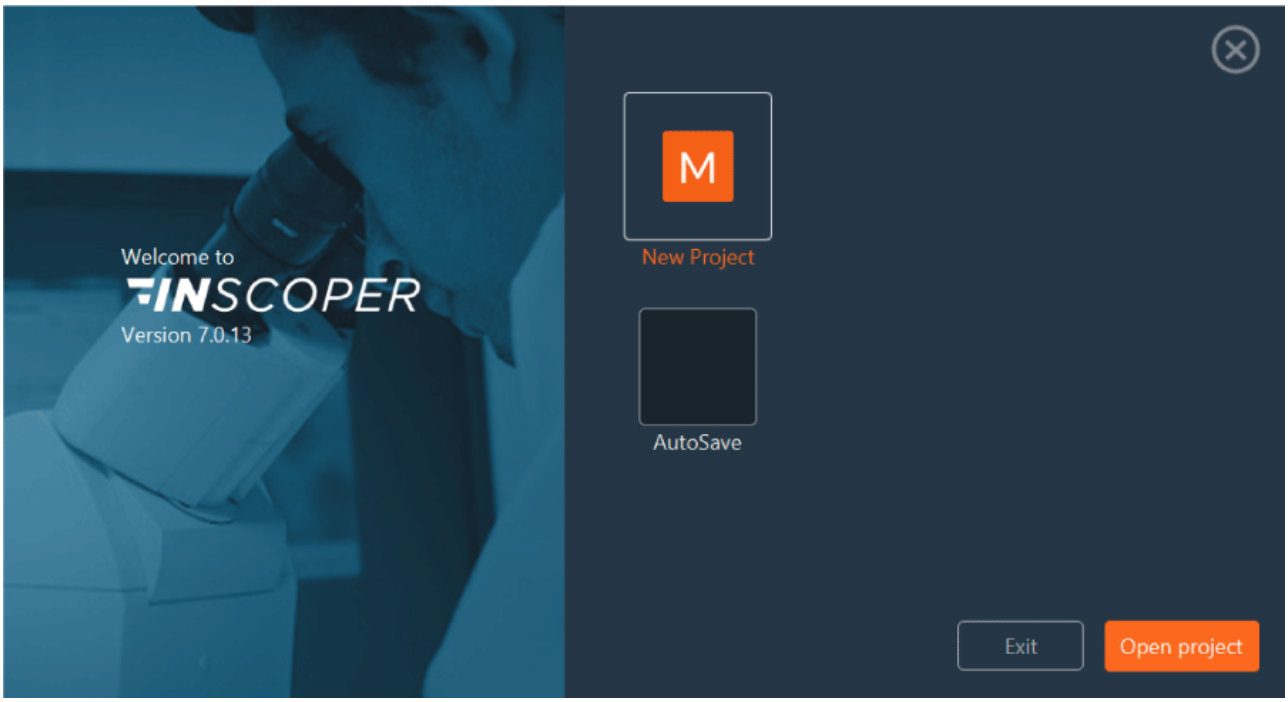
1.1.1. User journey

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:

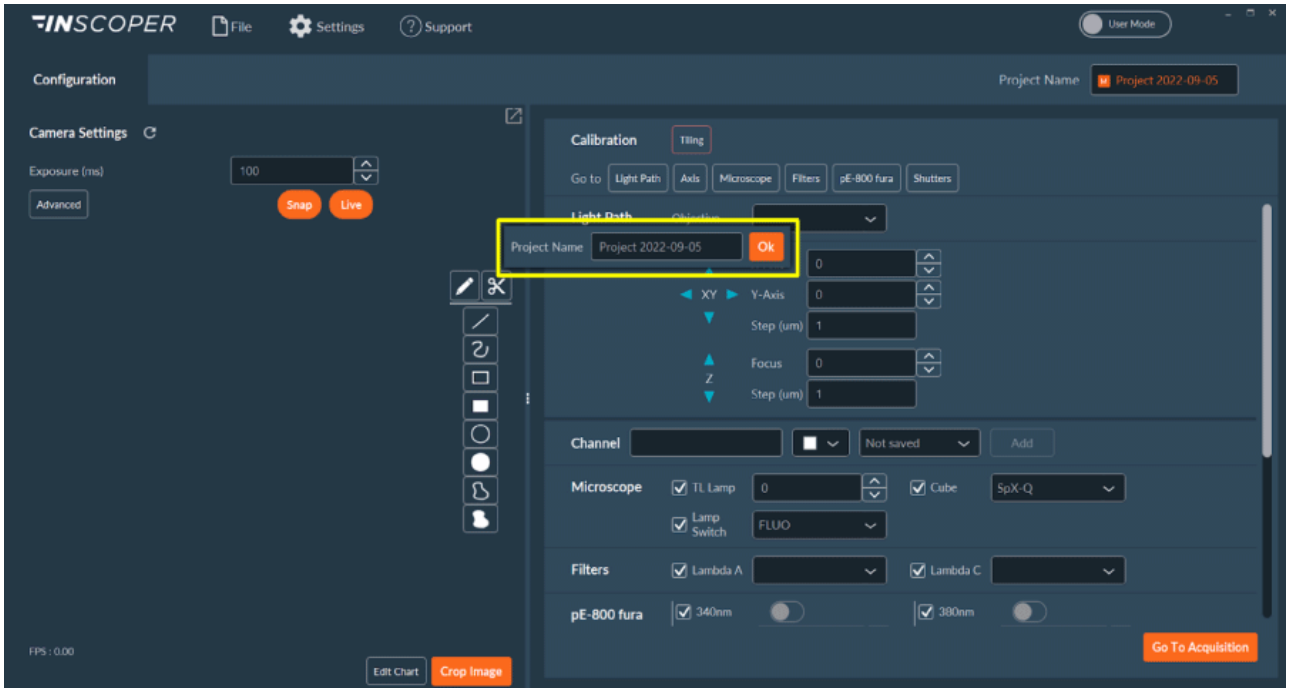
1. Configure the microscope's channels and general settings (Configuration Tab).
2. Define specific settings for the required image acquisition sequence. These settings are displayed as "dimensions" that can be combined and interlinked. Run the image acquisition sequence. Stop/Pause is possible.
3. View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

1.1.2. 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:

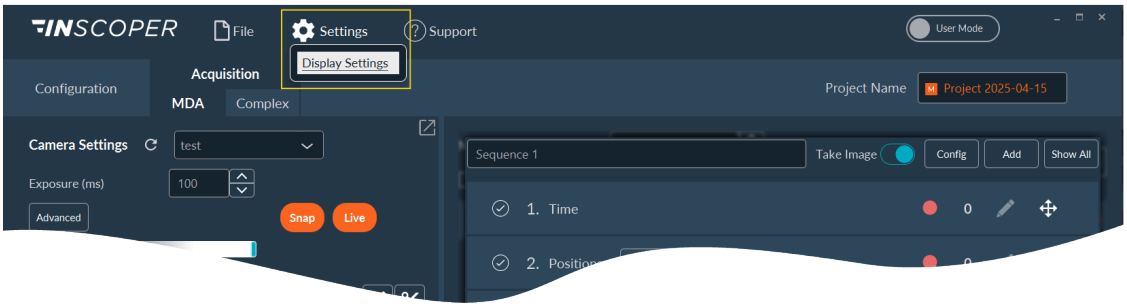
New Project	To create a new acquisition sequence, click on **New Project**. A loading screen will appear while the interface recognizes the devices of the microscope. Wait until it disappears before starting your project.
AutoSave	The **AutoSave** button opens the most recently created project, even 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.

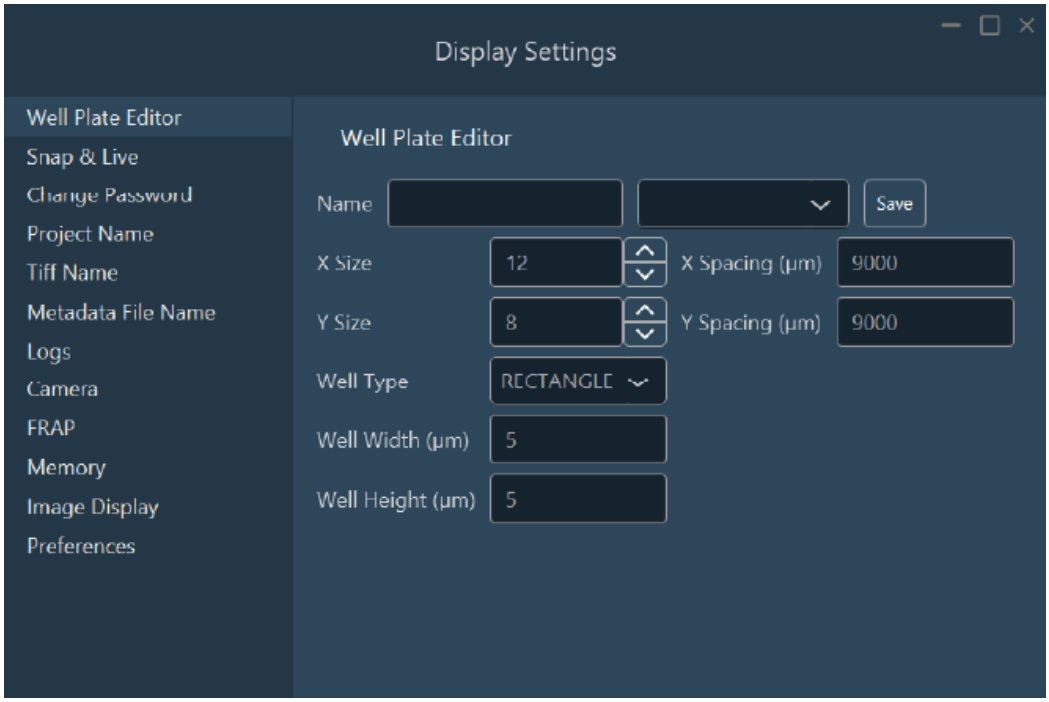
1.1.3. Display settings

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



This section allows you to adjust several settings. After each change you make, be sure to confirm it by clicking the orange **Save** or **Confirm** button. Below are some example settings.

1. Edit your well plate in the **Well PLate Editor** .



   a. Name the support.

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

   c. Enter the number of horizontal and vertical wells.

   d. Enter the horizontal and vertical spacing between the wells.

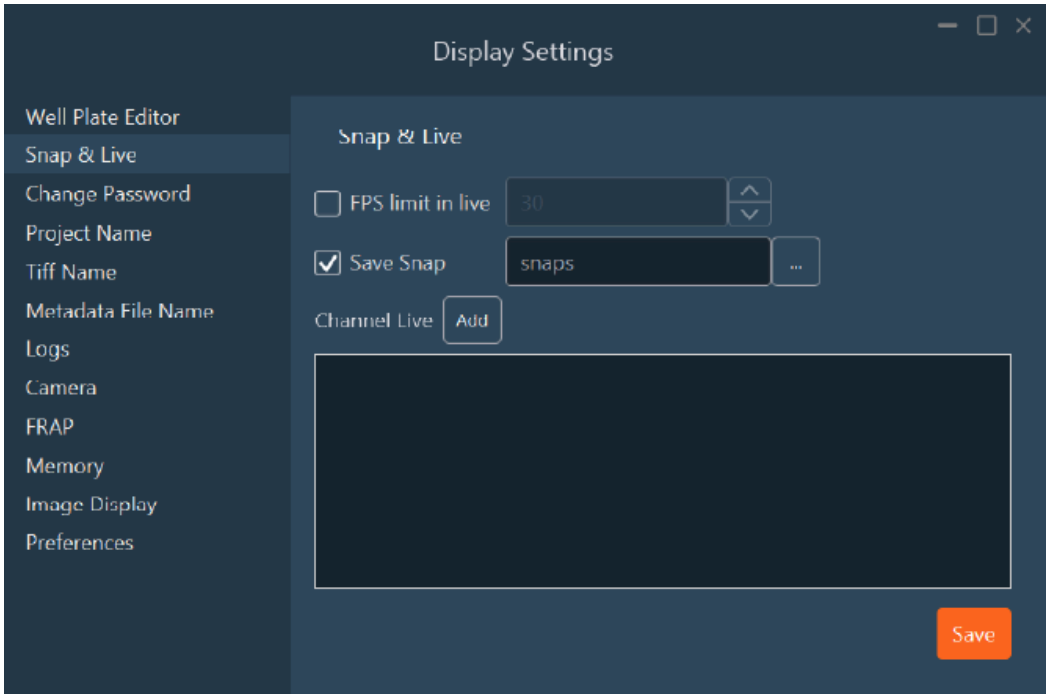
   e. Enter the well type (circle, square, rectangle, ...).

   f. Add the dimensions of the wells.

   g. Save this new well plate.

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

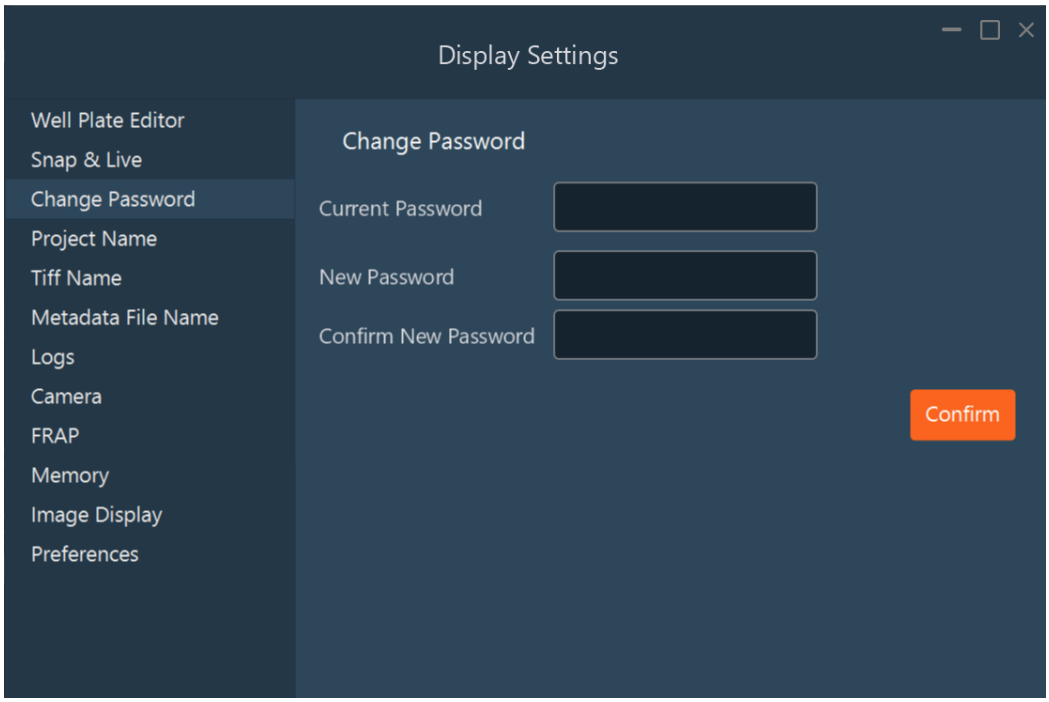
2. **Snap & Live:** Set options for the snap and live.



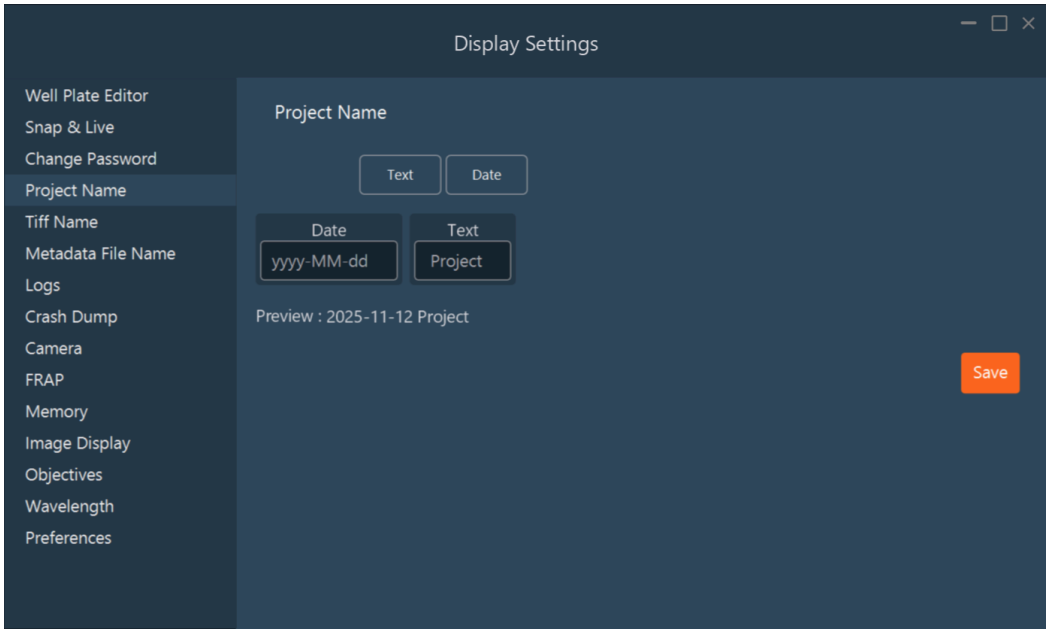
In this tab, you can:

a. Set a frame rate (FPS) limit for Live mode.
b. Change the directory where snapshots are saved.
c. Define a default channel for Live mode.

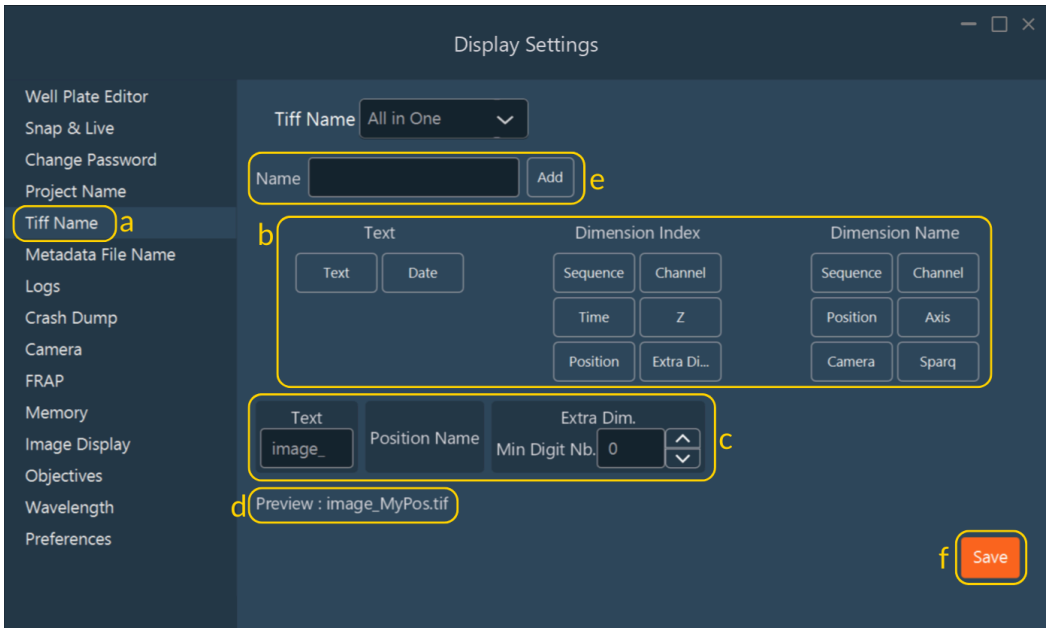
3. **Change Password**: Set or change the password to switch from user mode to expert mode.



4. **Project Name**: Set the default name of your projects.



5. **Tiff Name:** Change the format of the image saved.
   For example, by default, one file is saved per position (including all other dimensions). However, you can choose to save the image as one file per position per channel instead.



   a. Select the **Tiff Name** tab.

   b. Here are all the elements that can be automatically added to the standardized name of saved files. Add them by dragging and dropping them into the name construction tools below. Generally, the name structure is: text + dimension name + dimension index.

Note: The dimension index allows you to choose the number of digits following the dimension name.
For example: if you have 3 positions, the name of your files will be image_pos1, image_pos2, image_pos3.

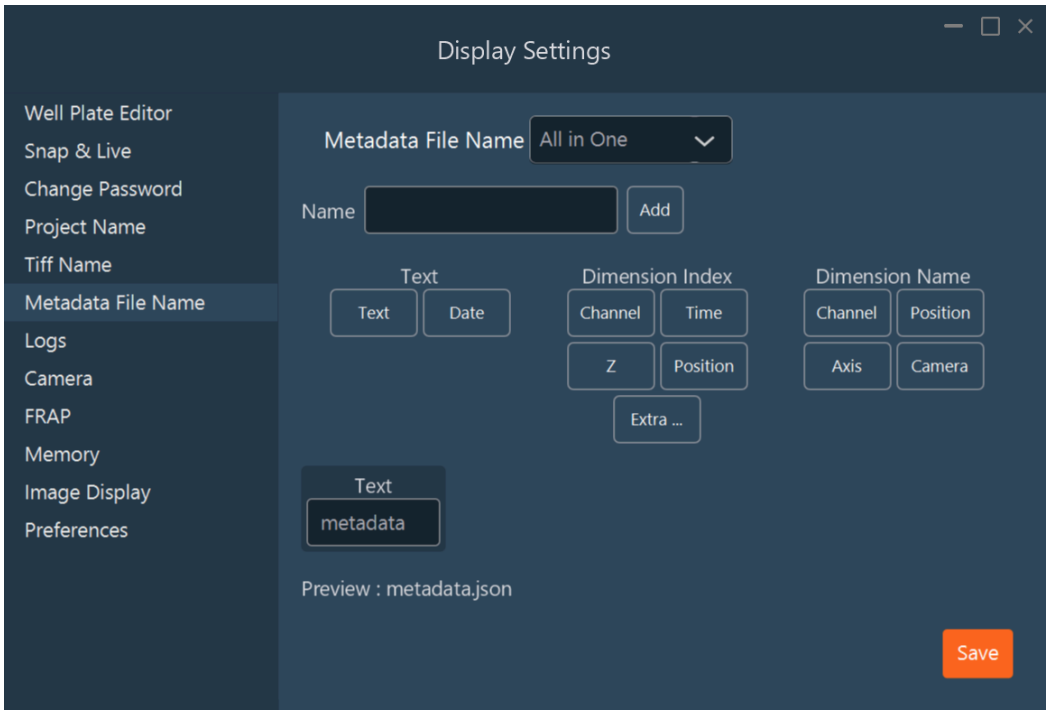
c. Name construction tool that shows schematically the name of the future saved file.

d. Name preview.

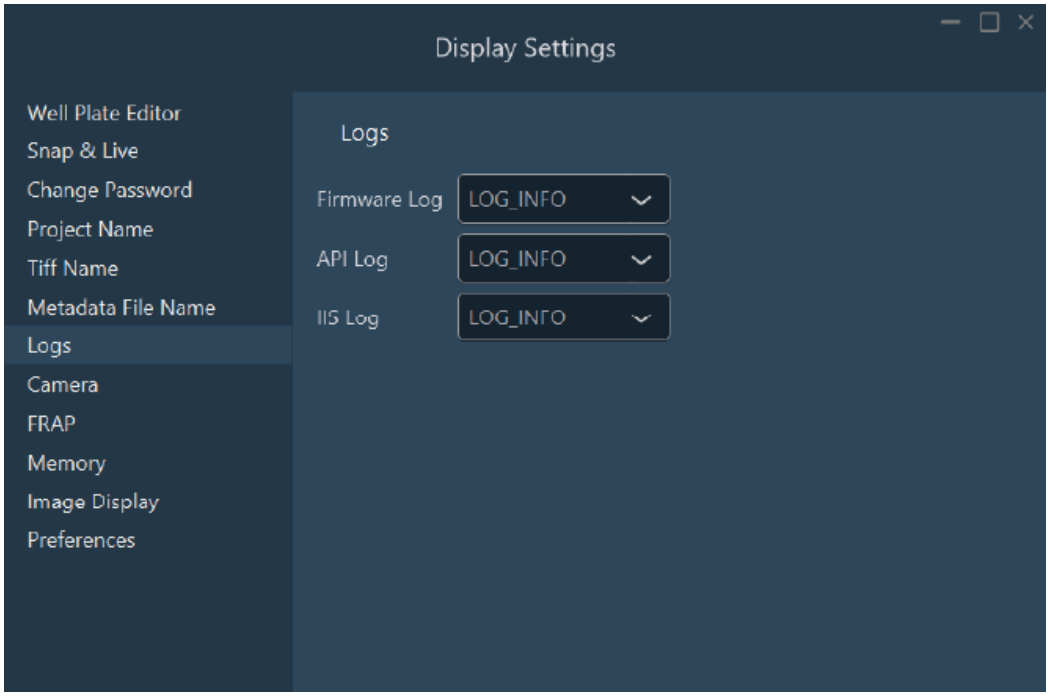
e. Enter a name and click on the **Add** to save this new default name.

f. When you like the name structure, click **Save**.

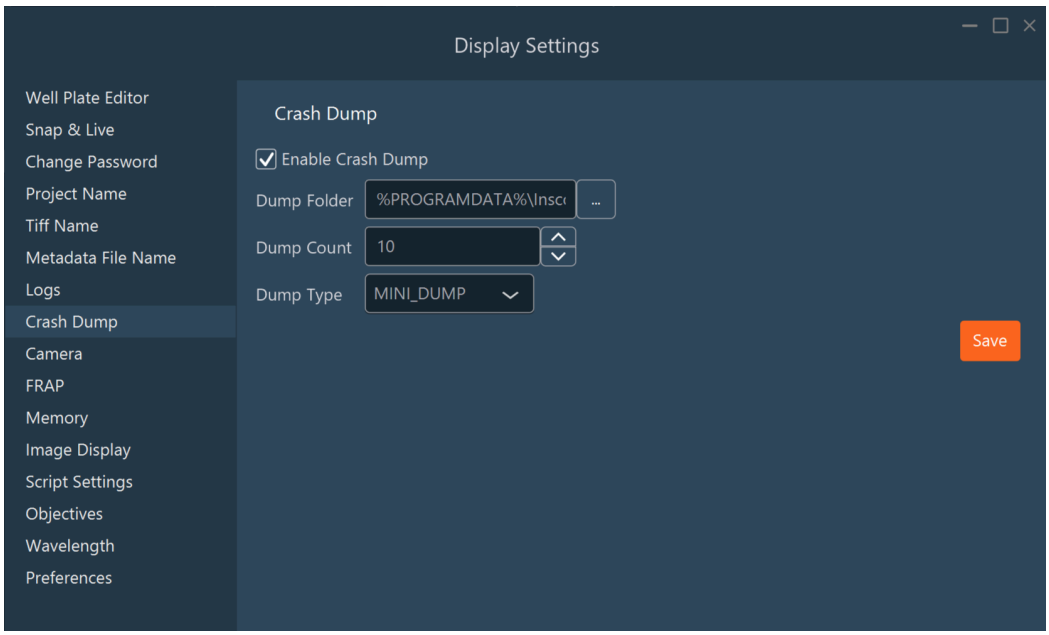
6. **Metadata File Name:** Change the format of metadata file saved.



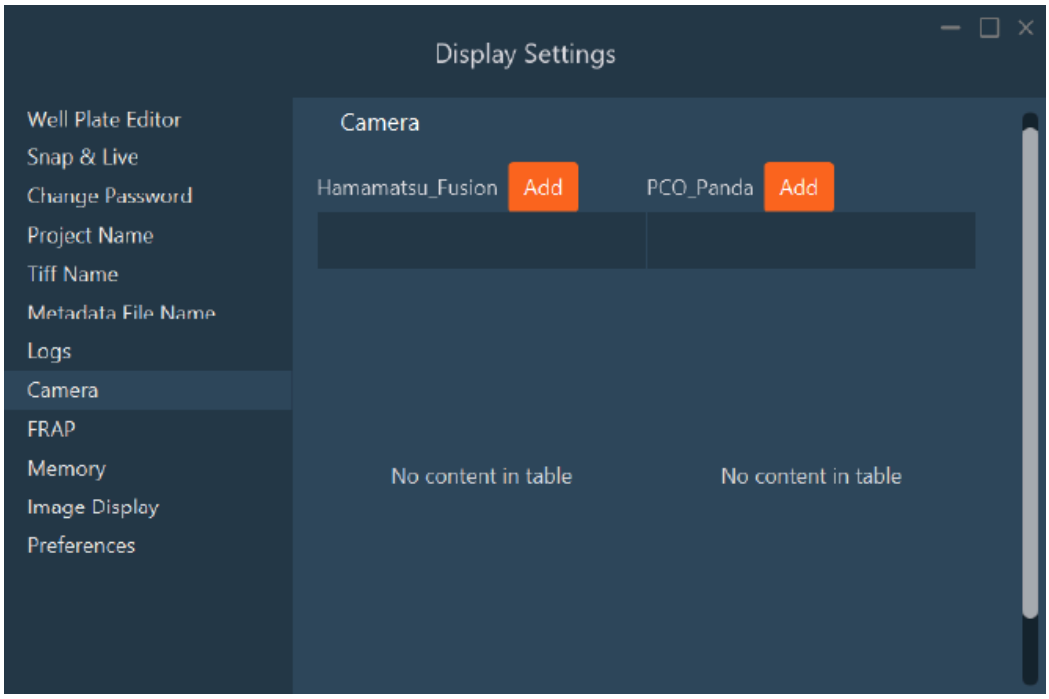
7. **Logs**: Modify logs level.



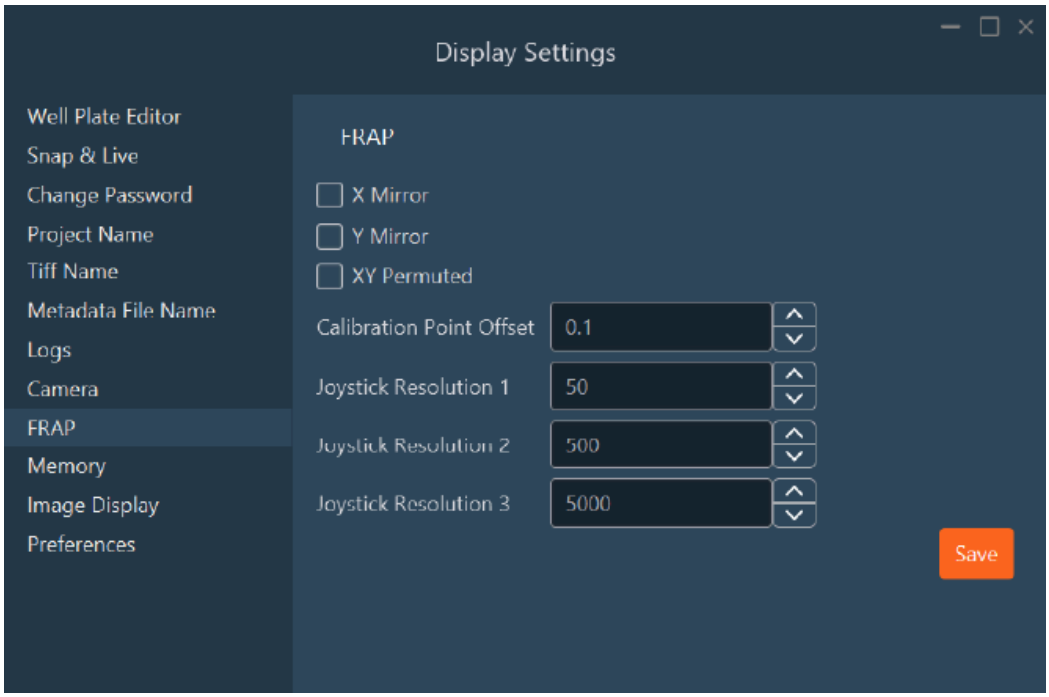
8. **Crash Dump:** Enable the creation of a Windows crash report if IIS crashes.
    - # Dump folder is the folder name where the report will be saved.
    - # Dump count: The maximum number of reports retained (10 by default, the oldest are automatically deleted).
    - # Dump Type:
        - # MINI_DUMP (by default): Saves only the essential information about the crash (sufficient in most cases).
        - # FULL_DUMP: Saves all memory used by IIS (takes longer to generate and uses more disk space).



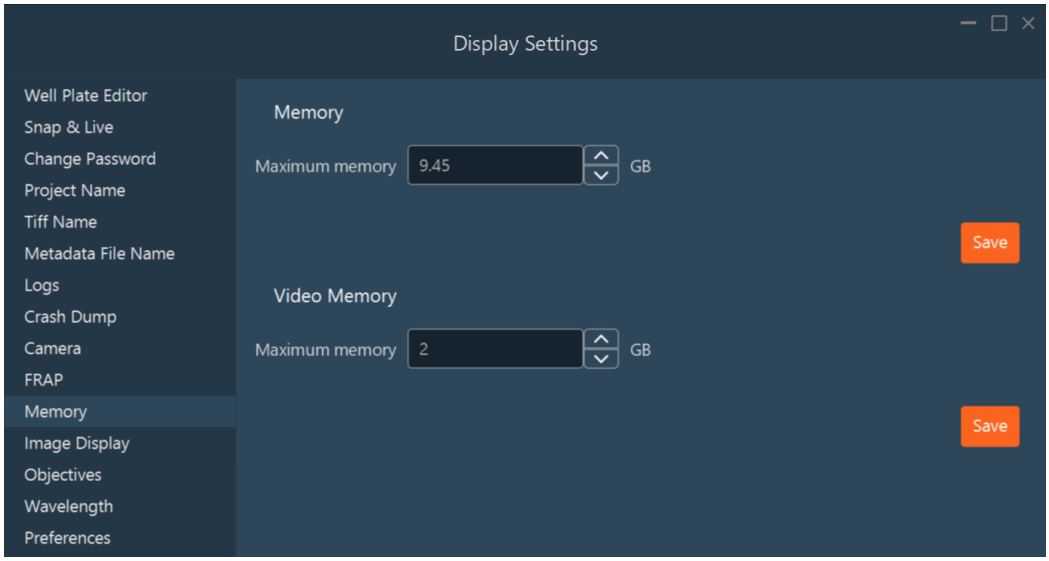
9. **Camera:** Find the camera installed on your system. If you have multiple cameras and need to rotate to see the same field of view, click the **Add** button to select the desired rotation.



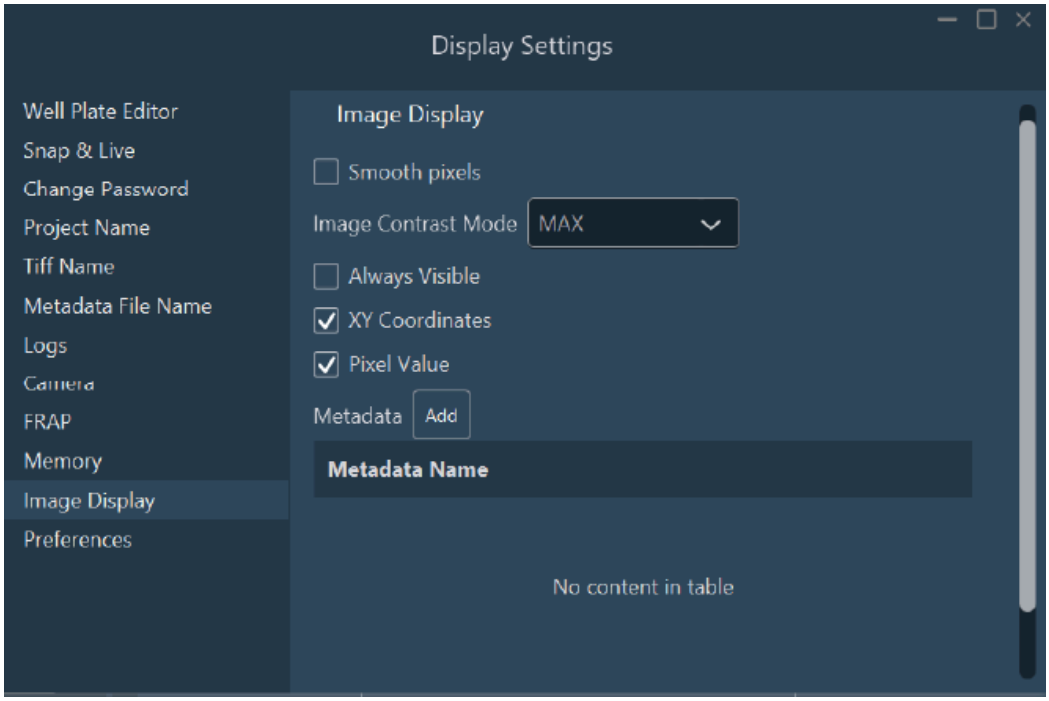
10. **FRAP:** Find the FRAP parameters and modify the sensitivity of the virtual joystick during the FRAP calibration.



11. **Memory**: Assign memory for the Inscoper software. By default, 2 GB is allocated to video memory, and 60% of the available RAM is allocated to the software.

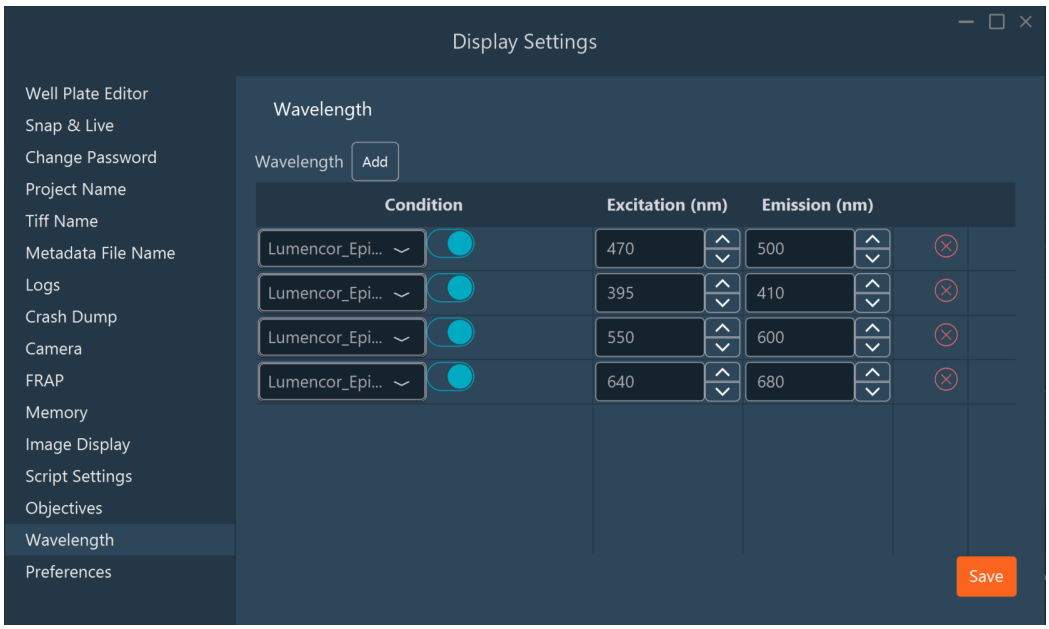


12. **Image Display:** Select which setting you want to apply



13. **Objectives:** Indicate the numerical aperture and refractive index of your objective. This information is used for the Nyquist calculation and other mathematical computations.

14. **Wavelength:** Indicate the excitation and emission wavelength of your light source or laser. This information is used for shading correction, Nyquist calculation, and various processes, and will also be added to the metadata.

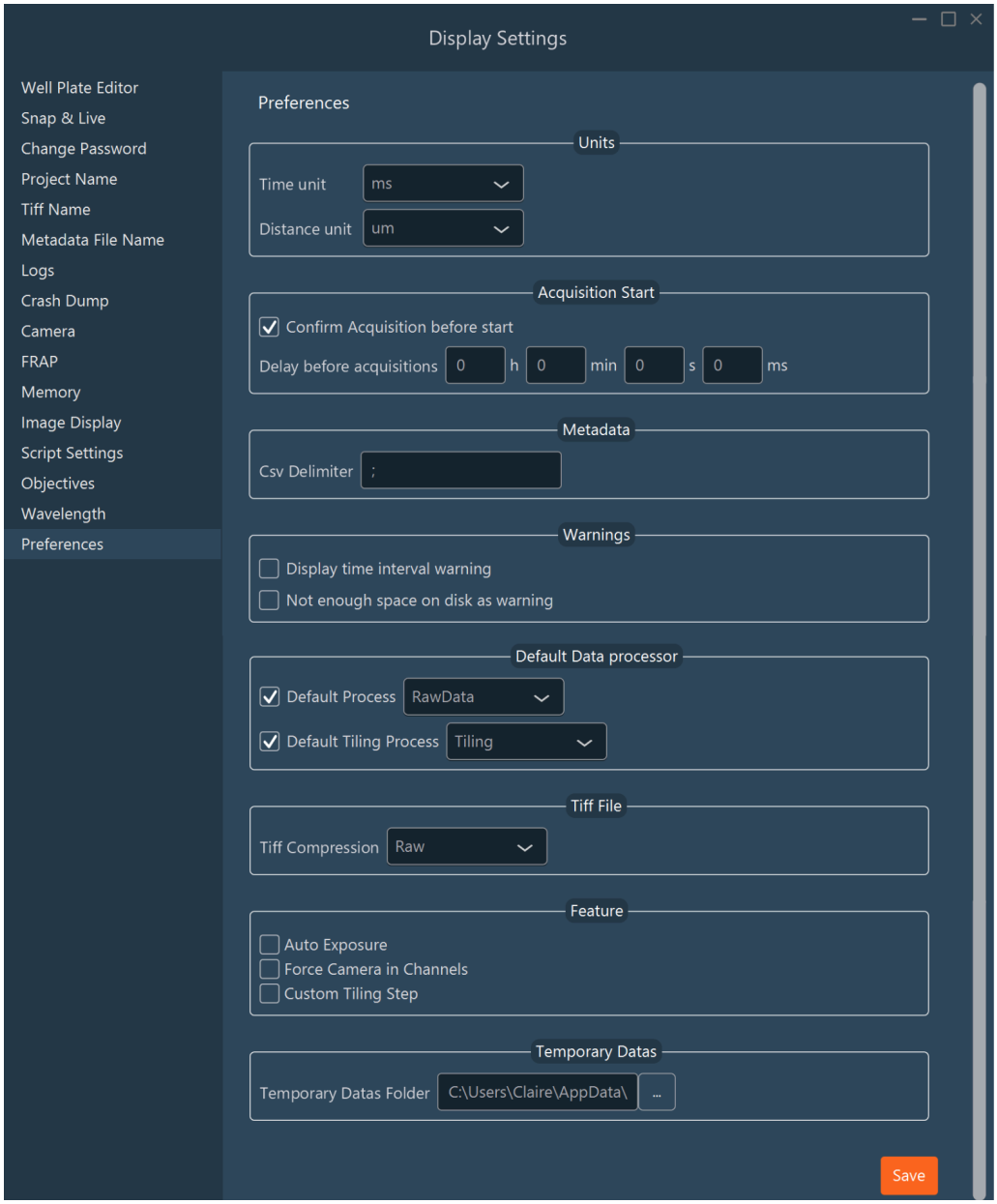


15. **Preferences**: Select and set up some other parameters (measuring units, acquisition delay, warnings, etc.).

Auto exposure: Allows access to the auto-exposure button.

Force camera in channels: Useful when using multiple cameras or a Maico module. When this feature is enabled, the selected camera is saved within the channel.

Custom tiling step: When enabled, you can specify a custom overlap (in µm) for your tiling acquisitions.

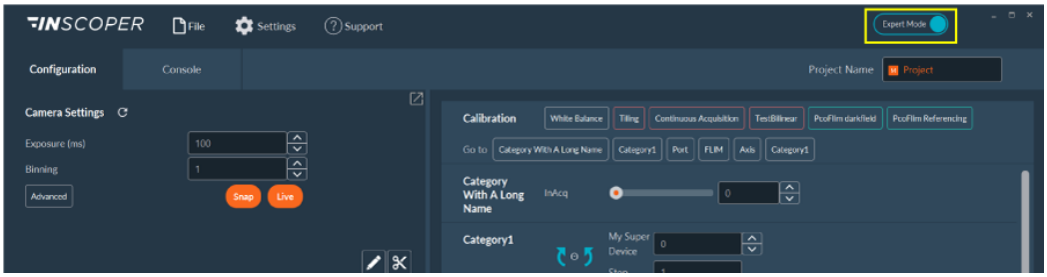


1.1.4. Configuration

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 different parts:

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



Expert Mode	**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	**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.

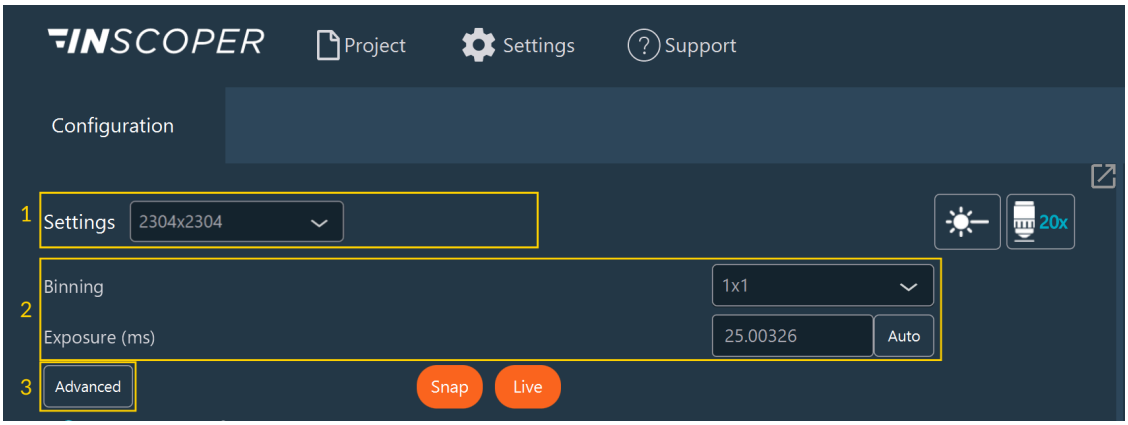
2. If you have two screens, you can split the interface by clicking this button. This allows you to display the camera view on one screen (with a larger image) while keeping the parameters on the other screen.
3. Access to channel and objectives.
4. Camera settings.
5. Live image viewing.
6. Tool to interact with the image.
7. If necessary, you can move the dividing bar between sections to enlarge either the camera view or the settings area.
8. Calibration protocols.
9. Access all motorized devices of the microscope, create channels, and save them for all your acquisitions.

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.

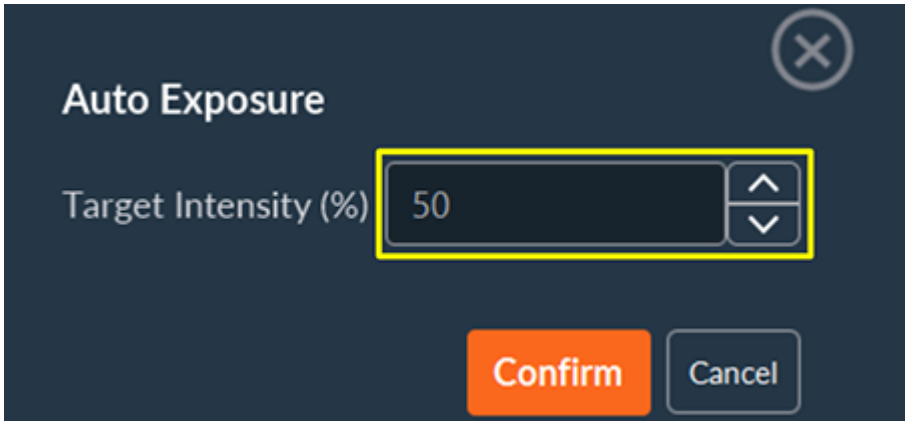
NB: the window display may change depending on the devices of your microscopy system.

1.1.4.1. Camera settings

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

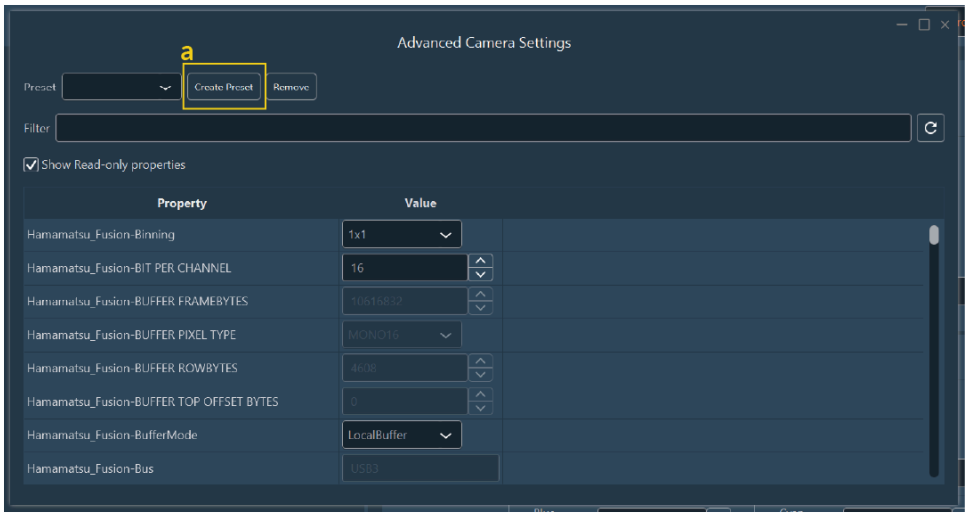


1. If you want to use the camera preset, you can select it from in the drop-down list.
2. Set the exposure time and binning. You can activate the auto exposure mode in the display settings. If it is activated, the **Auto** button will appear. This mode is useful for automatically adjusting the camera exposure time to avoid pixel overload. First, the option calculates the pixel intensity in the image in real time. The contrast is then adjusted to the value you specified.



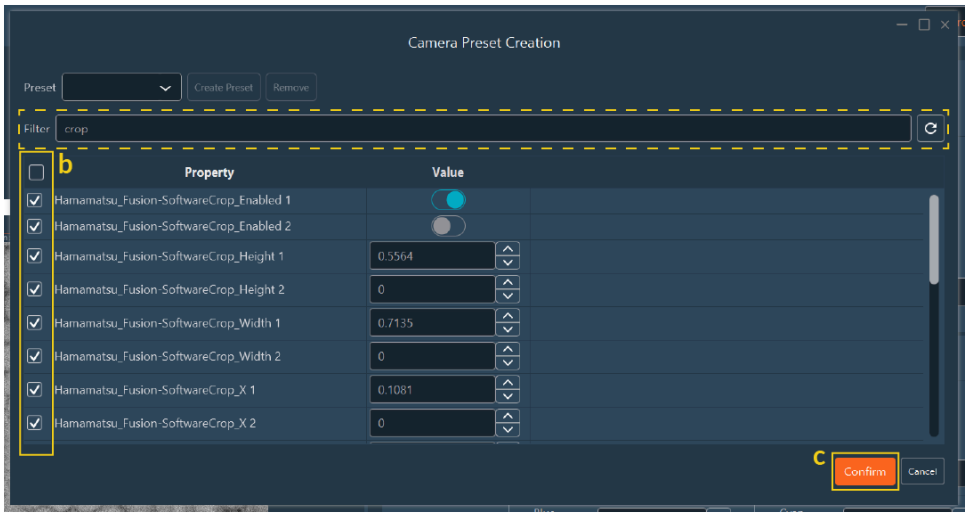
3. You can also have access to all **advanced settings** of the camera and create a camera preset:

   a. Click on **Create Preset**:

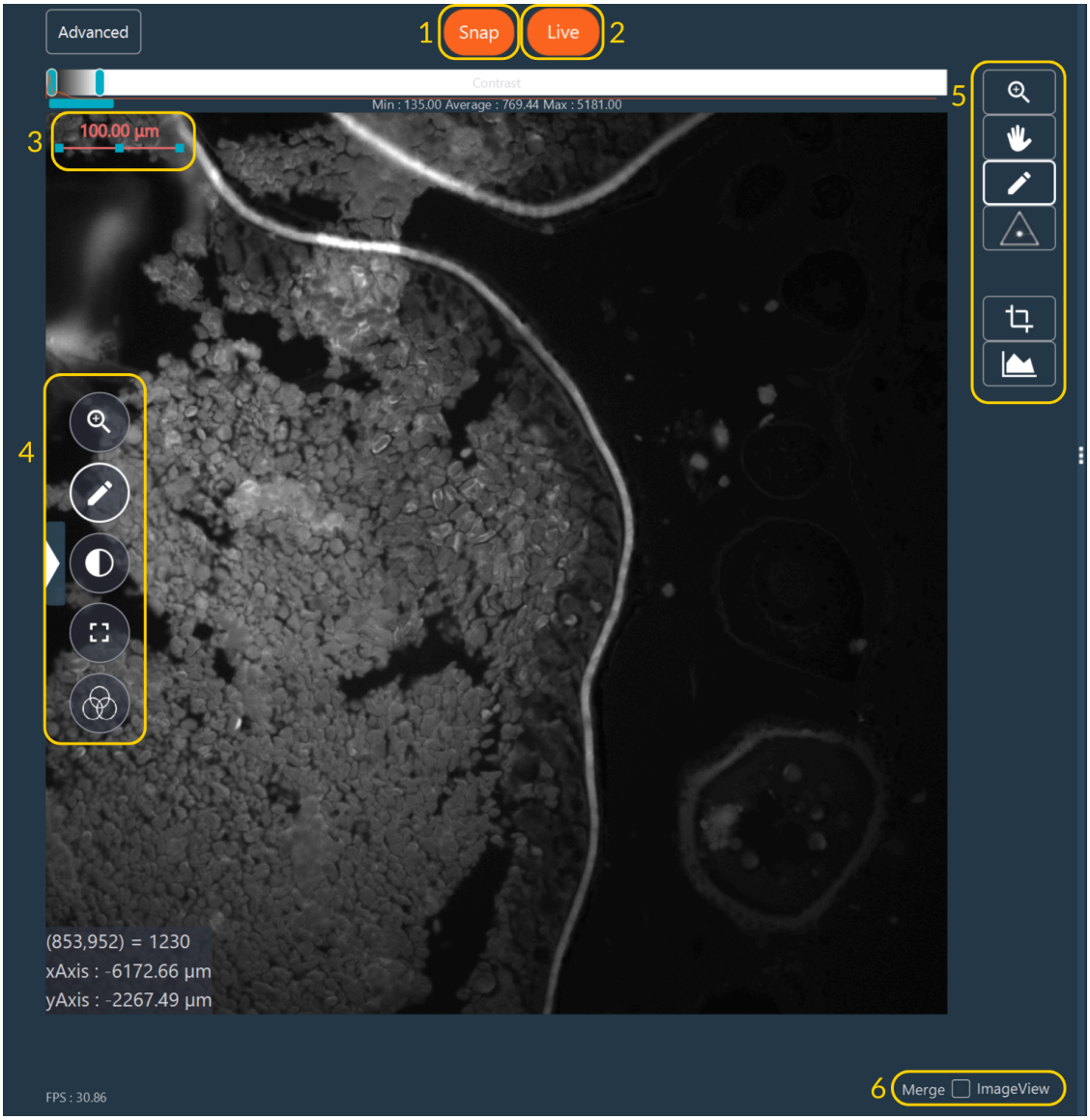


   b. Select the parameters which will be saved in the camera preset by checking boxes. You can filter the parameters by writing in the search bar.

c. Then Confirm and choose the name and location to save your camera preset:



1.1.4.2. 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 it to modify its orientation and length.
4. Tools to interact with the image:

	Numerical zoom.
	You can select this option to add ROI.
	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.

5. **Access tool to more advanced tools**:

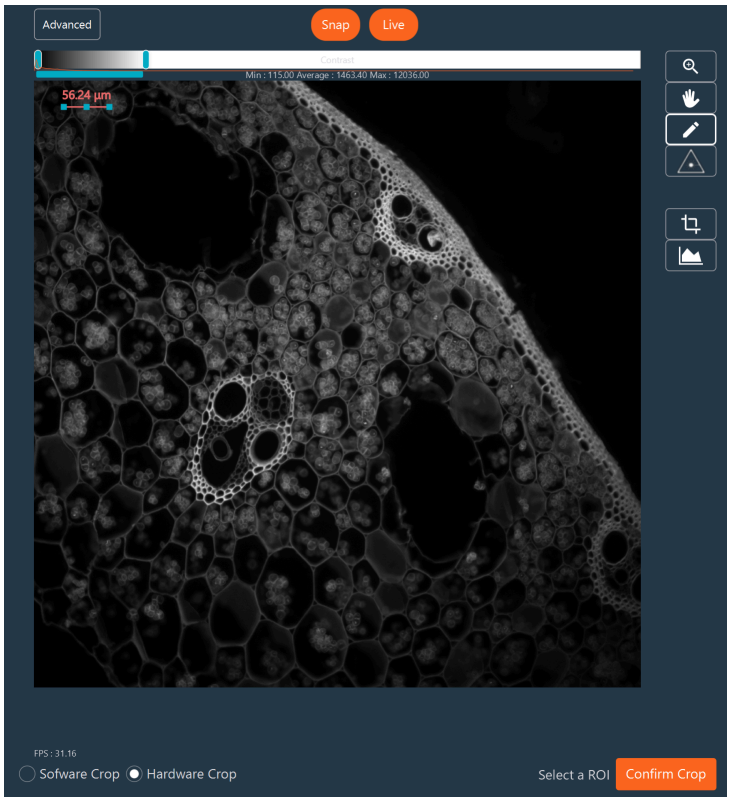
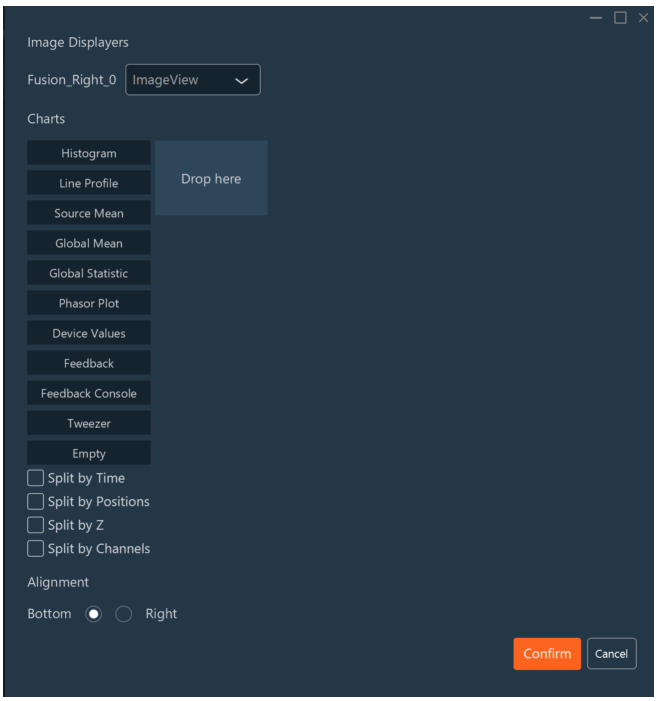
	Numerical zoom: Turn the mouse wheel over the image.
	Move the stage by a drag and drop directly on the image, and use the mouse wheel to navigate along the Z-axis.
	ROI access.

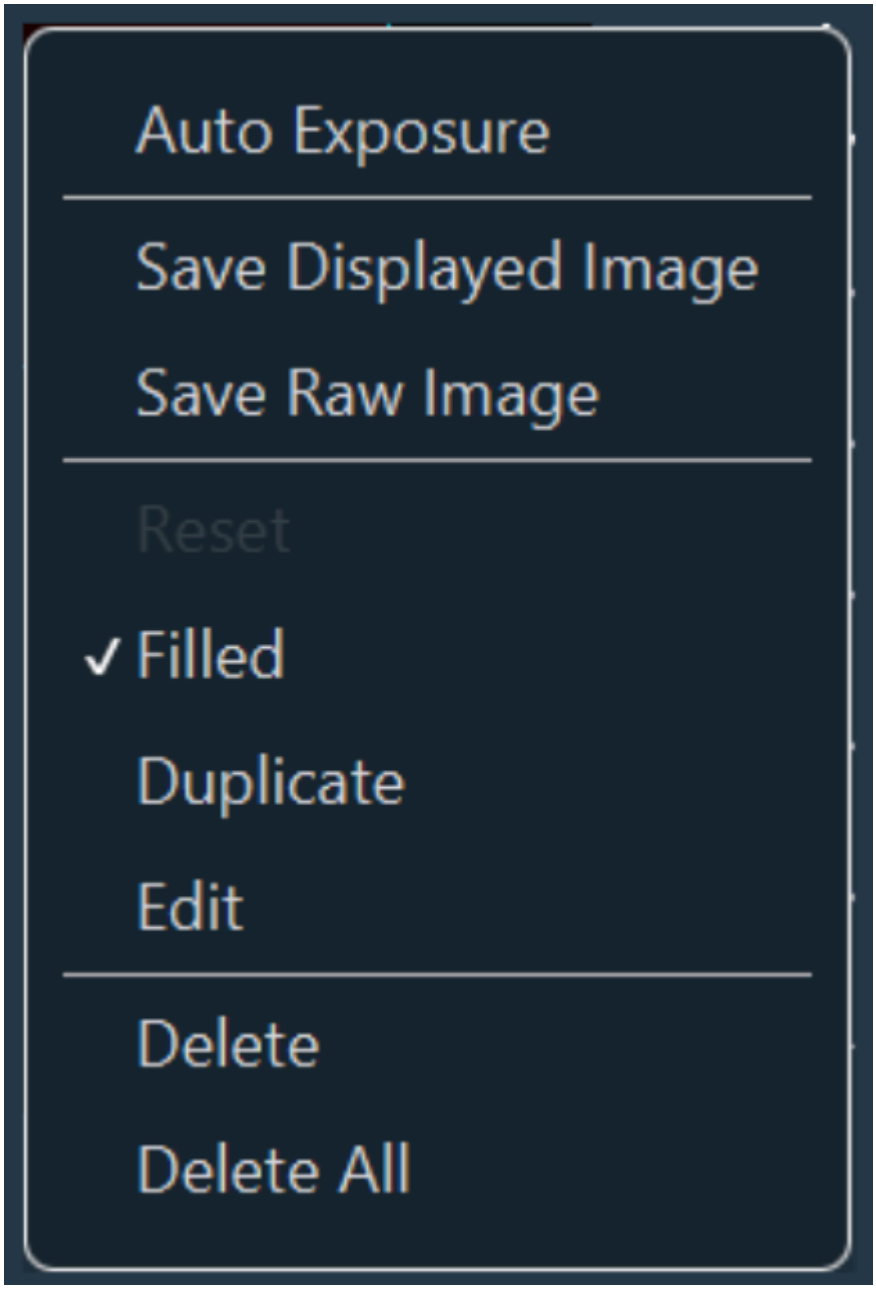
To create a ROI on the image, you can choose between two modes:

	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.

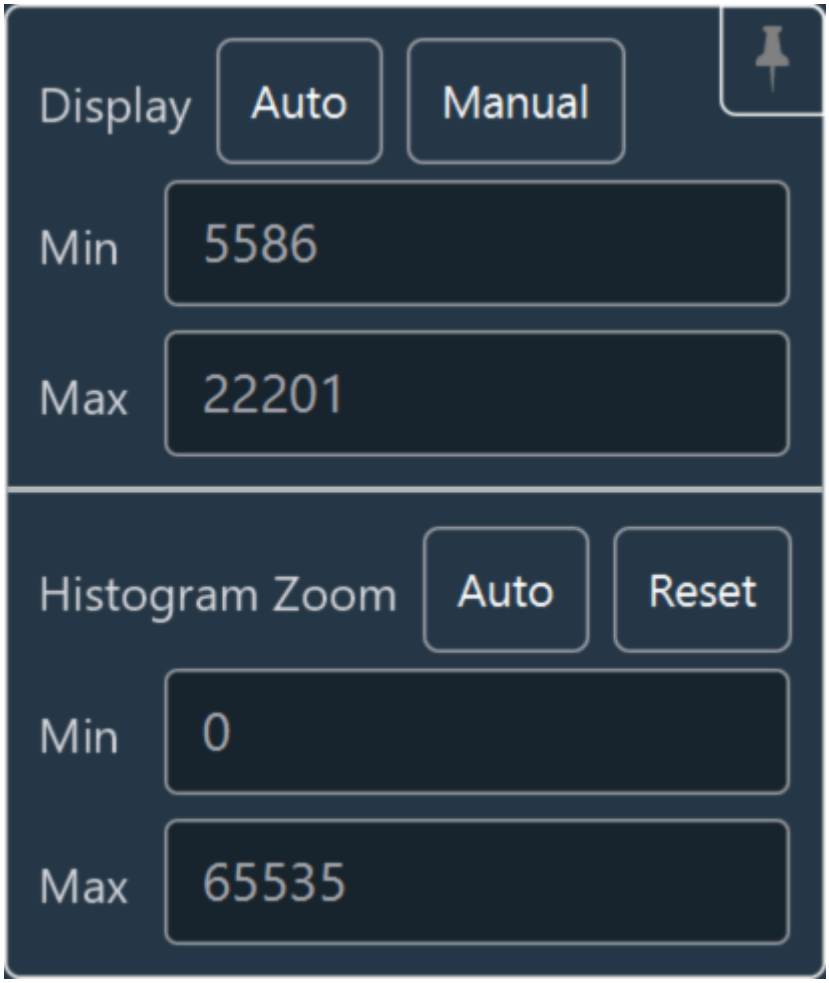
	Fire on Click function available only if the FRAP module is installed.
	Crop access. Can be set as either hardware or software crop. Draw an ROI, select **Hardware** or **Software** crop mode, then click **Confirm Crop** to apply. 
	Histogram access. Display of the histogram or other visualizations below or to the right of the image. Once it's confirmed, you can adjust the X axis according to the camera depth by placing your mouse at the top of the histogram. 



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.

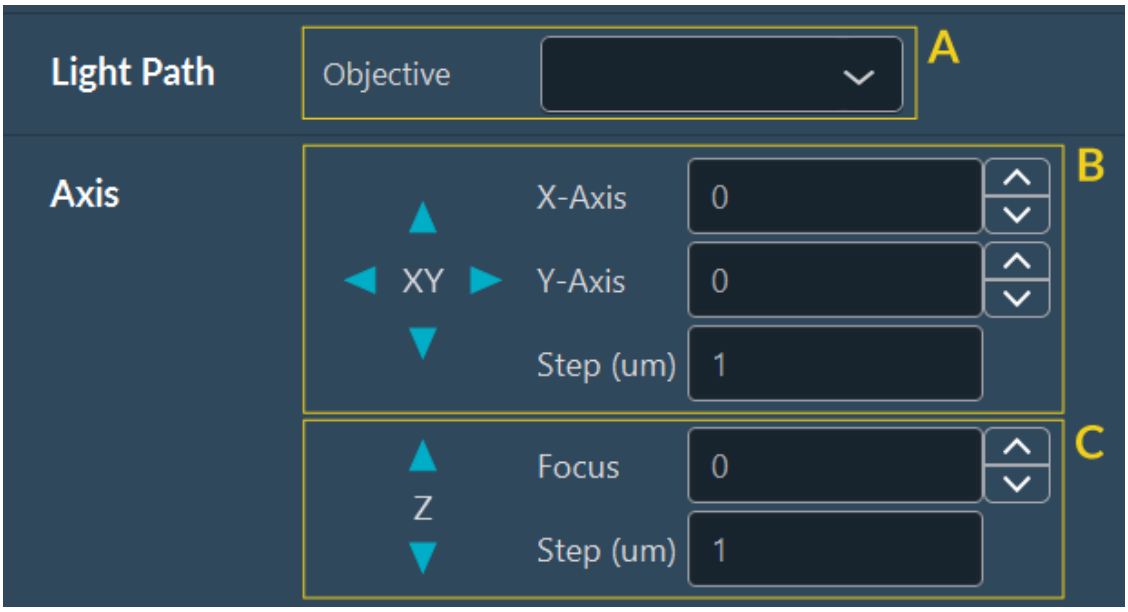
6. **Merged images**: If you have more than one camera, you can merge the images from all the cameras in Live.

The contrast adjustment tools can be accessed by hovering the mouse over the contrast bar at the top of the image. You can modify the minimum and maximum contrast values and zoom in on the histogram by adjusting these values.



1.1.4.3. Control of motorized devices

Interaction with motorized devices

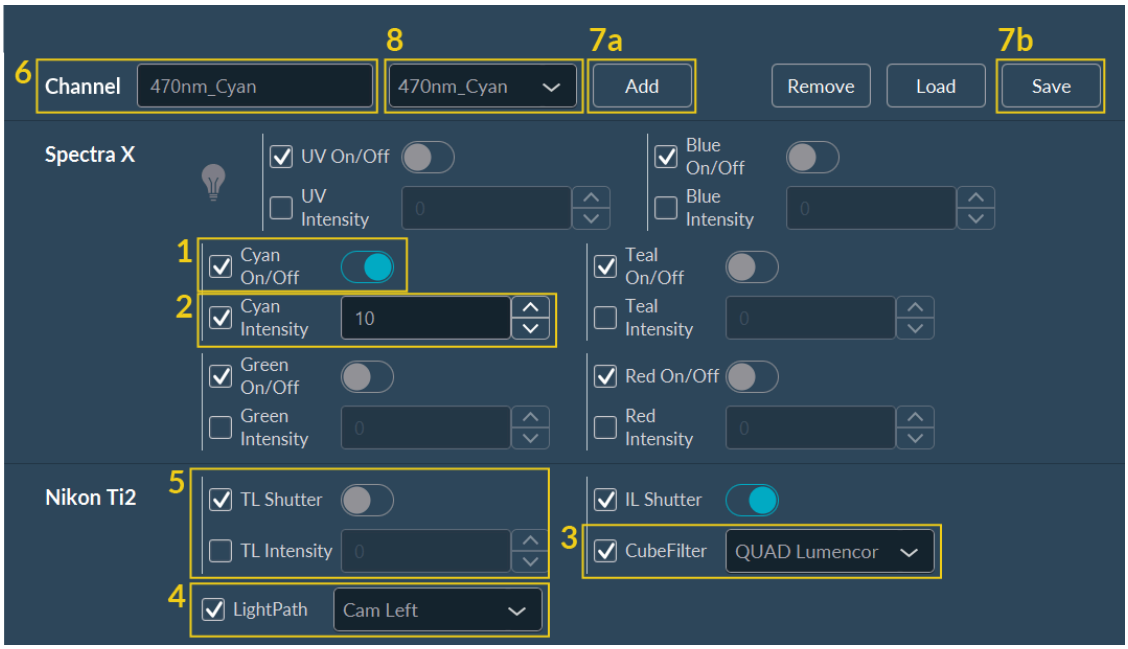


You have access to all devices controlled by Inscoper I.S., including microscope, stages, shutter, light source, wheel filters, piezo, microfluidic element, ...

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.

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.

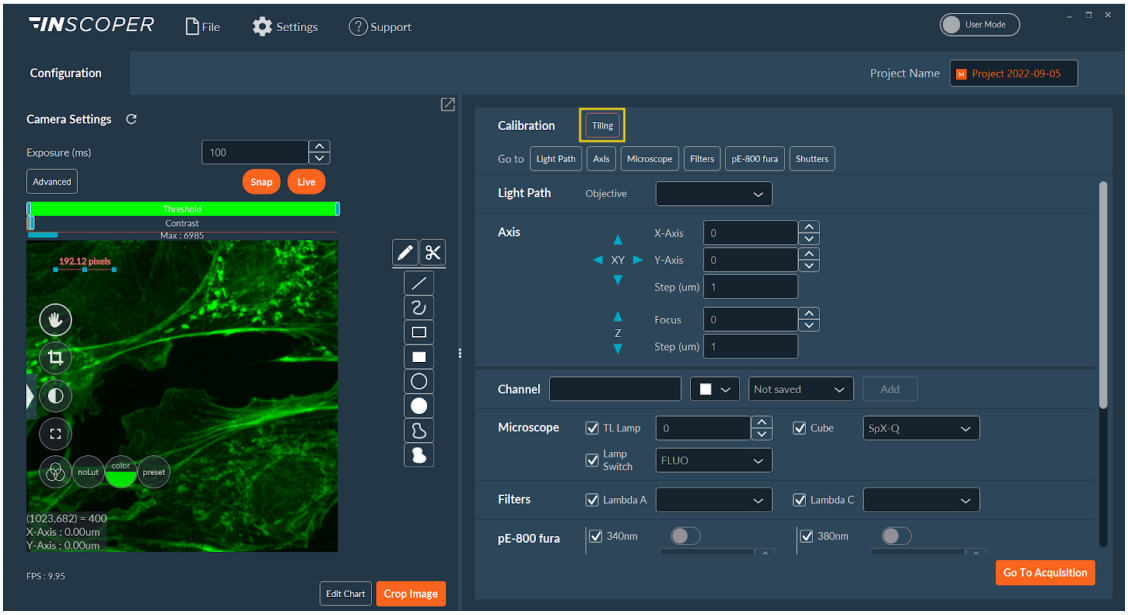
1.1.4.4. Calibration protocols

1.1.4.4.1. Tiling calibration

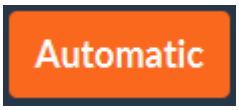
The tiling tool is used to image large samples. The whole final image is divided into several images, called "tiles", acquired one by one and "stitched" afterwards to constitute the whole sample.

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.

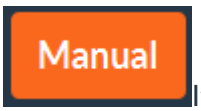
Click on **Tiling** in the Calibration section (if not yet calibrated, the button will be colored orange).



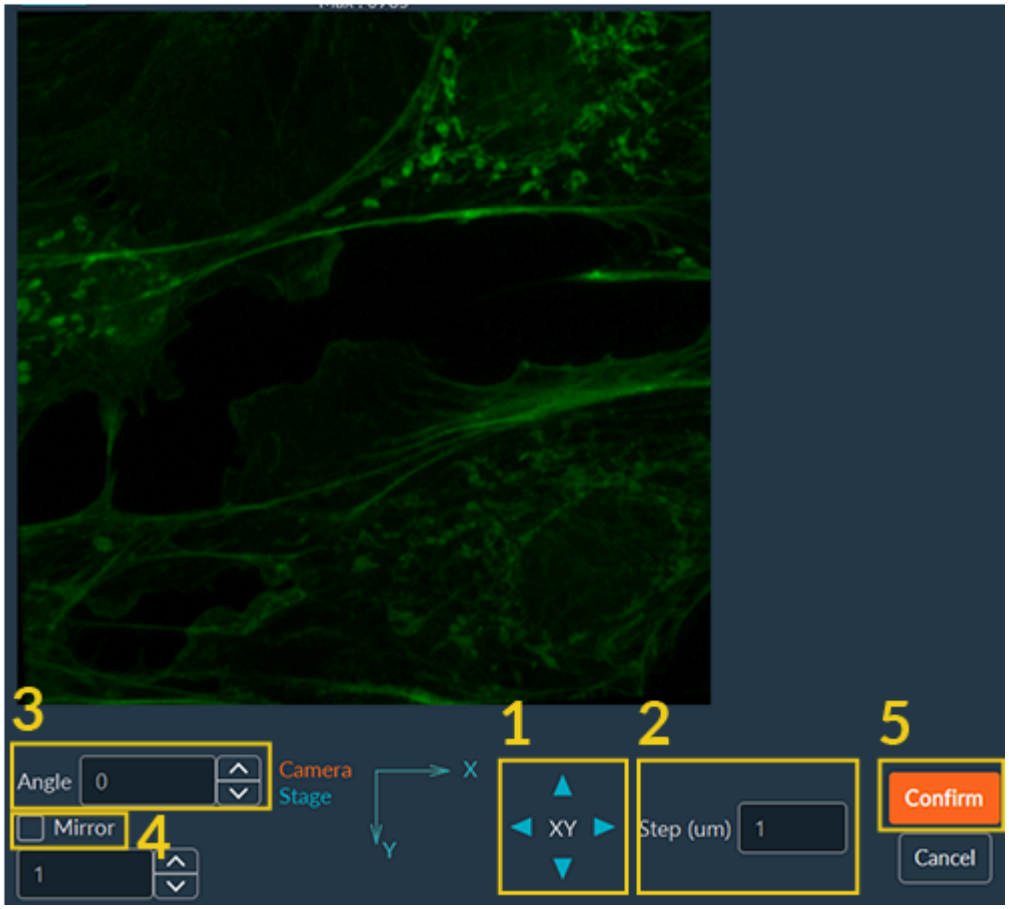
Calibration protocol can be performed according to two different approaches: **Automatic** or **Manual**.



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.

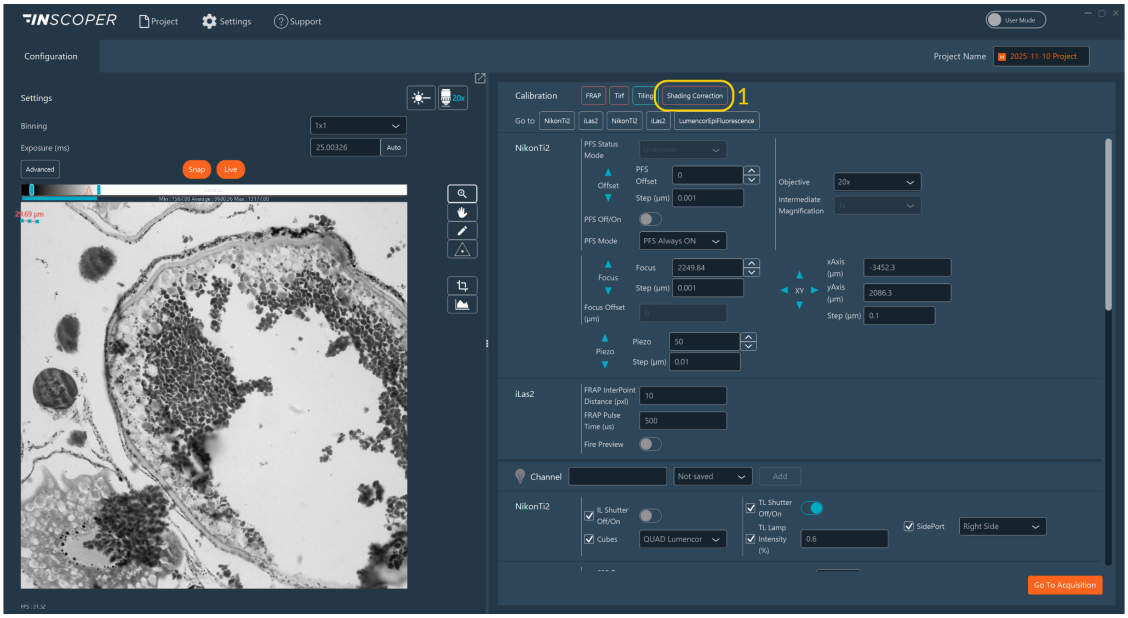


NB: First, be sure that the camera is switched on **Live**.

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.

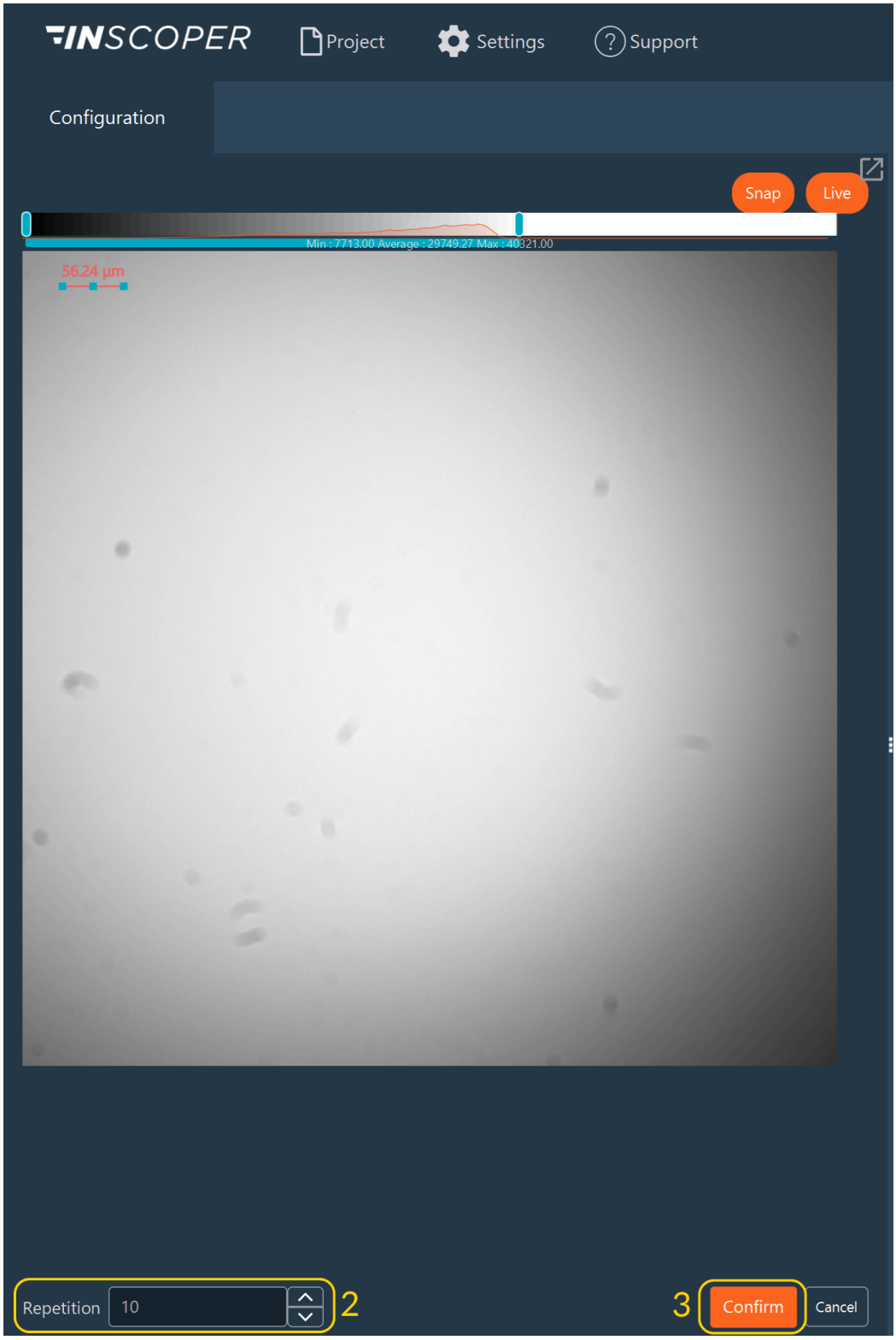
1.1.4.4.2. Shading correction

Shading correction is a tool used to ensure uniform illumination of the field of view across the image. Perform this calibration using a **chroma slide**.

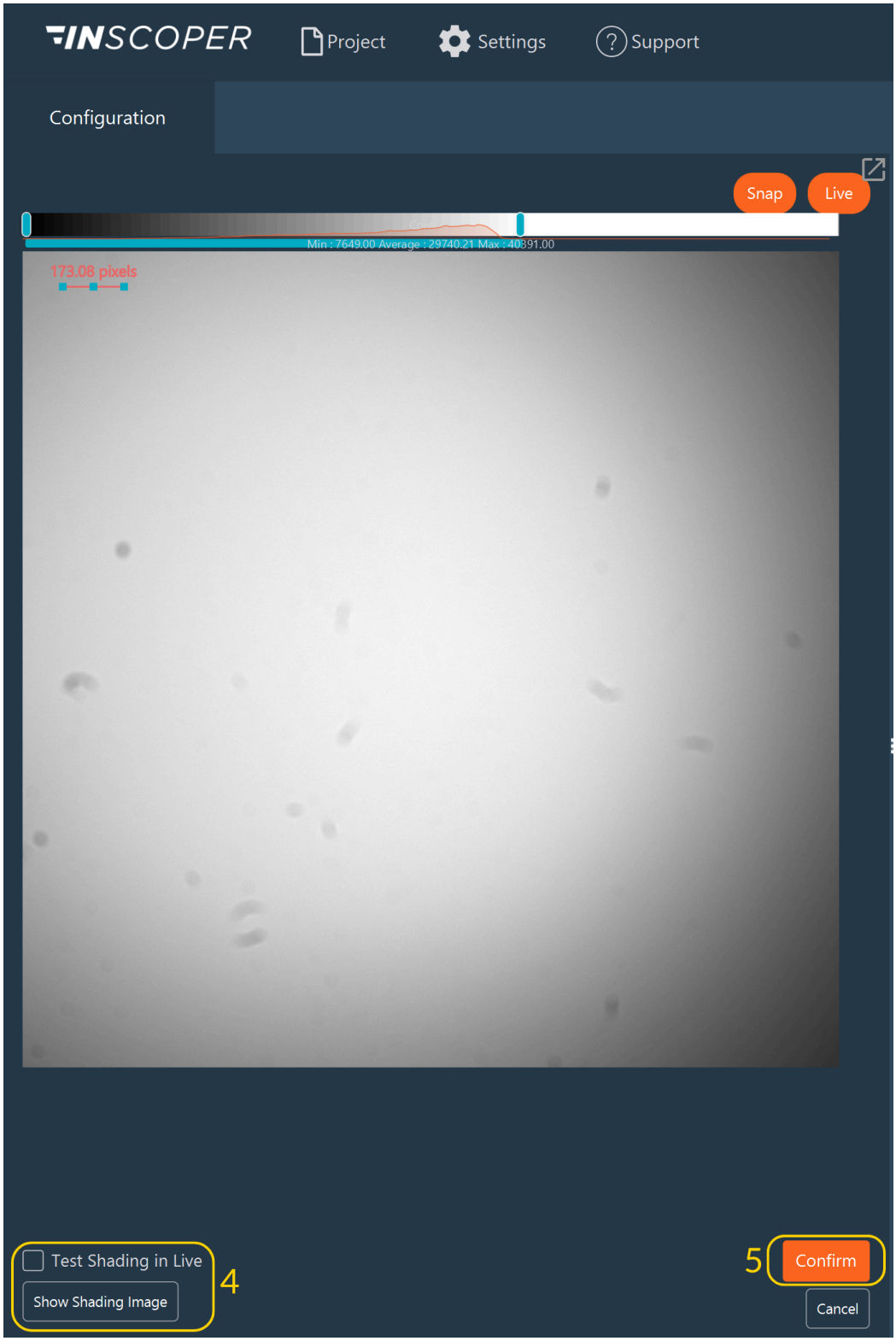


Performing a Shading Correction

1. Click on **Shading Correction**.
2. Specify the **number of acquisitions** — the shading correction image will be generated as the **average** of all captured images.



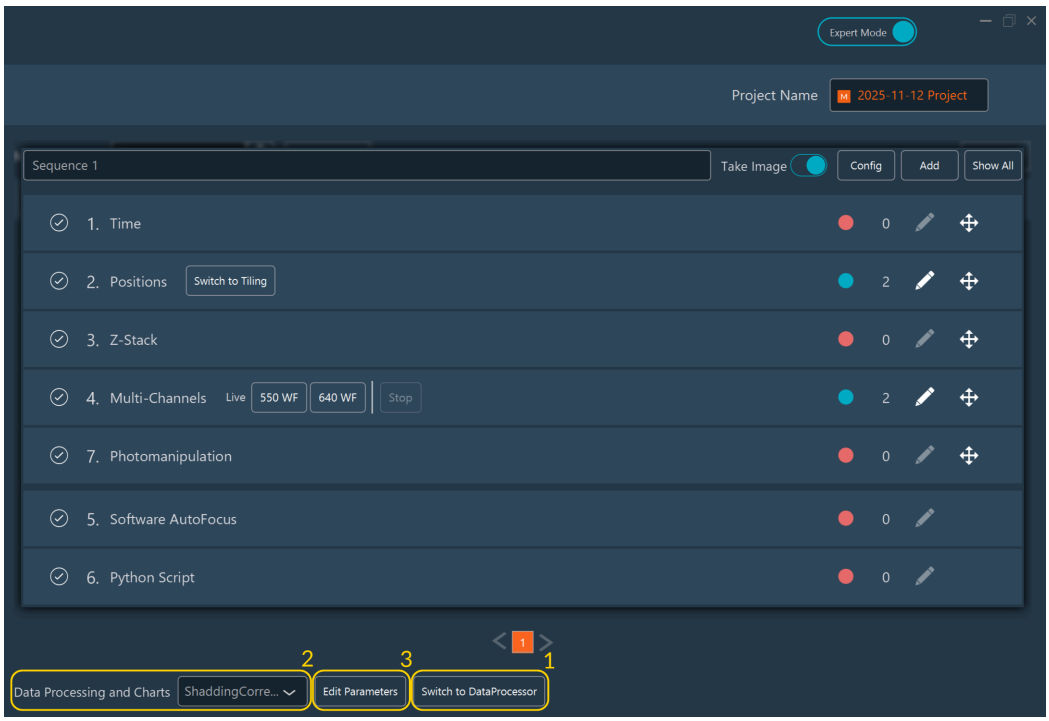
3. Click **Confirm** to start the calibration
4. Once the calibration is complete, you can:
    # Verify the result by clicking **Test Shading** in Live mode, or
    # View the generated shading image by clicking **Show Shading Image**.



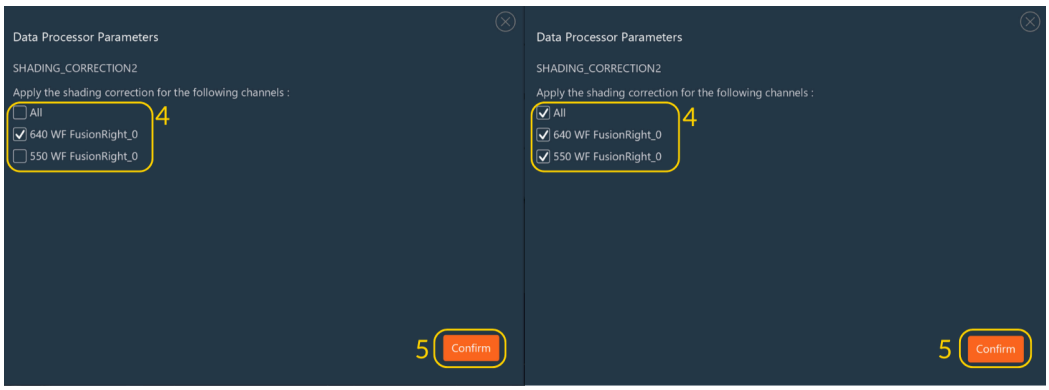
5. Click Confirm to validate the calibration.
6. When the shading calibration is successfully done, the **Shading Correction** button changes from red to green.

Using shading correction by channel with the data processor

1. Create your data processor (in Expert Mode) including the following nodes: **Raw Data**, **Shading Correction**, and **Tiling**.
2. Apply the data processor after configuring your sequence (during the data processor and charts selection step).
3. Click **Edit Parameters**.

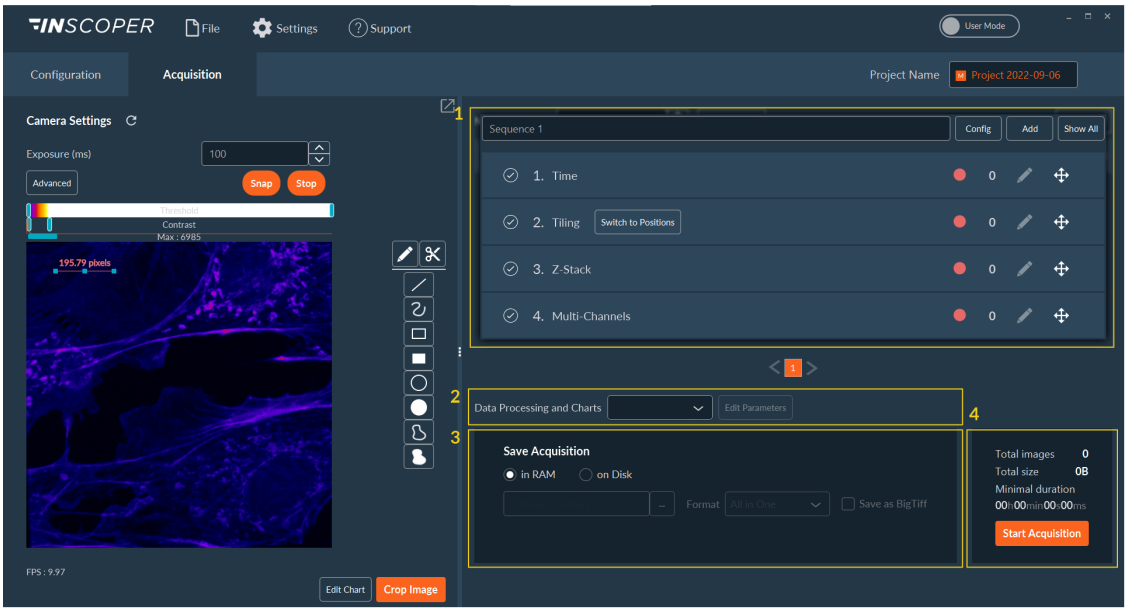


4. Select the channel(s) on which you want to apply the shading correction — it can be a single channel or all channels.
5. Click **Confirm** to validate your selection.



1.1.5. 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.

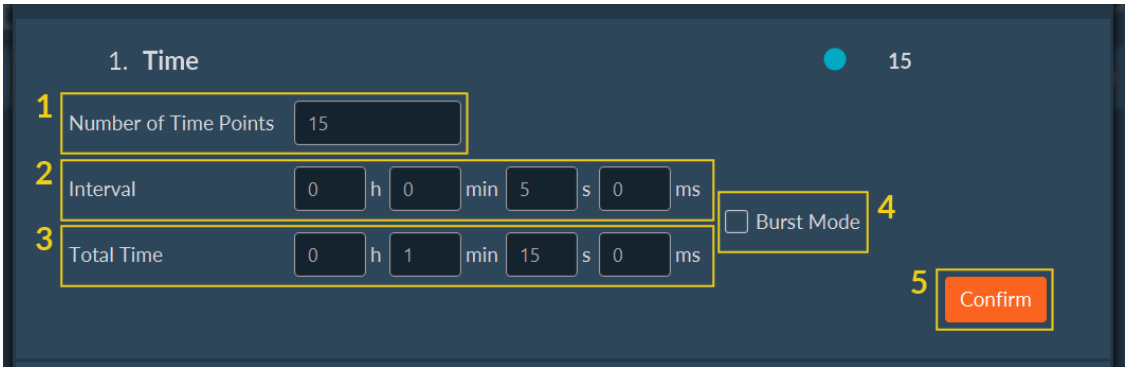
The interface may be different (especially the dimension's list) depending on the type of equipment in your microscope system.

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 deactivate.
	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.

1.1.5.1. Time

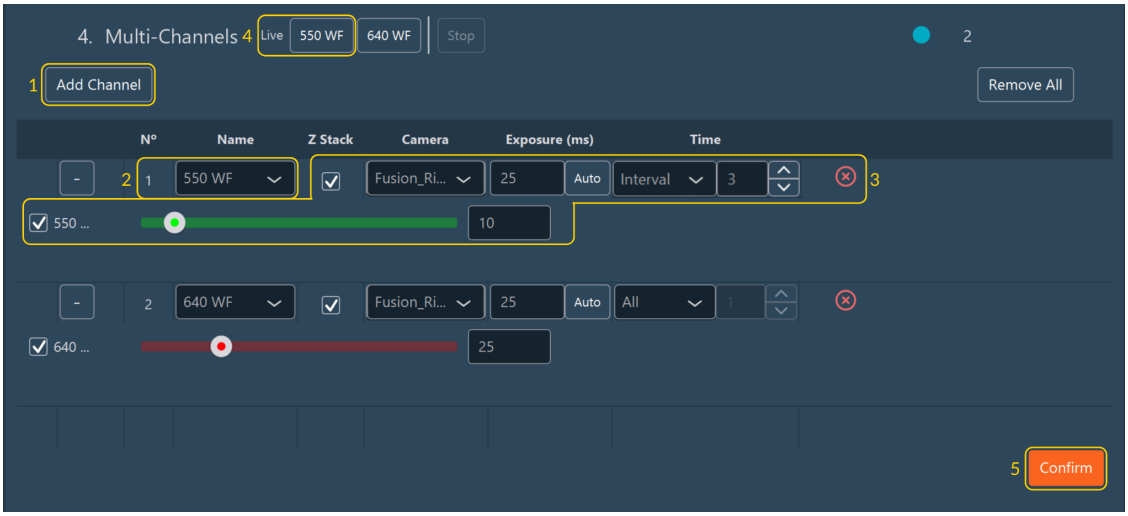
The **Time** dimension is used to run time-lapse and image cells over a long period of time.



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**.

1.1.5.2. 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:
    - **Z-stack**: Apply z-stack dimension.
    - **Z-Offset**: Apply a different offset if the focus varies.
    - **Shutter Blink**: Optimize shutter control to limit sample exposure and prevent photobleaching.
    - **Camera**: Select the one that you would like to use.
    - **Exposure(ms)**: Adjust exposure time.
    - **Time**: Select the time interval at which you want to acquire the channel. You can choose to acquire it at all time points, only the first time point, only the last time point, or at a specific interval.
    - **Intensity**: Adjust the excitation light (depending of the light source of your system).
    - Delete your channel by clicking the red cross
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 adjusting the exposure time, click again on **Live channel** to update the image.

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

1.1.5.3. Positions

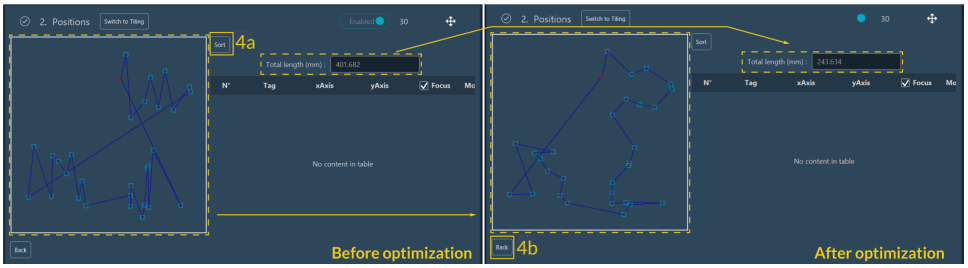
This dimension is dedicated to image different XYZ positions from a sample. You can switch from this dimension to the **Tiling** dimension by the button **Switch to Tiling**.

To use the **Positions** dimension:

1. Do a Preview of your sample.
2. Move the stage and the focus to the desired position.
3. Click on **Add position** to add the XYZ coordinates in the position list.
4. Click on the **Path** button if you need to optimize the **stage mouvement** when you have multiple positions.

    a. The stage moves following the order in which positions were added. You can view the complete movement path and calculate the total distance traveled. To optimize the path and reduce travel distance, click the **Sort** button.

    b. Once optimization is complete, click the **Back** button to return to the position dimension view.



5. If necessary use **Pattern Detection** tool which allows the recognition of patterns from the current live image or from ROI:

a. Click the **Start** button located in front of the Pattern Detection section.

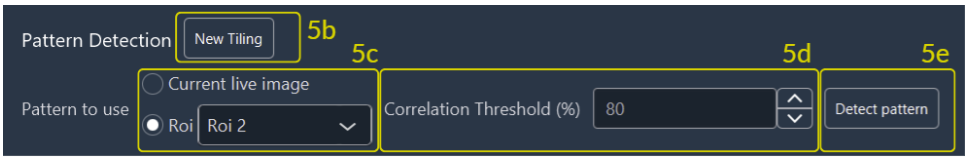
b. Create a new tile to find the pattern. Click on **New tiling**. Set it up as described in the Tiling dimension.

c. Select the pattern you wish to detect:

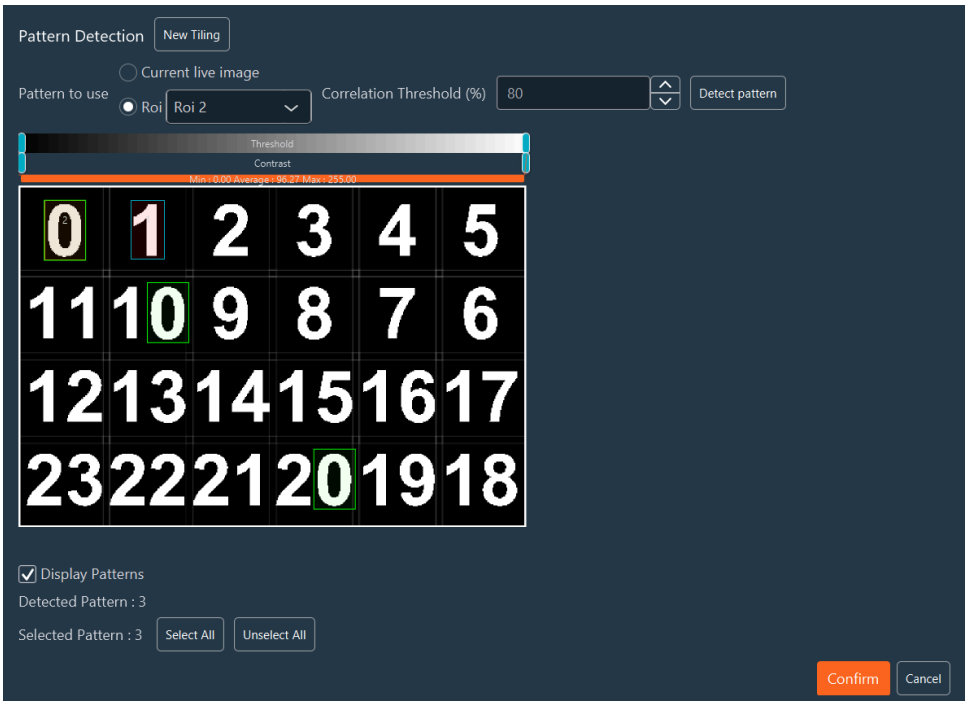
- **Current Live Image**: Use the pattern from the live image
- **ROI**: Draw an ROI around the specific pattern you want to detect.

d. Indicate the **Correlation Threshold** (that mean the percentage of similarity between pattern and other form).

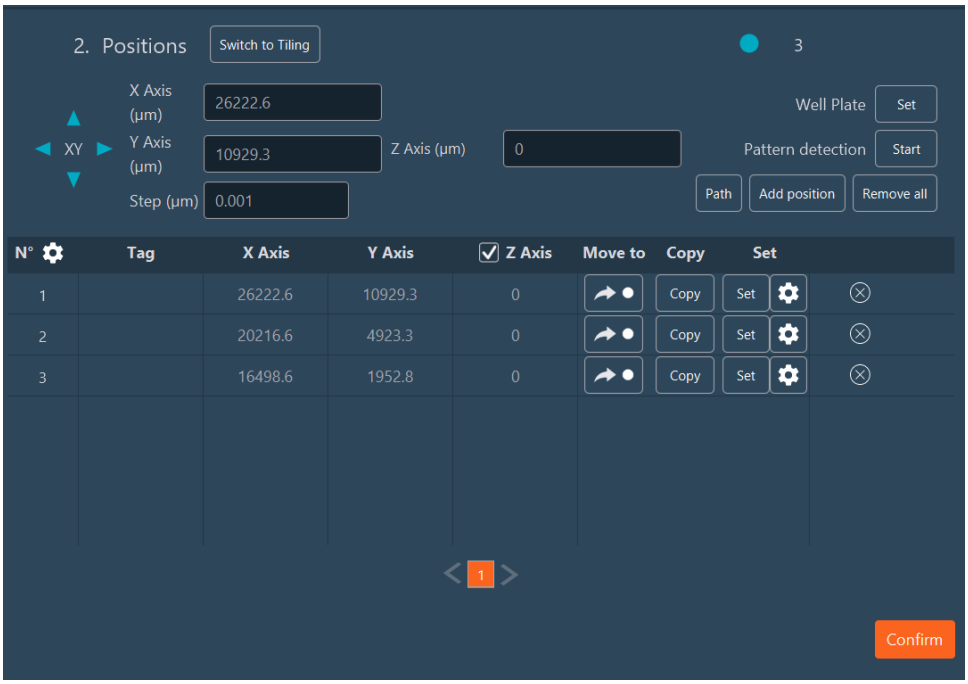
e. Click on **Detect pattern** to start the detection.



f. Once it's done, all patterns recognized are surrounded like ROI. You can select or unselect some ROI by clicking on **Select all** or **Unselect All** or by clicking directly on the ROI.

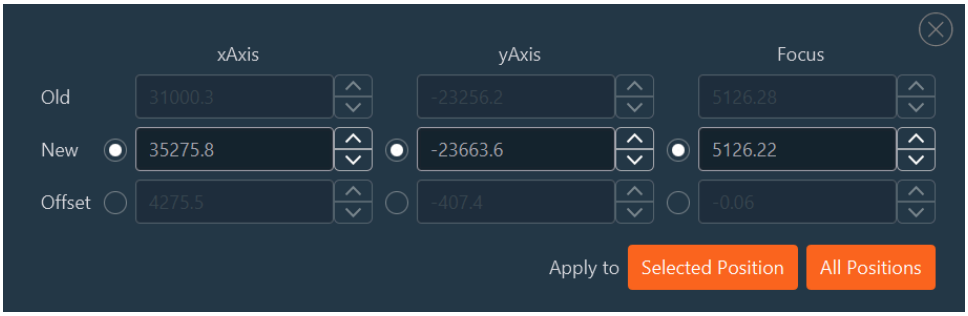


g. Then click on **Confirm** button and position of each pattern will be registered on the position list.

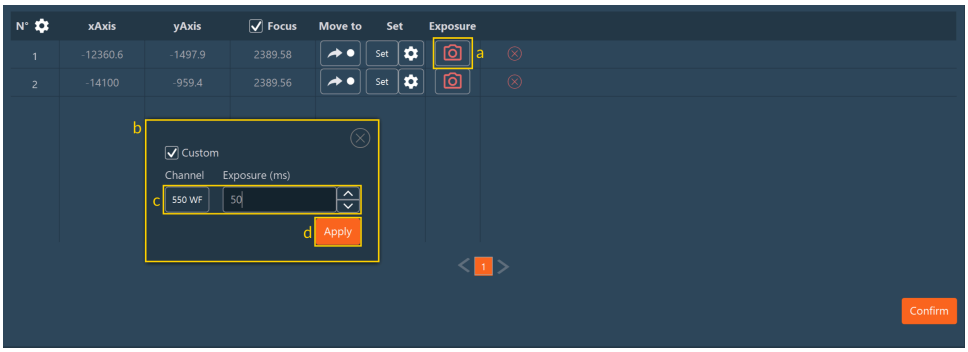


6. Adjust **Move Strategy** if necessary: When performing a multiposition acquisition with a significant distance between two positions, two options are available for the stage moving between these two positions:
   # **Fastest**: Move directly from position A to position B;
   # **XY Split**: Move between 2 positions with small steps. Indicate the step by filing the Step (µm);
7. Make other adjustments or manipulations if necessary:
   # **X-Axis/Y-Axis**: Change/adjust position if necessary.
   # **Focus** or **Z-axis**: 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.
   # **Move to**: Move the stage to this position.
   # **Set**: Update coordinate of the position by the actual XYZ position of the system; You can modify the axis (X,Y and focus), apply offset on the axis for all positions or for the selected

position by clicking on the  . You can select which axis you want to modify by checking it.

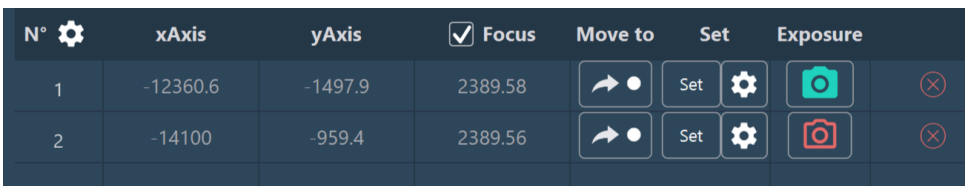


Adjusting Exposure Time by Position: This feature allows you to modify the exposure time for specific positions.



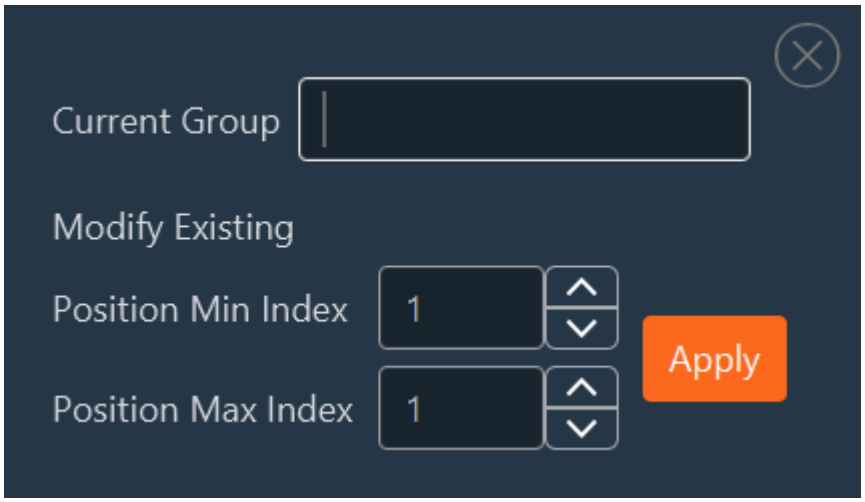
a. Click the **camera icon** on the line corresponding to the position where you want to adjust the exposure time.
b. Check the **Custom** box to enable exposure time modification.
c. Update the **channel value** you wish to adjust. The channel corresponds to the one defined in the channel dimension or if no channel is defined in the dimension, the **current channel** will be used by default.
d. Click **Apply** to confirm your changes.

When a line has been modified, the camera icon color changes from red to green, indicating that a custom exposure time has been set.



 : delete this position from the position list.

8. If you have multiple positions you can create different groups of positions. For this click the **gear icon** to open the settings menu:
    # Name your group.
    # Indicate the range of the position index that you want to group together.
    # Click on **Apply**.
    # Repeat those steps if you want to do multiple groups.

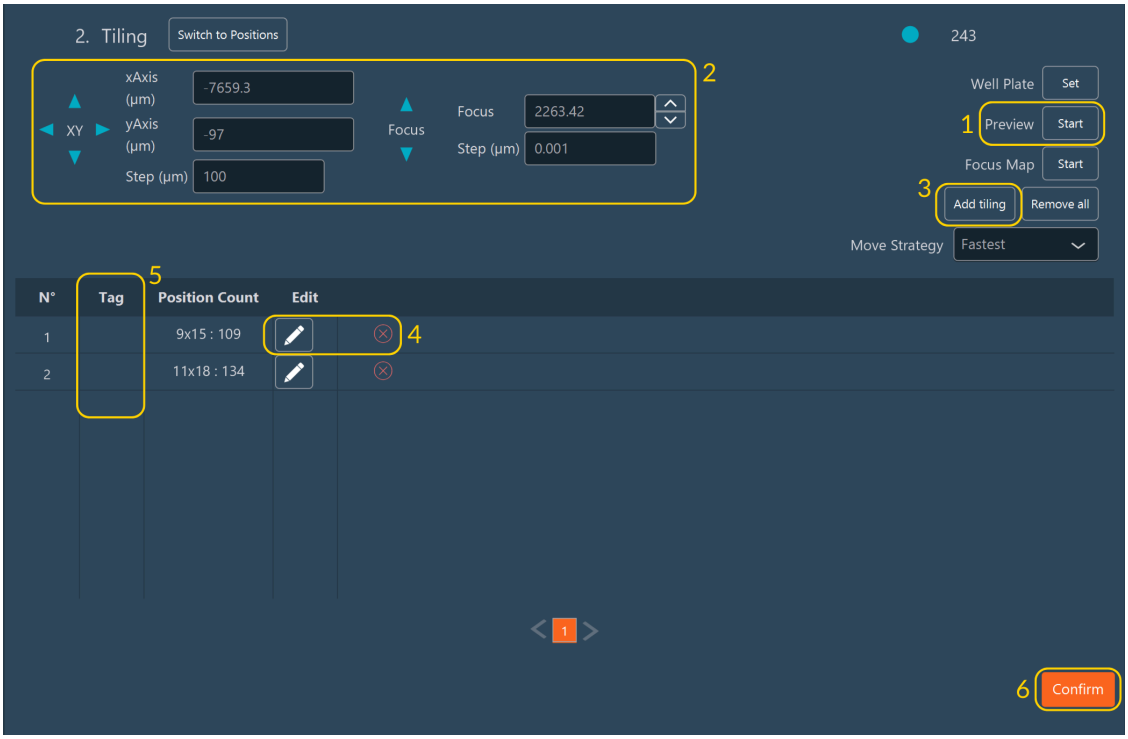


9. When all positions are set up, click on **Confirm**.

1.1.5.4. Tiling

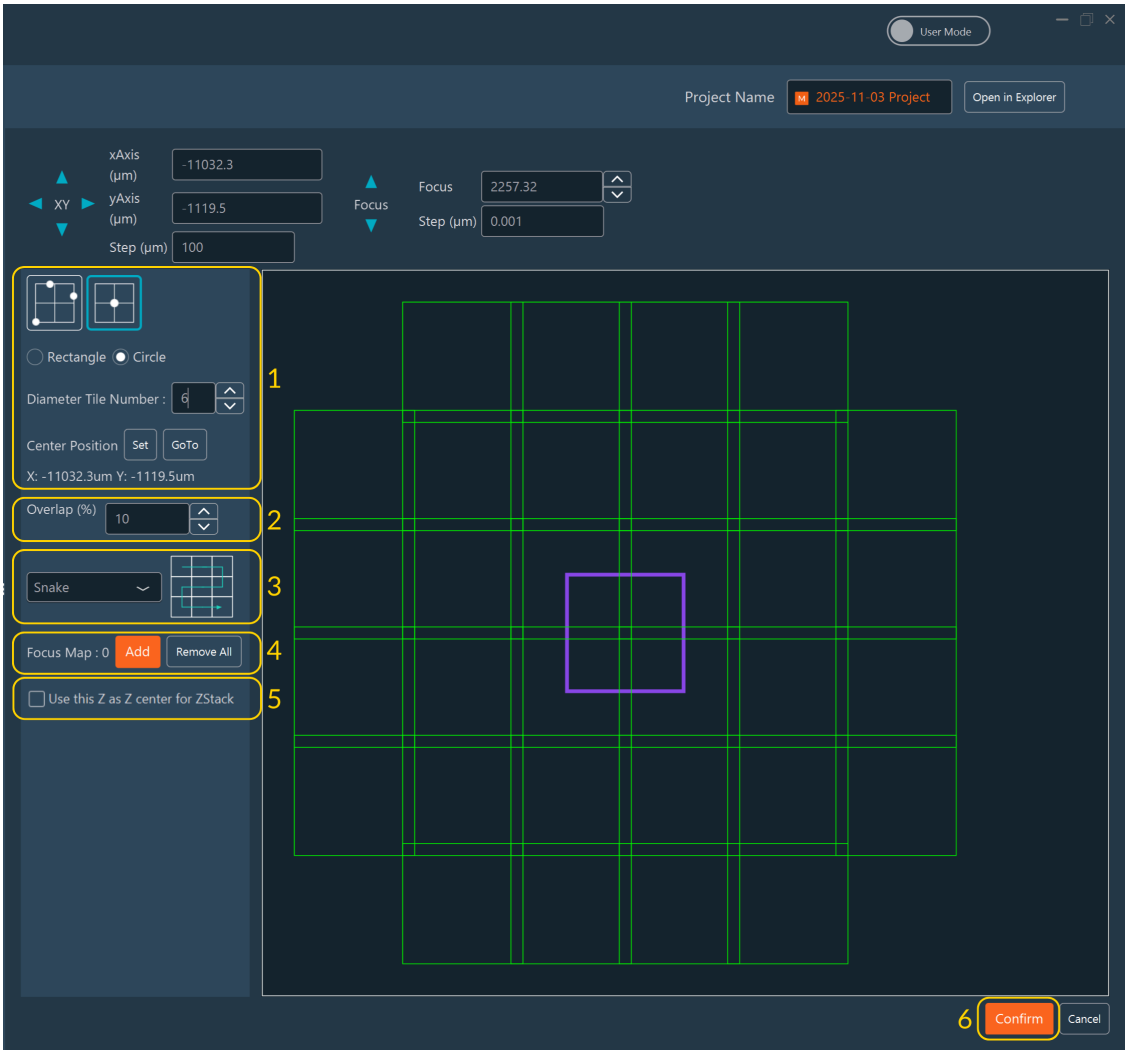
1.1.5.4.1. Tiling creation

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. Do a Preview of your sample
2. Move the stage and adjust the focus to the desired position.
3. Click on **Add tiling** to add a tiling in the list.
4. To edit/customize a tiling clicking the **Pen** icon (see the Tiling edition) or click the **Red Cross** to delete it from the list.
5. You can name each tiling in the **Tag** column by simply clicking on the **Tag** field.
6. Once all tilings are configured, click **Confirm** to finalize.

1.1.5.4.2. Tiling edition

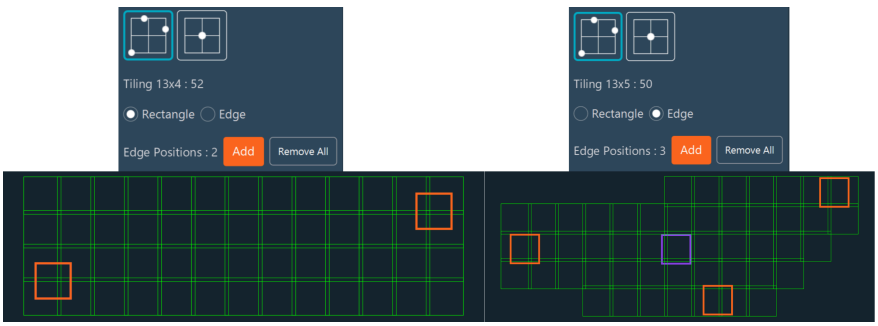


Setting up a Tiling

There are two ways to configure a tiling:

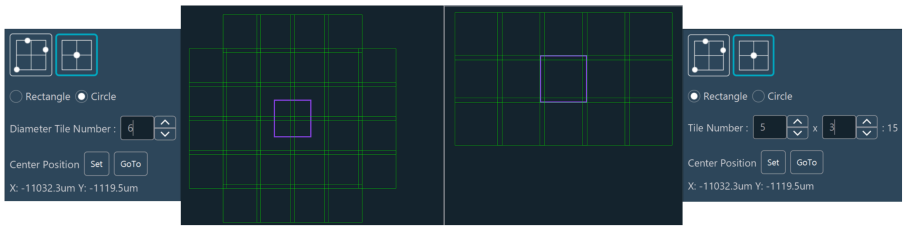
1. Choose how to create the tiling:
    a. Define multiple points to create either a rectangular tiling or a custom tiling by outlining its edges (not limited to rectangular shapes).

    To define points, click **Add**. You can remove all defined points at any time by clicking **Remove All** or remove one point by a right click on it.



b. Define the center of your tiling by clicking **Set**, then select whether you want to create a rectangle or a circle.

Enter the number of tiles to define the size of the tiling, or specify the diameter if you are creating a circular tiling.



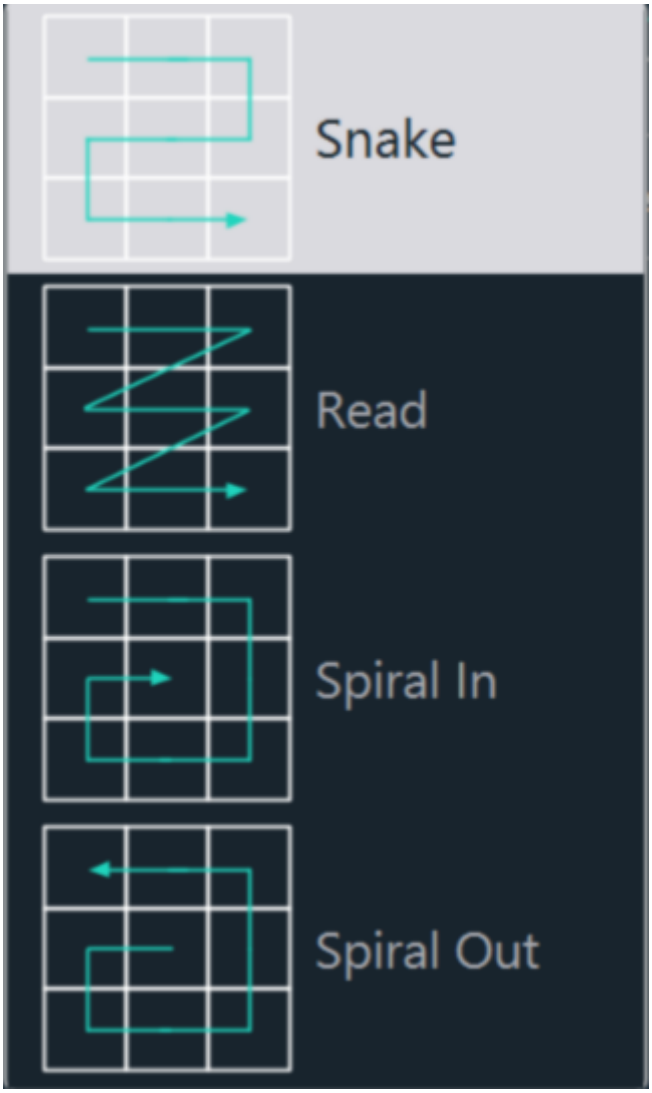
NB: You can interact with the right-hand panel to move the stage and delete defined points if needed.

2. Set the overlap percentage: Select an appropriate overlap between tiles (typically 10% is sufficient for final stitching).

Enable **Automode** to automatically calculate the exact overlap based on the coordinates defined during tiling setup. If needed, adjust the **Custom Step** (distance between the tiles), To do this, enable the option in the Display Settings.

This step is essential for accurate stitching.

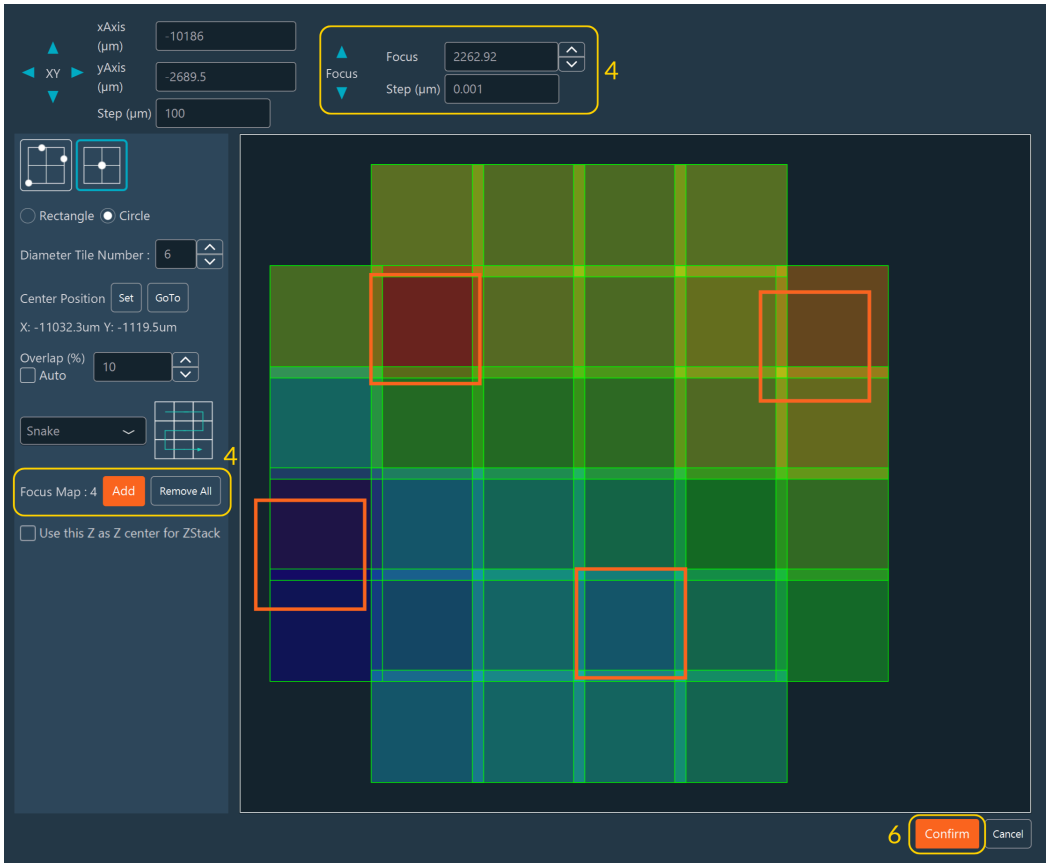
3. Select the acquisition order of tiles.



4. Create a focus map: Move on XY with the joystick (virtual or physic) or by clicking on the tiling. Adjust the Z position, then click **Add**.

   You can add as many focus points as necessary. By right clicking on the orange square you can remove one point of the focus map.

   The color displayed within the tiling indicates the Z-height differences between tiles.



5. Check the box if you want to use the Z position saved in the focus map or used to set-up the tiling as the center of your Z-stack. If the box is unchecked and :
   - # you do Z stack : the Z-stack will use the Z value defined in the Z-Stack Dimensions instead.
   - # you don't do Z stack, the Z for the tiling will be the Z defined in the Preview (if you have done it) or the current Z.
6. Click **Confirm** to validate and save your tiling configuration.

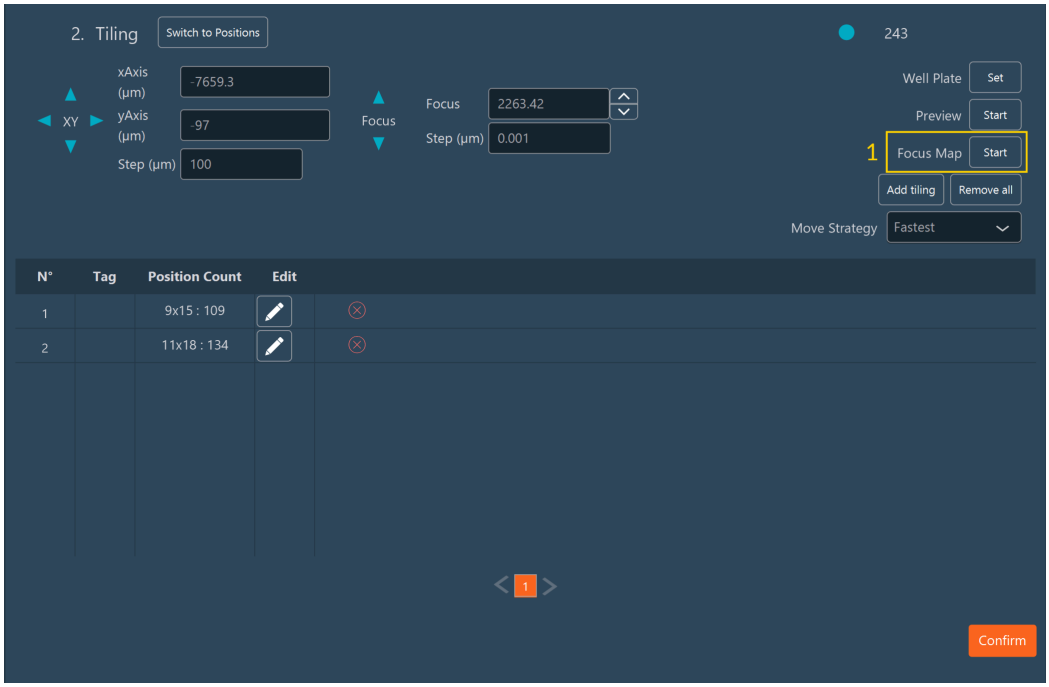
> NB: Orange squares represent the points used to define your tiling and the focus points of the focus map.
>
> The purple square indicates your current XY position.

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

1.1.5.4.3. Focus Map automated setup for simple and multiple tiling

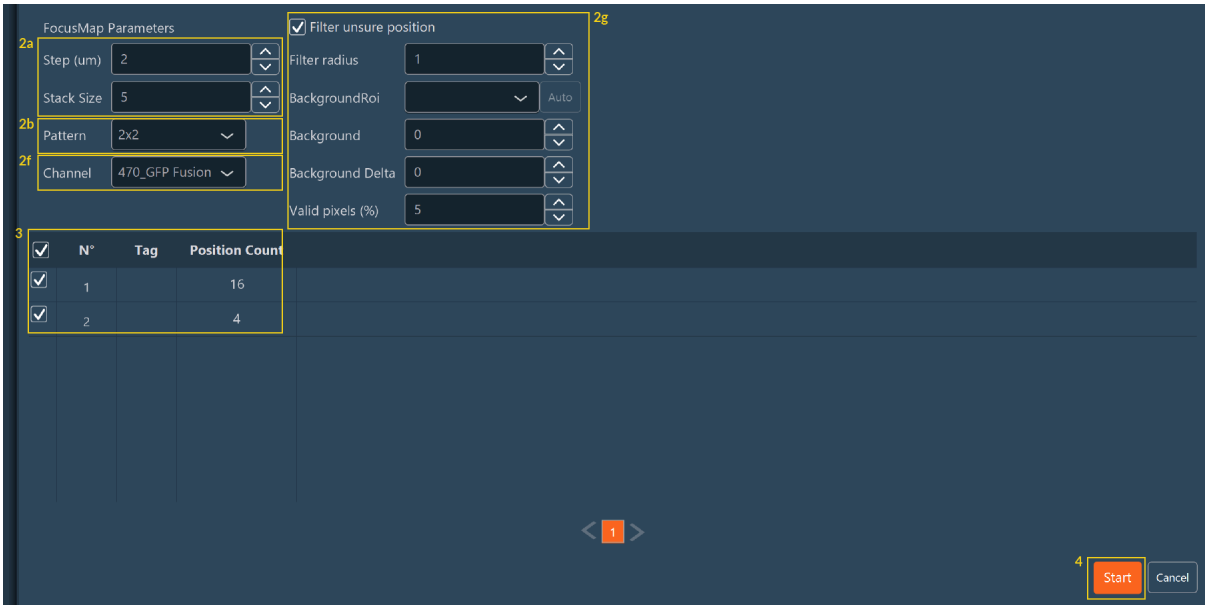
The Automated Focus Map is a software optional feature of the Inscoper I.S. that automatically generates a relief map of the biological sample when performing tiling imaging.

1. To start configuring: Click on **Focus Map Start** button in **Tiling Dimension**

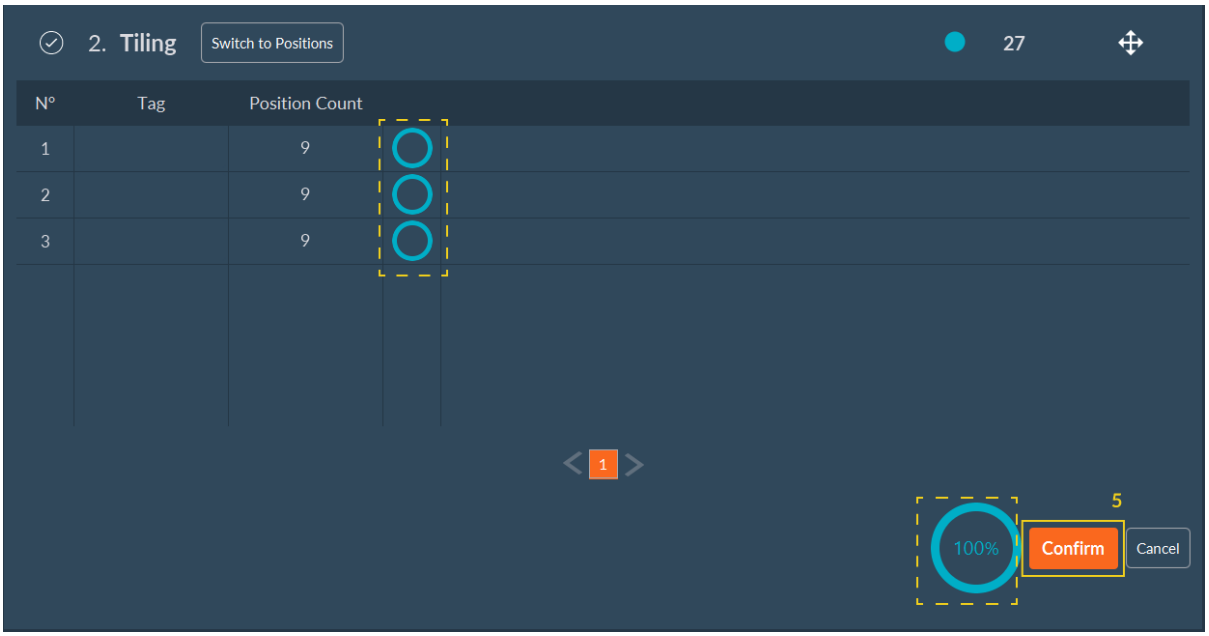


2. The following parameters must be set for both simple and multiple tiling:
    a. Choose the **step** and **size** of the stack.
    b. Select an existing pattern.
    c. Select channel to acquire the stack.
    d. Optional: Check **Filter unsure position** and set parameters:
        i. **Filter Radius**: 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, ...).
        ii. **BackgroundRoi**: ROI for auto-calculating background values.
        iii. **Background**: Mean background value.
        iv. **Background Delta**: Noise amplitude.
        v. **Valid Pixels**: Percentage needed to validate position.
3. Select the tiling for which you want to create a Focus Map.
4. Click on Start. The system will automatically scan all points to generate the Focus Map. A list of focus points will be displayed. The progression of the focus map by tiling is indicated with a progress bar. When the Focus Map

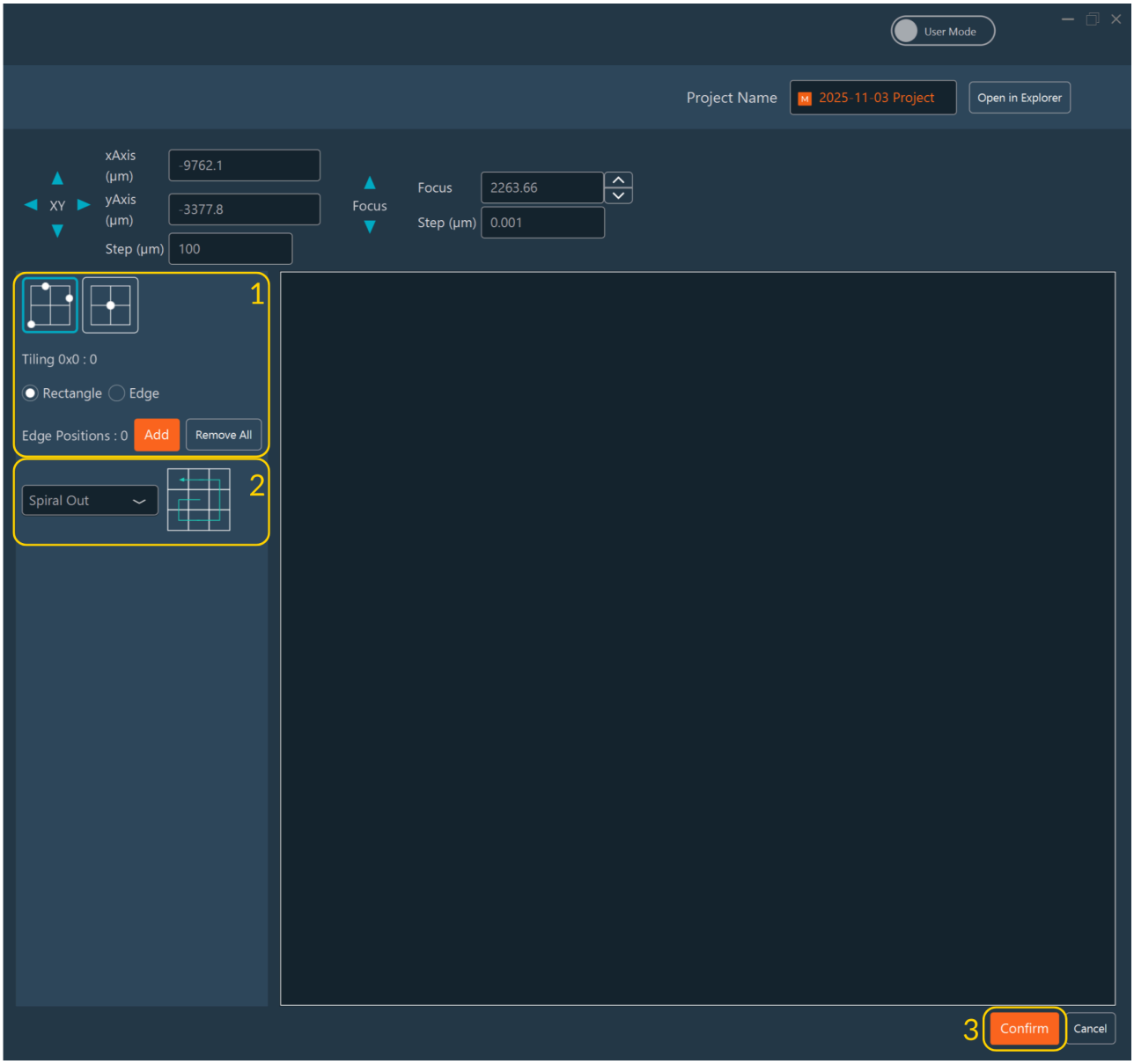
is ready: You will be automatically redirected to the Tiling Dimension screen.



5. Click **Confirm** to validate and proceed. You can continue to configure your acquisition sequence.

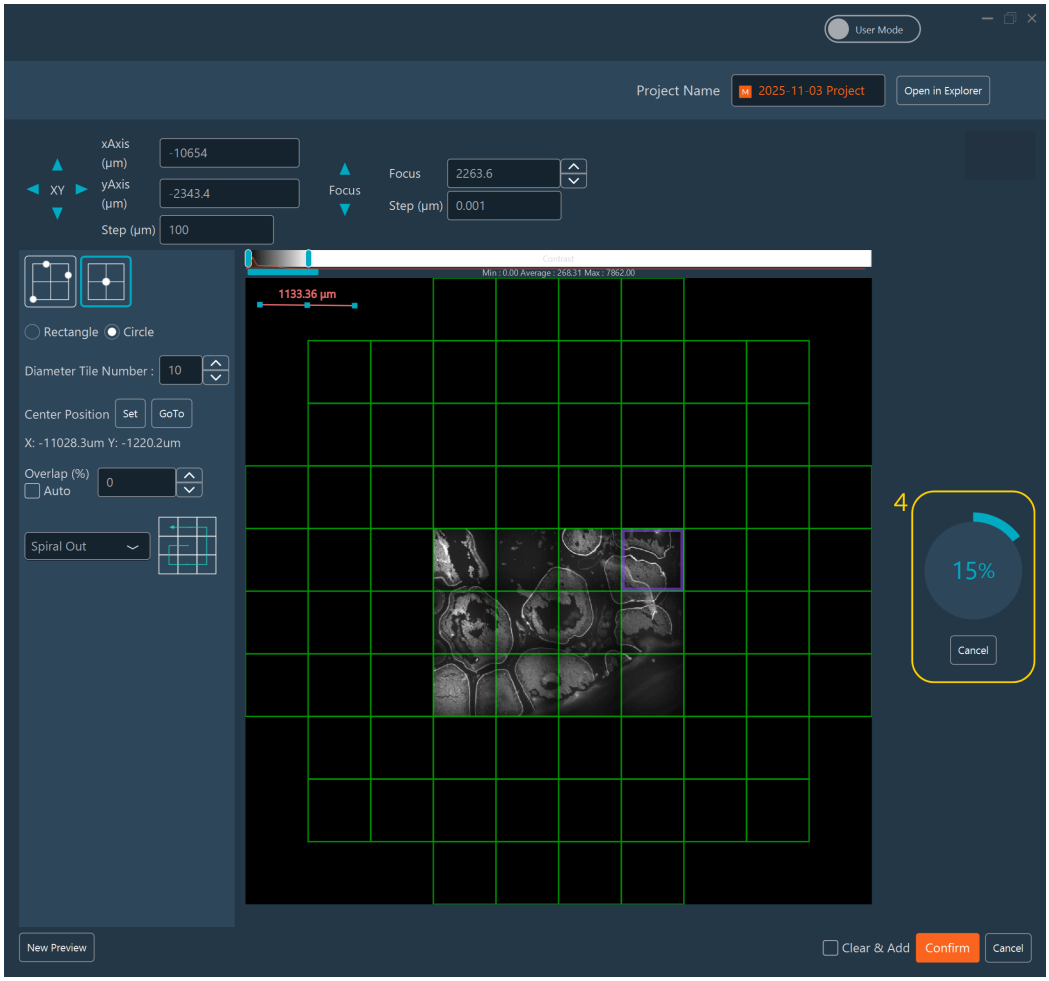


1.1.5.5. Preview feature in Position and Tiling dimension

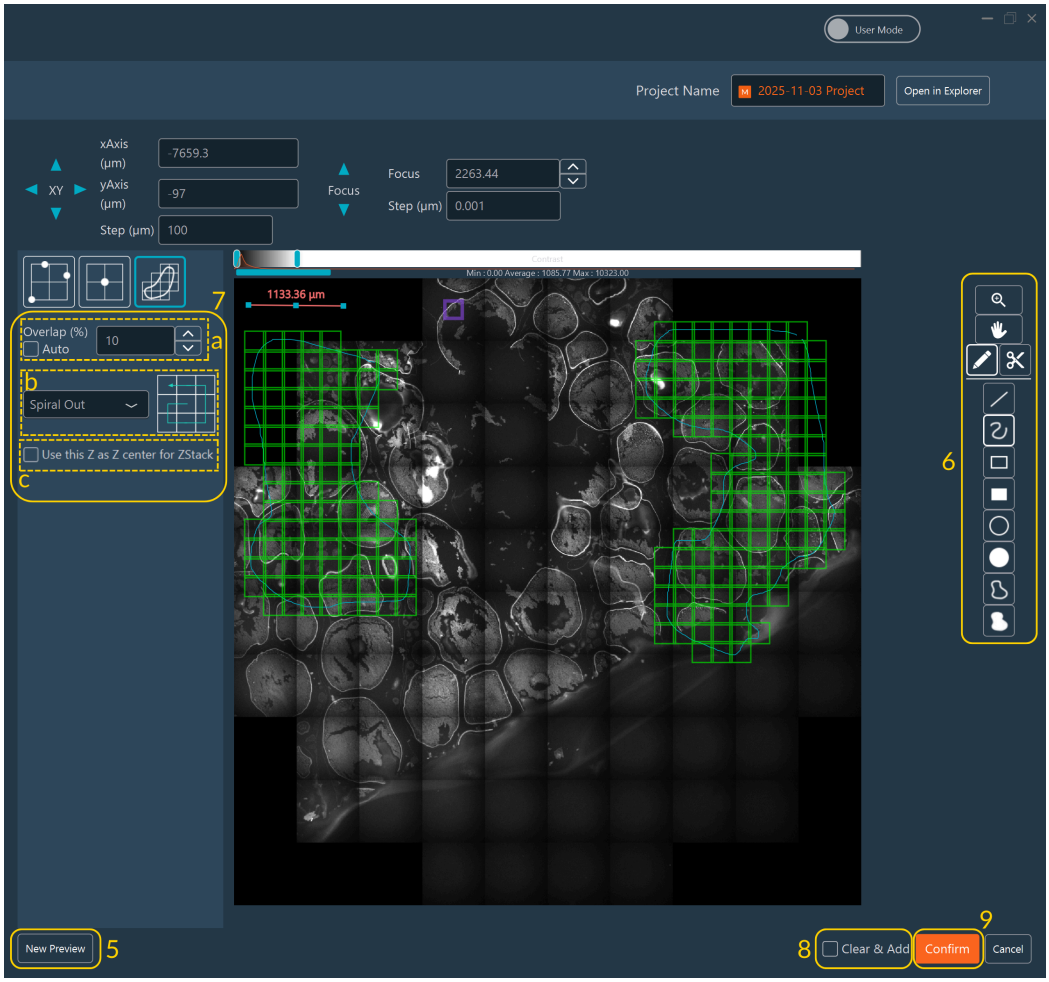


1. Configure the **Preview**: Similar to Tiling Edition, you can either:
    # Define multiple points to create either a rectangular preview or a custom preview by outlining its edges (not limited to rectangular shapes) by clicking **Add**, or,
    # Define the center of your tiling by clicking Set, then specify the **number of tiles**.
2. Select the **Read Mode** (Spiral, snake,..).
3. Click **Confirm** to validate your configuration.

4. Follow the preview process in real time. You can stop it at any time by clicking **Cancel**.

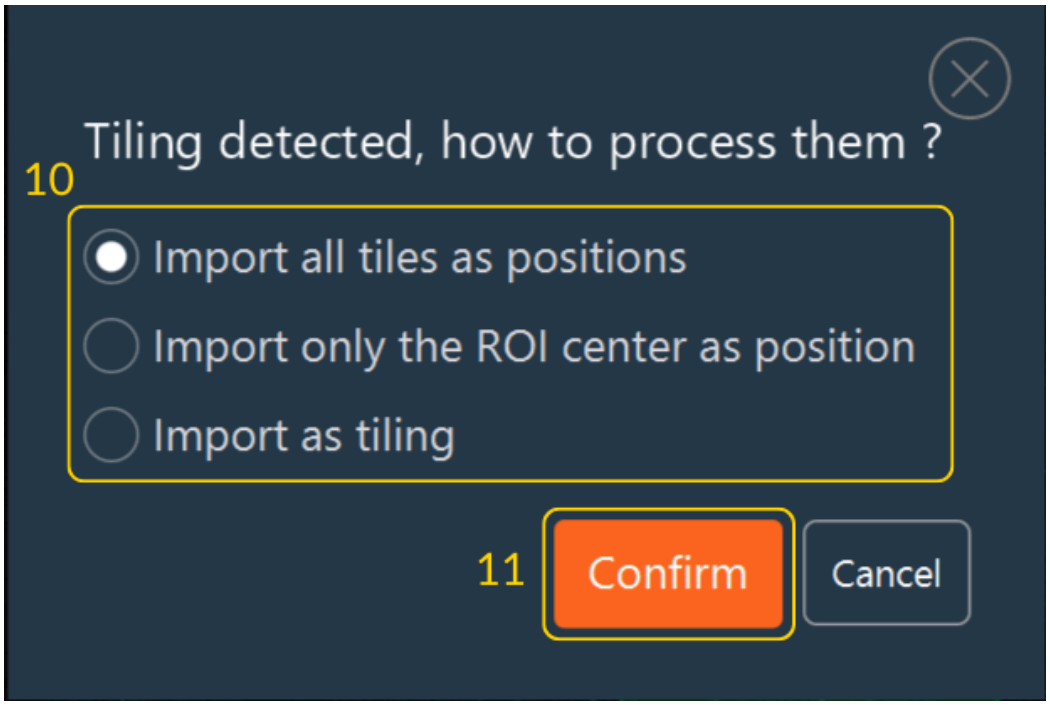


Once the preview is complete:



5. Click **New Preview** to start a new setup.
6. Draw multiple ROIs in various shapes: **point** (useful when the preview is created in position dimension), **freehand**, **circle**, or **rectangle**.
7. Specify the **tile overlap**.
    a. Enable **Auto Mode** to automatically calculate the exact overlap based on the coordinates defined during the tiling setup (available only when the preview is created in tiling dimension).
    b. Choose the **read mode** (only available in tiling dimension).
    c. Check the box if you want to use the Z position saved in the focus map or used to set-up the tiling as the center of your Z-stack. If the box is unchecked, the Z-stack will use the Z value defined in the Z-Stack Dimensions instead.
8. Check **Clear & Add** if you wish to replace existing tiling or positions. Leave it unchecked to add new ones.
9. Click **Confirm** to validate.
10. If your preview is done in **Position dimension** and you draw an ROI containing multiple tiles, a popup will appear asking how to import this ROI:

Import all tiles as positions: Creates one position per tile.
Import only ROI center as position: Creates one position per drawn ROI.
Import as tiling: Switches to tiling dimension, even if there is only one tile.

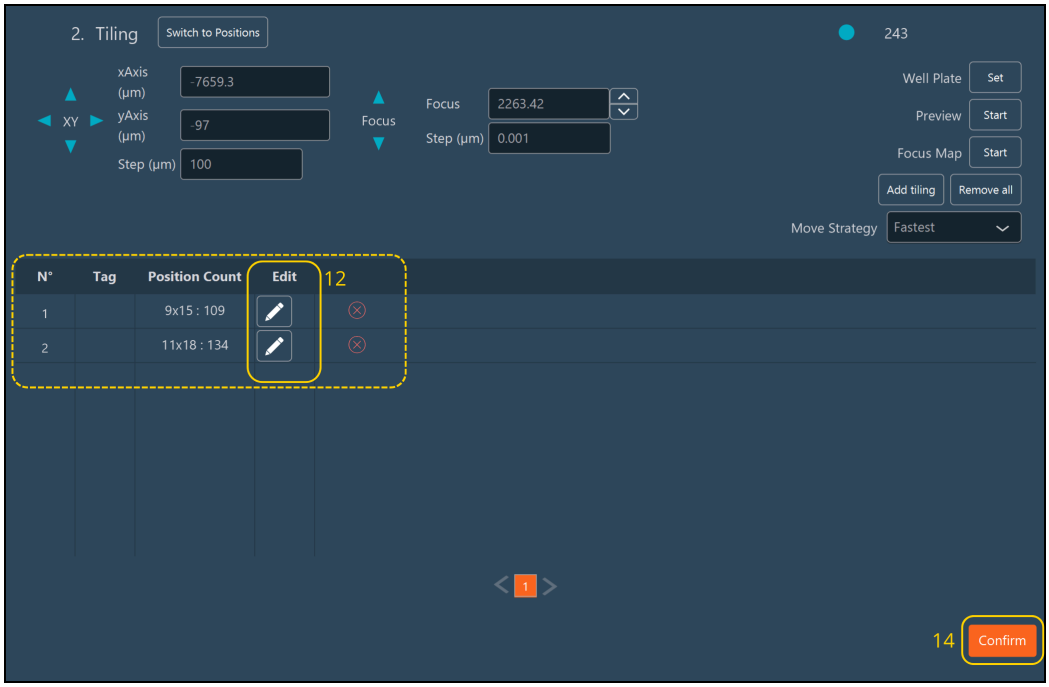


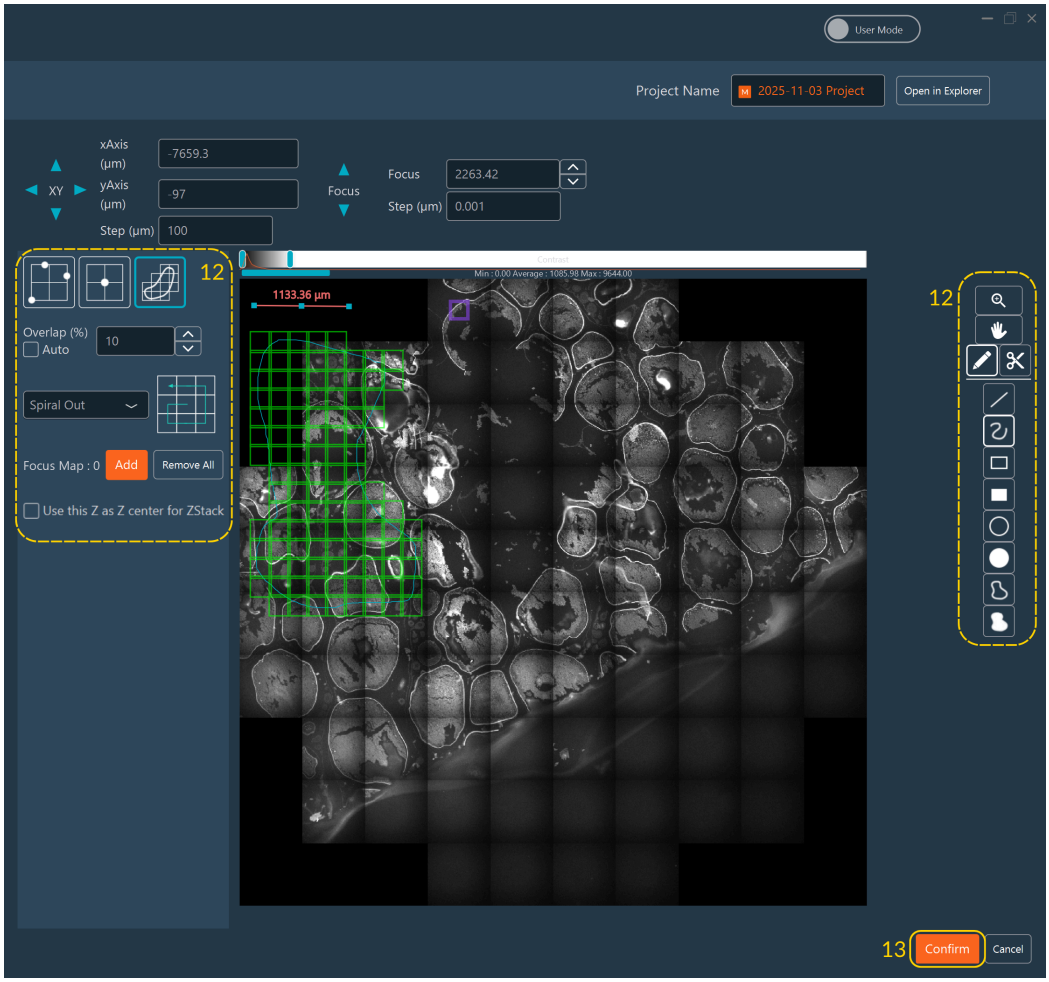
11. Click **Confirm** to apply your choice.

After confirmation, all created positions or tilings appear in the corresponding dimension.

12. Click the **Pen** to edit them: you will find the preview associated to the tiling:
    # **Move**, **modify**, or **delete** an ROI.
    # Change the **Read Mode**.
    # Add a focus Map if your sample is not flat or if you need to adjust the value of the Z.

Select if you want to use the Z position saved in the preview as the center of your Z-stack. If the box is unchecked, the Z-stack will use the Z value defined in the Z-Stack Dimensions instead.





13. Click **Confirm** to save your modifications.

14. Click **Confirm** to validate the **Tiling Dimension**.

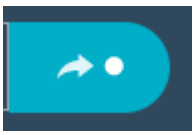
> NB: To save your preview, right-click on the preview window.

1.1.5.6. Multiwell plate mode

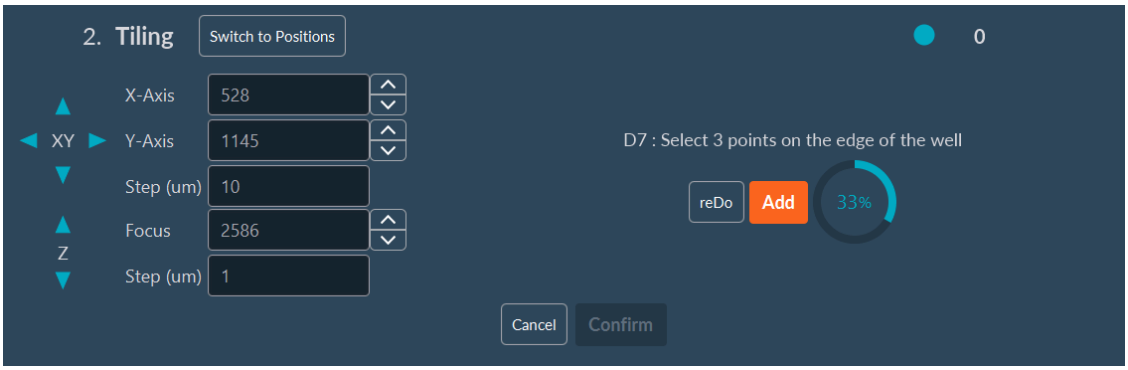
The Inscoper software has a strong feature for imaging multiwell plates and for some applications including High Content Imaging.The Multiwell plate mode of the Inscoper I.S. is compatible with all commercial or homemade supports (multi-well, multi-slide, etc.). An option **Well Plate Editor** allows you to simply add a large panel of supports. Click here to set it up.

1.1.5.6.1. 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

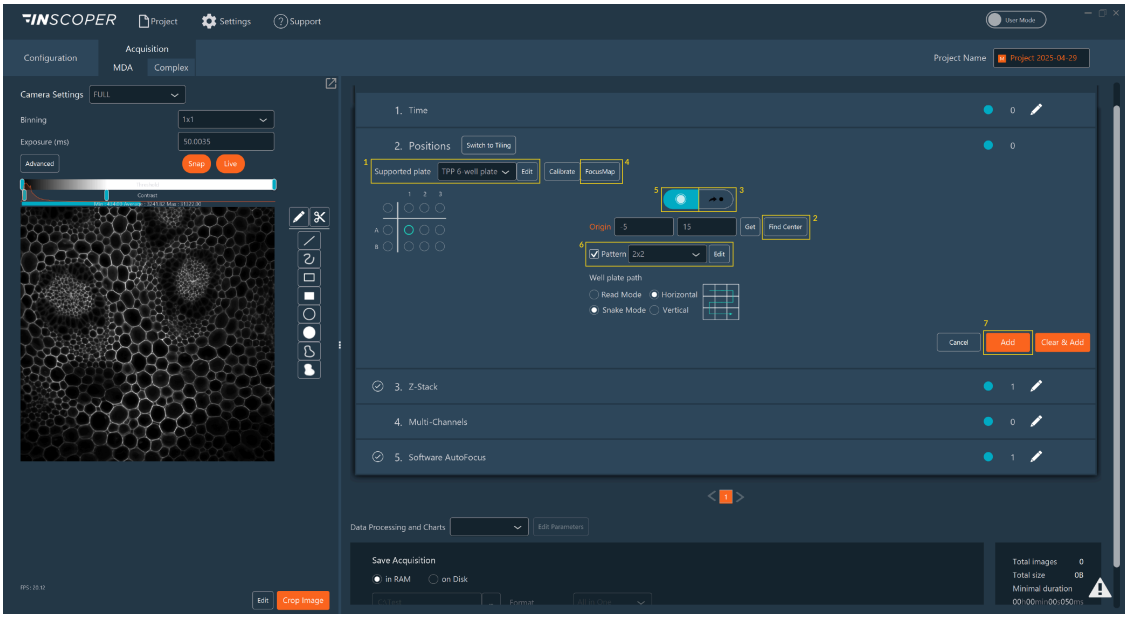
1.1.5.6.2. Multiwell plate calibration



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.

1.1.5.6.3. Multiwell plate mode in Positions dimension

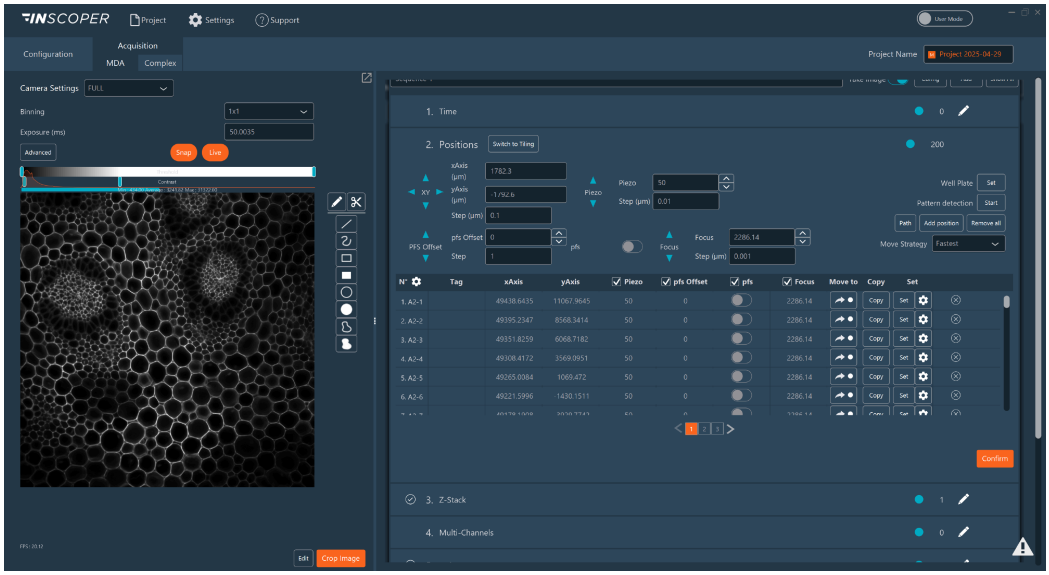


1. Use the **Supported plate** dropdown list to select the support you need.
2. Detect the well plate using the Find Center.
3. Automatically move the stage to some wells.
4. Create a Focus Map for some wells.
5. Select the wells that you want to image.
6. By checking the box **Pattern**, you can generate 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

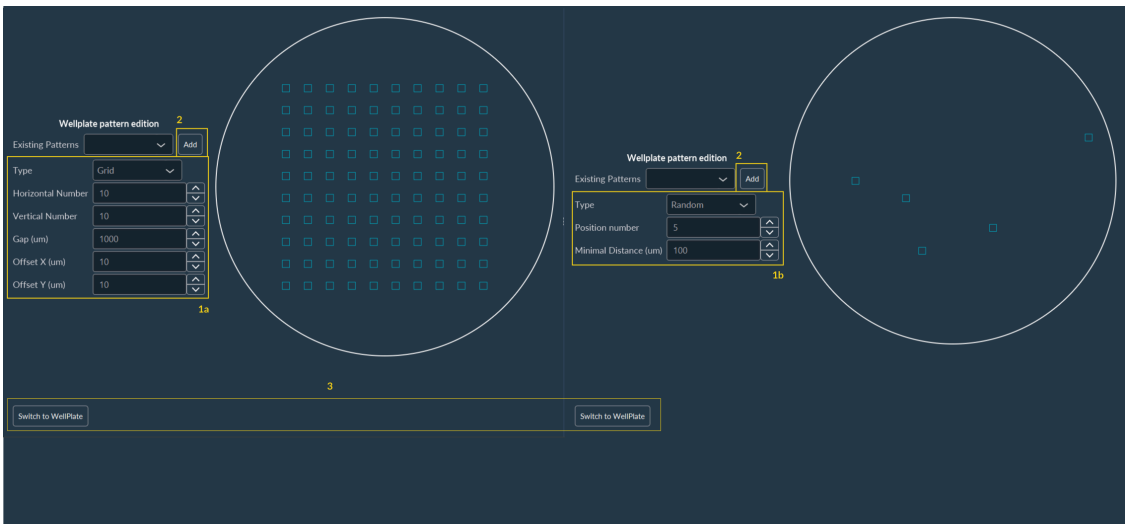
> NB: You can select multiple wells at once by holding down the mouse and moving the mouse over different wells.

You will find all your positions inside the dimension.

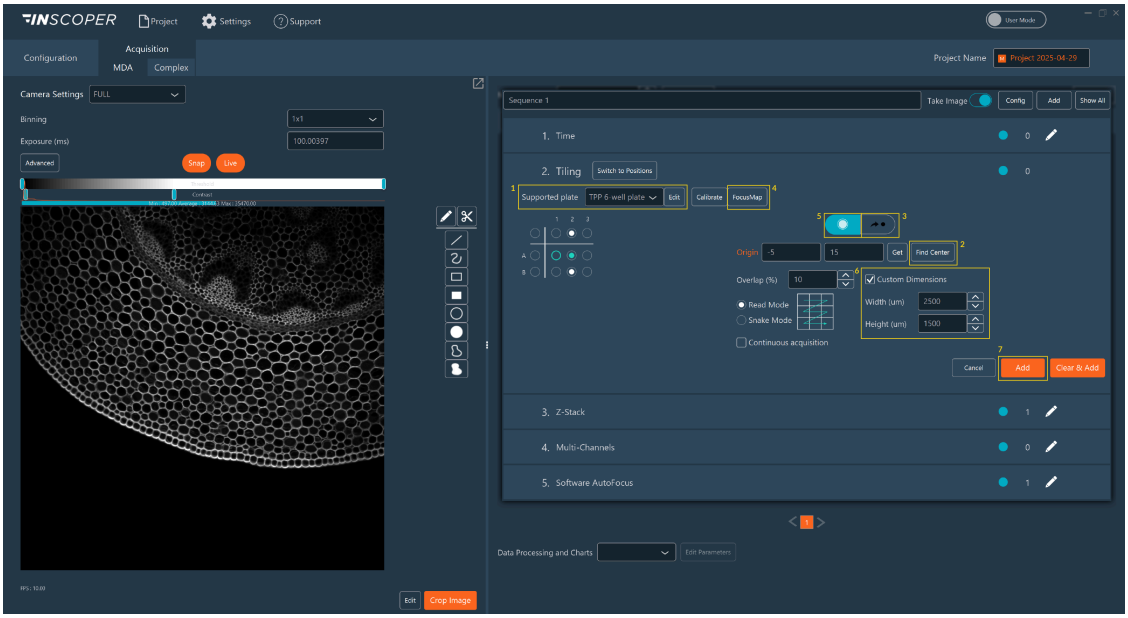


To edit pattern, click on **Edit**.

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**.



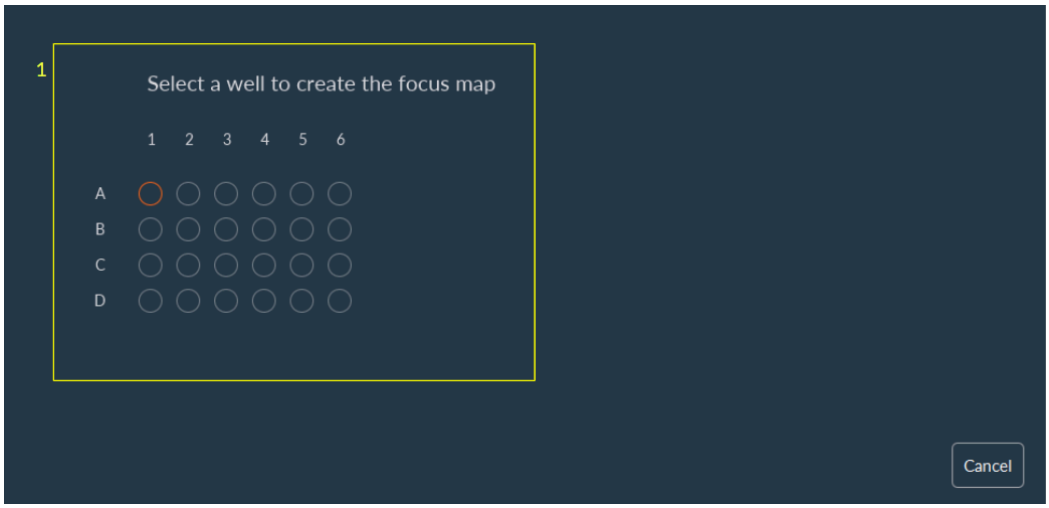
1.1.5.6.4. Multiwell plate mode in Tiling dimension



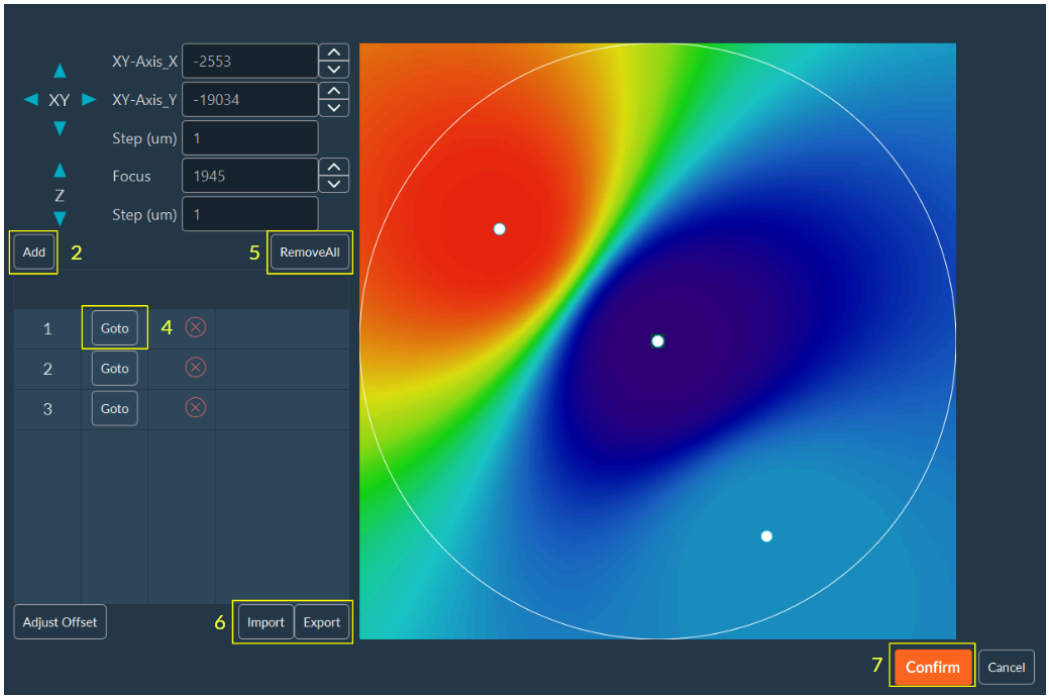
1. Use the **Supported plate** dropdown list to select the support you need.
2. Detect the well plate using the Find Center.
3. Automatically move the stage to some wells.
4. Create a Focus Map for some wells
5. Select the wells that you want to image.
6. By checking the box **Custom Dimensions**, you 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**.

1.1.5.6.5. Focus Map manual setup

1. Select the well to create the Focus Map.



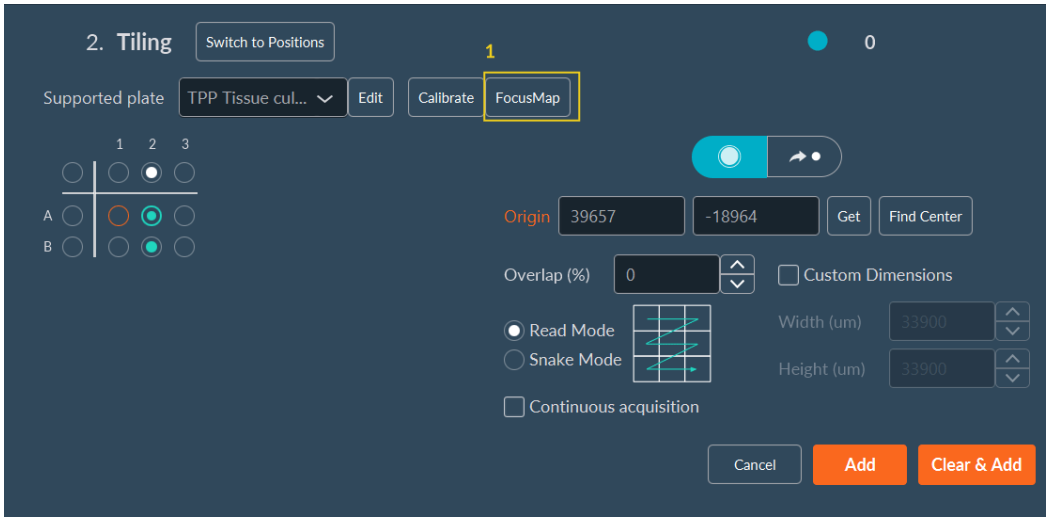
2. Choose the position in the well where you want to make the focus and click on **Add**.
3. Repeat this process until you are ok with the focus of your well.
4. Click on **Go to**, to move to the position.
5. Click on **Remove all** to delete all the points of your focus map if necessary.
6. You can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



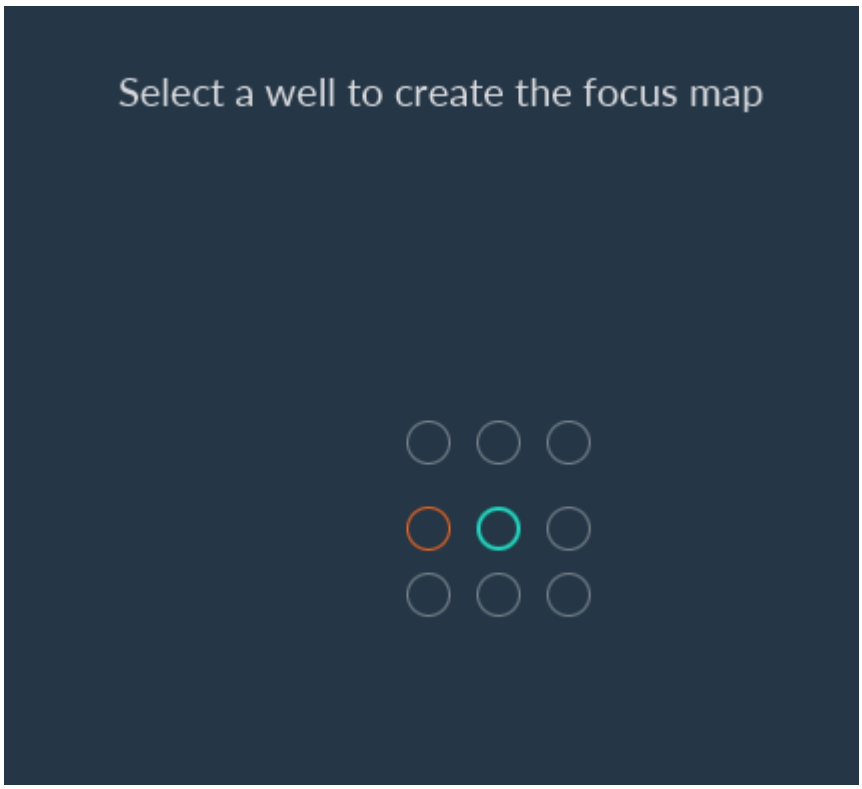
1.1.5.6.6. Focus Map automated setup

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

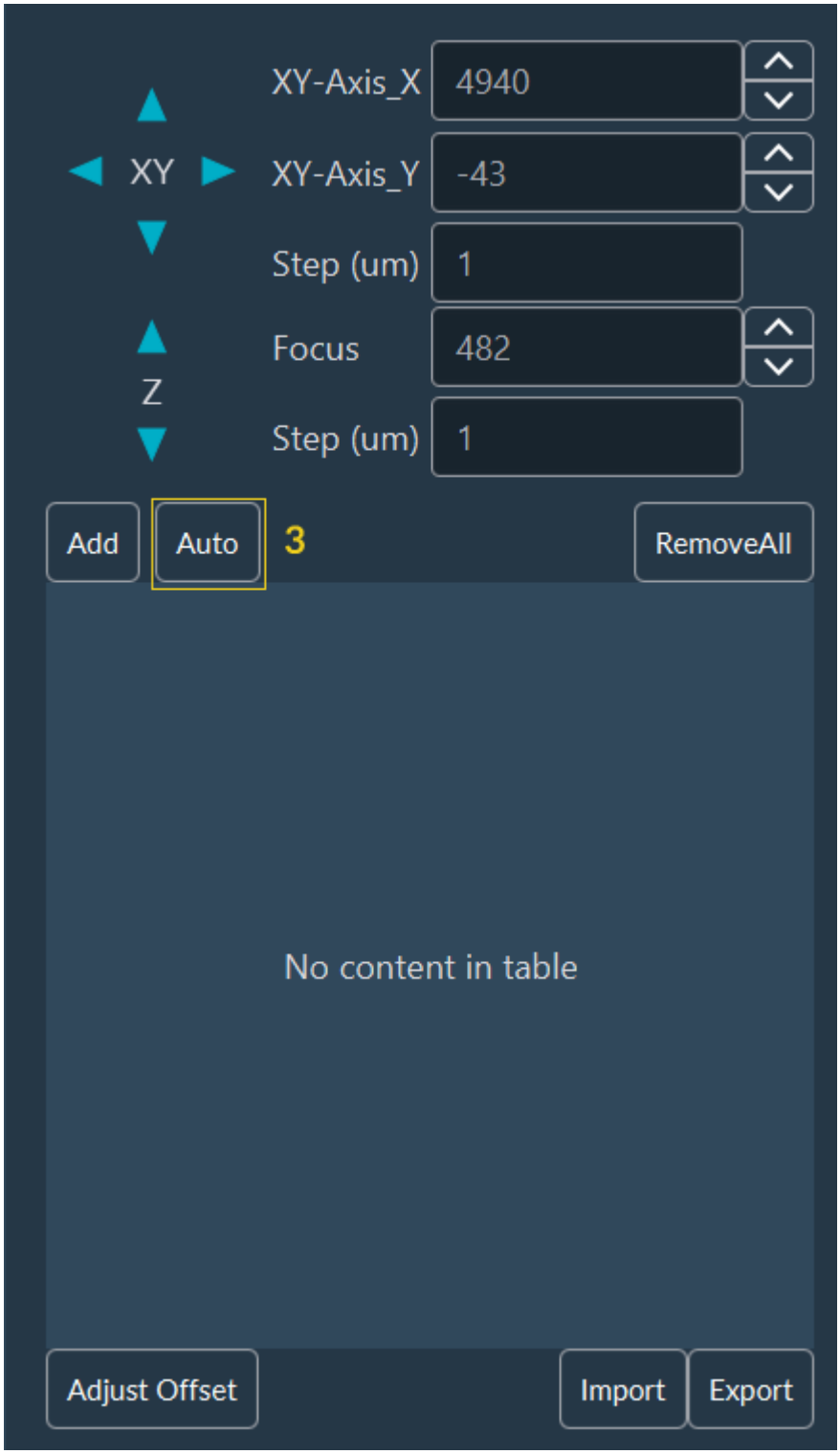
1. Click on **Focus Map** to configure it.



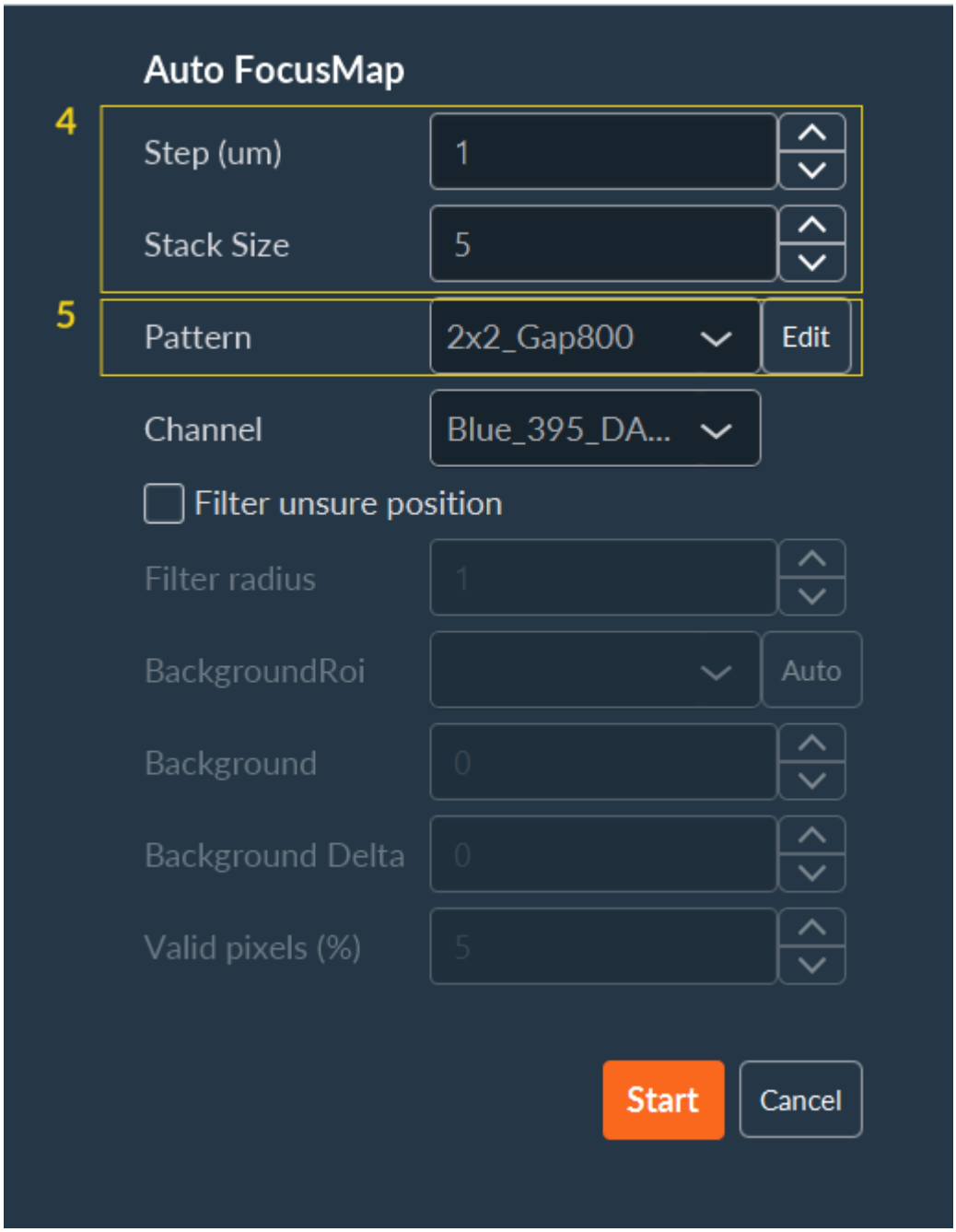
2. Select your well where you would like to create your **Focus Map** then click on **Confirm**.



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**.

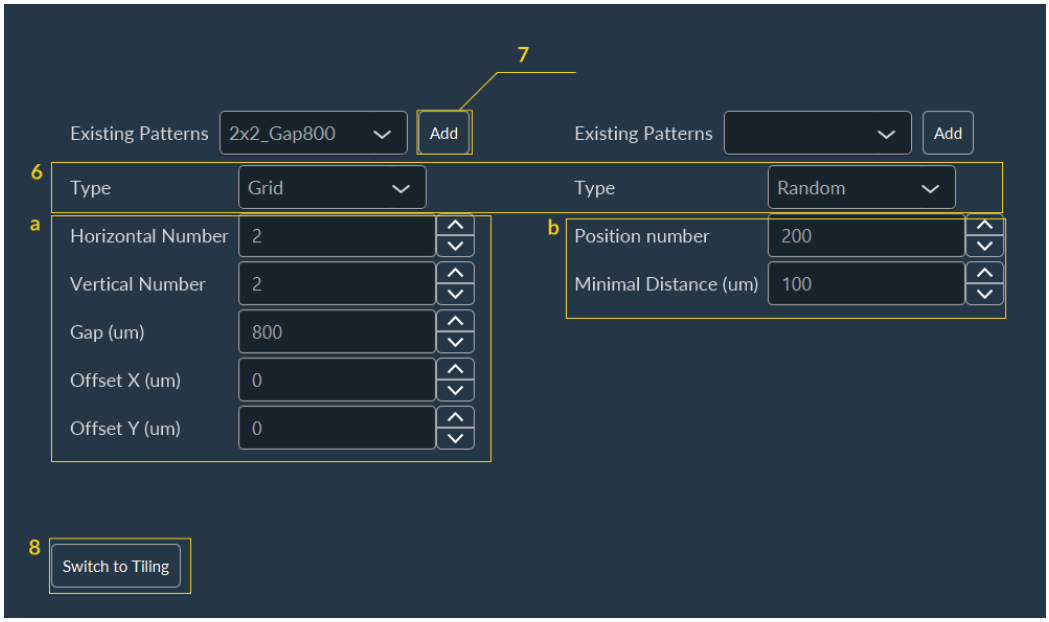


6. Select the pattern you want: **Grid** or **Random**:

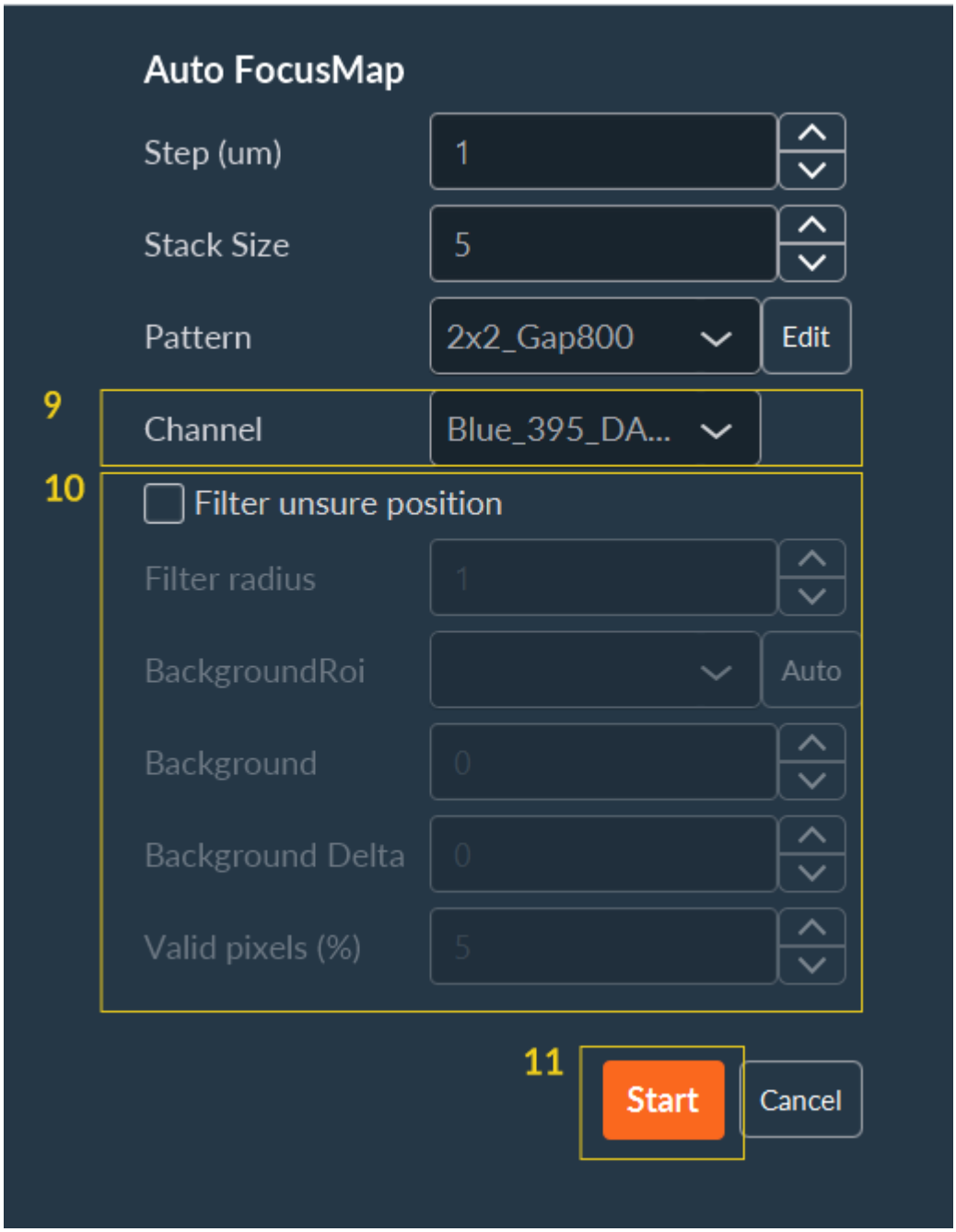
    a. For **Grid**, indicate the number of image you want to acquire horizontally and vertically, and the Gap (distance between 2 images).
    The offset X and Y allows you to move all your patterns in the well.

    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**.

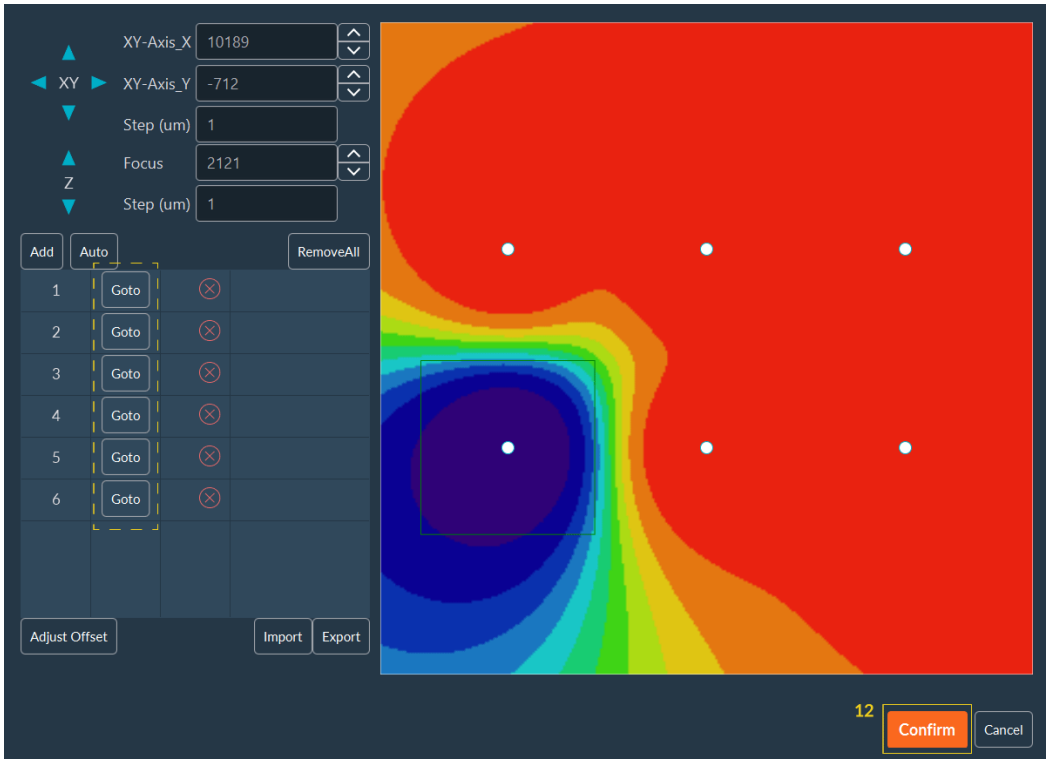


9. Choose the **channel** to acquire the stack.
10. You can apply some filters by checking the box **Filter unsure position**:
    - Filter Radius: filter to smooth the noise by replacing each pixel by the median of the neighbor (0= no smoothing, 1= median on 3x3 square around the pixel, 2= median on 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**.

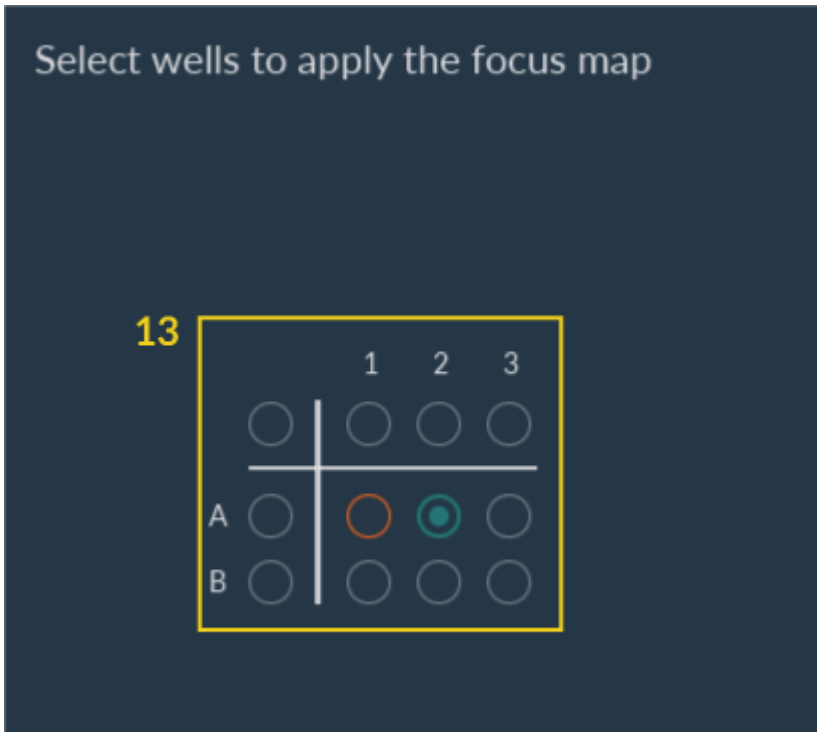


System will perform all points to create the Focus Map and you will obtain a list of points. By clicking on **Goto** or on the image, you can check the focus.

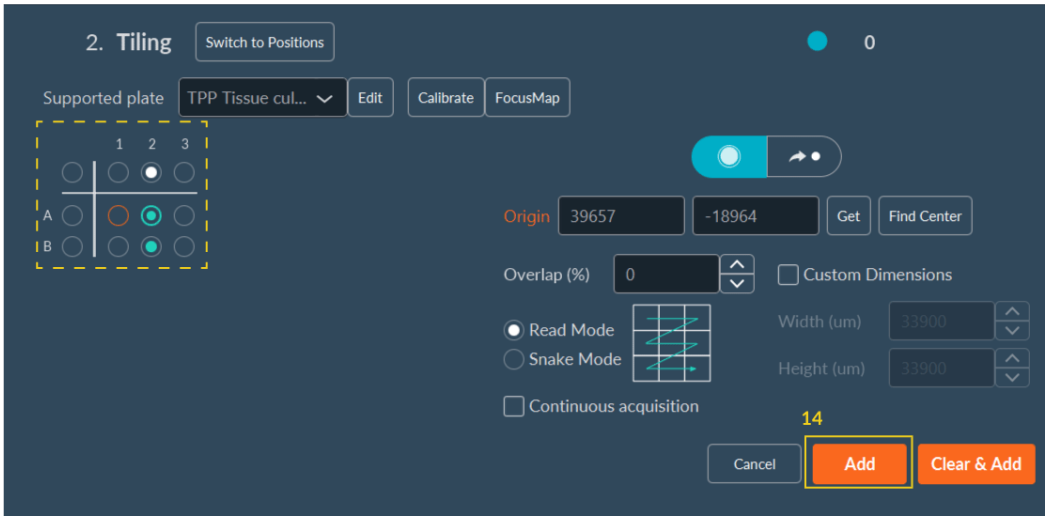
12. Click on the **Confirm**, the **Focus Map** is ready to use.



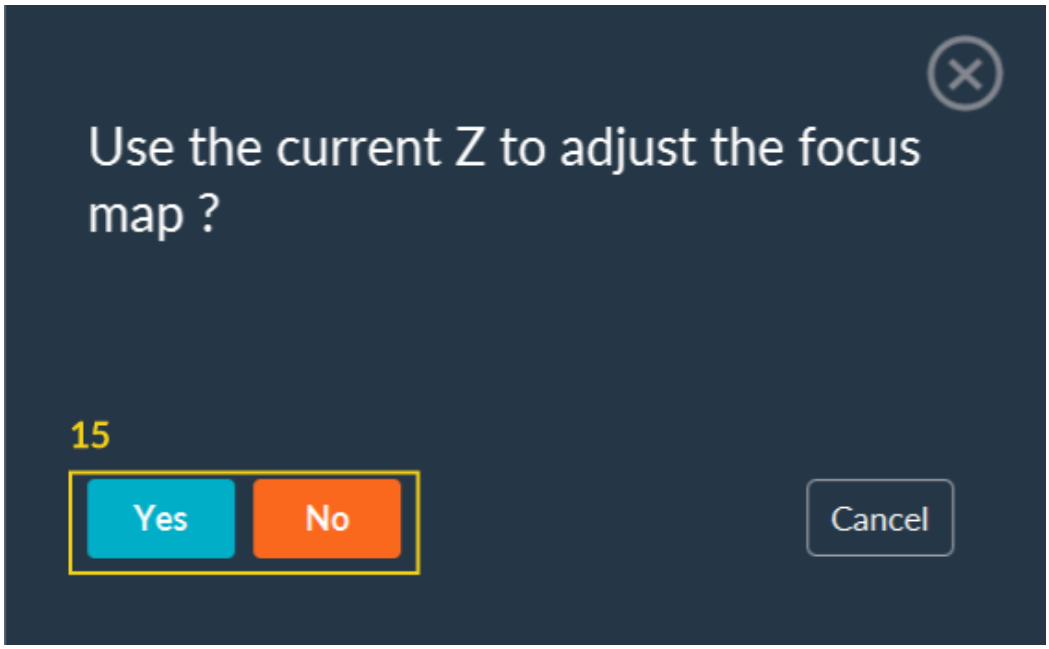
13. Select the wells where you want to apply the Focus Map then click on **Confirm**.



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.



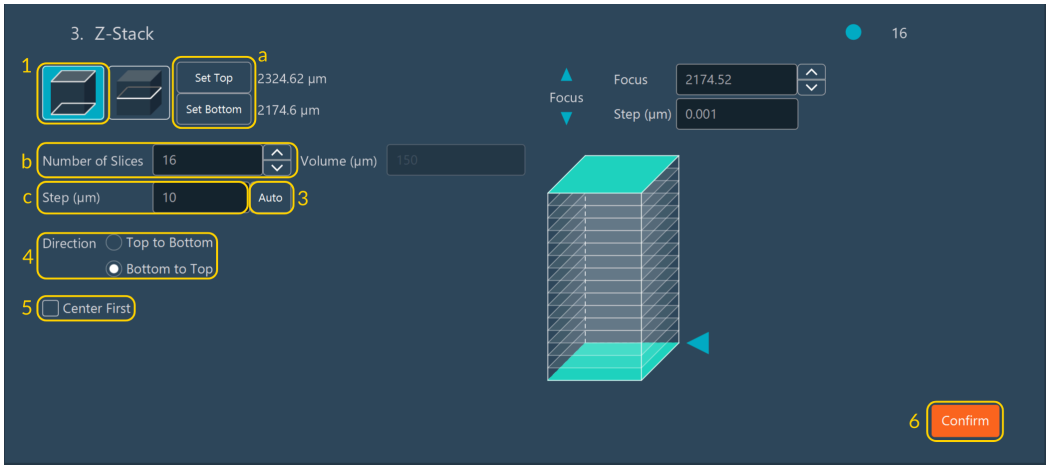
After this step, you can continue to configure your acquisition sequence.

1.1.5.7. Z-stack

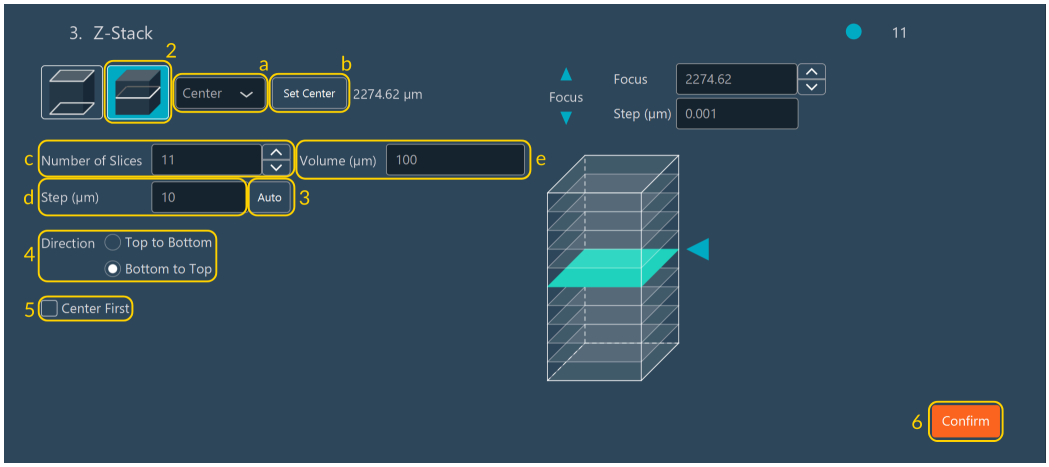
The volumetric imaging with Inscoper I.S. can be performed using the Z-Stack dimension.

You can configure your Z-stack in two ways:

1. By defining the **top and bottom limits**:
    a. Click **Set Top** and **Set Bottom** to define the upper and lower limits of your Z-stack.
    b. Enter the **number of focal planes** in the Number of slices field.
    c. Define the **step size (in µm)** between each focal plane.



2. By selecting a single reference plane.



a. Choose whether you want to define the **center**, **top**, or **bottom** of your Z-stack.

b. Click **Set Center, Set Top, Set Bottom** to select the focal plane.

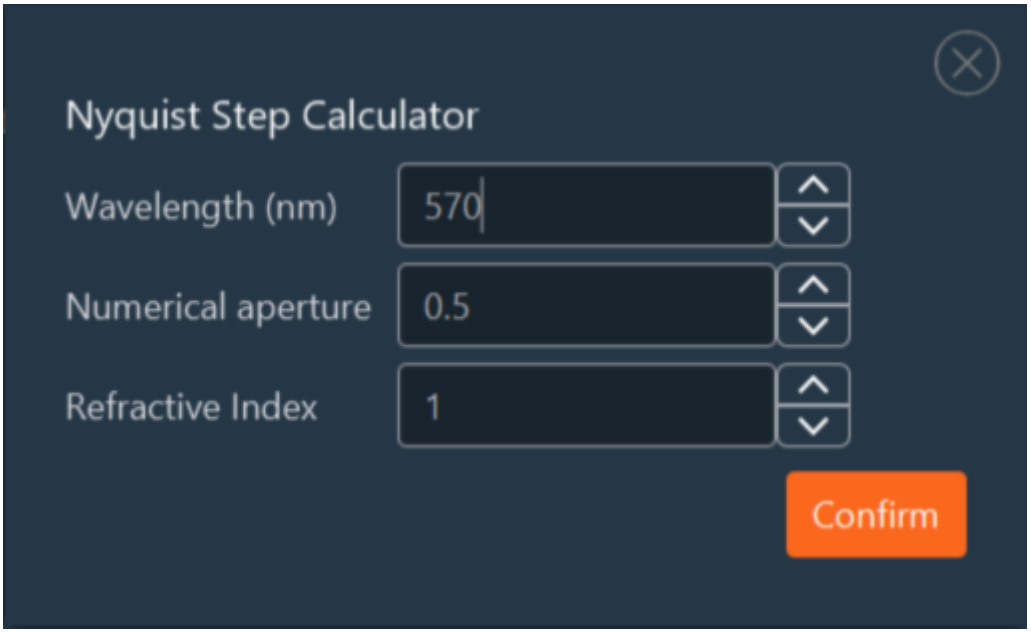
c. Specify the **number of focal planes** in the Number of Slices field.

d. Set the **step size** (in µm) between each focal plane.

e. Enter the **volume** of the Z-stack (in µm).

3. Click **Auto** to automatically calculate the optimal Z sampling for your Z-stack. The software uses the highest wavelength defined in the channel dimension to determine the optimal step size.

   All fields remain editable, allowing you to manually adjust the calculated step if needed. [Modify the emission wavelength, the numerical aperture of your objective and the refractive index of your objective immersion medium] then click Confirm to validate your settings.



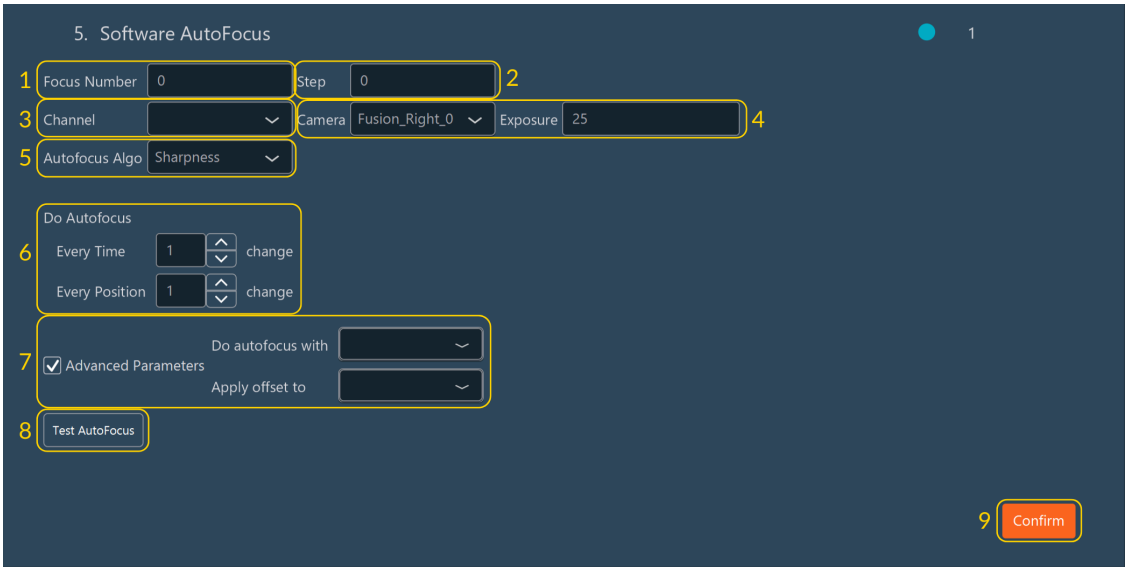
4. Choose the **acquisition direction**: from **top to bottom** or **bottom to top**.
5. Enable **Center First** if you want the acquisition to start from the central plane of the Z-stack. This option must be activated when using a hardware autofocus system such as **PFS**.
6. Click **Confirm** to validate.

NB: Use the visual **Z-stack diagram** to check your setup — you can either move the **blue arrow** or click directly on the diagram to preview your stack.

NB: If you set your ZStack with the center plan, the Volume, Step and Size are linked. If you have position dimension activated and the Z-axis is checked for your position, this focus will be the center of your Z-stack.

1.1.5.8. Software autofocus

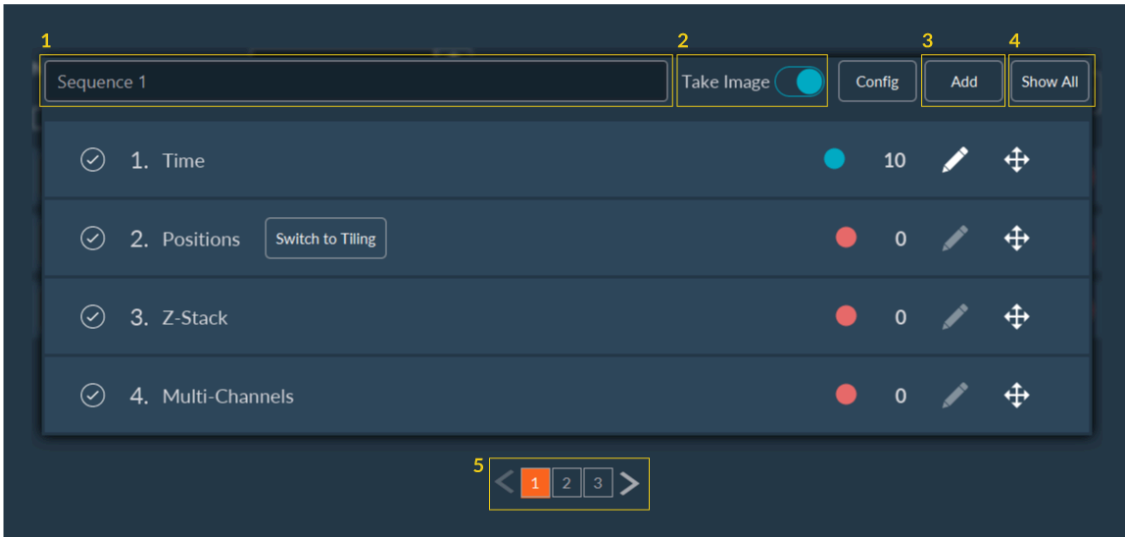
The Inscoper I.S. has a software autofocus mode to keep samples at the focus throughout the acquisition sequence.



1. Select the number of focus to perform to each position.
2. Select the step (µm) between each focus.
3. Select the channel to use.
4. Select the camera to use and its exposure time.
5. Select the autofocus algorithms to be applied: maximal intensity or sharpness (the best focus plane will be the sharpest).
6. Add Condition: interval of autofocus depending on dimension (for example, every 3 time points).
7. Check **Advanced Parameters** to set up the device to do the autofocus (for example, the piezo or the focus of the microscope) and if you need to apply offset to another device.
8. Test the autofocus protocol.
9. Click on **Confirm**.

1.1.5.9. 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.

When you click on Show All (4), the Summary tab below appears and provides the following options:

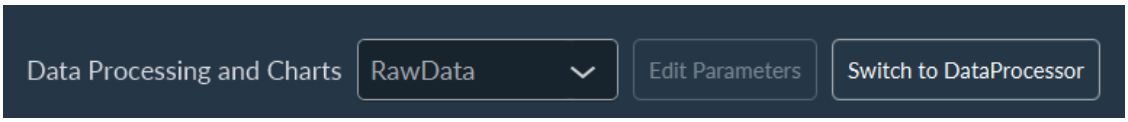
1. Adjust the loop number to determine how many times the entire acquisition sequence will be repeated.
2. Optional feature: Make intermittent sequences by checking the checkbox and specifying the dimension to consider.
3. Choose to take image (or not) during each sequence.
4. Activate/deactivate a sequence 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**.

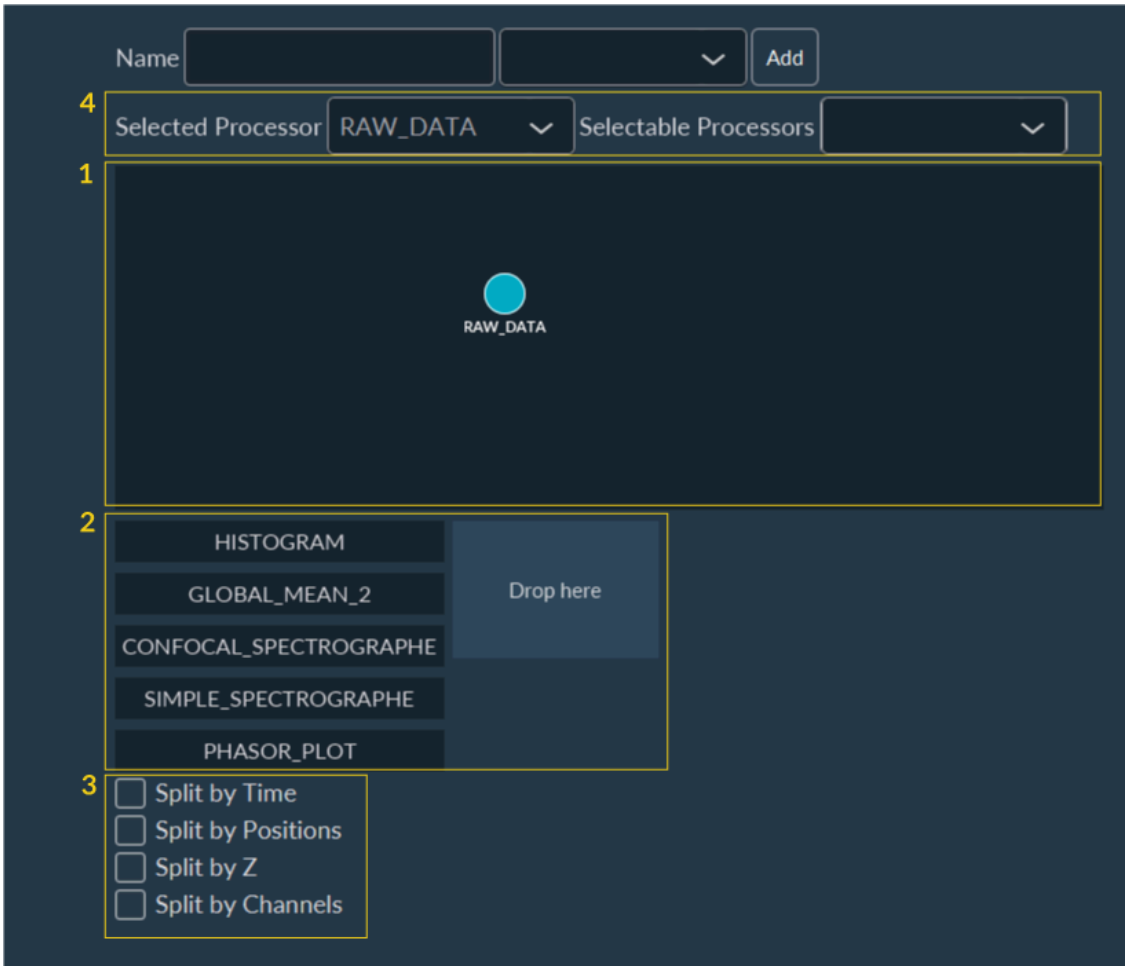
1.1.5.10. Data Processor

1.1.5.10.1. 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.



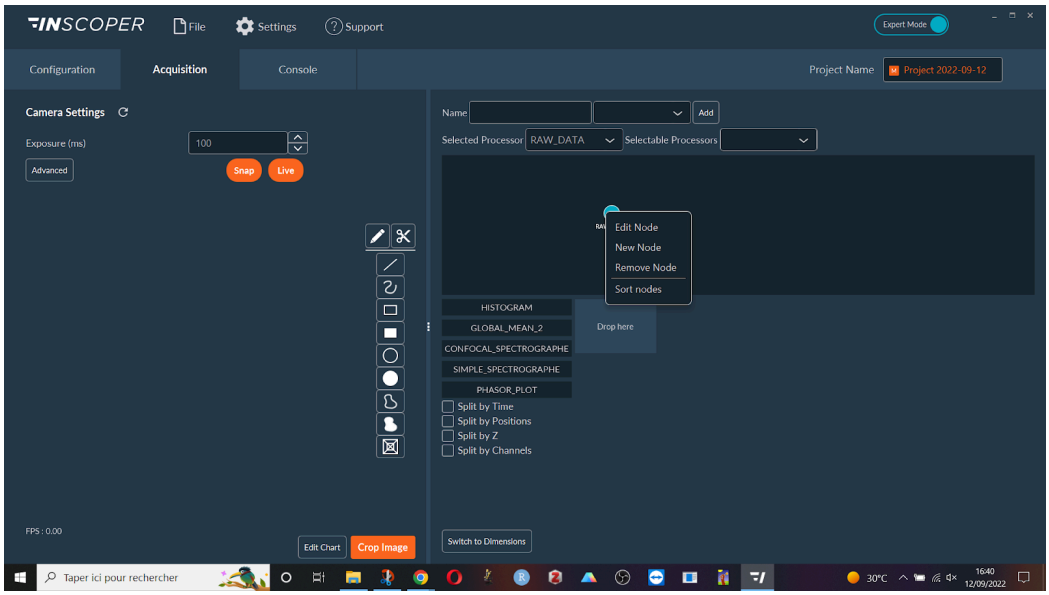
1. Space for creating data processing workflow.
2. Data visualization customizing tools.
3. Tools for analyzing data after splitting by dimension.
4. Tools to customize the image visualization after pre-treatments:

> 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.

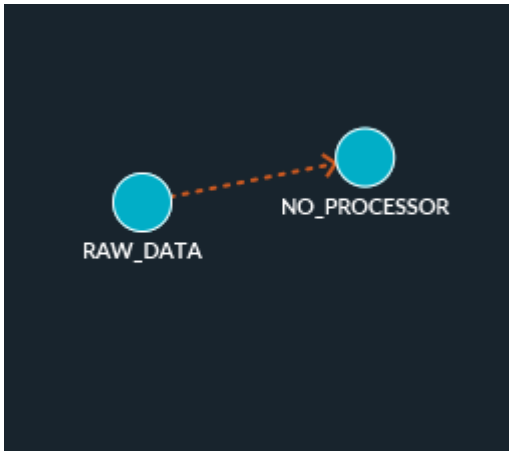
1.1.5.10.2. Create a new data processing workflow

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**.



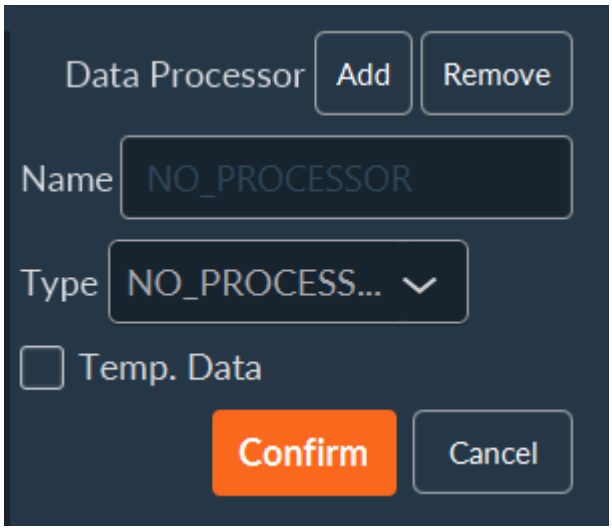
2. A new node will appear on the screen. At the moment there is no treatment associated with this node. To add one, double click on it or right click and select **Edit Node**.

> 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.). If you don't want to save this process to disk, check the **Temp. Data**

box. 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.

1.1.5.10.3. Data visualization personnalization

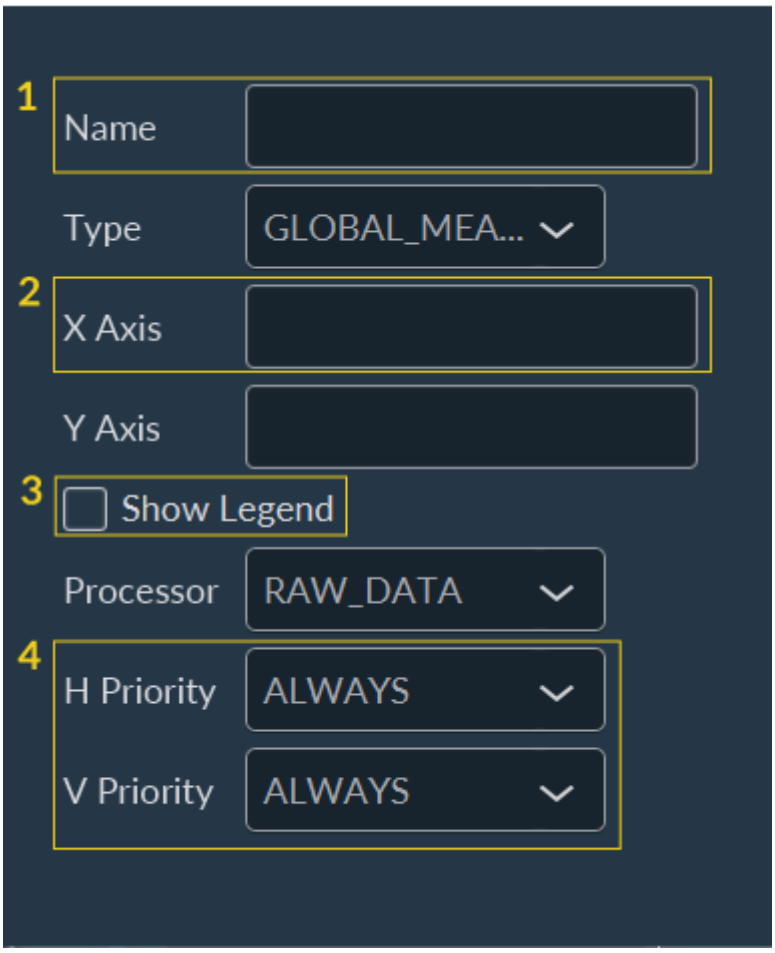
You can customize the display of the data during the acquisition, such as the evolution of the fluorescence intensity for example.

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.

1.1.5.11. Saving images and metadata

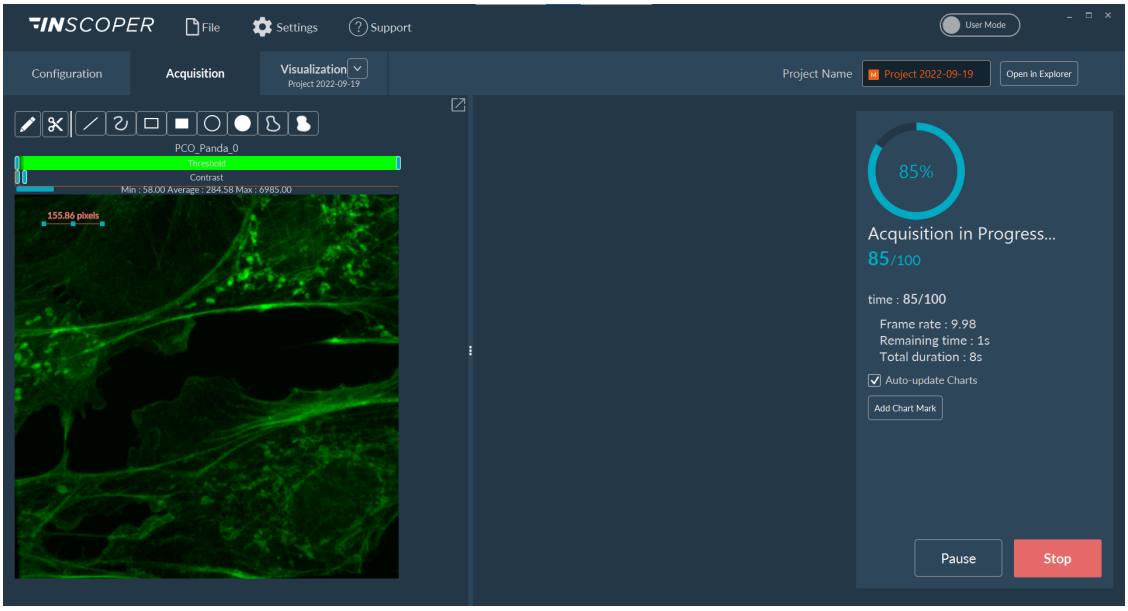
To save the acquired images and metadata:

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).

If you need to standardize the name of all saved data files before the acquisition, please, click here

1.1.6. Visualization

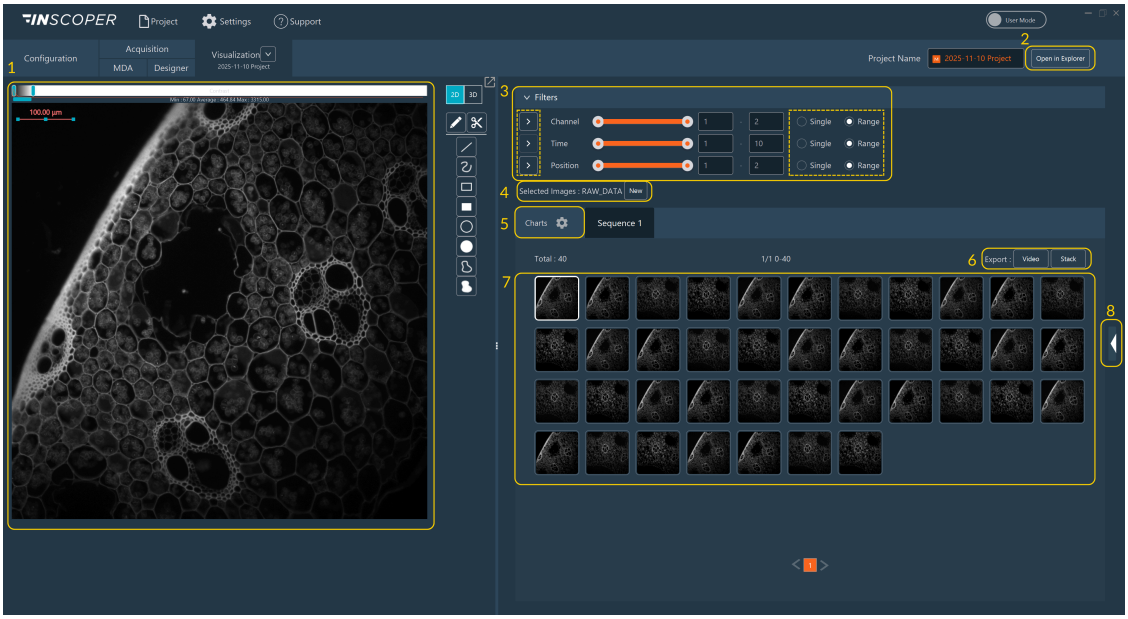
1.1.6.1. Visualization during acquisition



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.

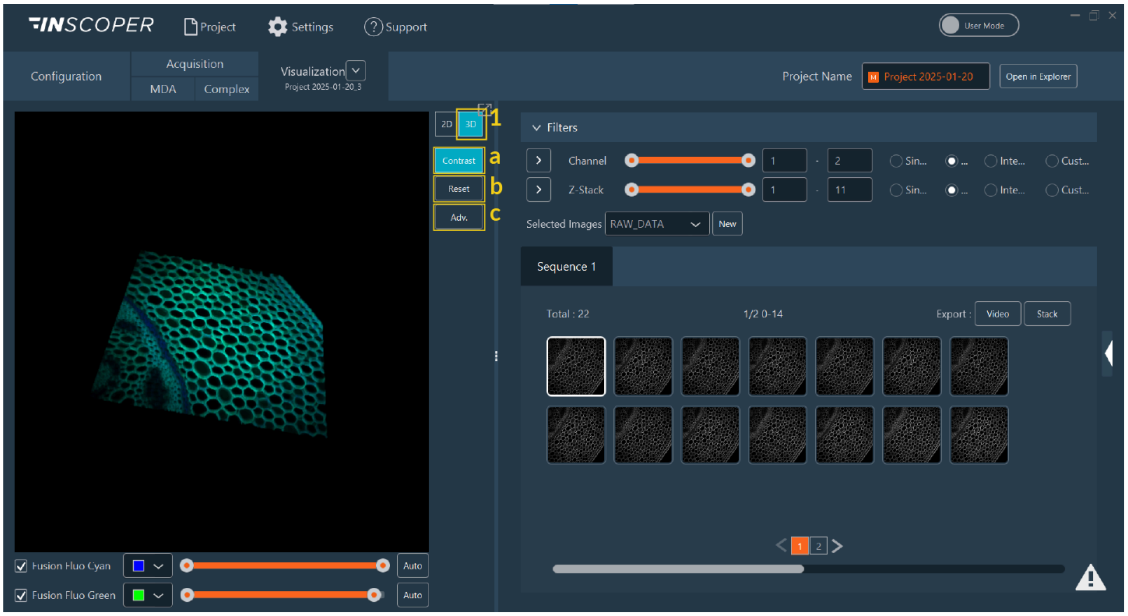
NB: For large image acquisitions, it is possible to see the total tiling with the different images implemented during the acquisition.

1.1.6.2. Visualization after acquisition

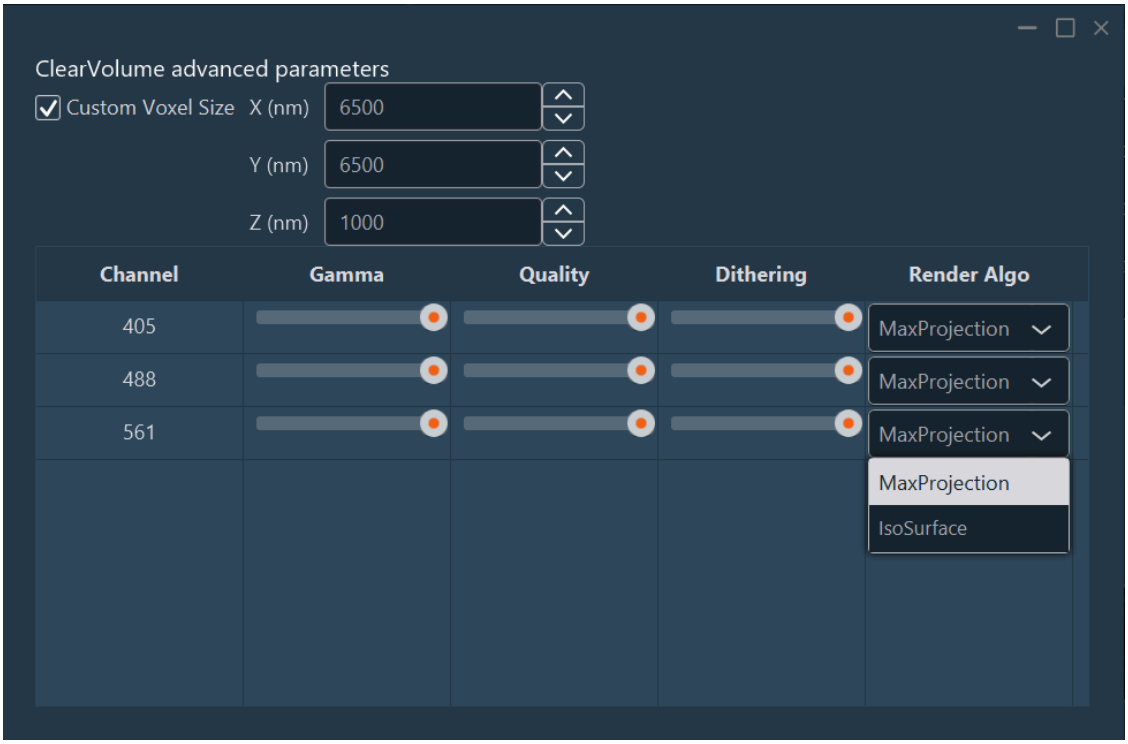


1. As in the rest of the software, this part of the screen is reserved for viewing images.
2. Opens the location where images are stored. Only works if you save your data to disk.
3. **Filters** to select images by dimensions: The Inscoper I.S. offers various tools to facilitate navigation between images, such as filters for example. In the **Filters** section, you can select a single image or all images. The **Play** button next to the dimension name allows you to replay the selected sequence.
4. Select image to visualize and create a new image processing.
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.

1.1.6.2.1. 3D viewer

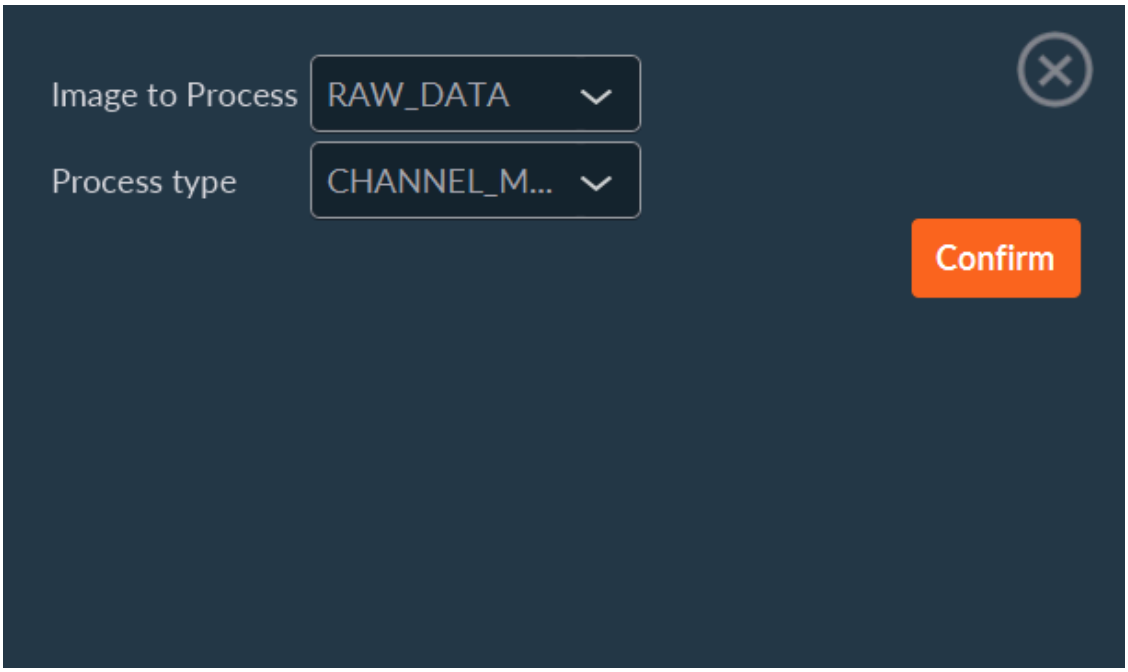


1. Click on the 3D button to have access to the 3D viewer.
2. Get access to 3D view of your data.
3. Adjust 3D options et parameters:
    a. **Contrast:** display the LUT and contrast bar below the 3D view.
    b. **Reset:** reset the view of your data by default
    c. **Advanced:** opens a new window to access the advanced parameters. By channel, you can modify:
        # **Voxel Size** (nm).
        # **Gamma:** apply a gamma factor to modify the contrast
        # **Quality:** decrease of the quality for a smooth navigation into the sample
        # **Dithering:** used to reduce visual artifacts, especially banding or aliasing, and to improve depth perception or rendering quality.
        # **Render Algorithms:**
            # **MaxProjection**: visualizes only the voxels with the maximum intensity encountered along each ray within volumetric data
            # **IsoSurface:** An isosurface is a 3D surface representation of points with equal values in a 3-D data distribution.



1.1.6.2.2. Image Processing

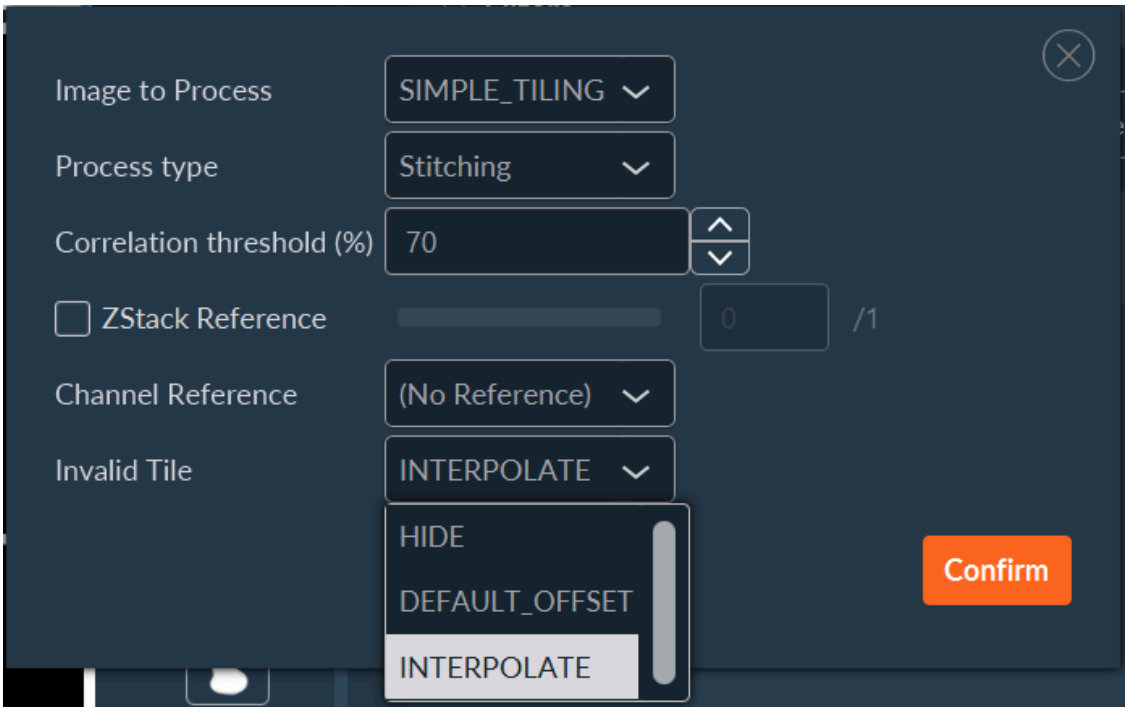
In this window you can process images acquired with processors of your choice.



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:



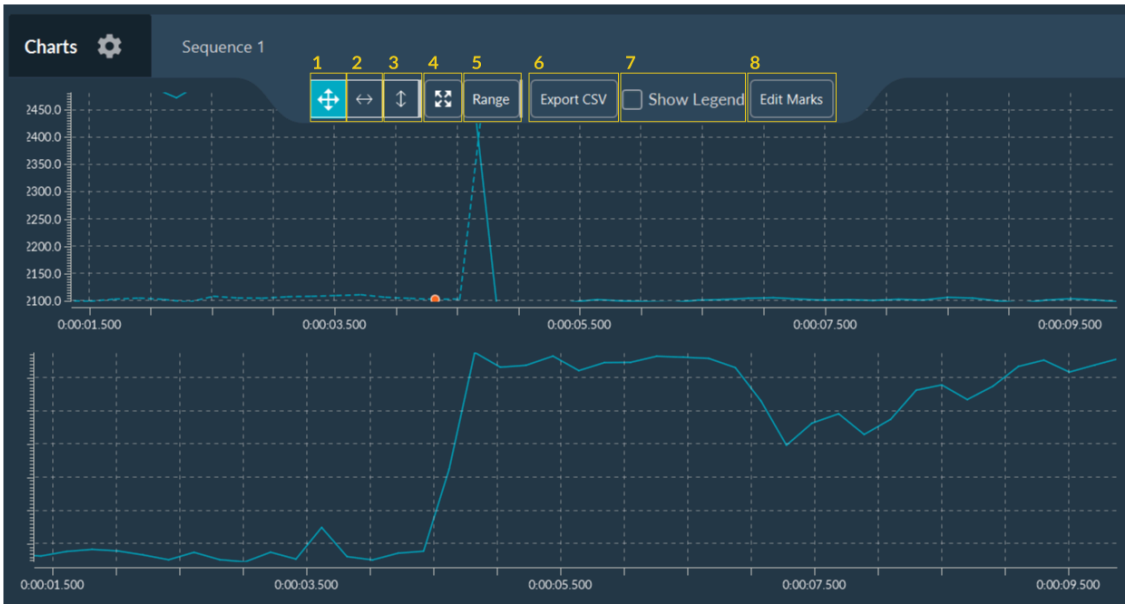
1. Select the image to process.
2. Select the process type.
3. Indicate the correlation threshold (%).
4. Check if you want to apply ZStack reference and select the good plane to calculate the stitching.
5. Select the Channel Reference if needed.
6. Select the action to apply to the invalid tile:
    # **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**.

1.1.6.2.3. 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.

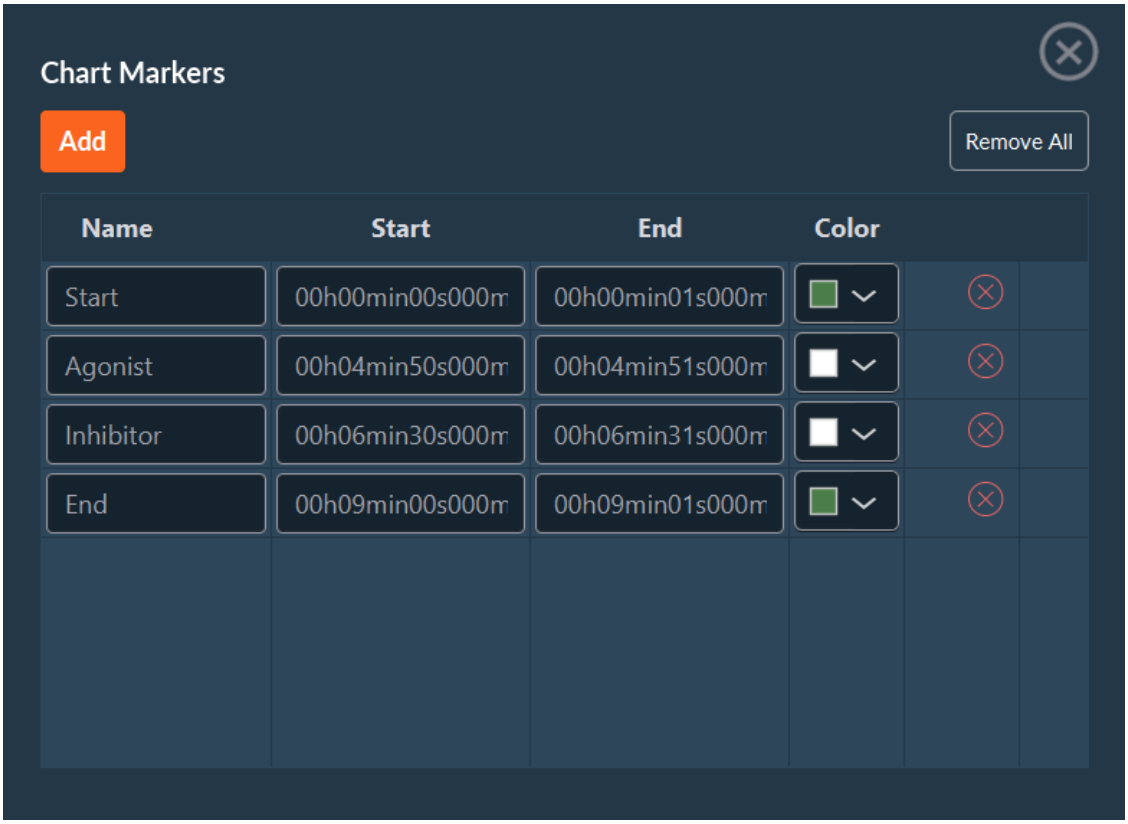
It is possible to:

- 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.

1.1.6.3. Data export

1.1.6.3.1. Video exportation

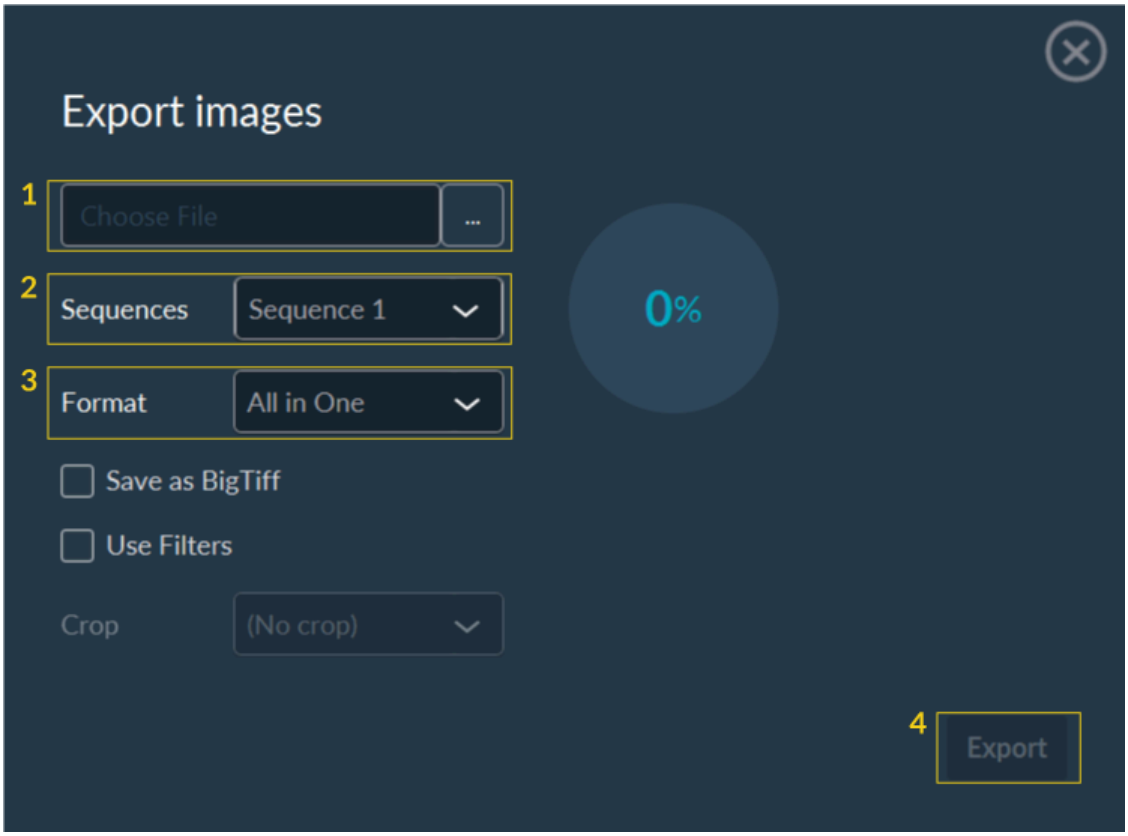
It is possible to export a video from the Inscoper I.S.. Select with filters all images to put together and click on **Video** of the “Export” list.

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**.

1.1.6.3.2. 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.

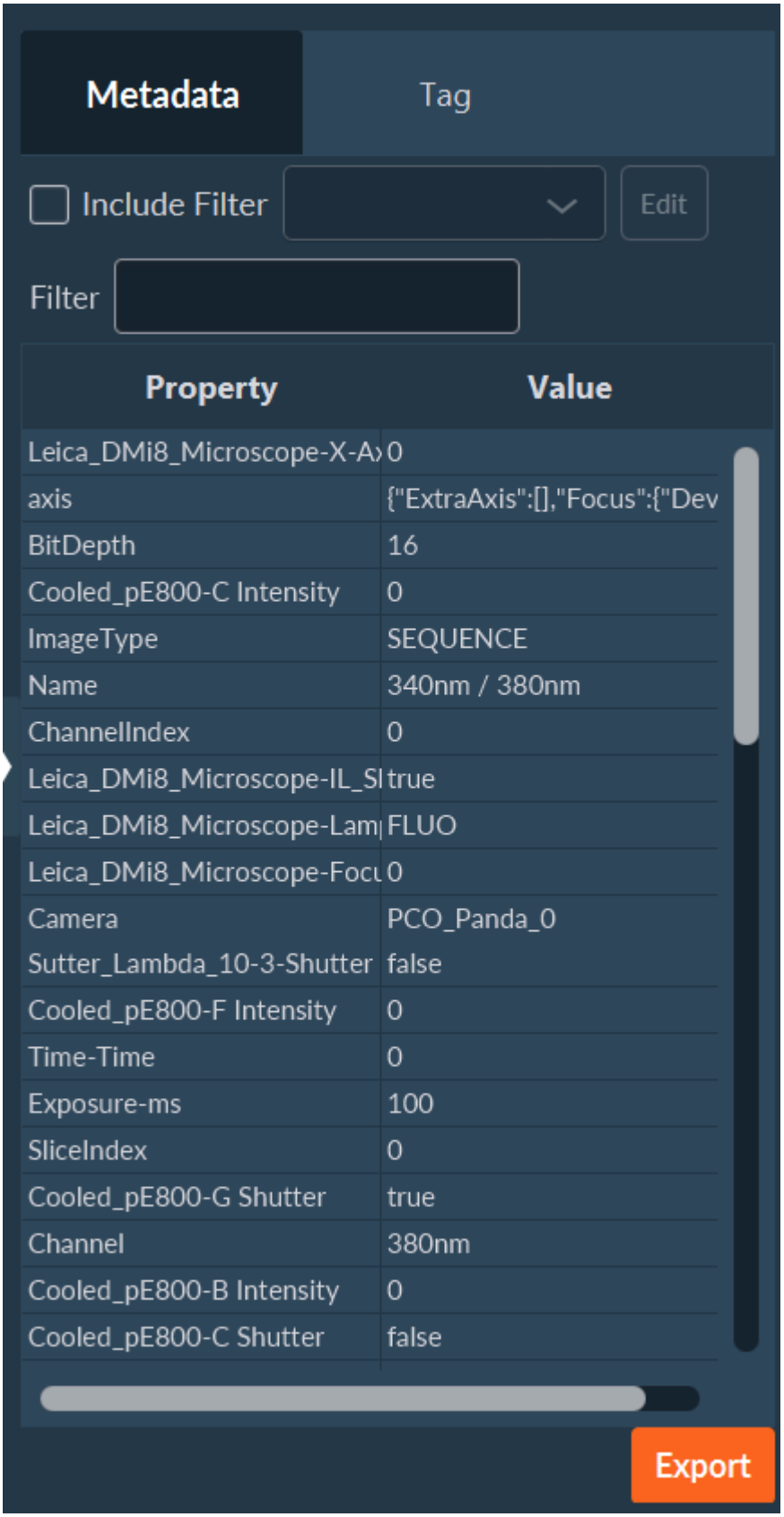


To export a stack:

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**.

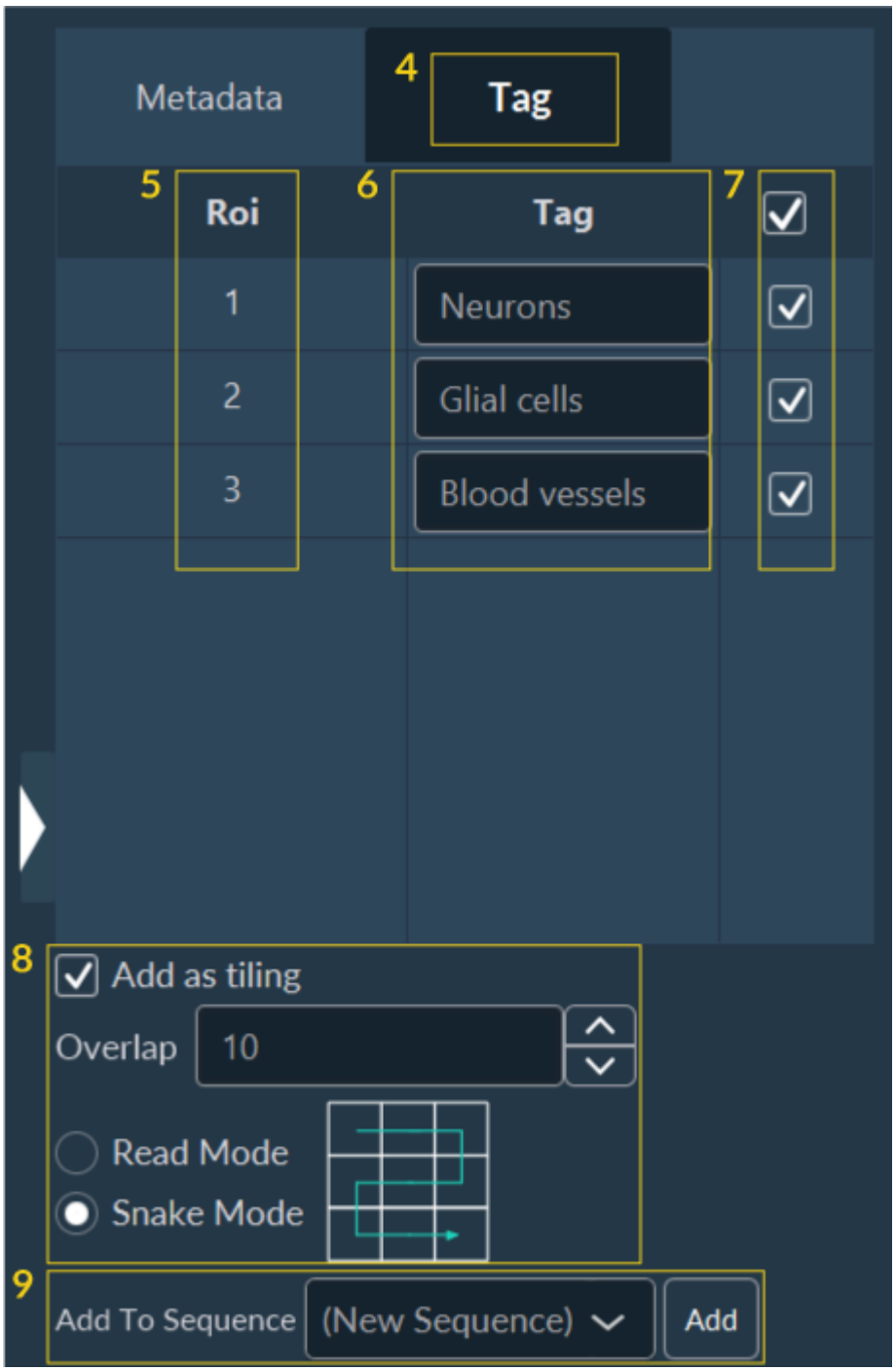
1.1.6.3.3. Metadata access

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

1.1.6.4. 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

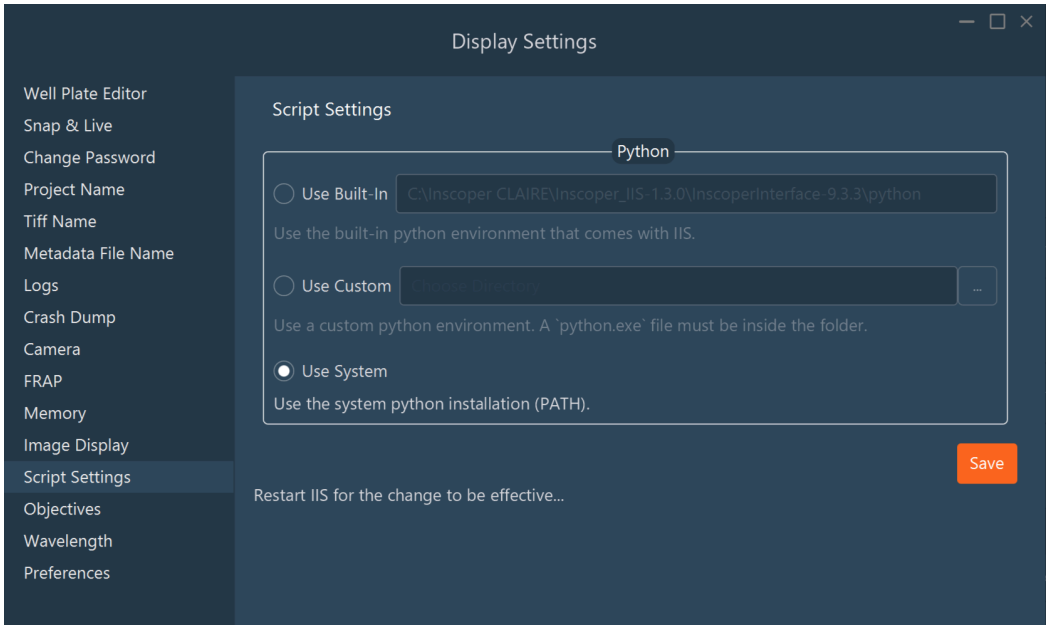
1.2. Custom Scripting module

You can use this module to call a Python script during the acquisition sequence. You must have at your disposal python scripts, structured with a template similar to that of the applications included in the inscoper-scripts resource provided with this module. In this guide, you may select any script provided as part of this resource.

Python Environment Setup

Before running scripts, you must define which Python engine the software uses.

1. Navigate to **Settings** > **Display Settings**.
2. Select **Script Settings** from the left-hand menu.



3. Choose your environment:
    a. **Use Built-In (recommended)**: Uses the Python version included with the installation. This contains standard image analysis packages.
    b. **Use Custom**: Select a local directory containing a specific `python.exe`.
        i. Critical Requirement: If using a custom environment (like Conda), you must ensure the package `jep` is installed, or the script will fail.
    c. **Use System**: Uses the global Python version installed on your OS.
4. **Restart I.I.S.** for these changes to take effect.

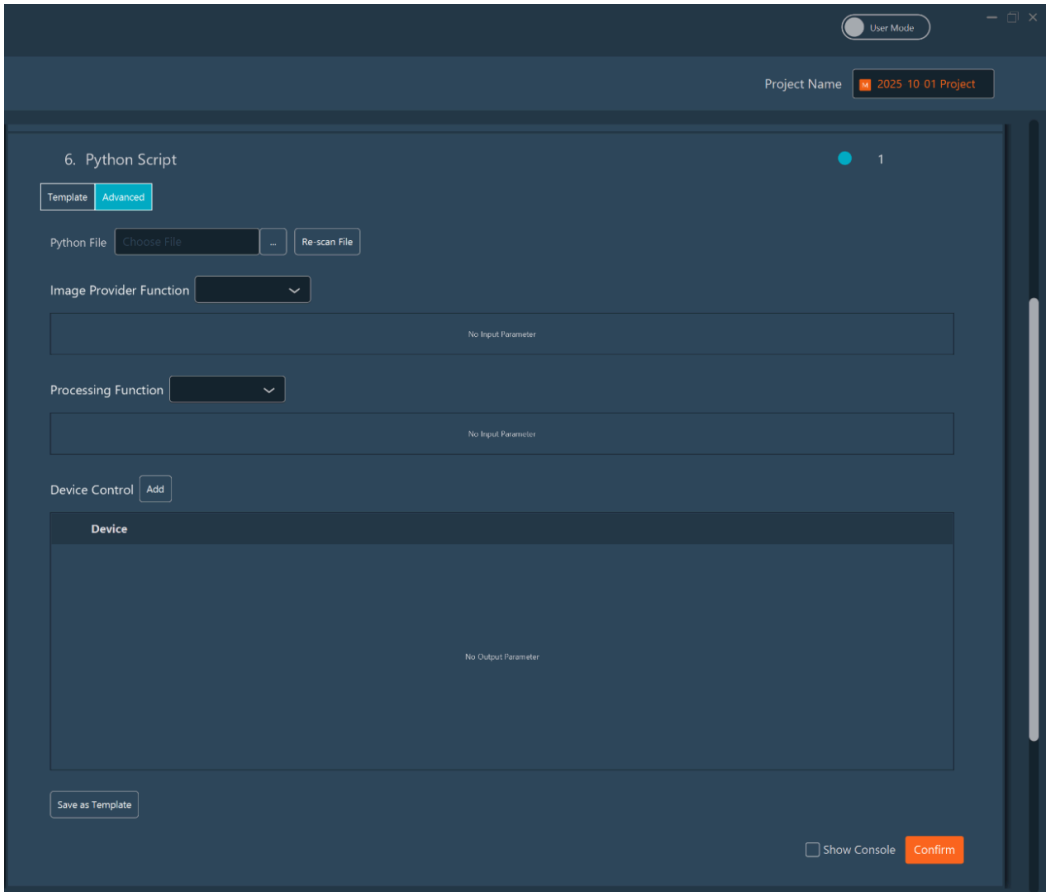
Note on Installing Packages: If you need extra libraries in the **Built-In** environment:

1. Open a console/terminal in the I.I.S. installation folder.
2. Navigate to the `python` folder.
3. Run: `./python.exe -m pip install <package_name>`

Creating a New Script Application

1. Go to the **Acquisition** tab and select **MDA** (Multi-Dimensional Acquisition).
2. Expand the **Python Script** module and toggle **Advanced** on.

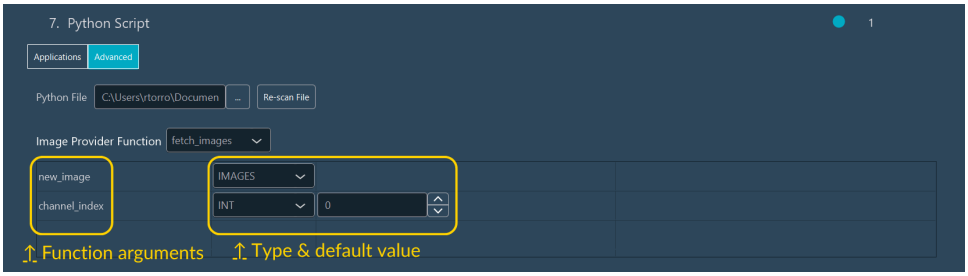
Figure 1. Advanced Script importation tab



a. Click the **"..."** button (next to "Re-scan File") and browse for your `.py` file.

3. **Map Python Functions to I.I.S. Functions**: You must tell the system which part of your code handles images and which part handles processing. Then you must map the script's arguments to I.I.S. hardware or settings.
   a. **Step A: Image Provider**: Select the function in your script that fetches images (e.g., `fetch_images`).
      i. Map Arguments: Pair the script variable `new_image` to the Type `IMAGES`. In practice this pattern allows the software to send the last acquired image to the script.
      ii. Pair `channel_index` to INT and set a default value (0). Channel indices coincide with the order of the channels in the multi-channels dimension.

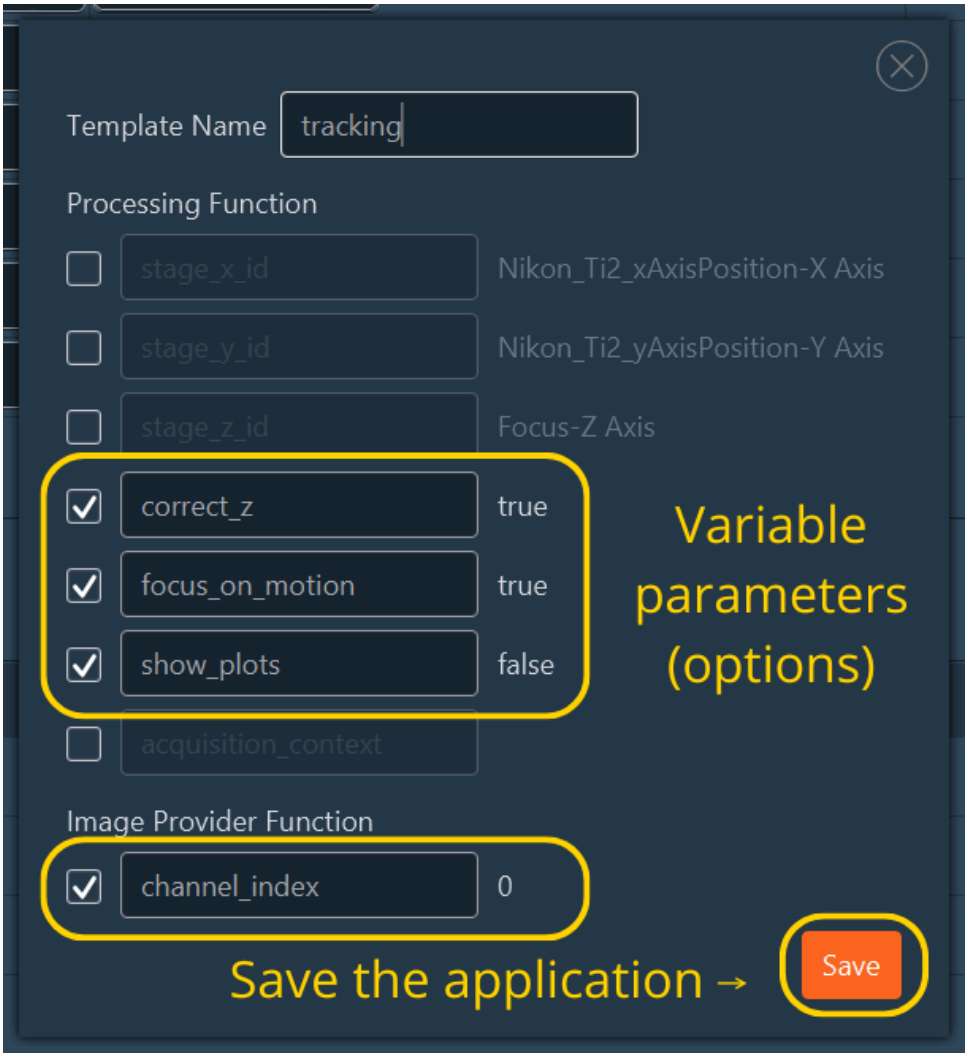
Figure 2. Pair function arguments to valid types and default values



b. **Step B: Processing Function**: Select the function that analyzes the data (e.g., `correlation_tracking`).
    i. Map Arguments: Pair the script variable `images` to the Type `IMAGES`. This variable received the images selected by the image provider function when entering the processing function.
    ii. Pair `stage_x_id` to SUBDEVICEID and set a default value to the name of your X axis subdevice. This variable tells explicitly the script the name of the X axis subdevice for this system.
    iii. Pair `stage_y_id` to SUBDEVICEID and set a default value to the name of your Y axis subdevice.
    iv. Pair `stage_z_id` to SUBDEVICEID and set a default value to the name of your focus axis subdevice.
    v. Pair `correct_z` to BOOL and set a default value.
    vi. Pair `focus_on_motion` to BOOL and set a default value.
    vii. Pair `show_plots` to BOOL and set a default value.
    viii. Pair `acquisition_context` to IIS_CONTEXT and set a default value. The IIS context allows the script to useful software functions (e.g. send a matplotlib figure to the software).
c. **Step C: Device Control**: Set the Device Control subdevices that should match with the outputs of the processing function. If the function does not update devices, it should not return anything and this part should be left blank. In the tracking example, add your three subdevices (`stage_x_id`, `stage_y_id`, `stage_z_id`).

4. **Save as Template**
    a. Once mapped, click **Save as Application Template**.
    b. A pop-up will appear. Tick the boxes for parameters you want visible to the end-user.

Figure 3. Generate an application template after importing a script



c. Unticked parameters will be hidden and locked.

If your script outputs graphs or logs, you need to configure the display panel.

1. Toggle **Expert Mode** (Top-right corner).
2. In the **Data Processing and Charts** section, click **Switch to Data Processor**.
3. Add the Console: Drag and drop the Feedback Console chart to the right panel. This displays Python `print()` statements.
4. Add Charts: If your script uses `display_figure(..., processor_name='name')`:
   # Drag a Feedback Chart to the panel.
   # Crucial Step: Rename this chart to match the `processor_name` defined in your Python script header (e.g., check the `DATA_PROCESSOR` variable in your code).
5. Name this configuration (e.g., "tracking_charts") and click **Add**.
6. Click **Switch to Dimensions** to return to the main view.

Running the Experiment

Once the template is saved, daily operation is simple.

1. Go to the **Application** tab.
2. Select your new template (e.g., "tracking").
3. Adjust the visible control parameters.
4. Ensure **Data Processing and Charts** is set to the layout you created in Part 3.
5. Press **Start Acquisition**.

Figure 4. Typical application view for the correlation_tracking_withZ.py script

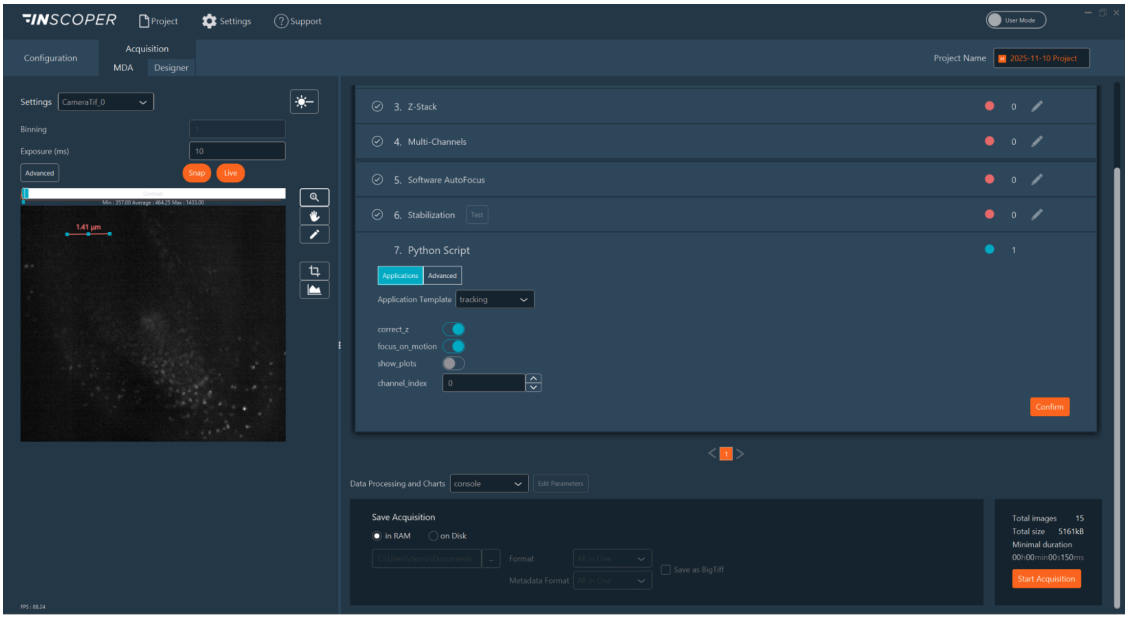
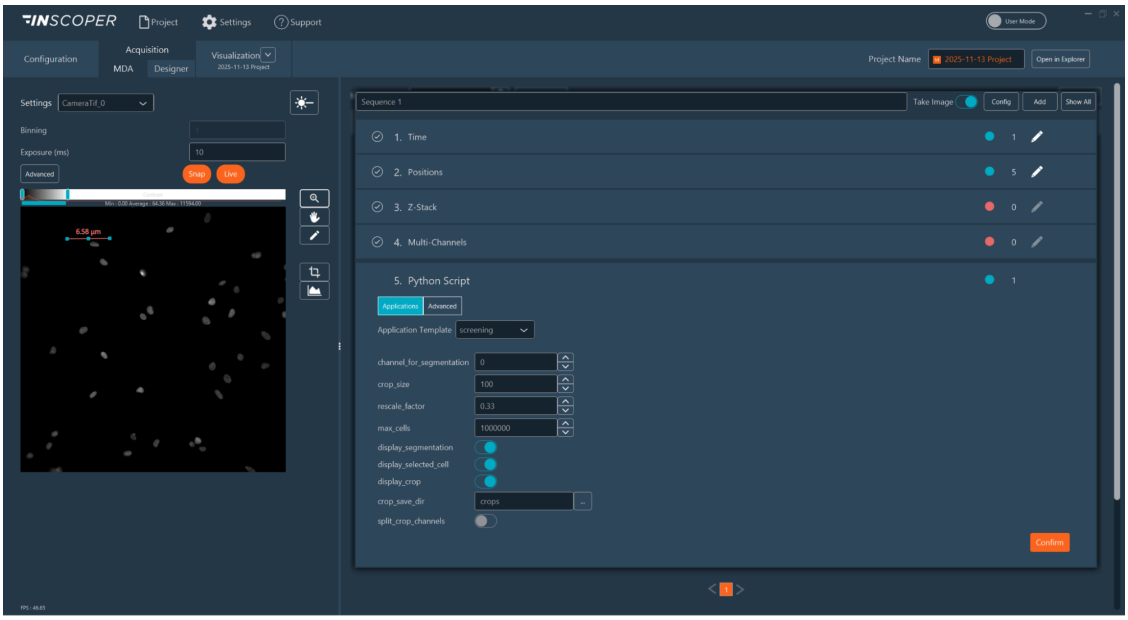


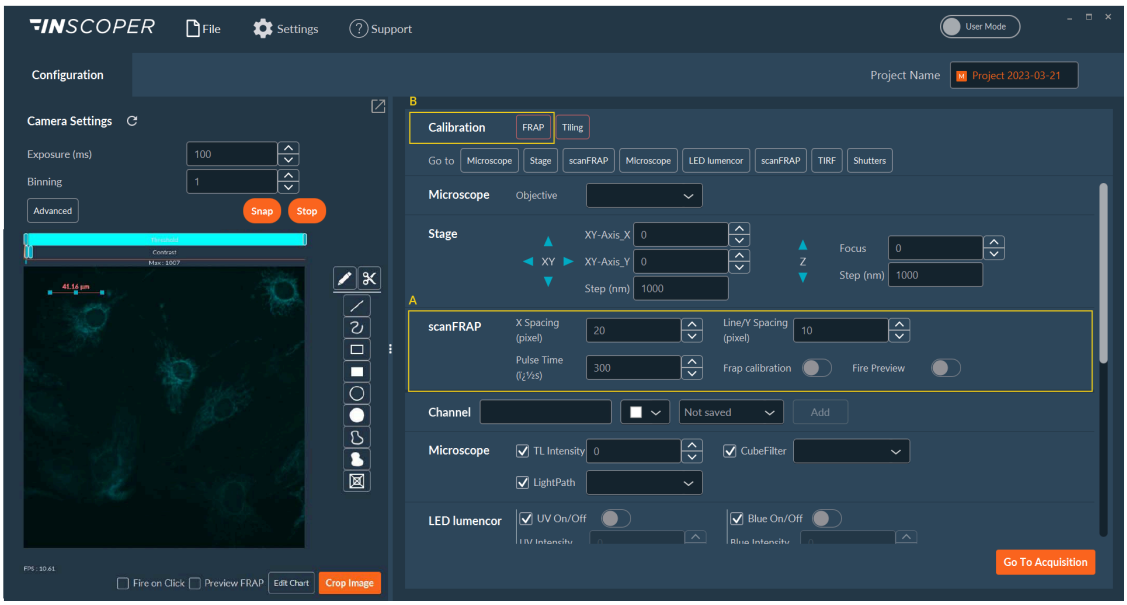
Figure 5. Typical application view for the single_cell_screening.py script



1.3. scanFRAP

The Inscoper scanFRAP solution is designed for photomanipulation and optogenetics experiments. Inscoper scanFRAP is fully integrated with Inscoper I.S.

1.3.1. Configuration & Calibration



The **scanFRAP** section (A) in the **Configuration tab** allows you to adjust the point density (space between points) for photomanipulation. The X and Y spacing refers to the density of filled forms. For lines or unfilled forms, the line/Y spacing is considered.

Fire Preview can be activated to test the scanFRAP settings. When activated, the laser will emit light on selected ROI.

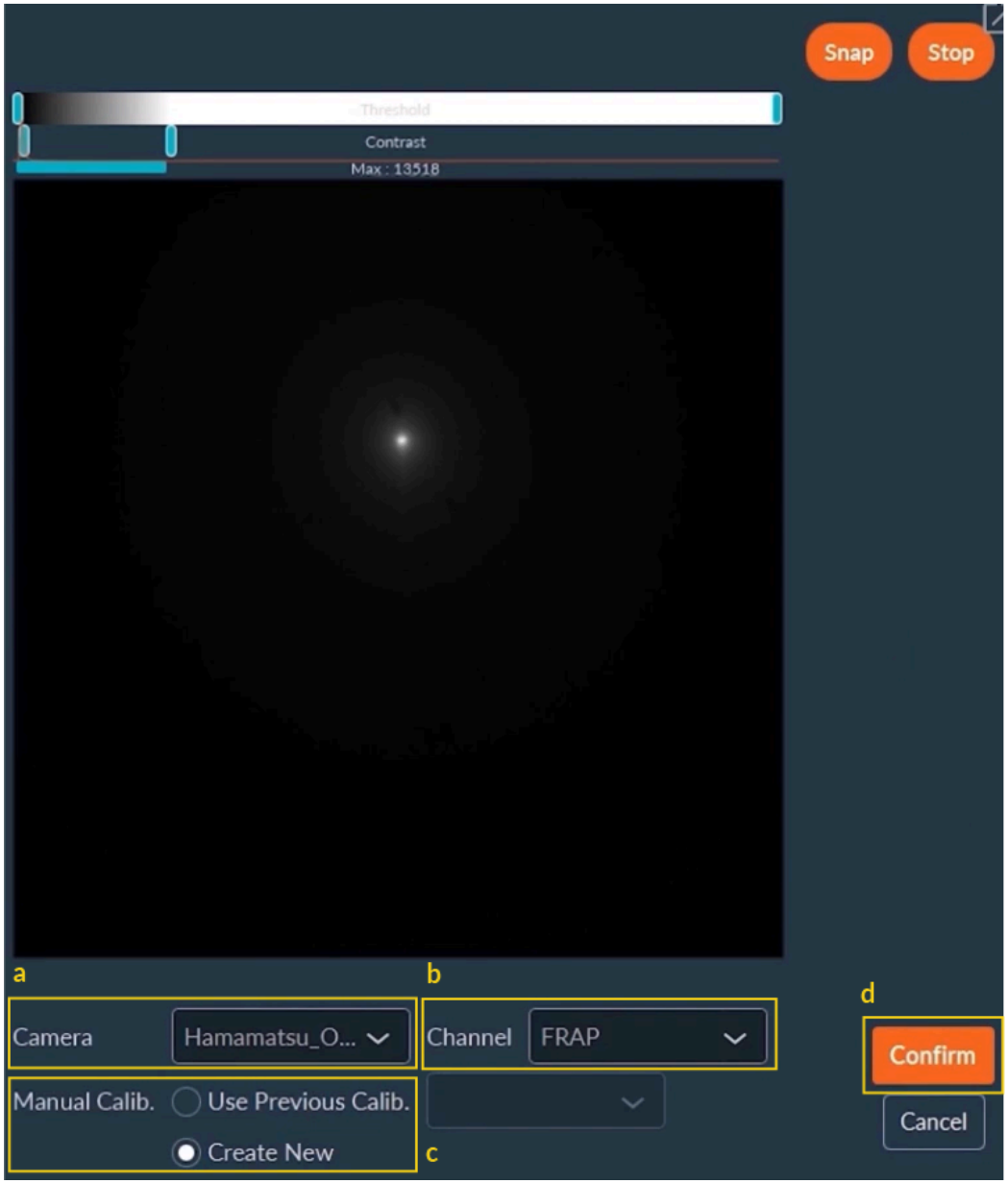
NB: The **Fire Preview** option requires (i) some ROI to be drawn on the image and (ii) the correct channel to be selected.

Before starting photomanipulation experiments, the scanFRAP must be calibrated. This semi-automated step is necessary in order to achieve a high level of accuracy on galvanometric mirrors.

1. **Manual calibration**

   After clicking on the **FRAP** in the calibration list (B), you have to:

a. Select the camera in the drop-down list.
b. Select the pre-saved channel to use
c. Select a previously done calibration or make a new one
d. Click on **Confirm**.



NB: It is recommended to perform this calibration using a fluorescent slide to obtain a strong signal and avoid laser phototoxicity.

NB: It is recommended to recalibrate each time the scanFRAP is used to optimise the accuracy of the system.

2. **Automatic calibration**

NB: It is important to ensure that the focus is good throughout the calibration process. The laser is most accurate when in focus. Do not hesitate to adjust the focus before the calibration to get the best result.

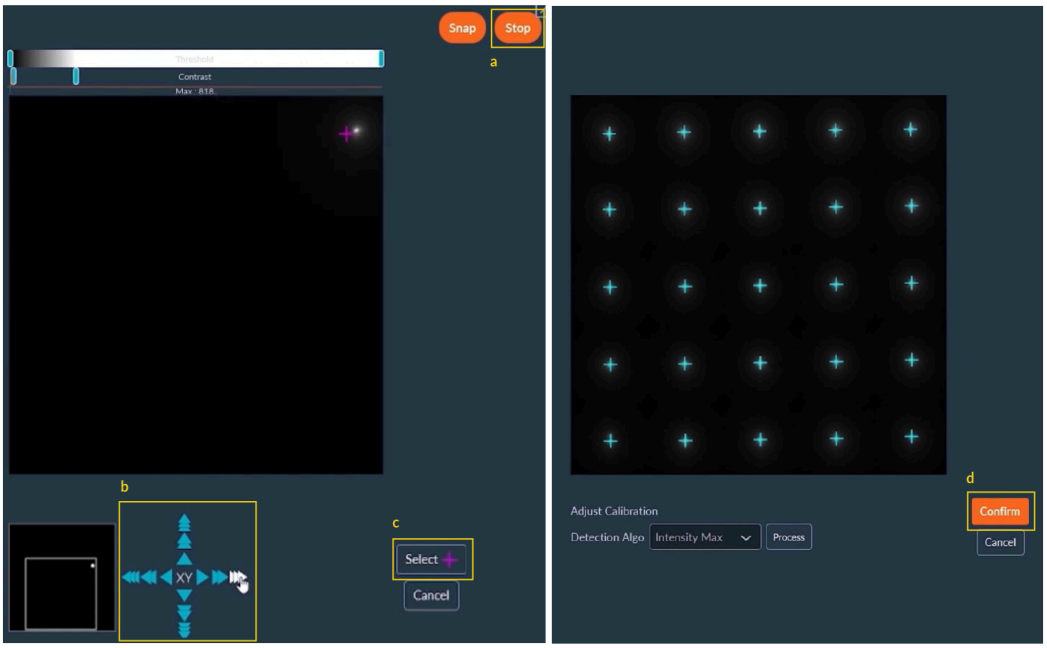
a. Click on **Live** if it is not already done (then you see **Stop**).

b. Use the virtual joystick to move the laser pointer to any of the virtual crosses on the screen.

c. When the laser pointer is on the cross, click **Select**. Repeat steps a and b three times.

d. A fully automated protocol will make a pattern on the image. You have to validate the regularity of this pattern and click on **Confirm.**

NB: If you find that the calibration is not good enough you can reprocess the calibration with the detection algo tab.



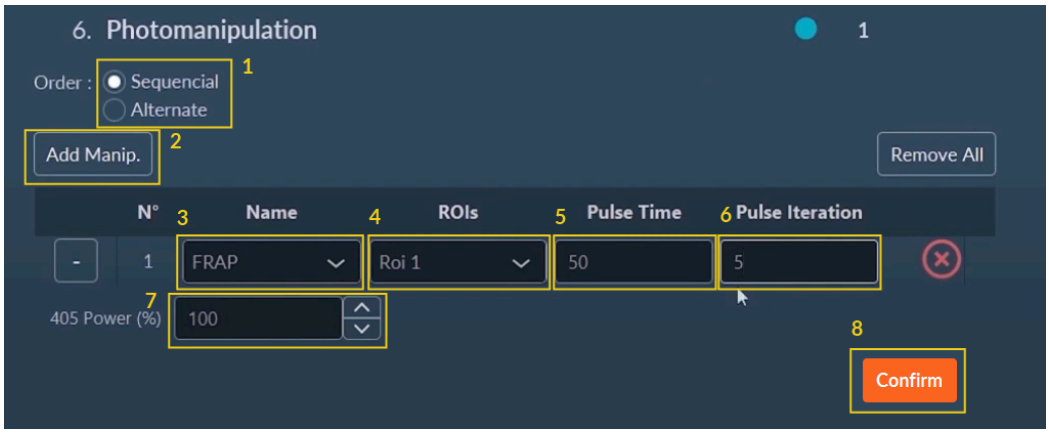
NB: To validate the calibration, do not hesitate to do some tests. For this, place some ROI on the live image and click on **Fire Preview**.

1.3.2. Acquisition

The Inscoper scanFRAP is integrated into the sequence for photomanipulation experiments. You will find a **Photomanipulation dimension**.

To use the **Photomanipulation** dimension:

1. Select the scanning order.
   The ROI can be scanned one at a time (**Sequential**) or alternately (**Alternate**).
2. Click on **Add Manip.** to add a photomanipulation event(protocol).
3. Select the pre-configured channel (name) to be used during the sequences.
4. Select all ROI to be bleached.
5. Adjust the pulse time (corresponds to the time needed to the movement from the previous point and the exposure time).
6. Adjust the number of iterations required.
7. Adjust the laser power.
8. Click on **Confirm** to validate all these settings.

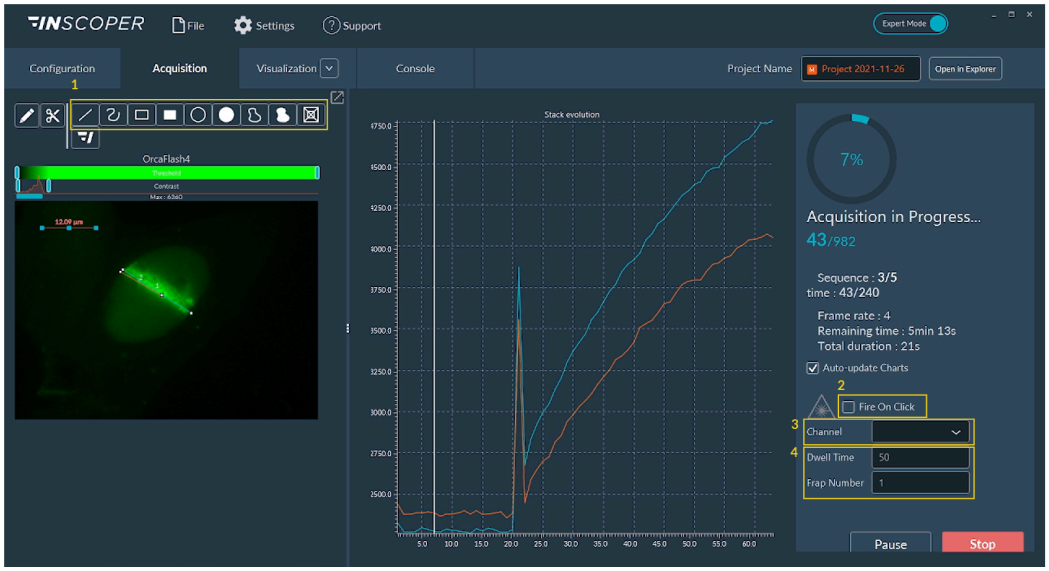


NB: Before starting a photomanipulation experiment, be sure that the scanFRAP is well calibrated.

1.3.3. Acquisition in progress

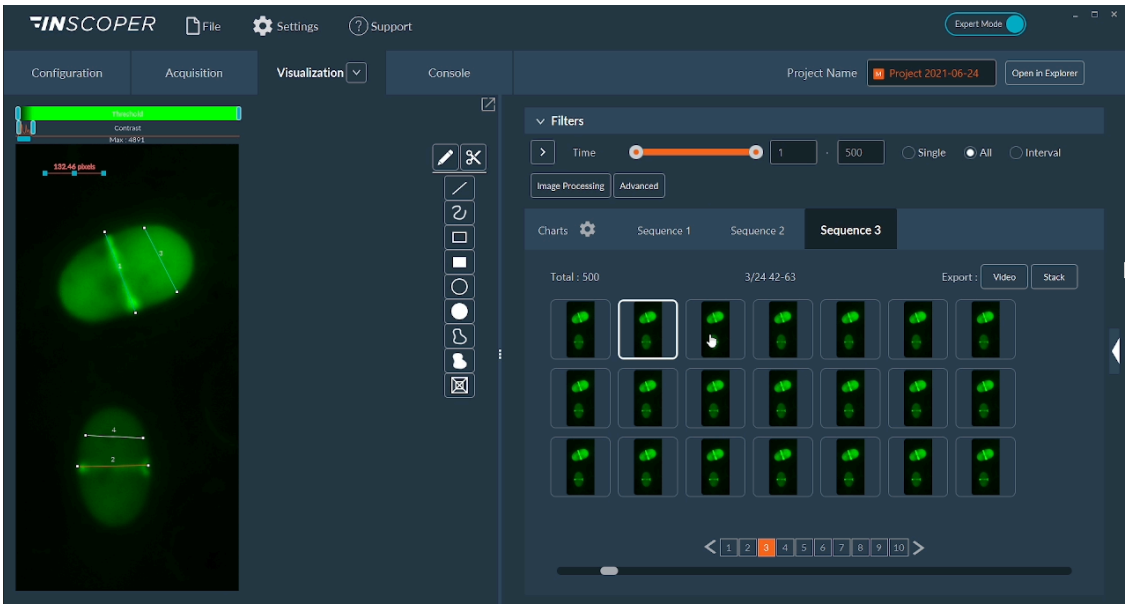
During acquisition, you can monitor the progress of your acquisition in real time. You will see the current images and graphs showing the raw data of each ROI. You can also add new area to photomanipulate during the sequence with the **Fire On Click** feature. To use the **Fire On Click**:

1. Create a new ROI to bleach.
2. Check **Fire On Click** box.
3. Select the calibrated channel to use.
4. Adjust the pulse time (**Dwell Time**) and the iteration number (**FRAP number**).
5. Click on the created ROI to make photomanipulation in live.



1.3.4. Visualisation

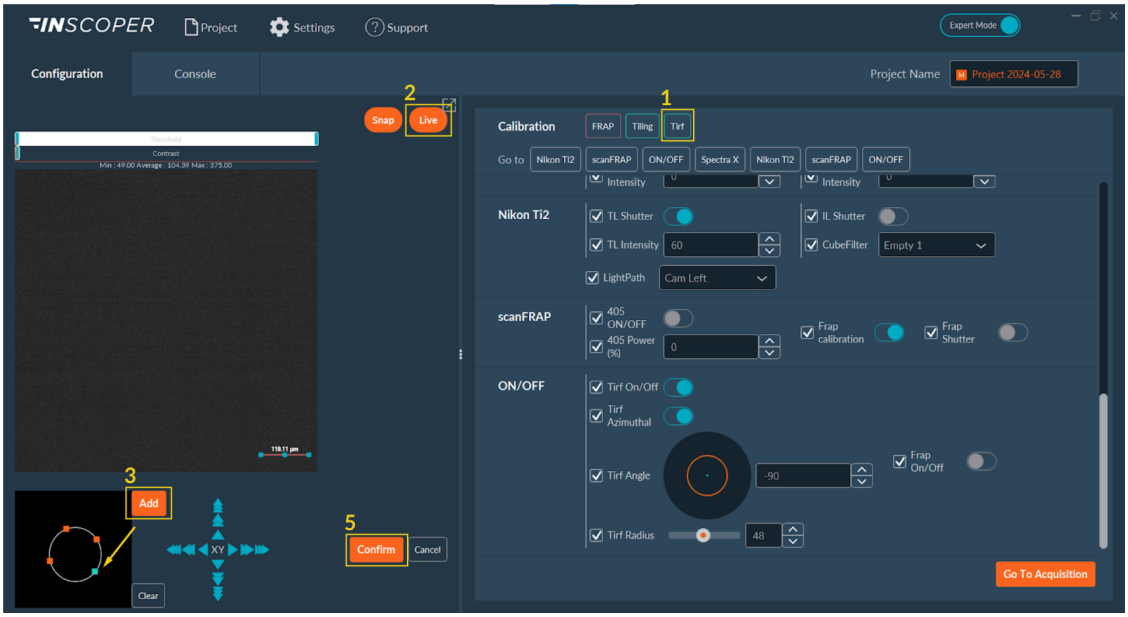
In the **Visualisation tab**, you will see the result of all sequences and the graph to follow your fluorescence intensity in the ROI.



1.4. TIRF

Total Internal Reflection Fluorescence

1.4.1. Calibration



1. Click on **Tirf** button in the Calibration section.
2. Start **Live**.
3. Click the **Add** button to add a point to the field of view. This point will be blue (i.e. selected) corresponding to the position of the Tirf laser, move this point (i.e. move the angle of the laser) until you get a Tirf image.
4. Repeat the previous step to add the desired number of positions.
5. Click on **Confirm**.
6. Repeat this calibration for each wavelength that you will use for your TIRF imaging.

1.4.2. Configuration

1. Activate or deactivate Tirf using the switcher.
2. Select **Tirf Azimuthal** (circle illumination) if you need.
3.
    a. If Tirf azimuthal is activated, you will see the diagram of the circle corresponding to the laser scanning in the Tirf angle.
    b. If Tirf Azimuthal is not activated, you will see the laser position (orange dot) and you can adjust the coordinates by changing the **Tirf Angle** value.
4. Adjust **Tirf radius** if you need (corresponds to the angle of the laser at lens outlet for WF - HILO - TIRF).
5. **FRAP** can be activated by clicking on the switcher.

NB: Those parameters (Tirf Azimuthal or point, Angle and Radius) need to be saved in the Tirf channel for Tirf Imaging

1.5. liveDRIM

1.6. liveSR

1.7. liveRATIO

The Inscoper liveRATIO solution is the feature which allows you to compare the evolution of two wavelengths by rationalizing them according to time. For that, the “Ratiometric imaging” data processor developed by Inscoper is the key element of this solution.

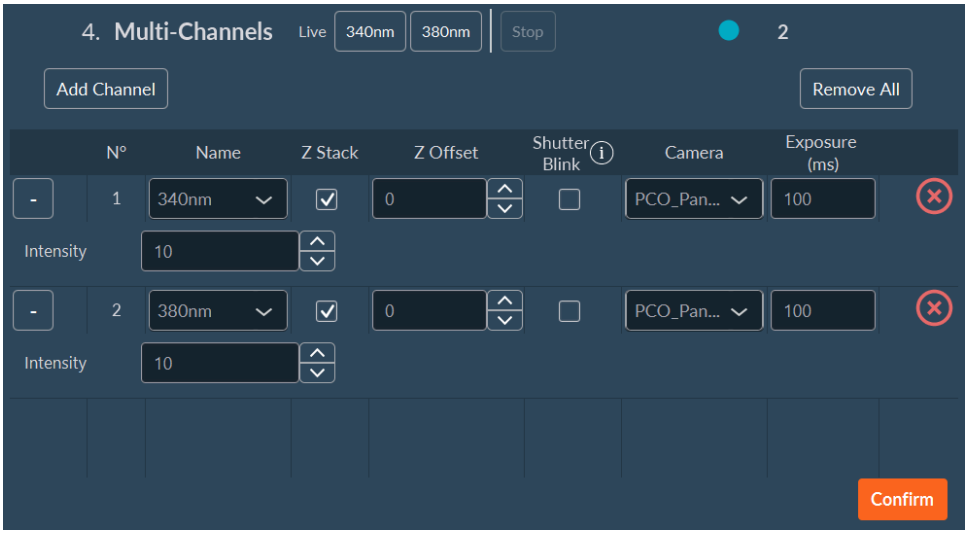
1.7.1. Configuration

For the **configuration** part, please refer to the configuration step of the Inscoper I.S..

1.7.2. Acquisition

1. Wavelengths set up

    a. In the **Acquisition** tab, go to **Multichannels** dimension.

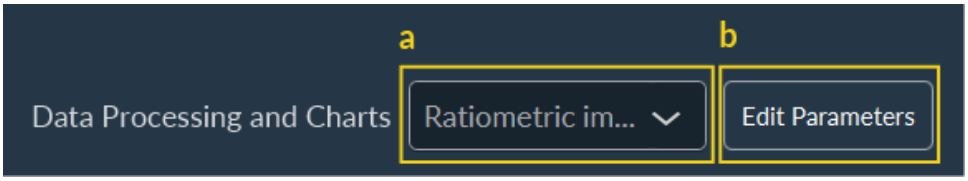


    b. Select two or more wavelengths.

2. Data processing

    a. Select the **Ratiometric imaging_Despeckle** in the **Data processing and Chart** drop-down list.

    b. Click on the **Edit Parameters** to set and to optimize the ratiometric module.



c. Select the ratio to measure during the experiment.

d. Check if you want to use the module to remove the background noise.

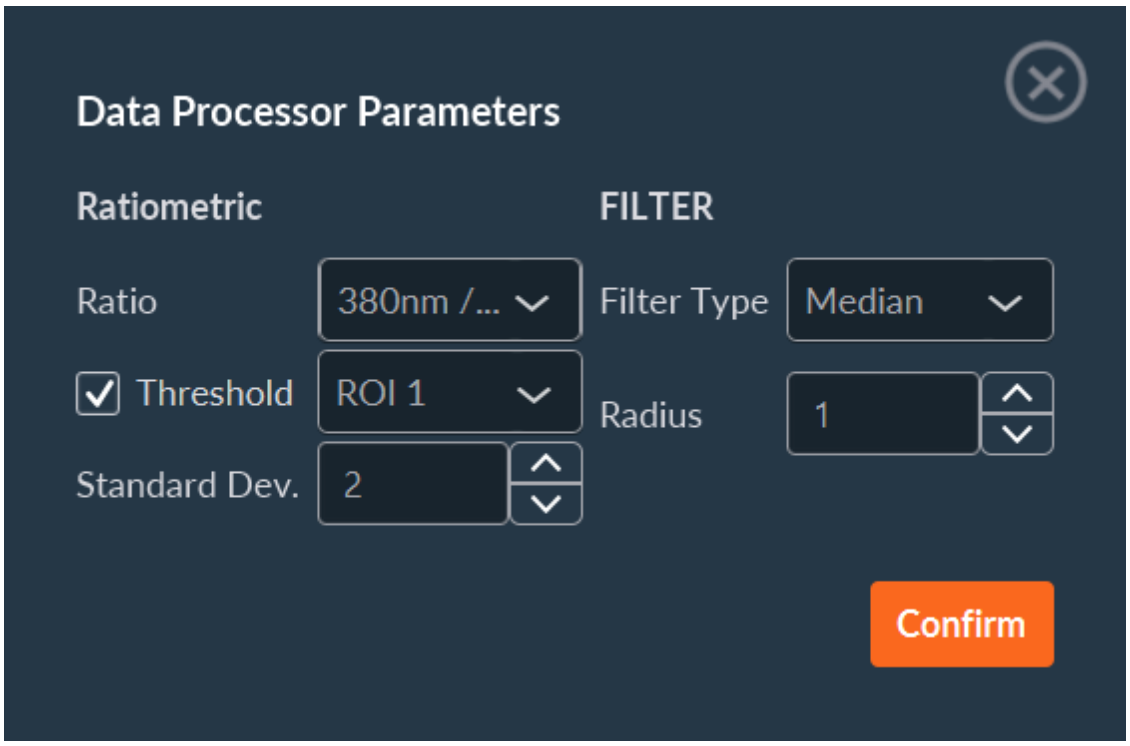
e. If checked, select a ROI without any signal.

f. If checked, select the number of times the standard deviation will be considered for the calculation.

g. Select the filter type to apply (median or average).

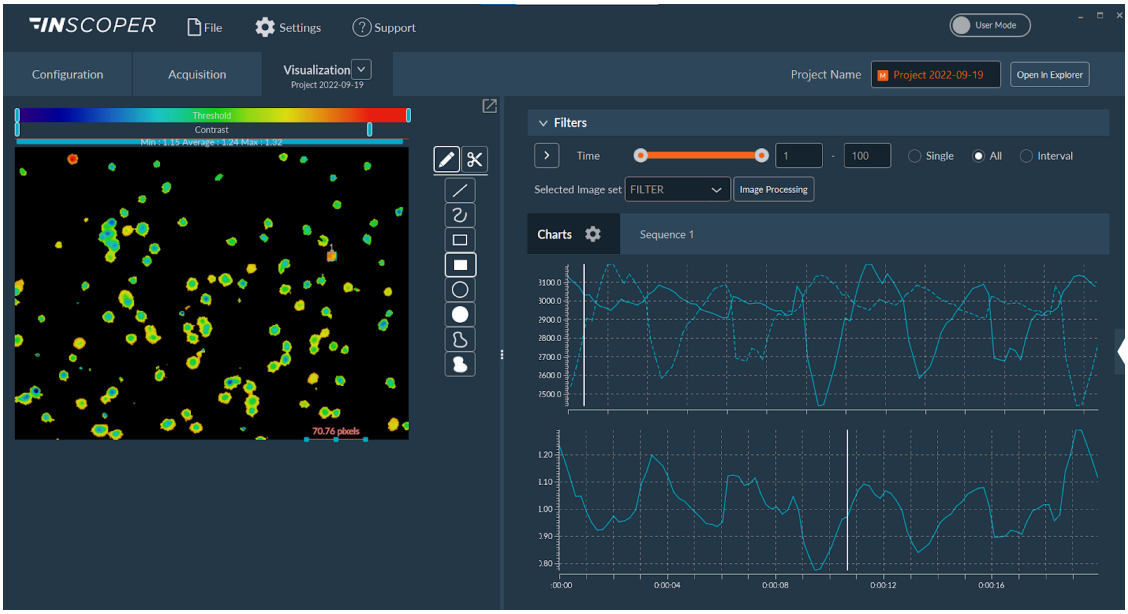
h. Select the radius to consider for this filter.

i. Click on **Confirm**.



1.7.3. Visualization

The **visualisation tab** gives you access to raw and rationalised data for each manually drawn ROI. The graphs are automatically updated whenever an ROI is added, modified or deleted.



1.8. MAICO I.S.

The MAICO I.S. presents an all-in-one answer for laser scanning confocal microscopy, designed to work seamlessly with microscopes from any brand. It serves as an ideal choice for routine microscopy imaging, whether it's used as a compact microscope on a lab bench or as the primary system in a core facility.

Before starting

Before opening Inscoper Software, you need to turn on and calibrate the MEMS unit:

1. Turn the Power key from Off to On. The led will go from orange light to green blinker light.
2. You need to wait 5 to 10 min to get a stable temperature. After this, the “laser calibration” button will be green.
3. You need to press it to proceed to the calibration.

> NB: the shutter needs to be open for this step.

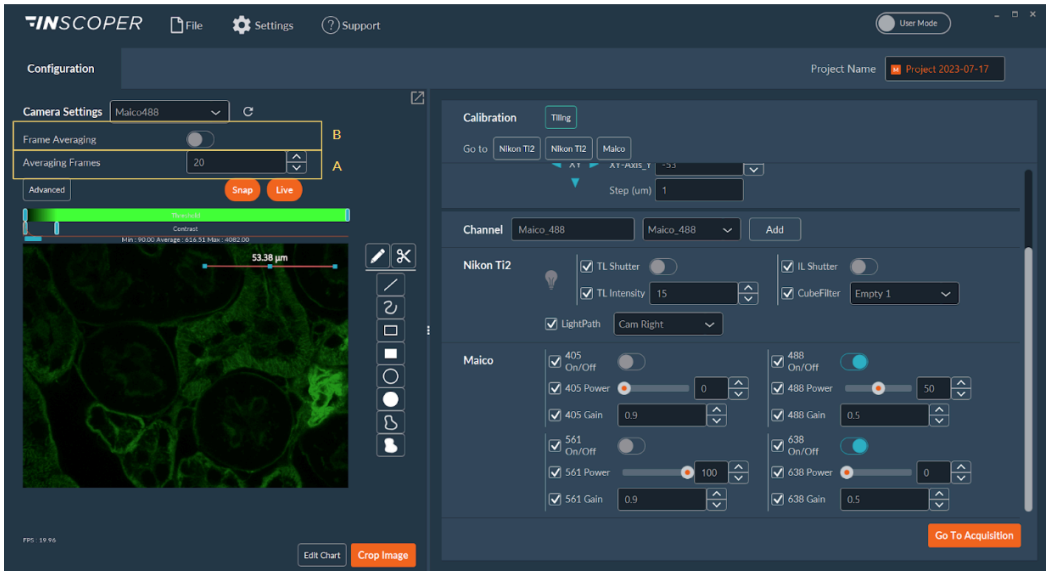
4. The green light of the button will be off when the calibration is done.
5. After this step, you can turn on the Inscoper Software.

1.8.1. Configuration

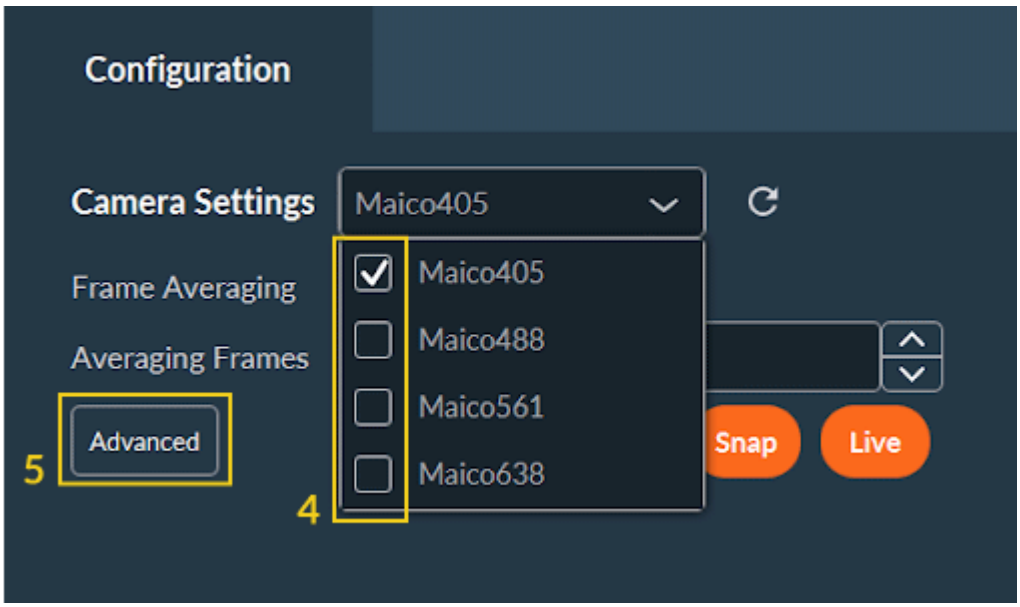
In the **configuration tab**, you have access to the laser and detector parameters. You can change the laser power and the gain of each detector. If you change the existing channel you can save it for your

experiment by clicking on the **Add**. If you want to create a new channel or save your channel on the disk, you need to pass from **User Mode** to **Expert Mode** and click on **Save** (See here).

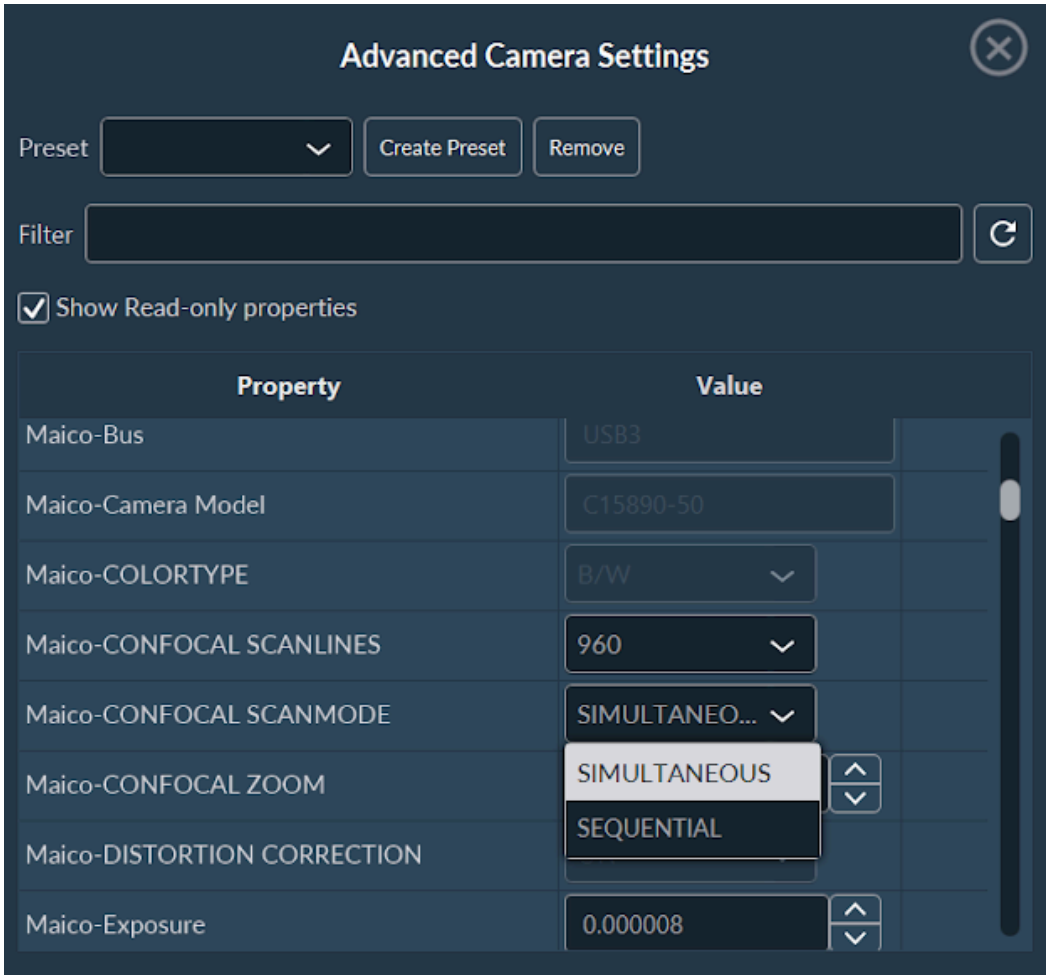
1. To do multicolor imaging, you need to create ONE channel with ALL laser lines that you will use during your acquisition even if you do sequential acquisition.
2. You can set the intensity and detector gain for each wavelength.
3. You can choose the frame averaging improve the quality of your acquisition : this averaging will be apply on all detectors:
    a. Indicate the number of the averaging frame.
    b. Activate the averaging.



4. When you have one or a multiple laser lines, you can manage the view of detection by checking the box of the detection of interest.



5. By clicking on **Advanced**, you have access to more options like:
    # Scan line: you can choose the number of lines that you want to scan (960, 480 or 240). This parameter allows you to increase the number of frame/s.
    # Scan Mode: Sequential ou simultaneous for the excitation.
    # Zoom: you have the choice between Zoom 1 or 2.



6. Once your channel is defined you can click on **Go to Acquisition** to set-up your acquisition sequence.

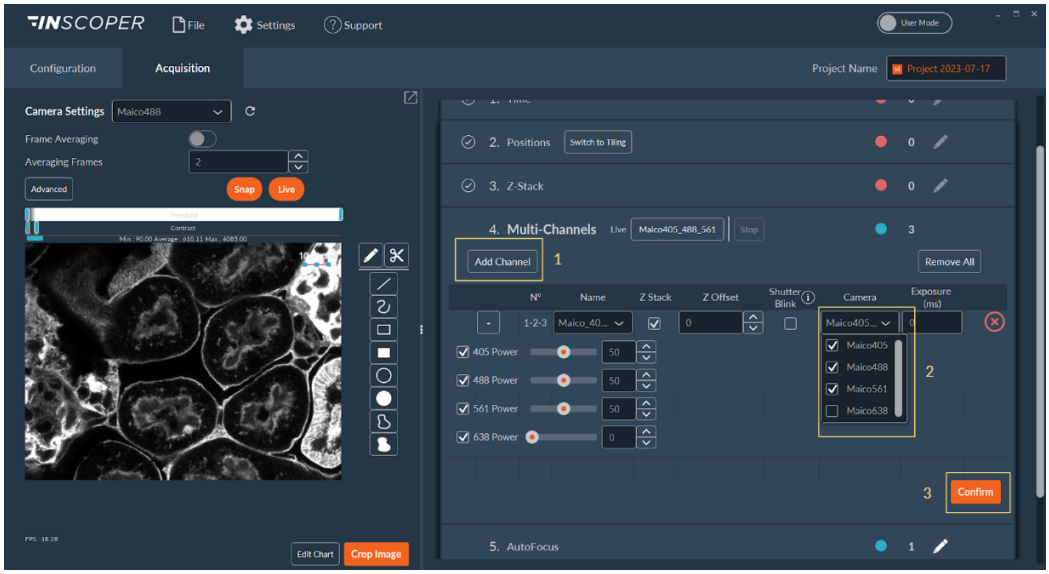
1.8.2. Acquisition

In the Acquisition tab, you can set up all your acquisition sequences as explained in the I.S. User Guide.

> NB: The only change for MAICO compared to I.S. usage is the multi-channels dimension.

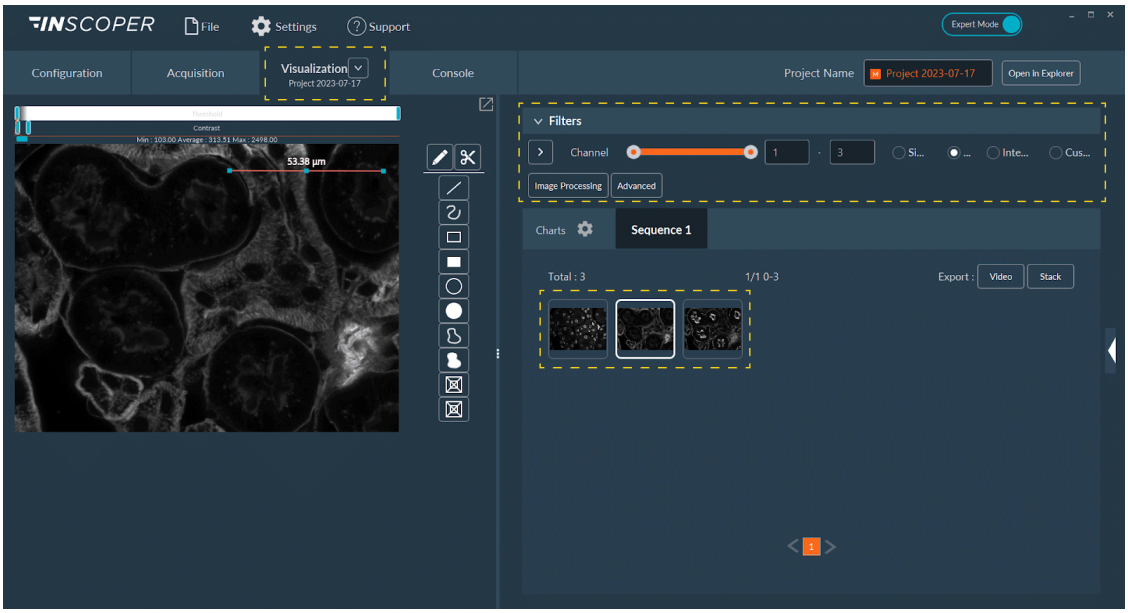
On the **Multi-channels** dimension:

1. You can add your channel by clicking on **Add Channel**(Add your channel including all laser lines that you will use).
2. You need to check the box of the detection you want to use.
3. Click on **Confirm** to validate your multi-channel dimension.



1.8.3. Visualization

After your acquisition, the **Visualization Tab** will appear. You will find your images and you can filter them by dimensions.



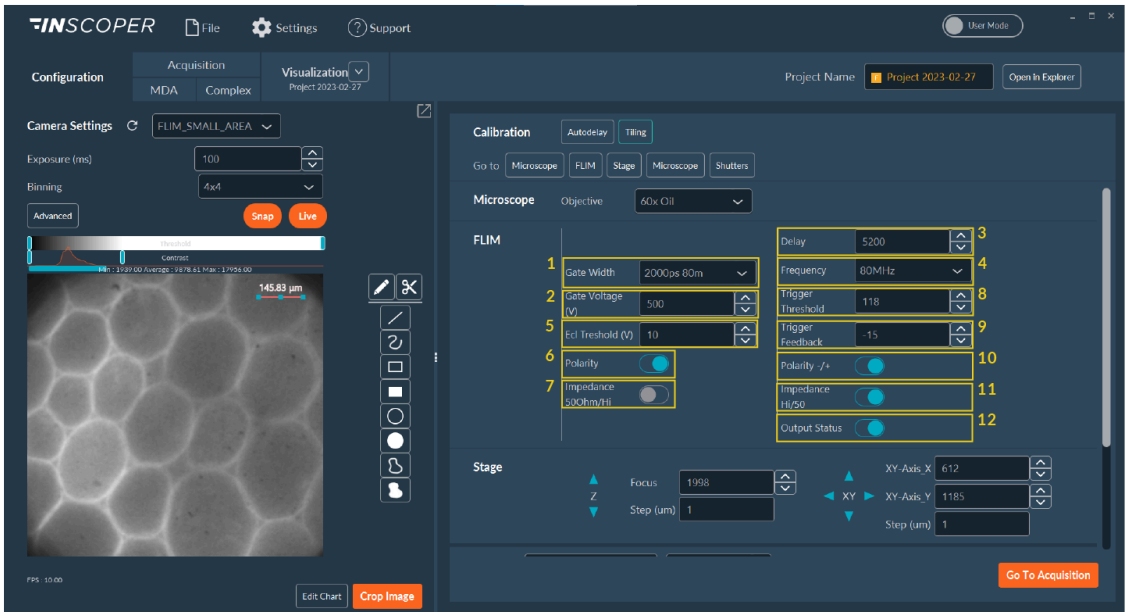
NB: If you have a Microvolution license, you can do your deconvolution in Inscoper I.S. by clicking on **Imaging Processing**. You have access to another image processing (ref Inscoper I.S. User Guide).

1.9. fastFLIM

The Inscoper fastFLIM is a turnkey system to measure the mean Lifetime with a camera based microscope.

FLIM measurement is compatible with **multi-positions**, **tiling**, **multi-channel**, **z-stack** dimensions.

1.9.1. Configuration



In the **Configuration tab**, you will have access to the following parameters of the FLIM:

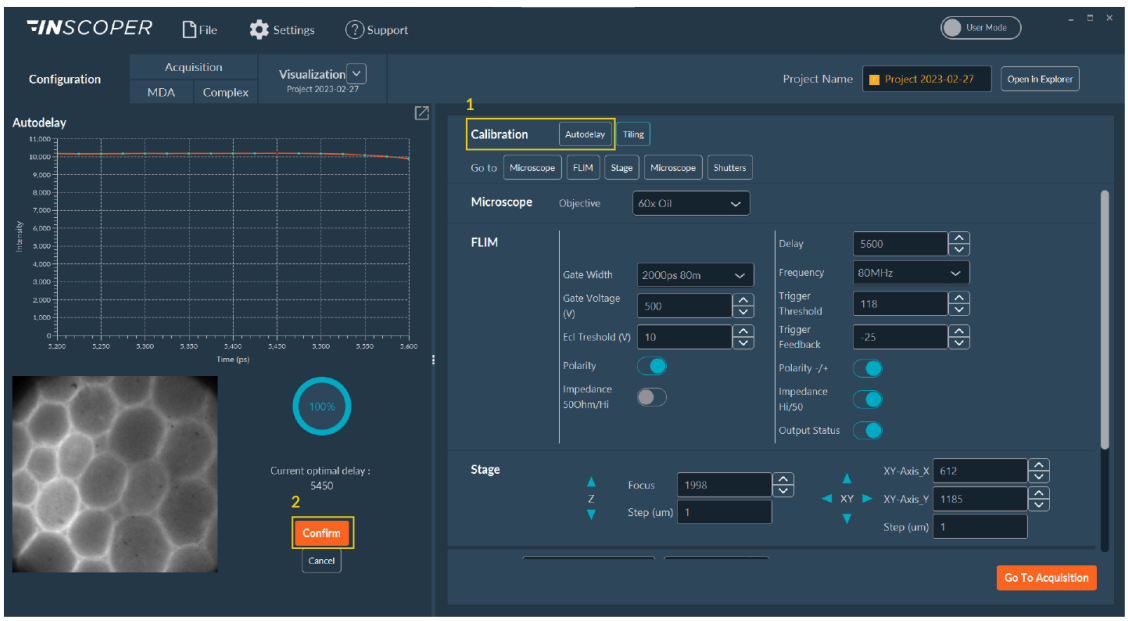
1. **Gate Width:** Width of the temporal gate.
2. **Gate Voltage:** Multichannel Plate (MCP) Voltage to amplify the input signal.
3. **Delay:** Time in picoseconds which corresponds to the delay generated regarding the synchronization signal of the pulsed laser.
4. **Frequency:** Frequency of the delay generator.
5. **Ecl Threshold:** Threshold to set the noise level on the input synchronization signal for the intensifier.

6. **Polarity:** Parameter to be set to ensure that the intensifier detects the delay generator synchronization signal
7. **Impedance:**Parameter to be set to ensure that the intensifier detects the delay generator synchronization signal
8. **Trigger Threshold:** Threshold to set the noise level on the input synchronization signal for the delay generator.
9. **Trigger Feedback:** Parameter for checking the synchronisation between the delay generator and the pulsed laser. The value must be close to 0 in this interval [-100 +100]. (Specific to the old generation of delay generators. Not present on the new generation.
10. **Polarity -/+:** Parameter to be set to ensure that the generator delay detects the laser synchronization signal.
11. **Impedance Hi/50:** Parameter to be set to ensure that the generator delay detects the laser synchronization signal.
12. **Output Status:** Shutter.

NB: Access to the parameters depends on the device selected for gating.

1.9.1.1. Autodelay calibration

NB: Before starting FLIM experiment, you need to calibrate the autodelay. The autodelay allows you to synchronize the pulsed laser and the temporal gate opening to get the max of intensity for the first gate.



To calibrate the autodelay:

1. Click on **Autodelay**.
   Calibration will start automatically.
2. Once a value has been found, click on **Confirm**.
   The value is automatically transferred to the interface - **Delay**.

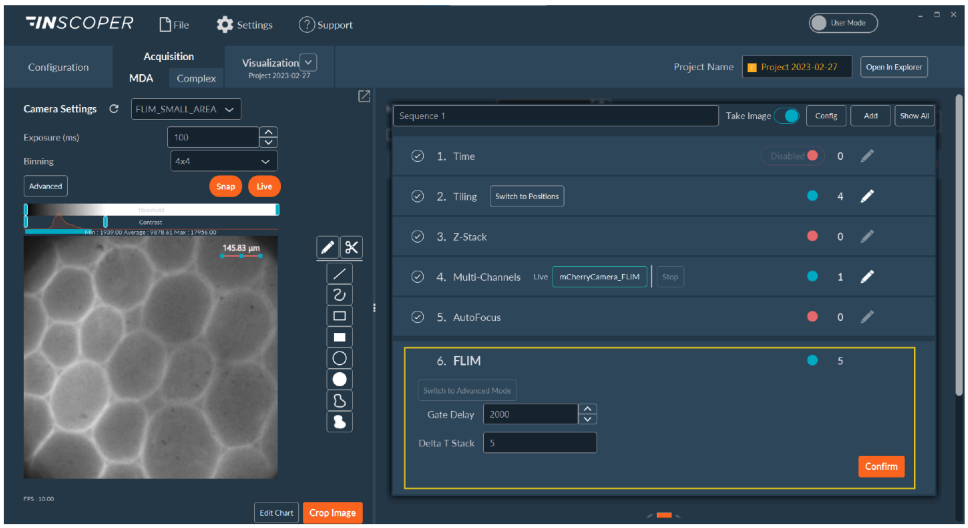
1.9.2. Acquisition

Once the calibration and configuration are done, you can do the acquisition.

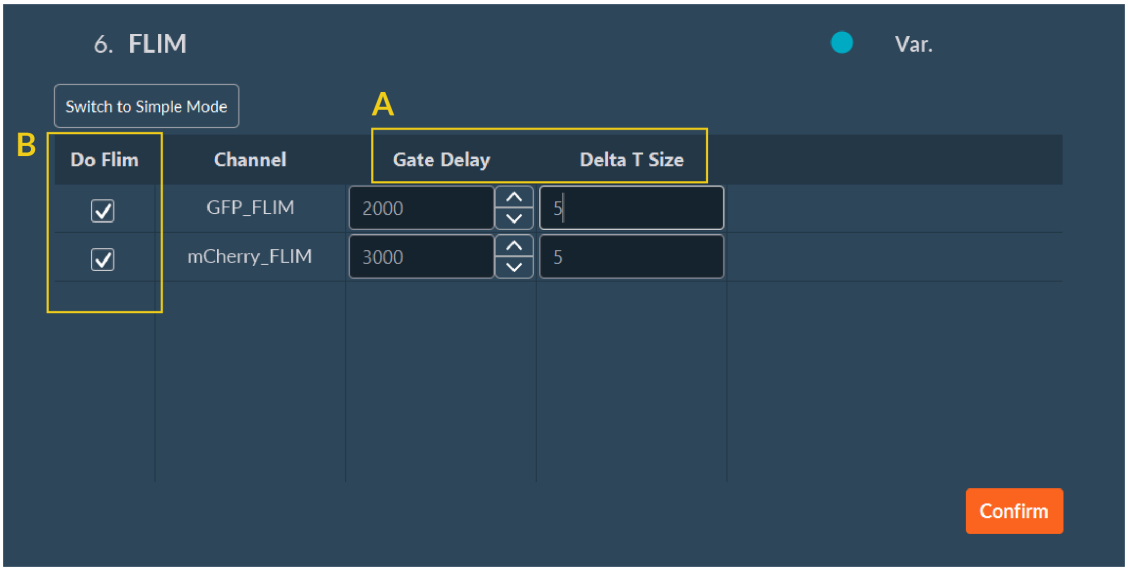
You will need to set your channel using the Multi-channel dimension. Thus you can set the FLIM and fluorescence channels.

Next you will find the **FLIM** dimension. In this dimension, depending on how many FLIM channels you set up, you will have 2 modes:

- **Simple mode:** if you have set one FLIM channel during acquisition. Here you will set:
  - **Gate Delay** (the time interval between 2 temporal gates)
  - **Delta T stack** (B - the number of images to be measured by FLIM).

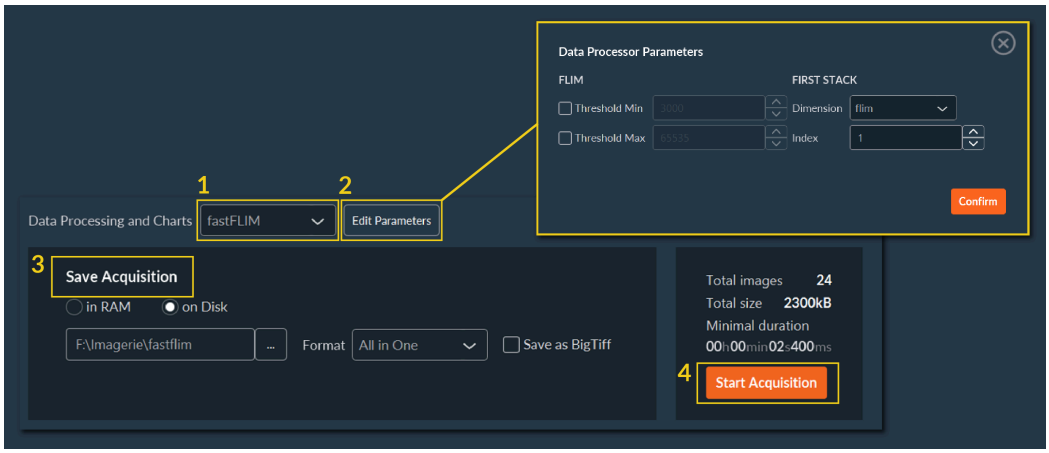


- **Advanced mode:** if you have more than one FLIM channel during acquisition. In this mode you can set:
  A. a specific **Gate Delay** and **Delta T Size** for each channel.
  B. Select the channel on which you wish to perform the FLIM measurement by checking the **Do Flim** box.



Once your FLIM experiment is set up, you have to click on **Confirm** to validate the setting. To finalize your acquisition sequence:

1. Choose **fastFLIM** on the **Data Processing and Charts** drop-down list.
2. By clicking on **Edit Parameters** you can set the minimum and maximum values for the **Intensity Threshold** for the FLIM calculation and click on **Confirm**.
3. Indicate the path to save your images.
4. Click on **Start Acquisition**.



1.9.3. Visualization

In the visualization tab, you have the result of your FLIM calculation and you have access to the FLIM, Intensity images, distribution of the lifetime and Intensity decay.

A. FLIM image

B. Intensity images used for the FLIM calculation

C. Intensity decay

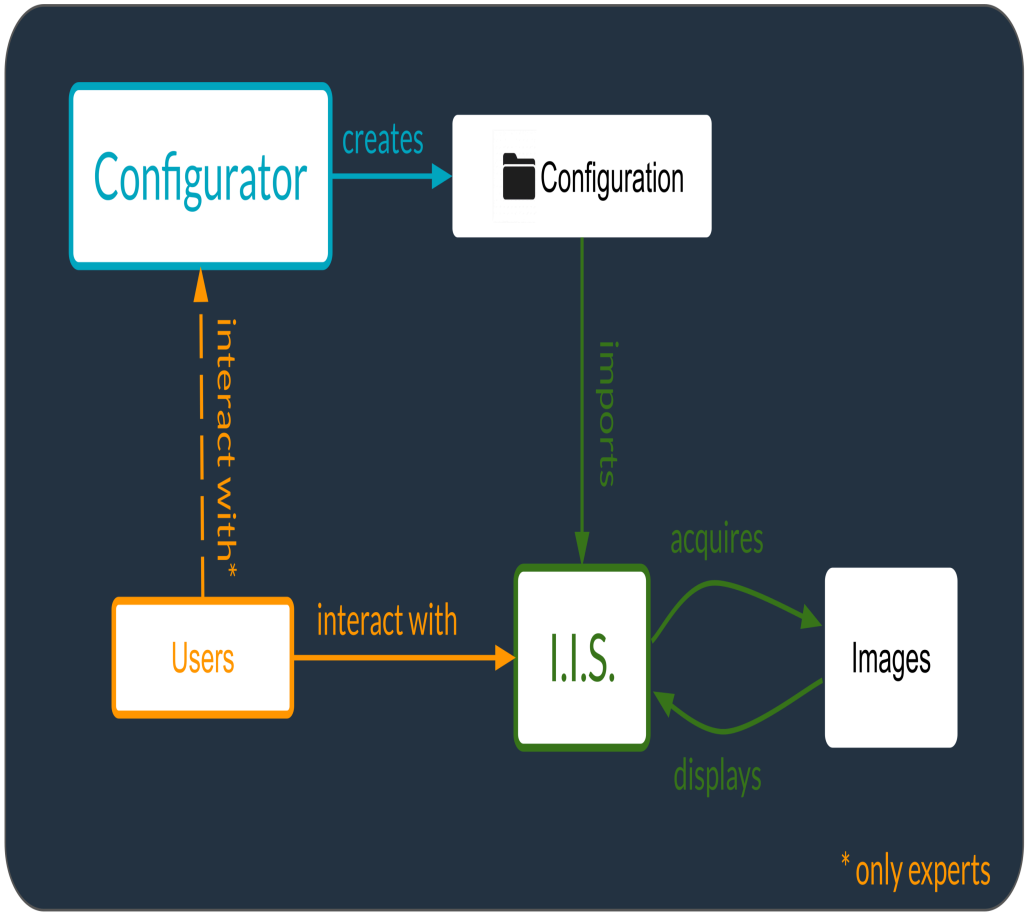
D. Lifetime distribution

All those images are saved in the folder that you choose.

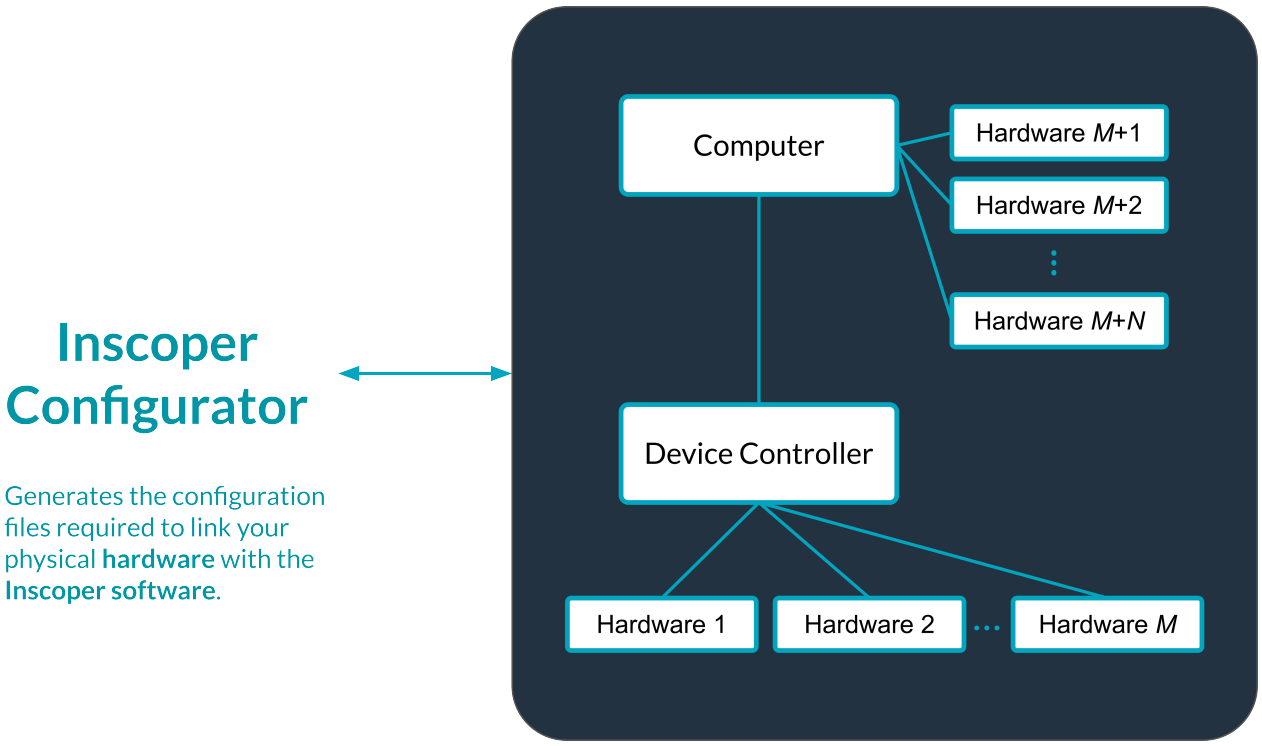
2. INSTALLATION

Once a certified installer has set up the Inscoper hardware, connected all components, and installed both software tools (Inscoper Imaging Software and the Inscoper Configurator),together, these elements form the operational basis of the Inscoper system.

User Interactions and Software Workflow: The diagram below shows how users interact with the Imaging Software and the Configurator. It highlights how these tools work together to produce images. The goal is to clarify the functional workflow from user input to image generation.



Hardware Structure and Configurator Integration: The diagram below presents the physical hardware setup and how each component is connected. It illustrates how the Configurator interprets this hardware layout. Its role is to convert the material structure into a configuration usable by Inscoper software.



2.1. Inscoper Configurator

The Inscoper Configurator is a powerful tool that simplifies the setup of complete microscopy systems built around the Inscoper Device Controller. It lets you define, connect, and manage all system devices to create a fully operational configuration tailored to your research needs.

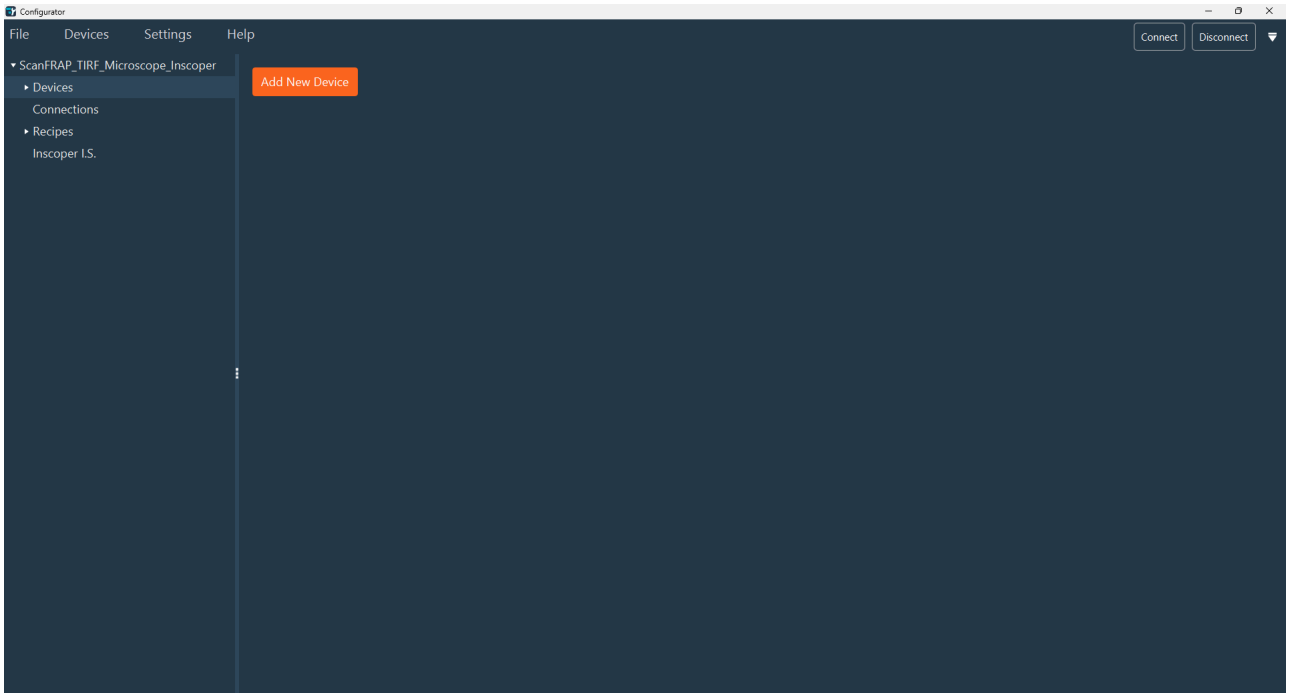
The Configurator outputs a ready-to-use system configuration compatible with the Inscoper Imaging Software (Inscoper I.S.) and the standalone Inscoper API.

Who is it for?

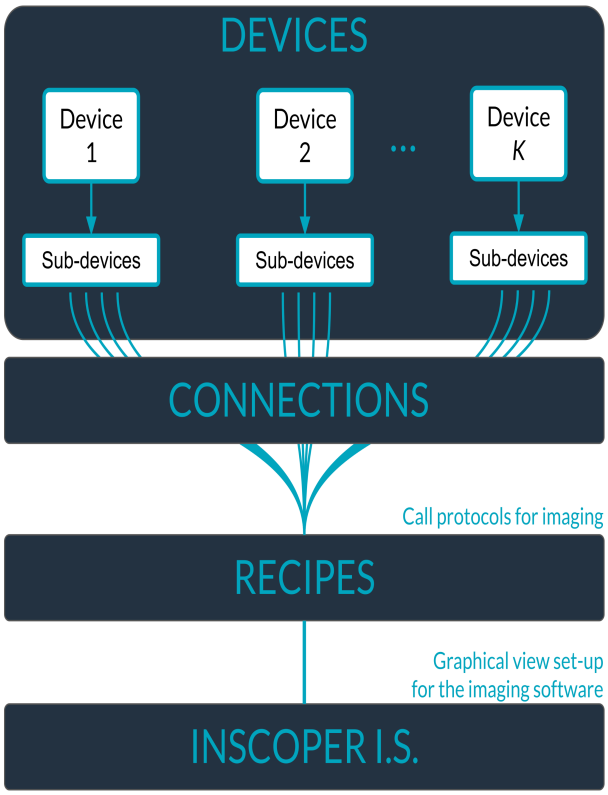
- System integrators installing microscopy systems controlled by the Inscoper Device Controllers.
- Advanced users performing hardware updates or adjustments (with appropriate precautions).

What will you find here?

This documentation offers step-by-step guides to help you perform key configuration tasks, from defining connected devices to pre-configuring the IIS.



Main Steps for Creating a Configuration:The diagram below shows the key stages involved in creating a configuration with the Configurator. It outlines the typical user workflow from start to finish. The goal is to provide a clear overview of the essential steps in the configuration process.



2.1.1. Getting started

The main interface is divided into three sections:

I - Toolbar

II - Configuration section (detailed here)

III - Device Controller (DC) status & actions

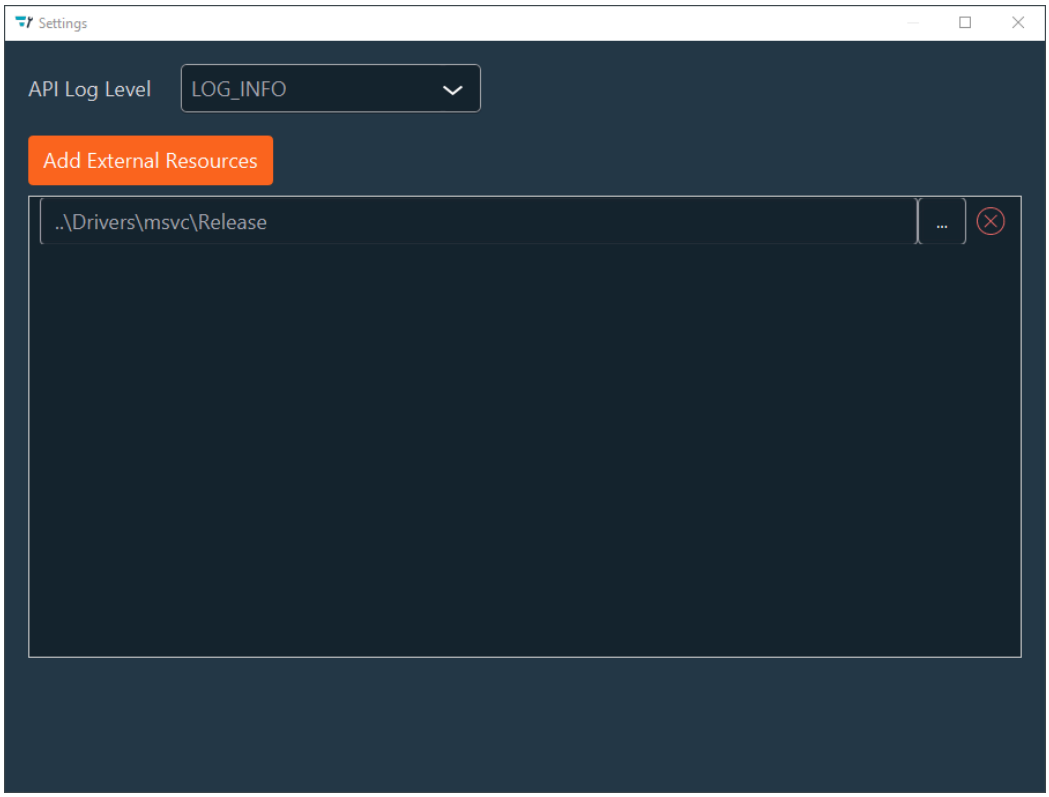
1. **File** allows to:
    - # **New:** Create a new configuration
    - # **Open:** Open an existing configuration
    - # **Save:** Save current configuration
    - # **Exit:** Close the Configurator window.
2. **Devices** options are:
    - # **Reload Devices:** Reload devices information from the DC and external drivers (micromanager drivers and custom drivers).

> NB: When the checking is done, a popup window appears in the bottom of the Configurator window indicating you the number of found/loaded devices (Inscoper, Micromanager or custom [which is no inside the DC])
>
> 
>

    - # **Load Devices from File:** read and import the devices information (settings, configuration, etc.) from stored file.

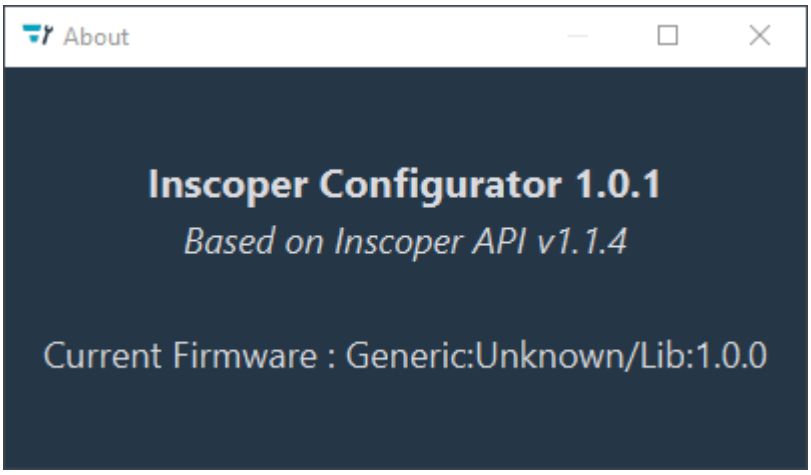
- **Save Devices to File:** export the devices information to a new local file.
- **Display Devices:** Display of the previously mentioned popup window.

3. **Settings**: You can specify the directory where the Micromanager and custom drivers are stored on your computer. You can add several directories by clicking **Add External Resources** and delete them by clicking on  . You can specify the **API log level** used by the configurator in the drop-down menu. When you are done, you can close the window and all the information will be saved automatically.



4. In the **Help** menu, you have the following options:
   - **Update Firmware:** Open explorer window to upload the firmware file
   - **Online Help:** Open the Configurator or Inscoper User Guides
   - **Visite Website:** Open the Inscoper website

About: Open popup window with all information about the Configurator (Configurator version, API number, Firmware Version)

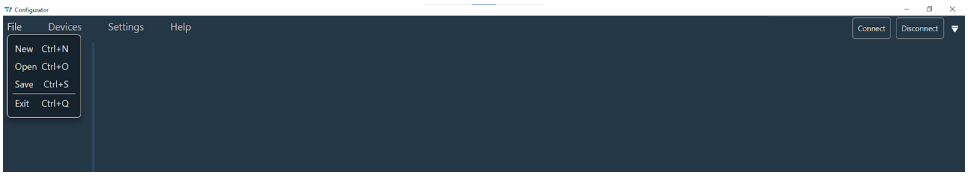


The **DC actions section** allows you the following actions:

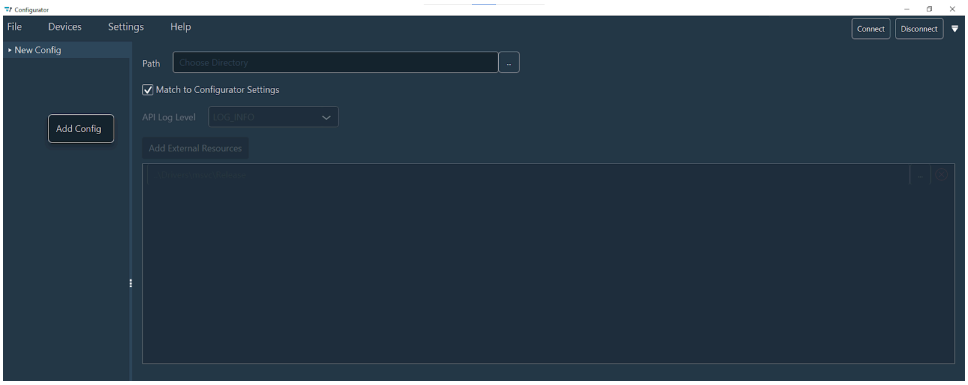
1. **Connect:** Connection of Device Controller
2. **Disconnect:** Disconnection of the Device Controller
3. **Restart Firmware:** Restart the Firmware
4. **Reset Inscoper Box USB view:** Triggers the Inscoper Box to rescan all USB devices connected to its ports. For example, if a device is plugged in while the Configurator is open, this action updates the detected USB devices within the DC.

2.1.2. Create configuration

1. There are three ways to create a configuration:
   # Click on **File** and **New**.

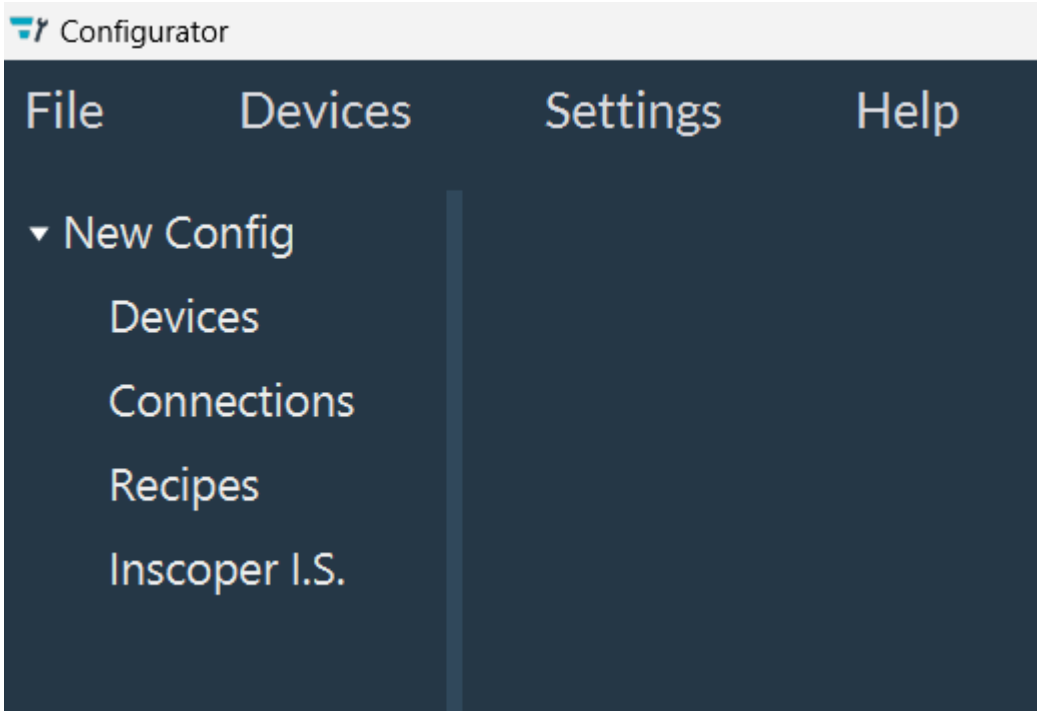


   # Right click inside the Configuration section (left part of the window) then click **Add Config**.



Use the key combination: **Ctrl + N**.

When a new configuration is created, it will appear in the Configuration section with a default name **New Config**.



2. To **save** your configuration, there are three ways:

Click **File** then by clicking on **Save**.

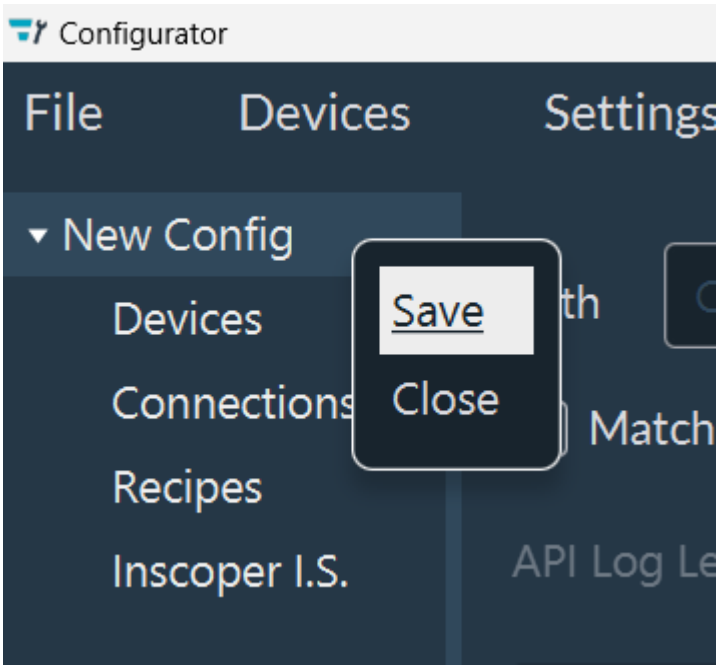
to select the folder. The name of your configuration will be the name of the folder.

Right click on **New Config** (or renamed config) then click on **Save**.

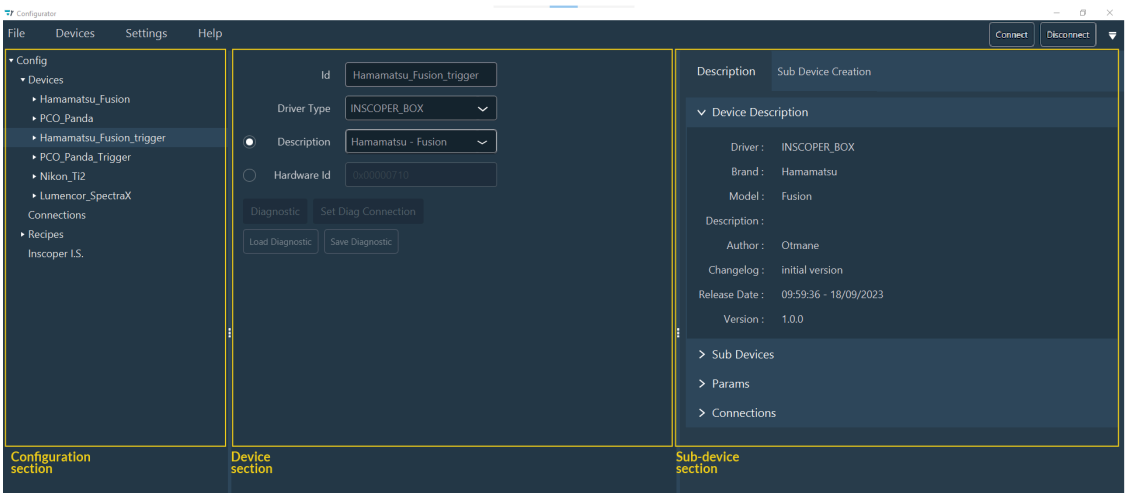
Use the key combination: **Ctrl + S**.

NB: If the **Path** field is not filled in, a file explorer opens to allow the user to choose a save directory. Upon confirmation, the configuration is saved.

NB: If the **Path** field is filled in, the configuration is automatically saved in the specified directory.



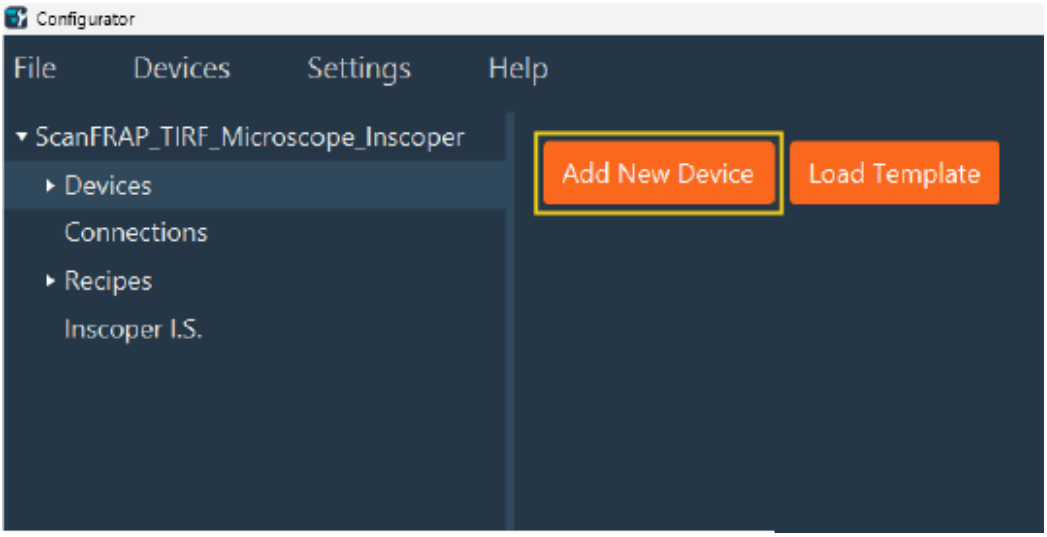
2.1.3. Devices setup



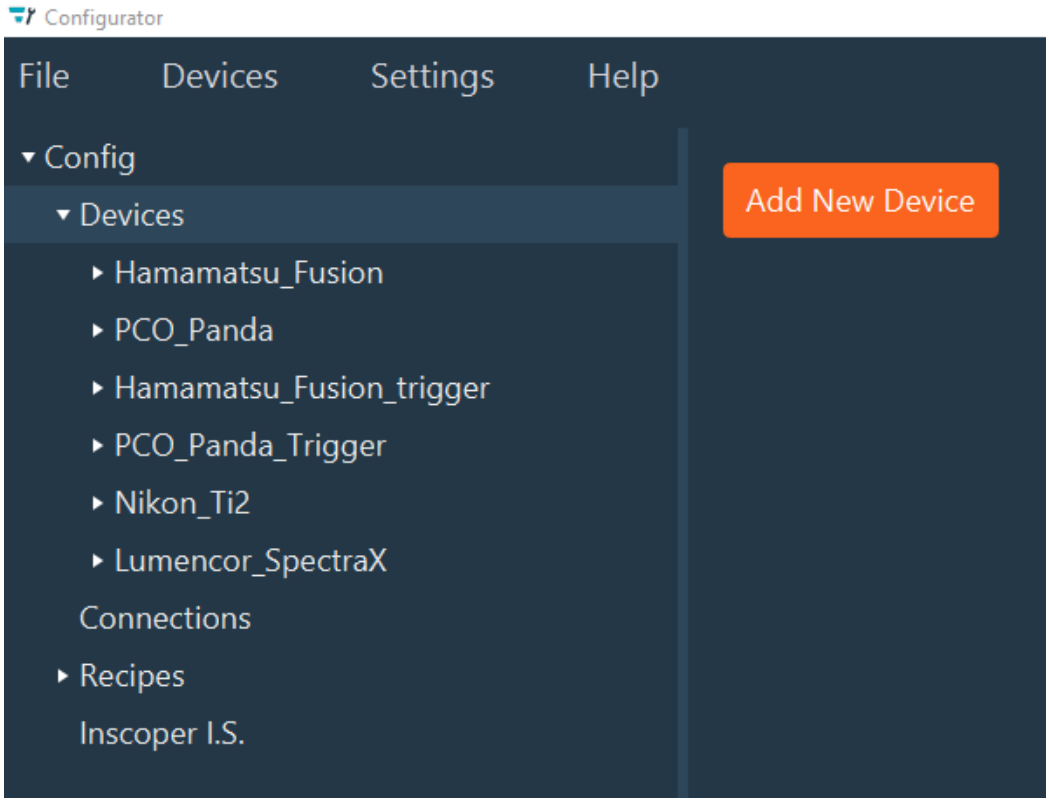
When you create your configuration, all the subsequent levels are automatically created [Devices, Connections, Recipes, I.S. Inscoper] in the configuration section.
In this stage, the main Configurator window is divided into 3 parts:

- **Configuration** section (already explained here)
- **Device** section: where you will configure all the devices
- **Sub Device** section: where you will create Sub Devices for the device. This section has two tabs: one for creating Sub Devices and the other for the device description.

1. Add Devices to Your Configuration
   For this, click the **Add New Device** button OR right-click on the **Devices** in the Configuration section and click **Add Device**.



   As a result, you'll see additional fields appear with the parameters to be filled in for this device and its Sub Devices.



2. Configure your device. Here, you can:

   a. Rename your device in the **Id field**.

   b. Specify the **Driver type** in the Driver Type field. For example : driver in Inscoper Box, custom driver or micro_manager.

   c. Depending on the chosen Driver type, fill in

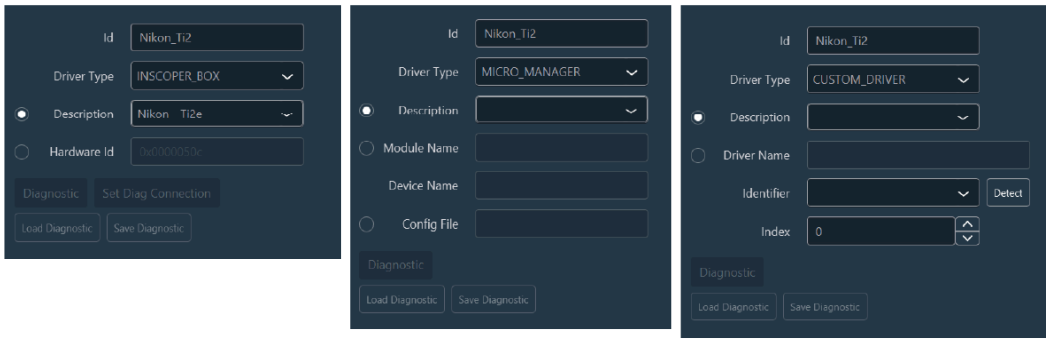
      # **For Custom Driver**: Enter the device **Description** or **Driver Name**, along with the device Identifier. You can click **Detect** to automatically find the device identifier. If multiple devices of the same type are present, add the device Index (starting from 0).

      > NB: In practice, the Identifier and Index fields are only useful when the same device is used multiple times, and only one of them should be used.

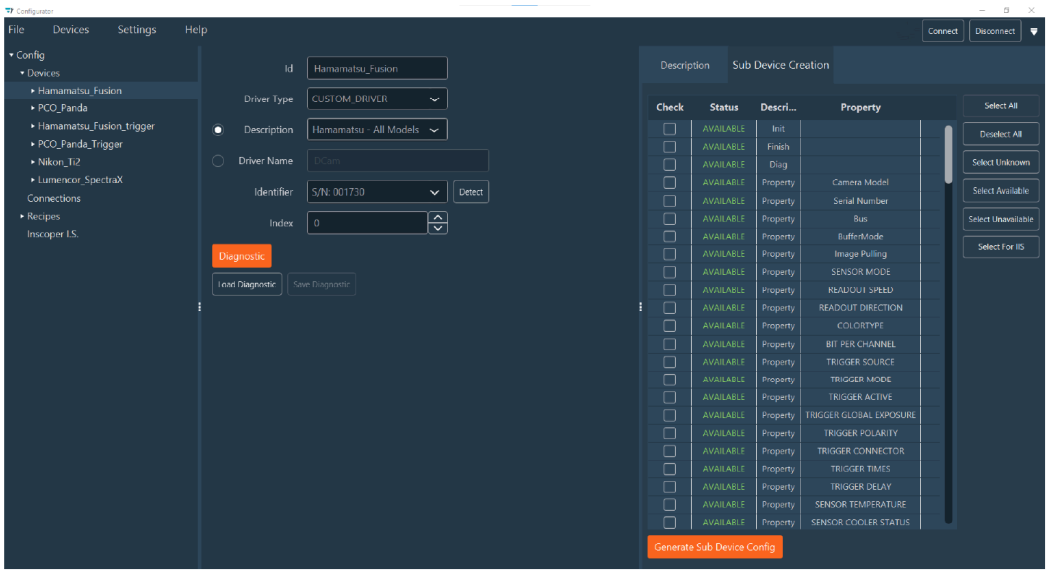
      # For **Inscoper Box**: Fill either the **Description**or the **Hardware Id** (using the Hardware ID requires knowing the exact identifier of the device to be added).
      # For **Micro manager**: Fill either the **Description** (preferred) or the **Module Name** and **Device Name** or **Config File** (rarely needed).

d. For the **Description**, find your device in the drop-down menu.

> NB: Items are listed in alphabetical order, but you can also search by typing the beginning of a word.

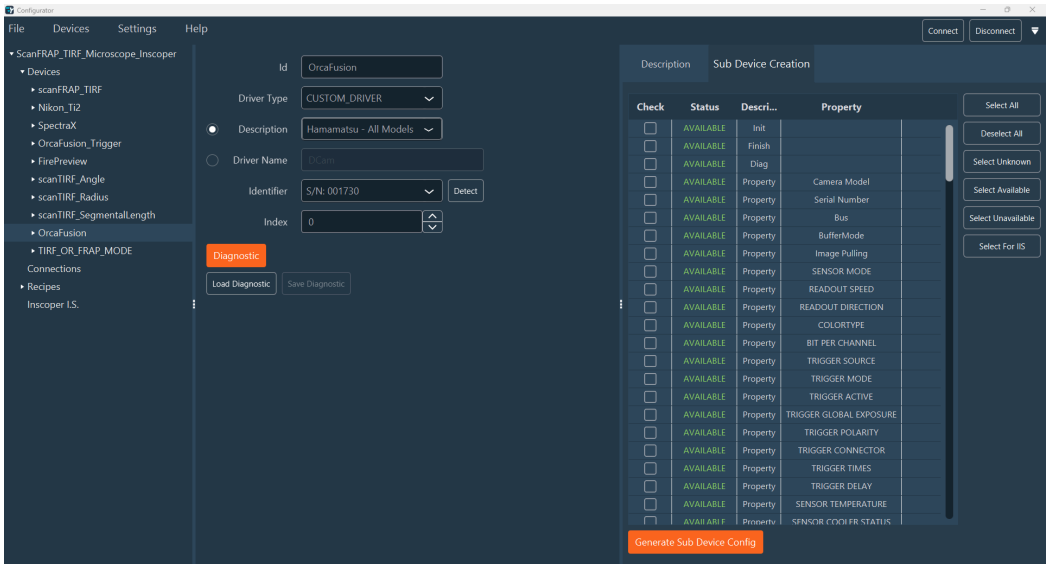


3. Before clicking on **Diagnostic** be sure that the device is connected to the system (either to the computer or the Inscoper Box). This function retrieves additional information beyond the initial description loaded via **Reload Devices** (brand, model, author, changelog). For Custom and Micro-Manager drivers, Diagnostic requests the device to provide its list of properties. It also updates the status of Sub Devices (Available, Unavailable, or Unknown) and may refine associated constraints.
   All the information about the device will be found in the **Description tab** of the **Sub Device section**.

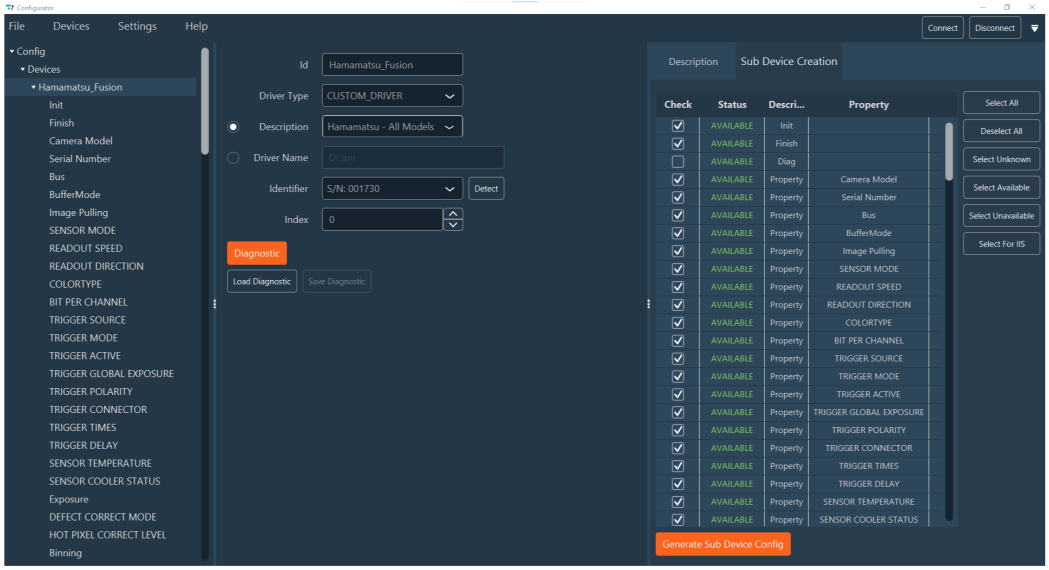


4. After running the Diagnostic, you can check the status of each Sub Device in the Sub Devices tab of the Sub Devices section. The table contains four columns:

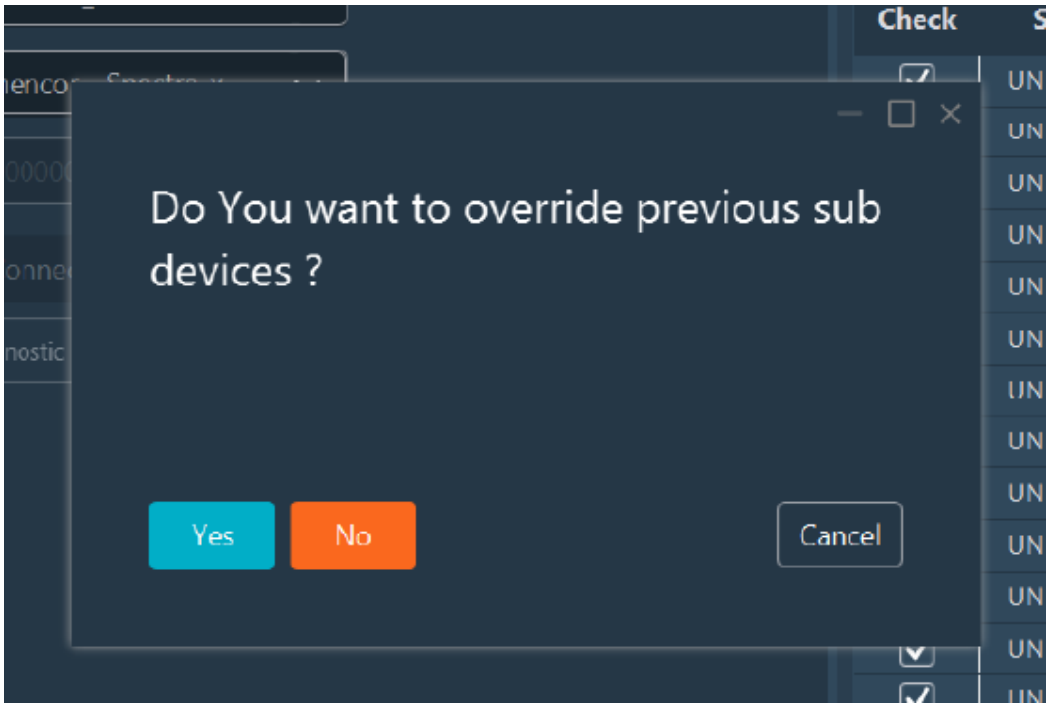
- **Check:** checkbox to select the Sub Devices.
- **Status:** indicates if the Sub Device is **available**, **unavailable** or **unknown** (after the diagnostic the driver could not verify the status).
- **Description:** indicates the name of the Sub Device.
- **Property** (Custom and Micromanager only)**:** property of the Sub Device.



5. To the right of the window, you have access to different buttons to interact with the Sub Devices list:
   - **Select All:** Select all Sub Devices
   - **Deselect All:** Unselect all Sub Devices
   - **Select Unknown:** Select all Sub Devices where the status is UNKNOWN
   - **Select Available:** Select all Sub Devices where the status is AVAILABLE
   - **Select Unavailable** Select all Sub Devices where the status is UNAVAILABLE
   - **Select for IIS (only for camera devices):** Select all Sub Devices needed for your Inscoper interface
6. When you have selected all Sub Devices needed, you can add them in the configuration by clicking on the **Generate Sub Device Config** button. All Sub Devices will be inserted below the Device Name on the Configuration section.



7. If some Sub Devices are missing, you can select them and click the **Generate Subdevice Config** button. A pop-up window will appear asking you if you want to override the previous Sub Devices. If you answer **Yes**, your selection will replace your previous Sub Devices, if you click **No**, your Sub Devices selection will be added to your previous Sub Devices list.

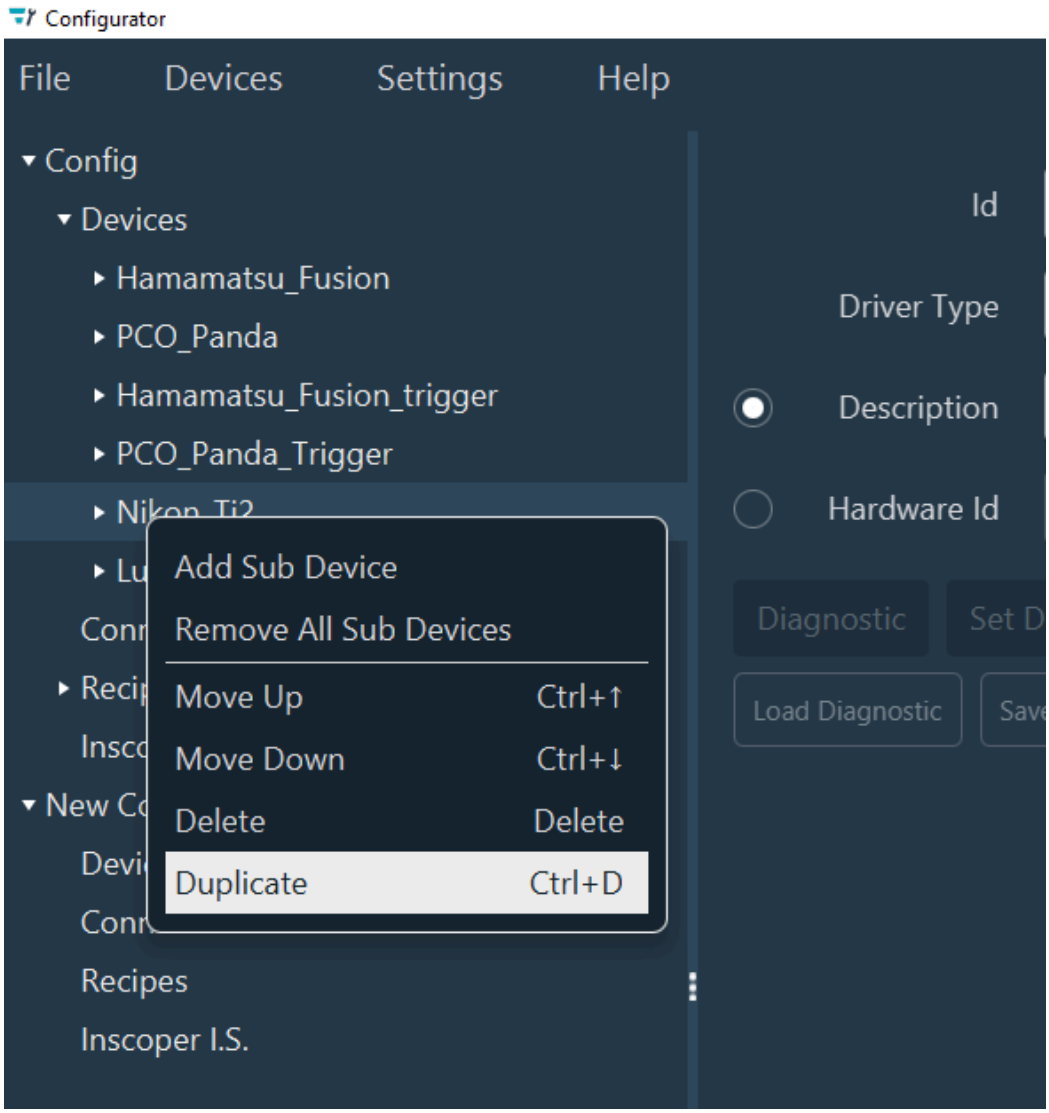


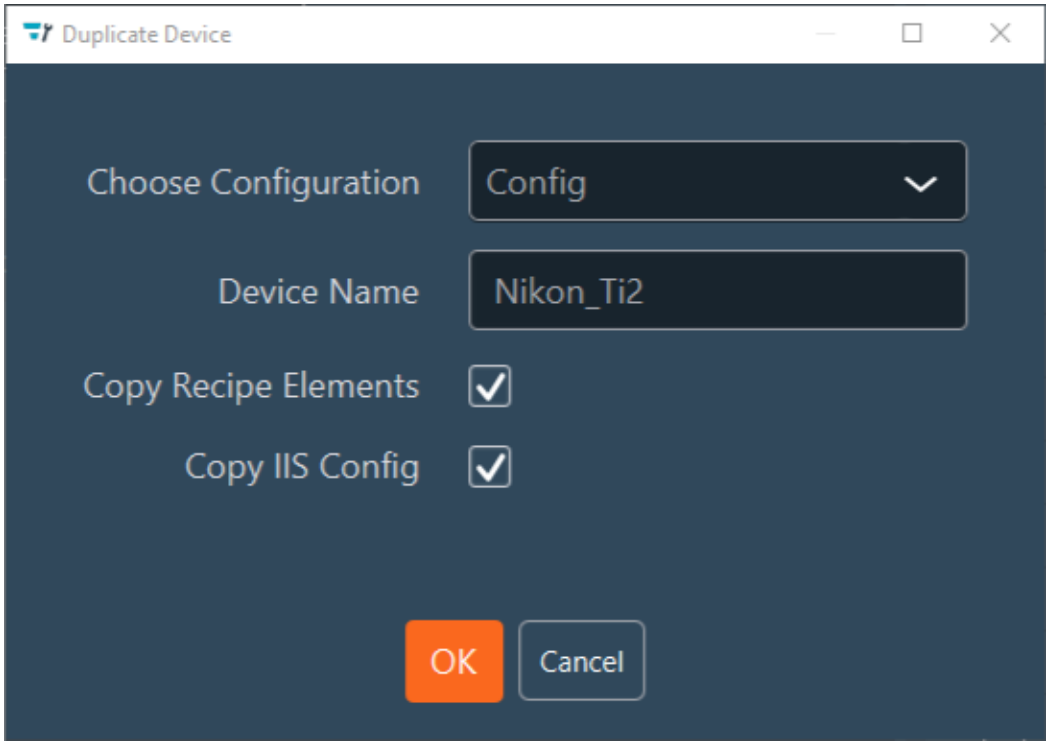
8. Right clicking on the device will allow you to:
    # **Add Sub Device:** Add a subdevice to the device
    # **Remove All Sub Devices:** Remove all subdevices from the device
    # **Move up:** Move the device up
    # **Move Down:** Move the device down
    # **Delete:** Delete the device and all its Sub Devices

Duplicate: Allows you to fully copy a device within a given configuration. This is useful, for example, if you have multiple identical devices or if you want to reuse an already configured device in a new configuration.

NB: It is possible to duplicate the device with all its recipe elements and elements related to the configuration of the Inscoper I.S.

NB: Impossible to duplicate the device with the same name to the same configuration (error message will appear).





9. If you manually add a Sub Device (with the Add Sub Device function), you will need to fill:
    a. The **Id** of the Sub Device
    b. Either the **Description** or the **Tag** of your **Sub Device**.
    c. [OPTIONAL] **Recipe Id**: Define a Recipe Id to group several Sub Devices in one unique Recipe Element.

    > NB: For example, if you define a Recipe Id "Shutter", you could apply it to all your shutter Sub Devices. Thus, you can later create a Recipe Element with this Recipe Id and all your shutter Sub Devices will be called with this Recipe Element.

    d. [OPTIONAL] **Post Init**: Check this if you want your Sub Device to be ignored by the Create Recipe function and the Initialize or Update Display Data. This also allows you to force the Sub Device to be called at initialization if the SET function has no param (for Sub Sevice with an editable param, a default value is required).
10. If your Sub Device has one or multiple parameters, you can add a default value to your parameterss by clicking on **Add Param**:
    a. Select either your param or the corresponding tag.
    b. Select if the param is fixed or not
    c. Define your default value

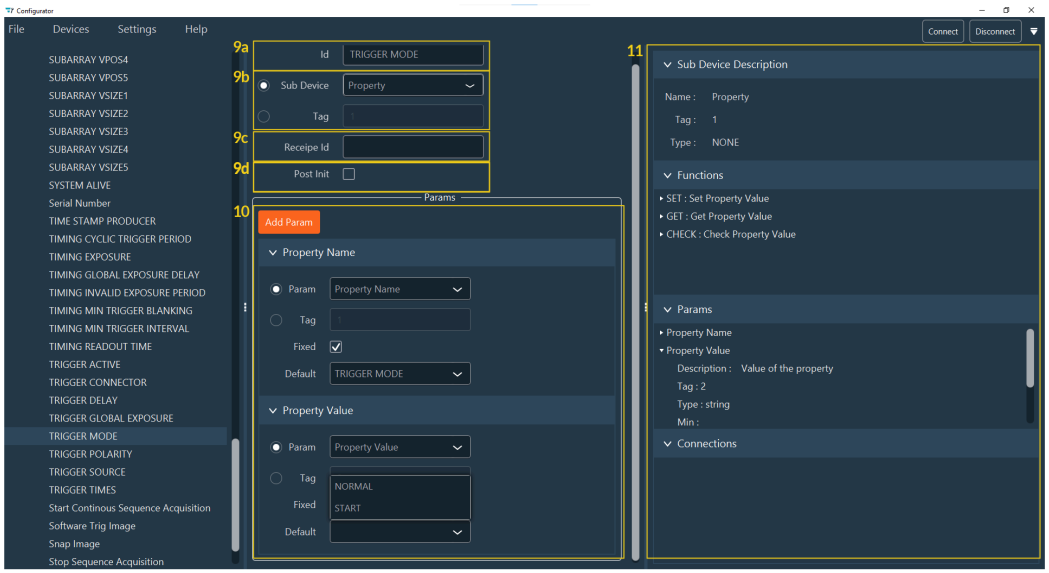
NB: In many cases, we want a Sub Device to carry one (or no) editable param. For Sub Devices with several params, you need to fix the value of several params to keep just one editable param.

Example: the Property Sub Device (defined for all external drivers) has 2 params : Property Name and Property Value. If you fix the Property Name value, the Property Value value will be the editable param of your Sub Device : Property Name = "Exposure" (fixed) -> never changes Property Value = Editable value

NB: If no param exists for your Sub Device, the Add Param button is not visible.

11. If you click on a **Sub Device**, you will get all information about it in the Sub Device part like **Description, Function, Parameters**.

NB: [FOR DRIVER_CUSTOM and MICRO_MANAGER ONLY] In Parameters (**Params**) you can see the **Property Name** and the **Property Value**. In the Device part you will always find the **Property Name**. This property is fixed. If you notice that a parameter (Property Value) is missing, you can add it by pressing the **Add Params** button. In the drop-down menu you can select the property that is not fixed. You can select the default value by selecting it in the drop-down menu.



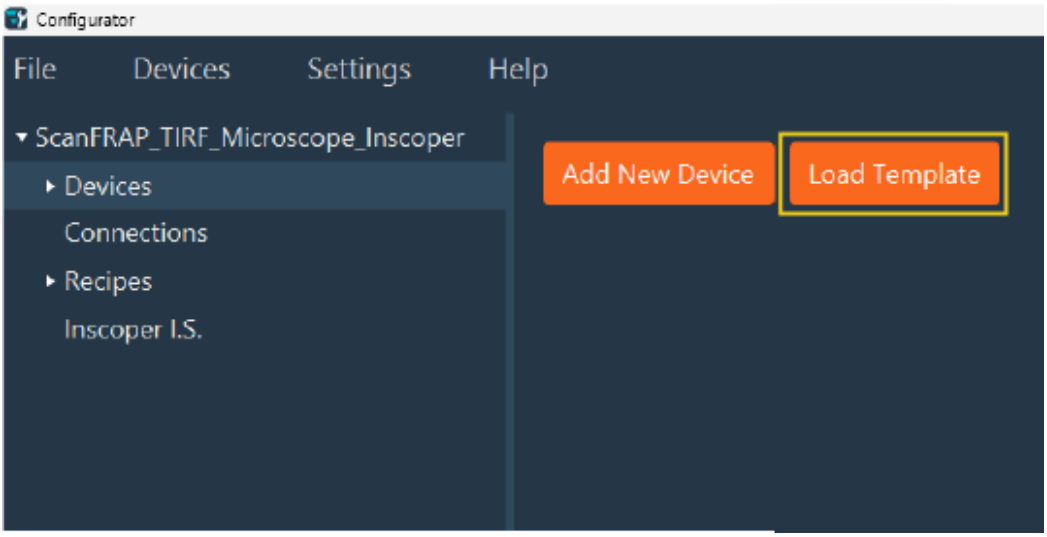
12. Repeat these steps for each device of your system.

When all devices are added to your configuration, you can go to the Connections step.

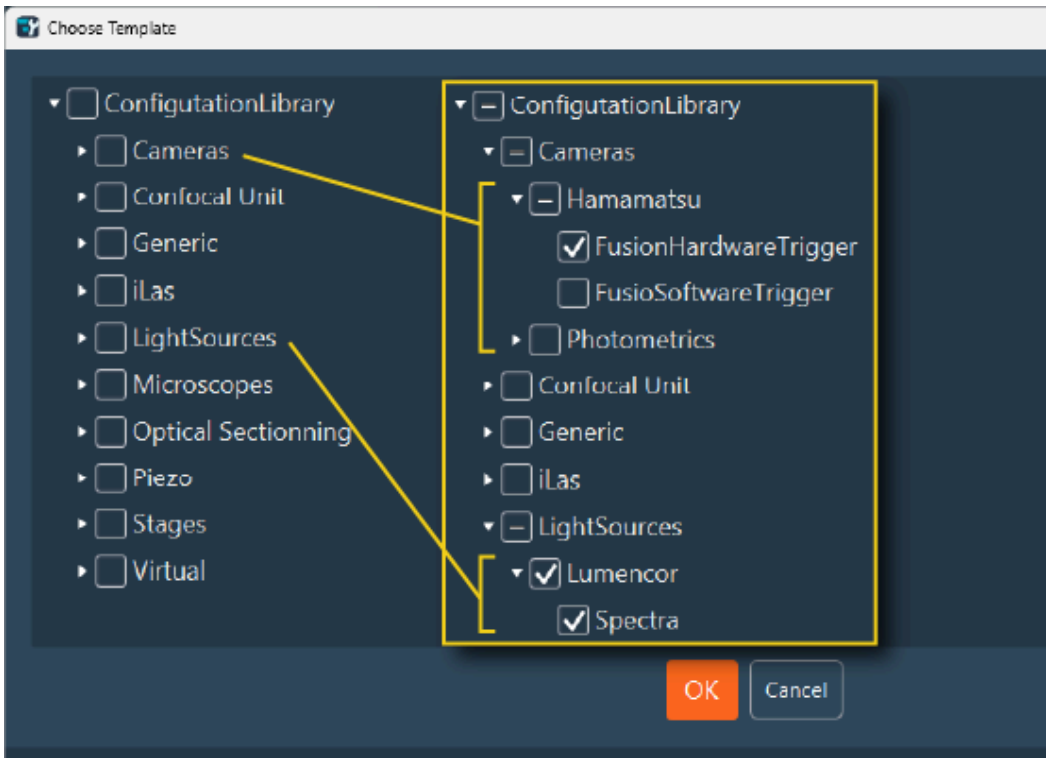
2.1.4. Template use

A quicker way to prepare your configuration and set up devices is to use templates: use devices that have already been added by our technical staff to the Configurator library. Templates are small configurations by device. Each configuration is tested and validated.

1. Click on **Load Template**.

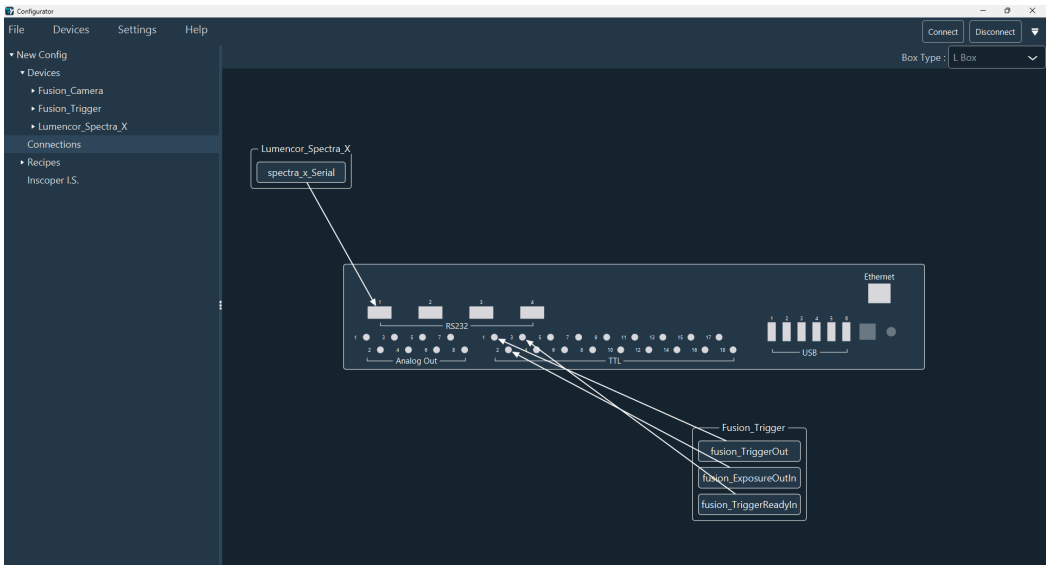


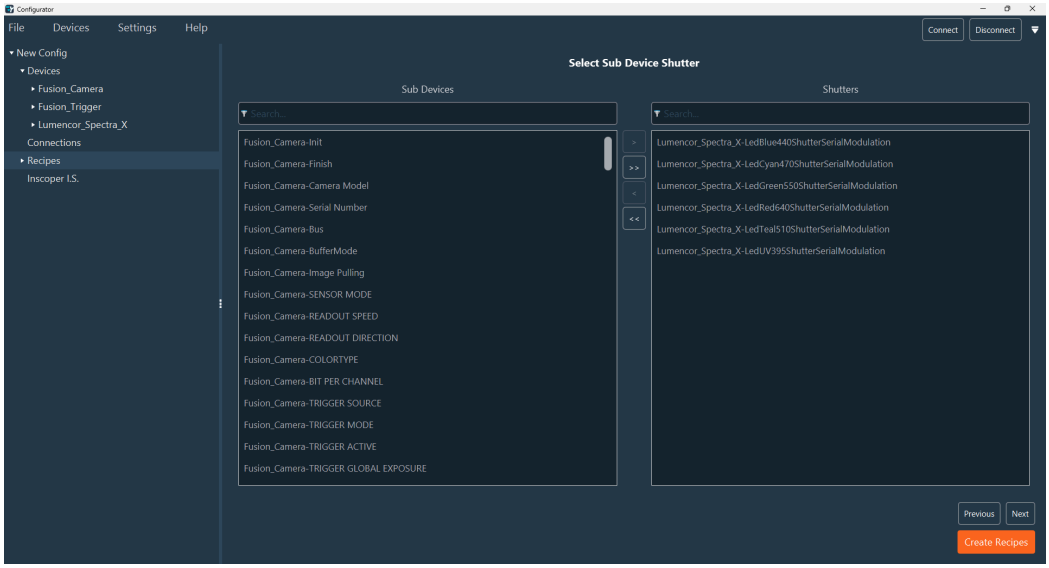
2. Select the devices in the library you want to load by checking the boxes.



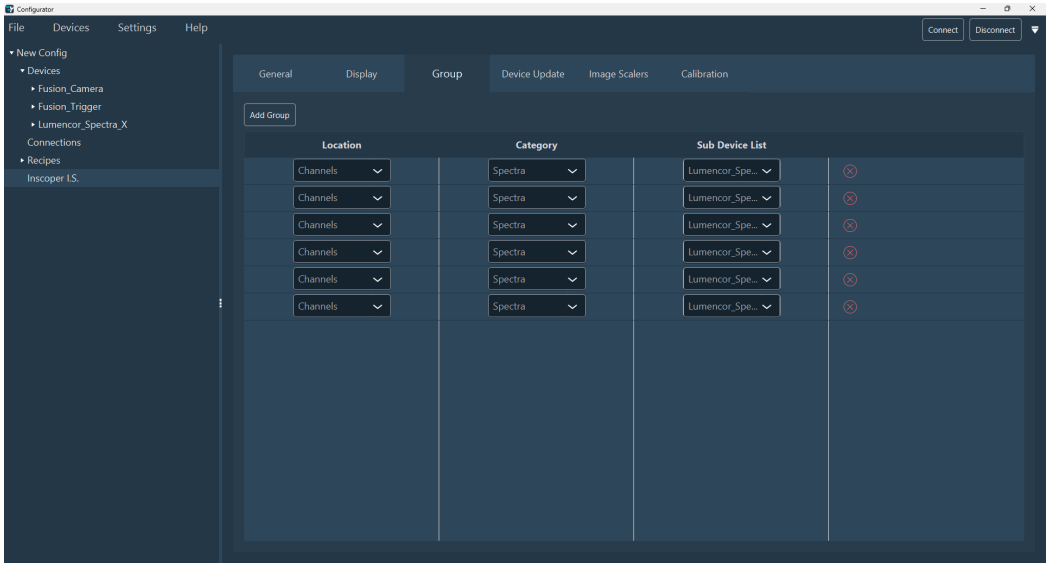
3. Click **OK** to load the devices into your configuration.
4. Check the connections and recipes of your devices and change them if necessary. The templates automatically import all the information.

> NB: In general it is necessary to modify the connections of the devices (especially if you do not have the same type of box and therefore not the same number of connectors).





5. You will then be able to check and modify the display of your devices within the Inscoper software by reviewing the Inscoper I.S. You will be able to modify all of the tabs by following steps described in Inscoper I.S. configuration.



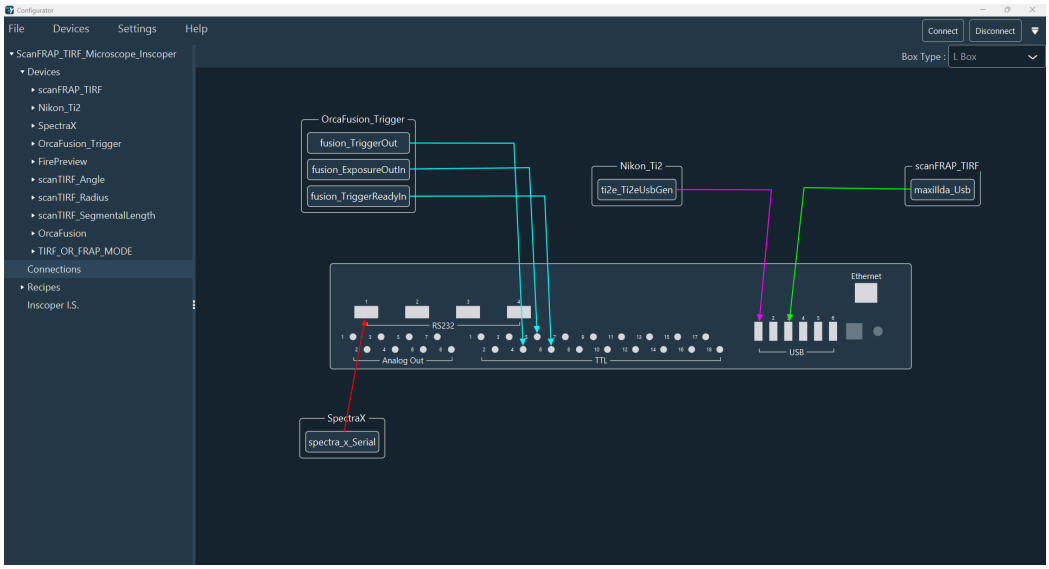
2.1.5. Connections setup

This stage is the link between the devices and the Device Controller (DC).

1. Click **Connections** inside the Configuration section, you'll get a view of the box and the devices that need to be connected to the DC.

2. The box type (S, M, L, XL) is recognized automatically. To select the box type manually, click on the **Box Type** drop-down menu in the top right-hand corner.

The drawing is automatically updated according to your choice.



3. Indicate where you have connected your device to the DC: right-click on the device you want to connect to change the mode from **Move Cell** to **Link Cell**.

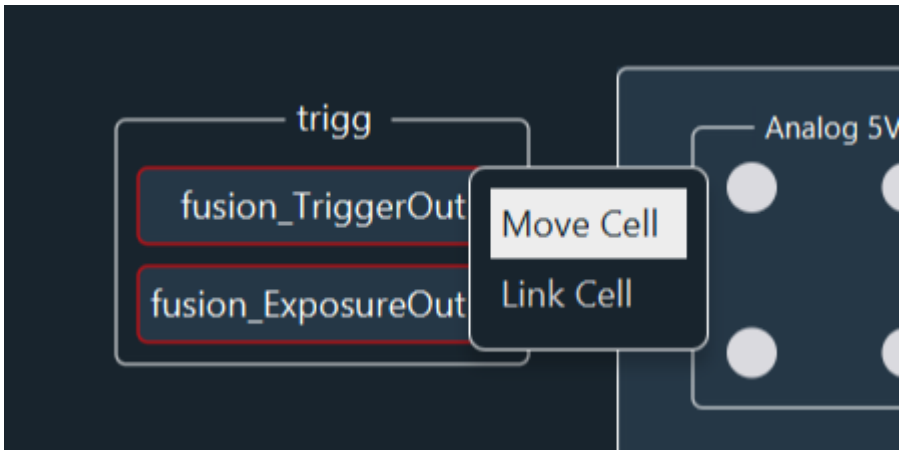
NB: **Move Cell** button allows to move the device on the diagram.

NB: The box diagram can be moved by simple click on it.

NB: Click and hold the mouse down to move the entire diagram (box and devices).

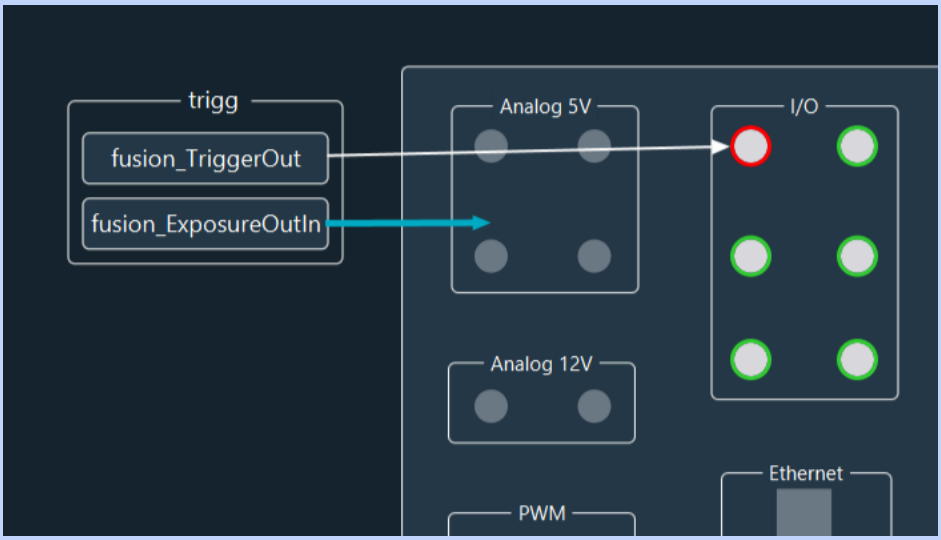
NB: Zoom in and out with the mouse wheel.

NB: double click on the mouse wheel resets the size of the entire diagram.

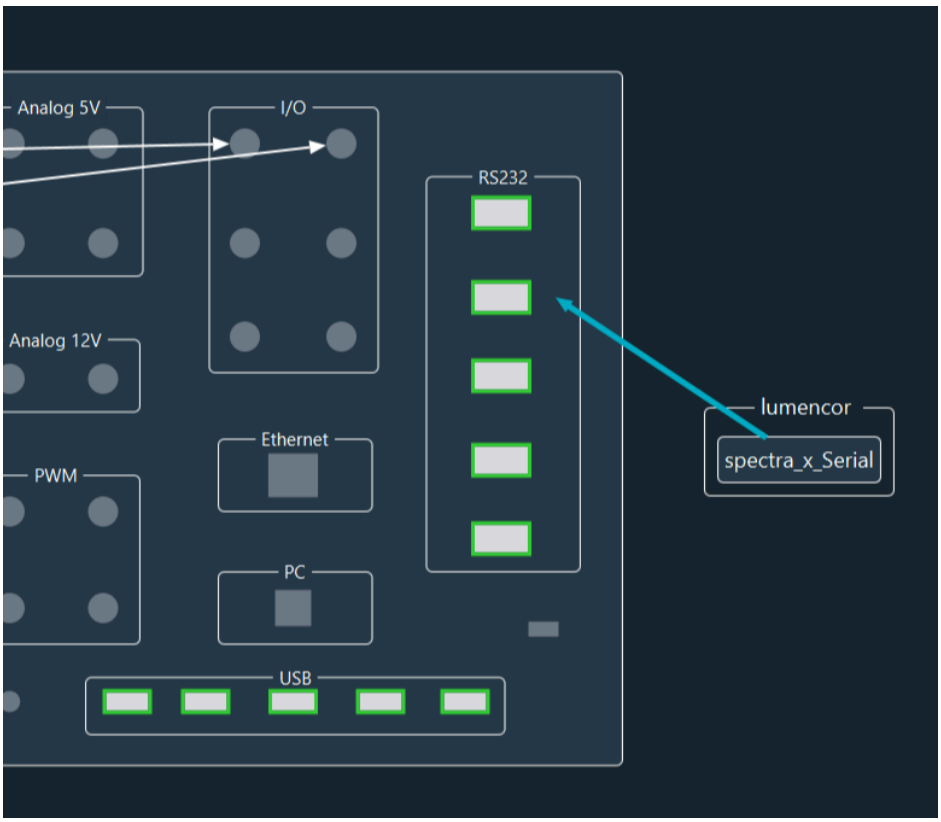


4. Link the connection by holding click from the connection to the DC. Repeat this step for each connection

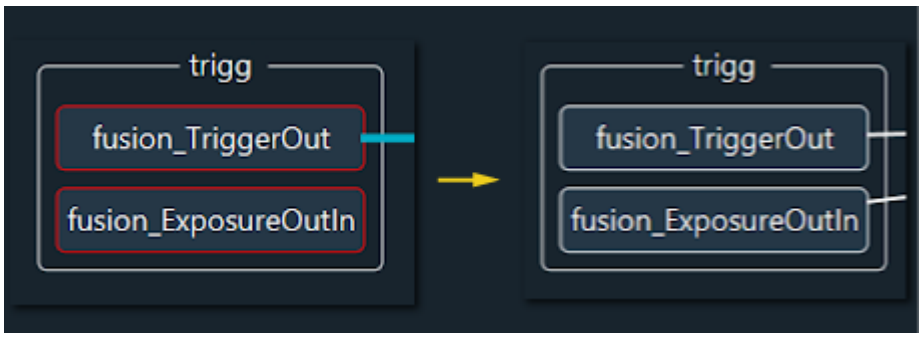
NB: The available ports for this connection are highlighted in green.When the connectors are occupied, it is surlight in red.



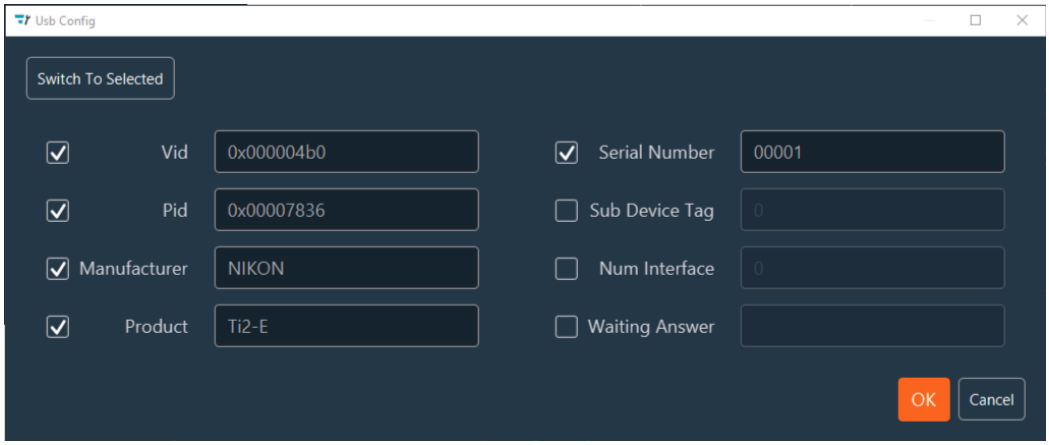
NB: Depending on the type of connection used, the connectors are automatically recognized. For example : the light source Lumencor Spectra X can be connected to the device controller by RS232 or USB connections.



When the connections are linked to the device controller (DC), the colour of the box around the Sub Device name changes from red to white.

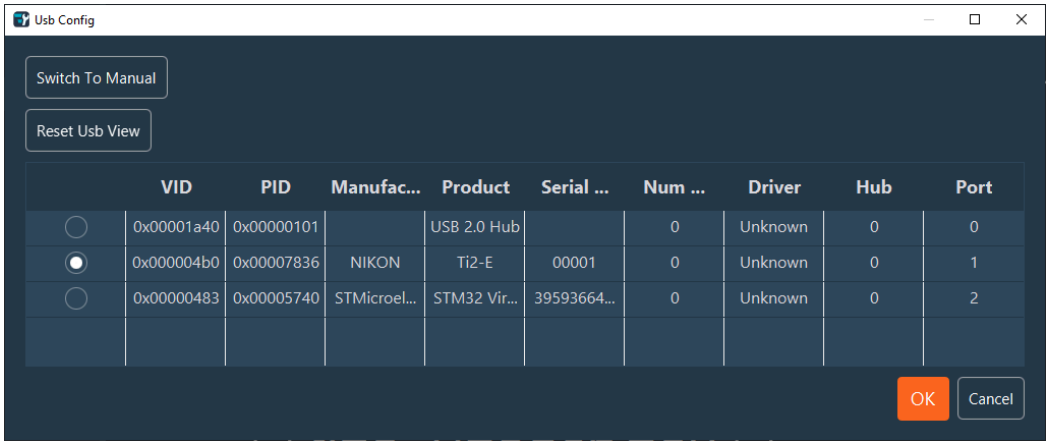


5. If the color of the box around the connection name is yellow, it means that a parameter is missing. To change it, double-click the connection name. A popup will appear and you can fill in the empty field. For example, for the Microscope Stand Ti2, you will get the window below and you need to enter the Pid and Vid numbers by checking the box of them.



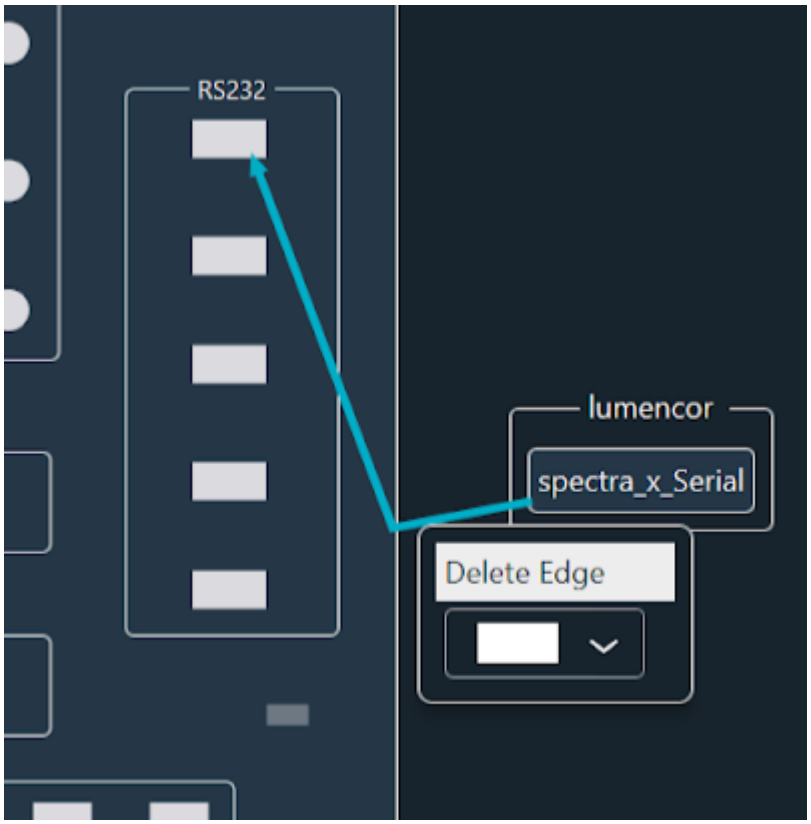
6. You can also detect all connected devices and select which port you need. **Switch To Manual** and **Switch To Selected**

   By clicking on **Rest Usb View**, The box will rescan all USB-connected devices.

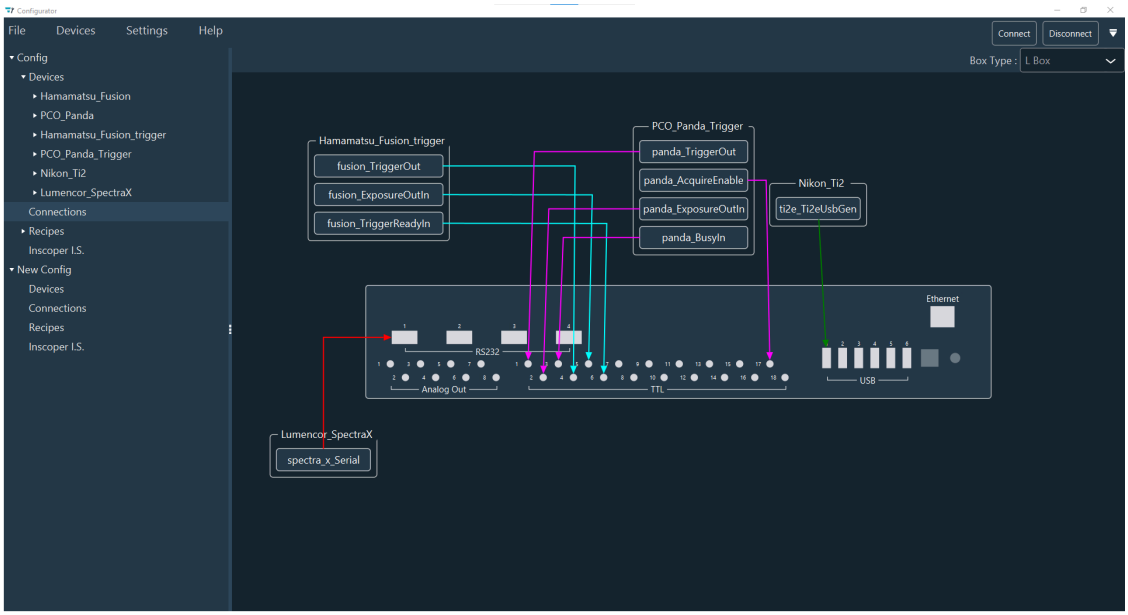


NB: In the list of USB ports, the first line corresponds to the USB hub inside the box (not valid for S type box). This is given for information only, to check that the box is working correctly.

7. You can modify the color of the arrow (**Color Box**) or delete it (**Delete Edge**) by a right click on it. If you click on the arrow you create a spot and you can move it to make an angle (like the example). To delete it, make a right click on the spot.



Once all your devices are connected to the DC, the diagram will look as follows:



After, you can go to the next step, which is the Recipe creation.

2.1.6. Recipe creation

There are two ways to create recipes:

1. Manual recipe creation
2. Automatic recipe creation

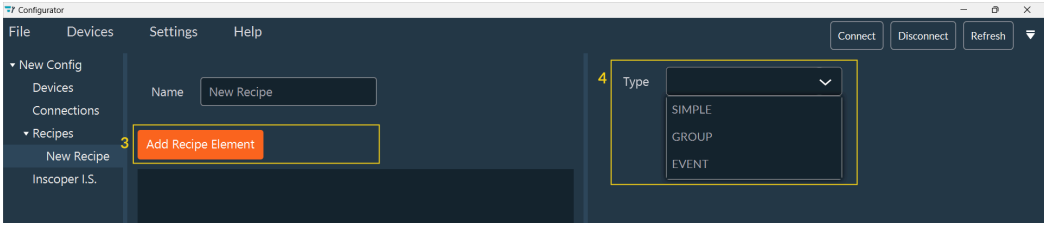
2.1.6.1. Manual recipe creation

1. Add recipe to your configuration
   For this, right-click on the **Recipes** in the Configuration section and click **Add Recipe**.

   NB: If you want to delete all the recipes, select **Remove All Recipes.**

   NB: You can duplicate the recipe by clicking on **Duplicate**, then select the Configuration and indicate the recipe name and validate.

2. If necessary, give this recipe a name in the **Name** field.
3. Then click **Add Recipe Element**.
4. Choose the **Type** of this recipe element:
   - **SIMPLE:** Recipe for a Sub Device (or a list of Sub Devices if recipe Id is selected)
   - **GROUP:** Recipe call another recipe
   - **EVENT:** Option that triggers a specific action at a defined moment in the acquisition sequence (e.g., stop or pause). This ensures, for example, that shutters are closed when the system is paused. This applies to both configurations with and without the device controller (DC).



5. If you choose **SIMPLE** option, you can:

   a. Modify the **Name** of this recipe element.

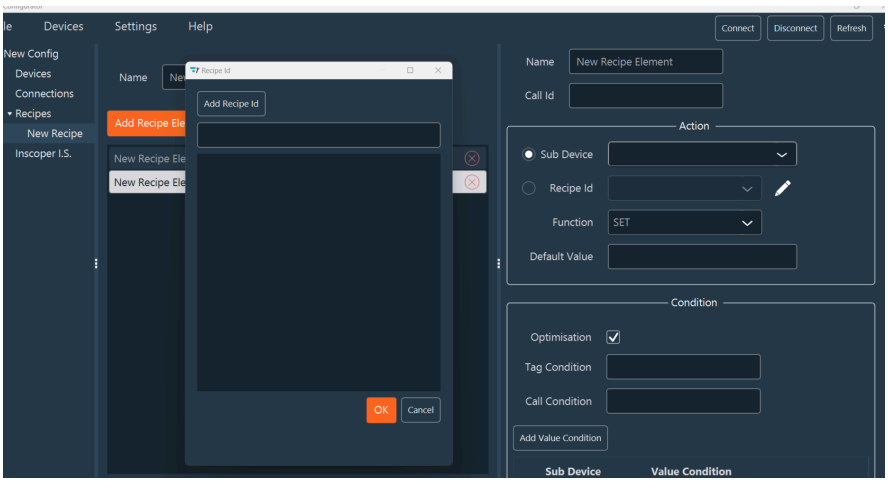
   b. Indicate the **Call ID** which is the ID of the recipe.

   c. In the **Action** part, you can:

      # Indicate the **Sub Device** involved in this recipe element or if you want to call the **Recipe Id** defined in the Sub Device during the configuration.

> NB: click on the pencil to open the pop-up window to **Add Receipe Id**

> NB: Recipe ID created at device level can not be removed or modified. All recipe IDs created at recipe level can be created, deleted, and modified.



      # Choose the **Function** that you want to call:

         # **SET**: send a value

         # **GET**: give the current value

         # **CHECK**: wait until the Sub Device is in the good status

      # Define a **Default Value**

   d. In the **Condition** part, you can:

Check the **Optimisation** box if you want to call the function only if the value changed

Define **Tag** and **Call Condition**:

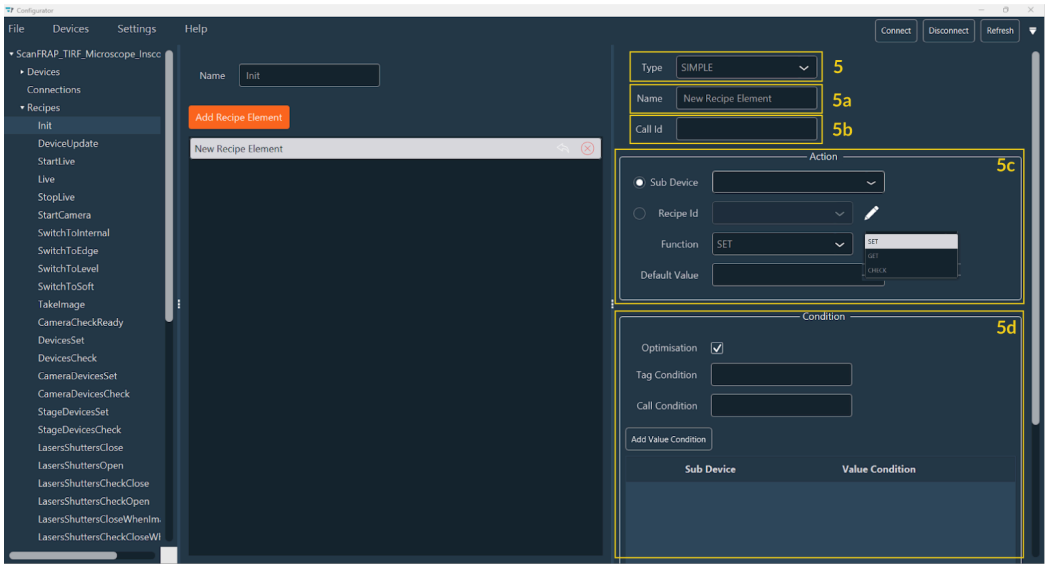
Tag Condition: Boolean expression about the presence or absence of a tag

Call Condition: Boolean expression to check if a recipe element with a specific callId has been called previously. For example : TI2xAxisPosition || TI2yAxisPosition

Add value condition by clicking **Add Value Condition**

Select a **Sub Device** and indicate the **Value Condition** for this Sub Device

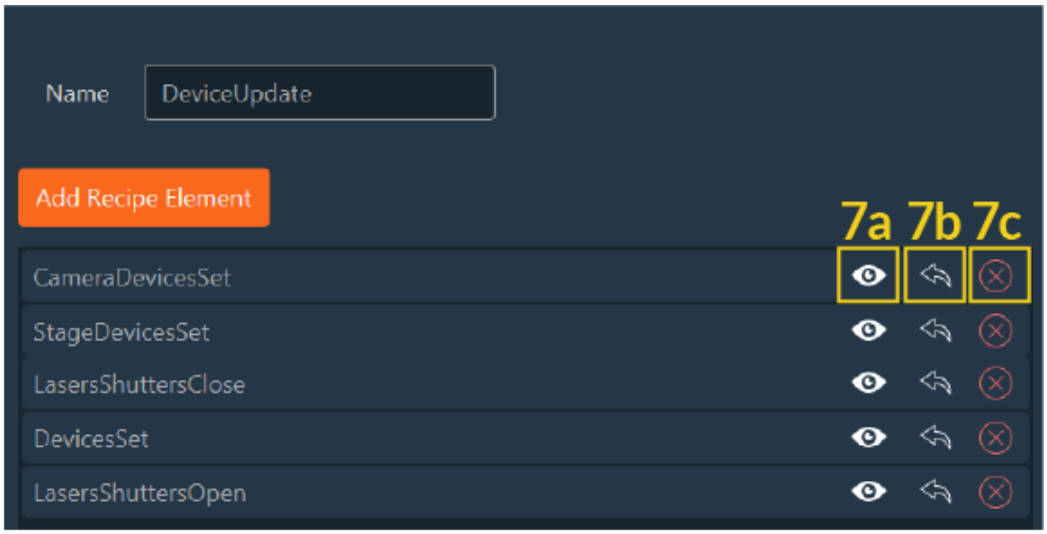
You can add several value conditions.



6. If you choose **GROUP**, you can add many recipe elements by clicking on **Add Recipe Element** and repeat the previous steps to configure them.rec
7. Recipe elements tools allow to:
   a. View details of this element (valid only for a Recipe Element Group).
   b. Move the Recipe element to another recipe.

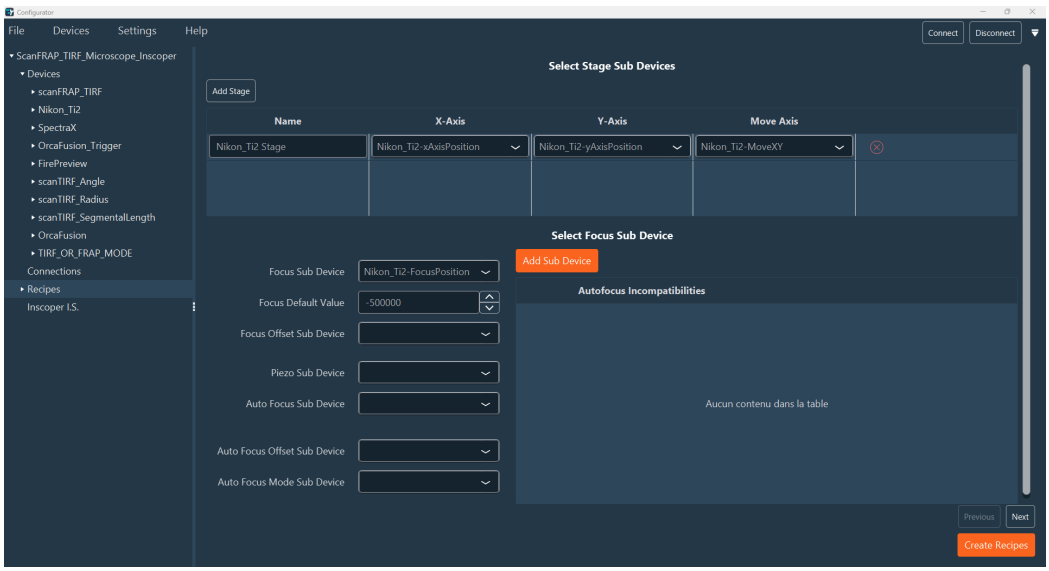
c. Delete this Recipe Element.

d. Drag and drop the Recipe Element to reposition it.



2.1.6.2. Automatic recipe creation

1. In the Configuration section, select the **Recipe** line. For each function, you must verify the details of the selected Sub Device. This Sub Device definition is required to generate the recipe. By default, the fields are prefilled.
2. For the **Stage Device**, you can enter the name of the stage, modify the Sub Devices.If you have multiple stages on your system, you can add a stage by clicking on the **Add Stage** button. You can delete a stage by clicking on the red cross.
3. You will find all properties for the focus device. You can enter the piezo and autofocus Sub Devices. When it's done, click on **Next**.



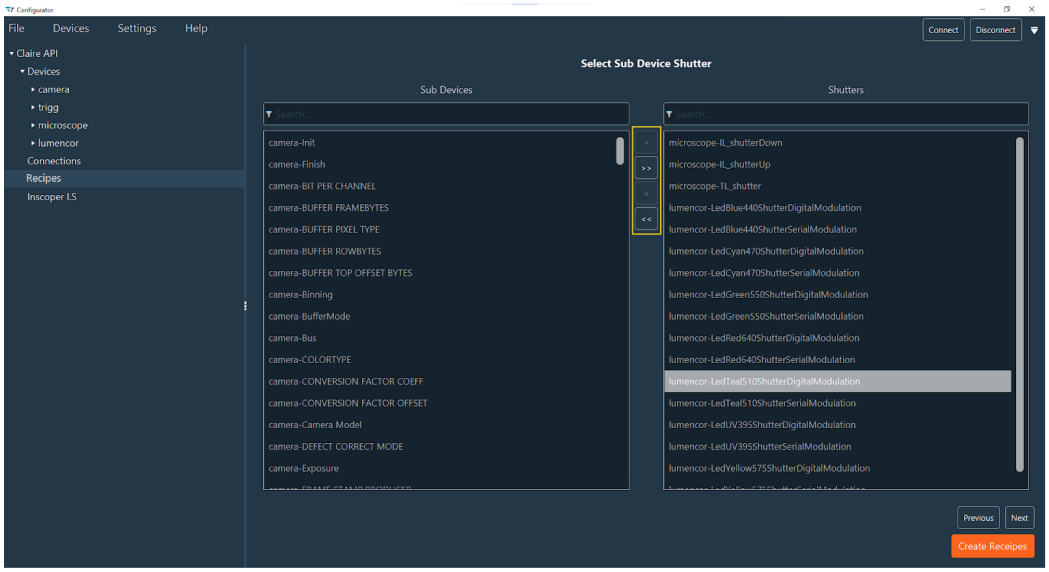
4. Enter all the shutters in your system. If one is missing, you can search for the name of the shutter in the search field of the left column and move to the right column by clicking the arrow.

   >> : All Sub Devices on the field are moved in the shutters list

   > : Only the selected Sub Device is moved in the shutters list

   << : All Sub Devices on the field are removed from the shutters list

   < : Only the selected Sub Device is removed from the shutters list

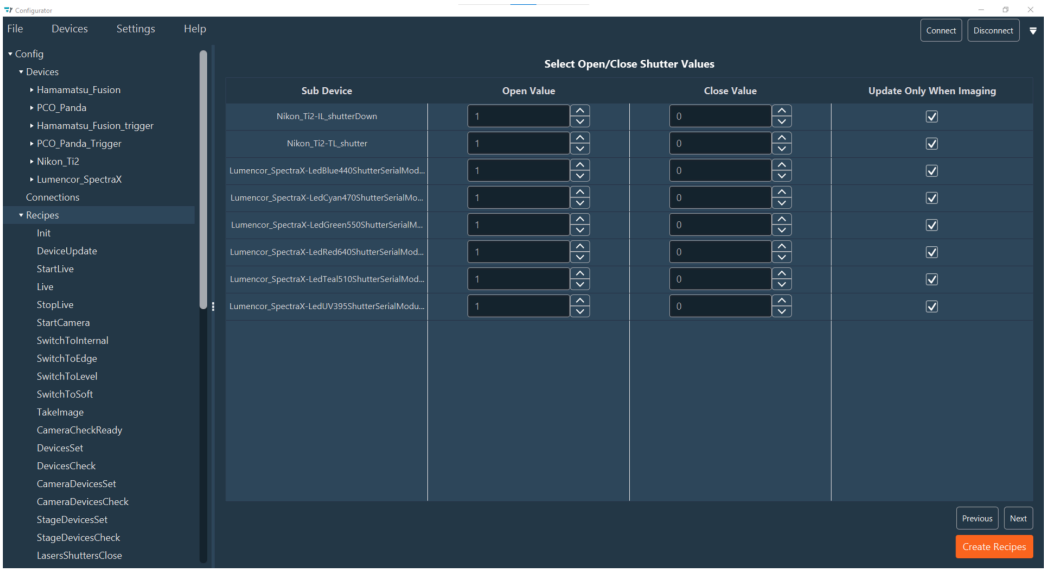


NB: Use the search line to find the needed element.

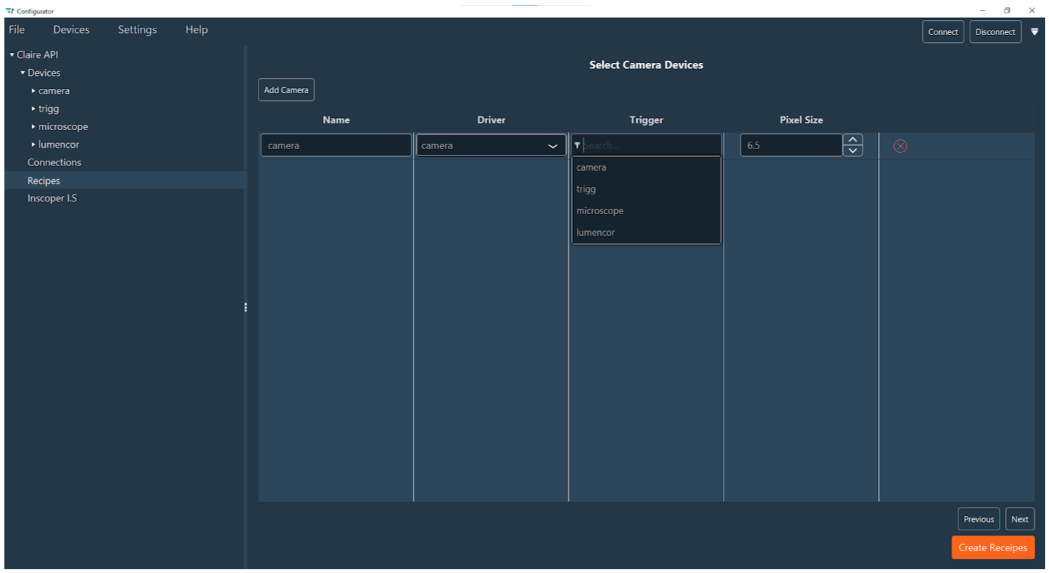
You can come back to the previous device by clicking on **Previous**. Once all your shutters are in the list, you can click **Next**.

5. Verify if the open and close values of the shutters are correct and click **Next**.

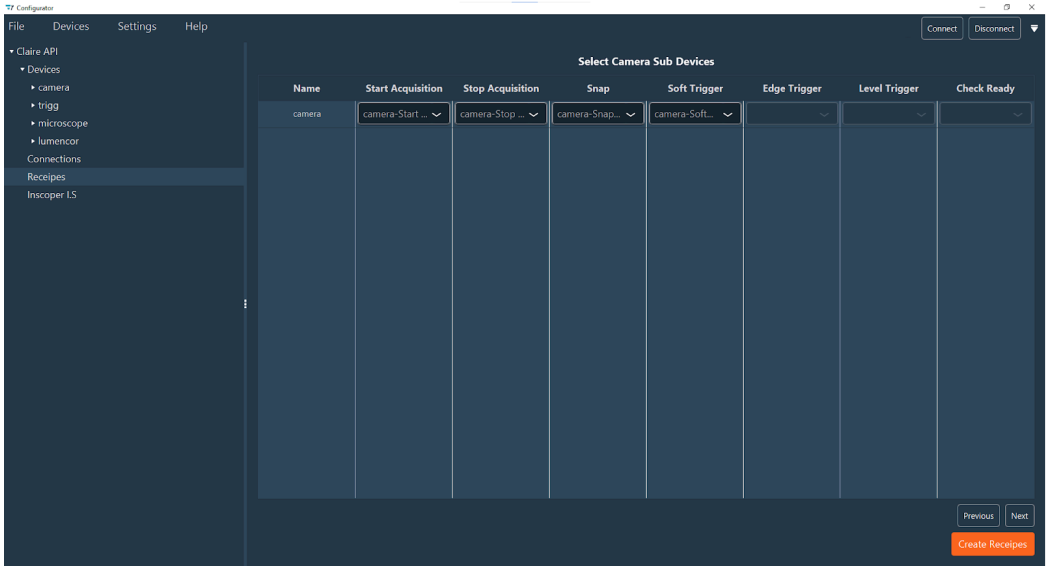
NB: By default 0 means close shutter and 1 means open shutter. Check if necessary "Update Only When Imaging". If you check the box of **Update Only When Imaging**, the status of the shutter will be only in **Live** or during the acquisition. If the box is unchecked, you can modify by yourself the status of the shutter.



6. Enter all your cameras. The camera is automatically detected. You can add many cameras by clicking on **Add Camera**. For each camera, you need to select or modify the **Name**, the **Camera Driver**, the **Trigger Device** (only if you want to trigger the camera with the device controller) and indicate the pixel size. You can delete the Camera by clicking on the red cross. Click on **Next**.

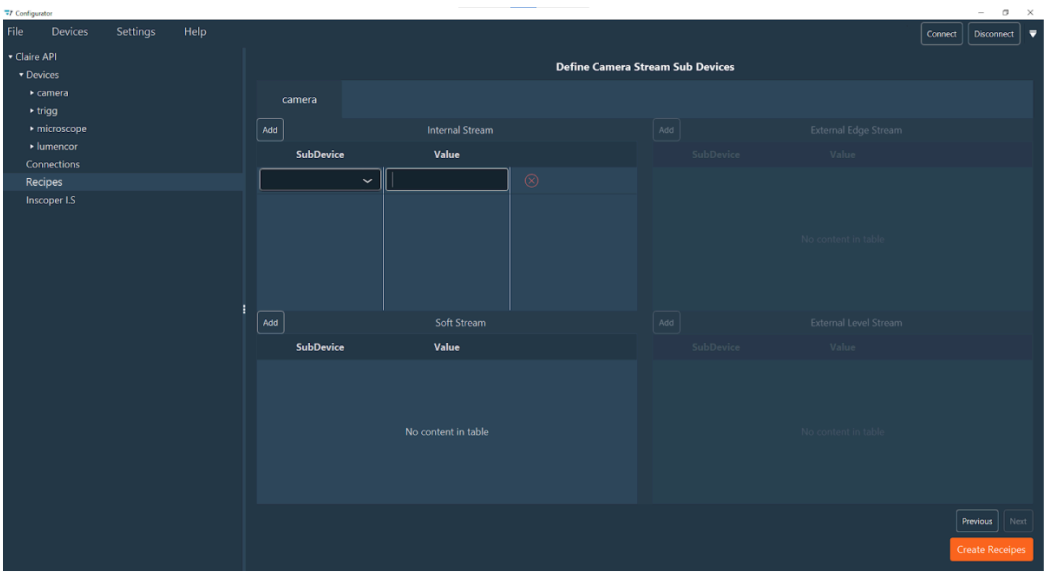


7. **Start**, **Stop**, **Snap** and **Soft Trig** Sub Devices are pre-filled. They are Sub Devices of the camera **Driver Device**. If the **Trigger Device** has been filled in, **Edge**, **Level** and **CheckReady** are available and pre-filled. Otherwise, the fields are disabled. Click **Next**.

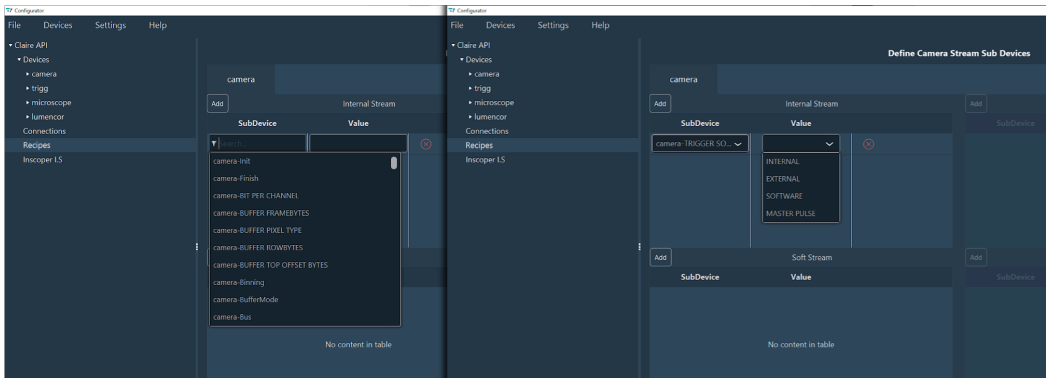


8. Define the **Camera Stream Sub Devices**: Indicate the parameters applied for the different modes of the camera. 4 fields need to be filled in:
    - **Internal Stream:** This mode I used for Live or Snap.
    - **Soft Stream:** In this mode, the camera is triggered via software by calling the previously defined Sub Device in the **Soft Trigger** field. This field is available only if **Soft Trigger** was specified in the previous view.
    - **External Edge Stream:** In this mode the camera is triggered by the DC with TTL. With this mode, you can't use different exposure times in one sequence. This field is available only if **Edge Trigger** was specified in the previous view.
    - **External Level Stream:**In this mode the camera is triggered by the DC with TTL. With this mode, you can use different exposure times in one sequence. This field is available only if **Level Trigger** was specified in the previous view.

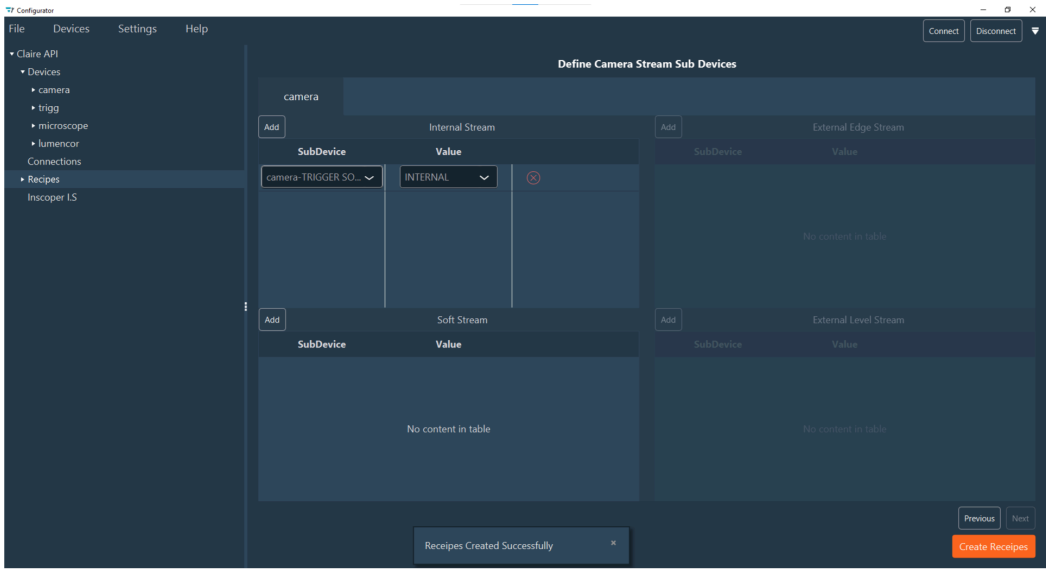
The Sub Devices responsible for those properties depend on your camera.



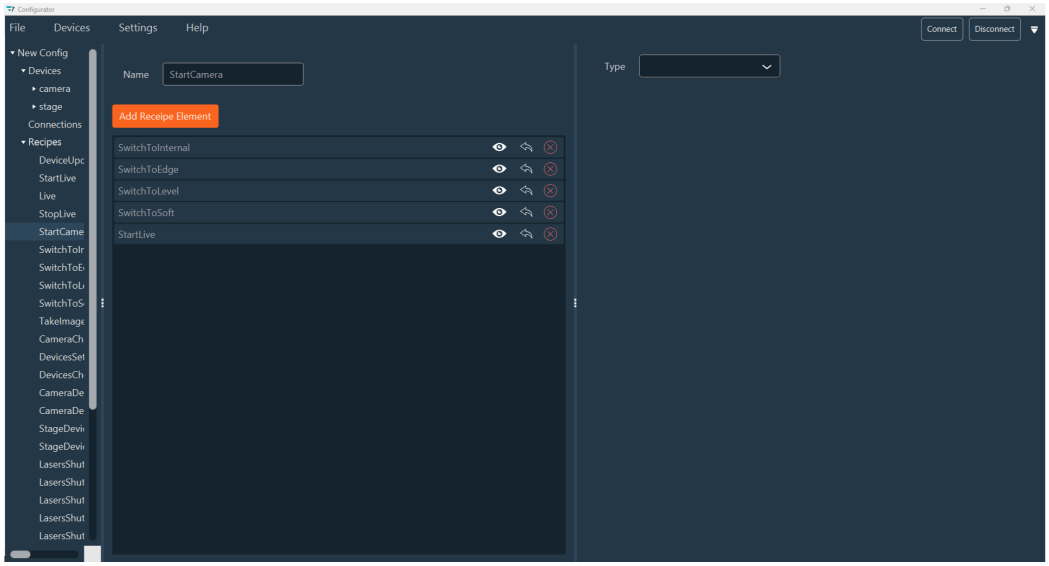
9. For each field, select the correct **Sub Device** and **Value** using the search box. Once your Sub Device is selected, you will get the values specific to this Sub Device in the Value drop-down menu.



10. [This is a optional step for all system with special techniques using ILDA connector (FRAP, TIRF) or FLIM option. If so, check ILDA functions]
11. When all the properties are filled in, click on **Create Recipe**. Once done, you will get a popup window "Receipes Created Successfully" and you will find your receipe in the configuration part (under Receipes line).

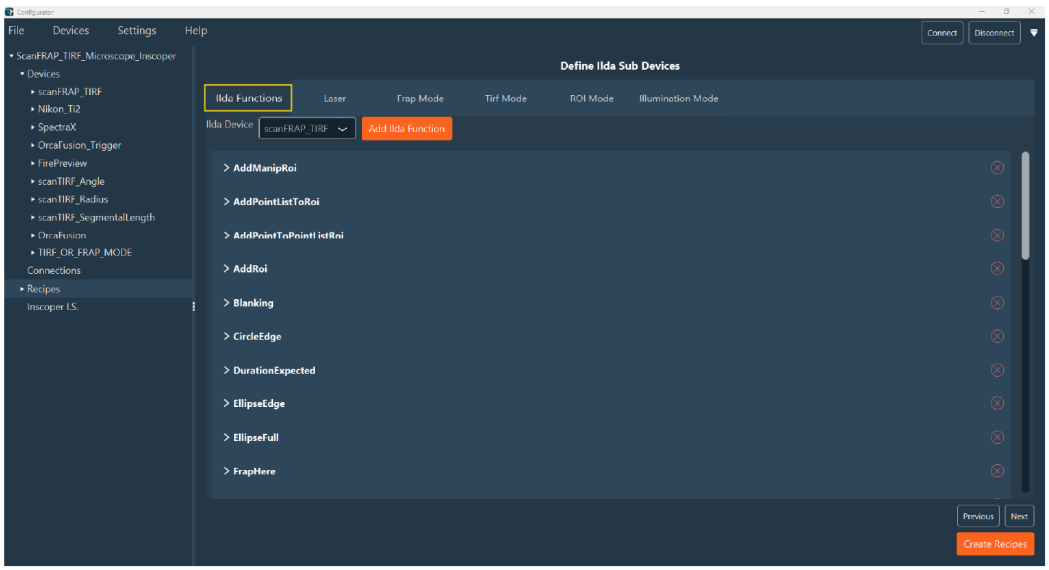


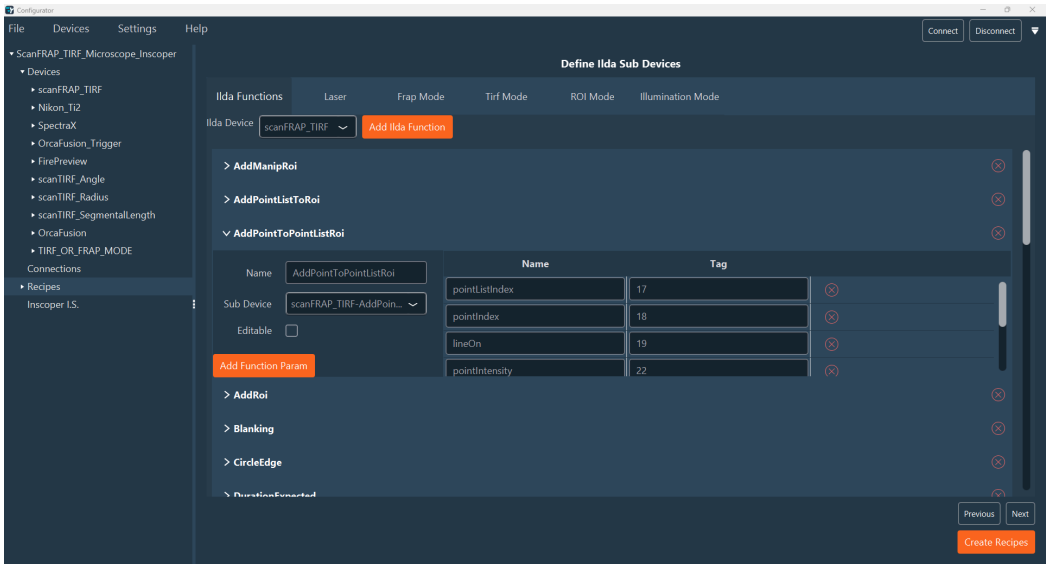
12. Normally you don't need to change the recipe, but if you want to, you can drag and drop the recipe function to change the order.



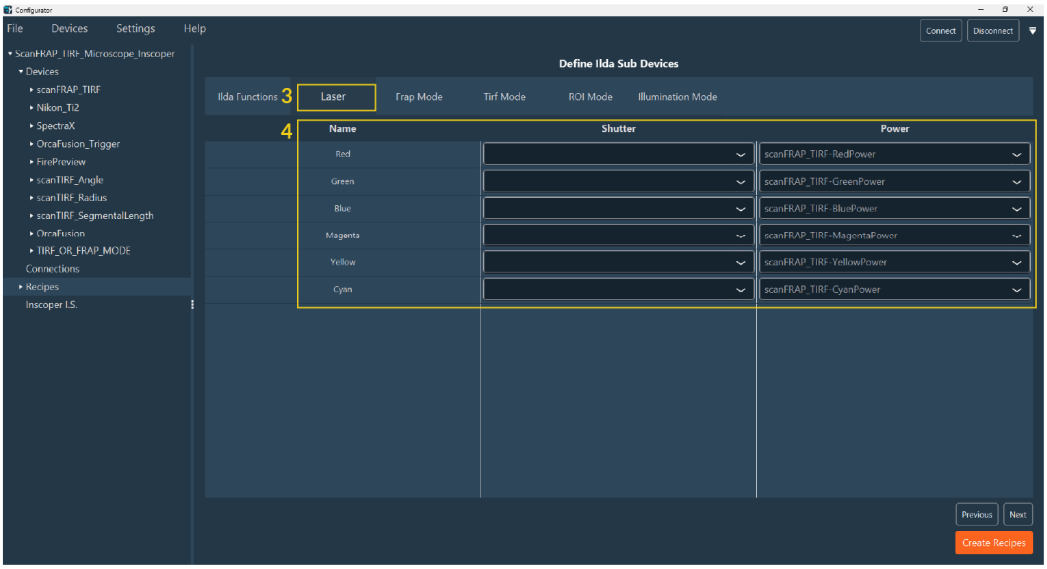
2.1.6.3. ILDA functions for FRAP, TIRF, FLIM modules

1. If you have an additional module such as a FRAP, TIRF or FLIM module, ILDA fuctions will need to be configured. You can continue setting up the recipe by clicking **Next**.
2. In the **Ilda Functions tab**, select your device connected to the Ilda connector (only available for the XL box type).





3. All Ilda functions and all parameters in each function are automatically loaded. Check if all functions and parameters are correct, then click on the **Laser tab**.
4. In the Laser tab, you will find a table where you must specify the shutter and power Sub Devices for each laser line. Select the Sub Devices by clicking on the drop-down menu in each column.



5. Then click on the **FRAP Mode tab**.
6. Select **Active Frap Mode** to access this option in the interface (if the box is unchecked, no FRAP parameters appear in the interface):

    a. Select the sub-device that determines the frap density and the scanRate.

    b. Specify the density index, which must match the FRAP diameter value set for the sub-device in the Device tab.

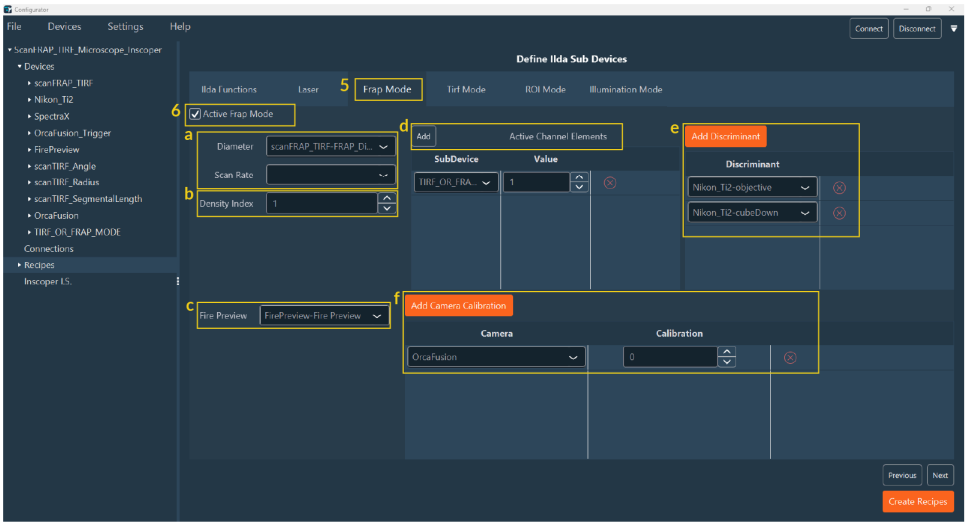
    c. Select the Sub Device that determines the Fire Preview.

d. Active Channel Elements shutter for FRAP (Allows to indicate which channel is a FRAP channel).

e. Add the discriminant for the frap calibration like Objective, filter cube.

f. Add camera calibration.

> NB: If you have several cameras indicate if you want to use the same calibration for all cameras or a different one. If you want to use the same calibration indicate the same number in the calibration column for all cameras



7. When it's done, then go to the **TIRF Mode tab.**
8. Select **Active TIRF Mode** to access this option in the interface (if the box is unchecked, no TIRF parameters appear in the interface):

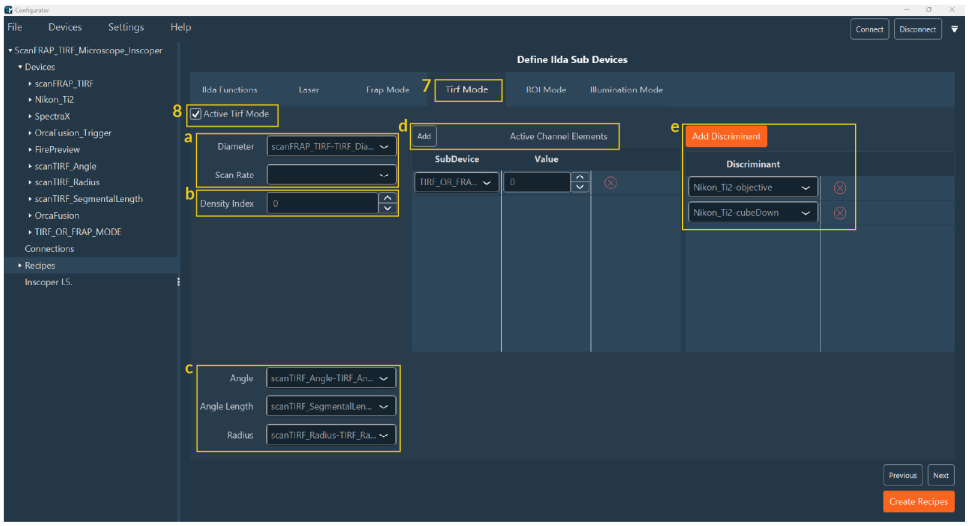
a. Select the Sub Device assigned to the TIRF density.

b. Specify the density index which need to be the same number than in the subdevice TIRF diameter in the Device tab.

c. Select the Sub Device responsible for the Angle, Angle Length and the radius. Those parameters will help you to configure as you want a TIRF experiment.

d. Active Channel Elements for TIRF (allows to indicate which channel is a TIRF channel).

e. Add discriminant for the TIRF calibration like Objective, filter cube, lasers.



9. If you have a lightsheet system, go to the **ROI Mode tab (for ROI scanning).**

a. Select **Active ROI Mode** to access to this option in the interface.

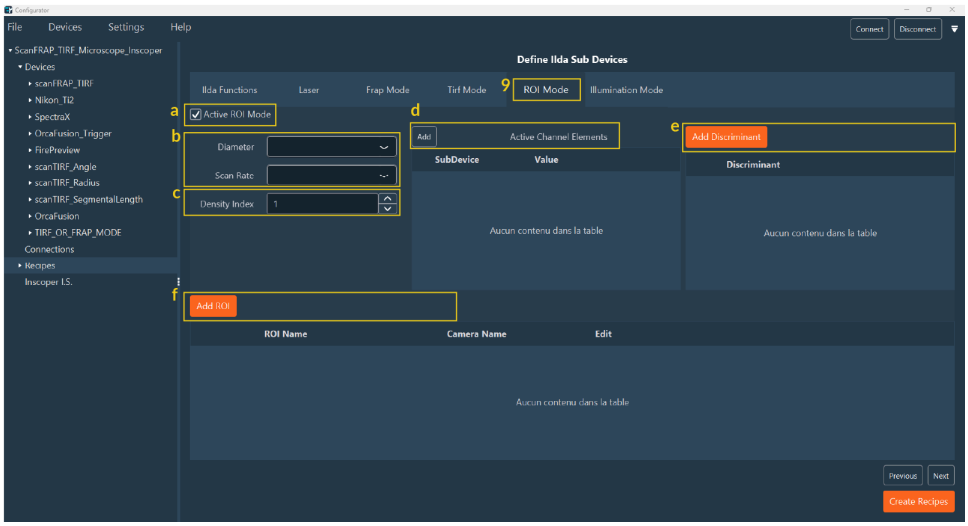
b. As with FRAP, select the sub-device that determines the density and the scanRate.

c. Specify the density index which need to be the same number than in the subdevice frap diameter in the Device tab.

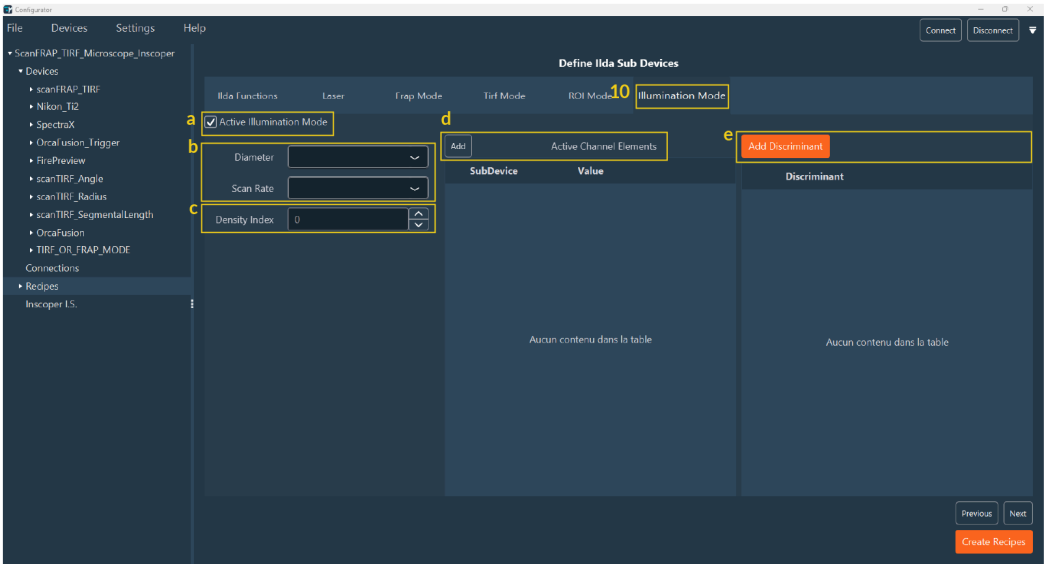
d. Add active channel elements (if you activate one element in the channel that means you are in the ROI mode).

e. Add discriminant.

f. Add ROI.



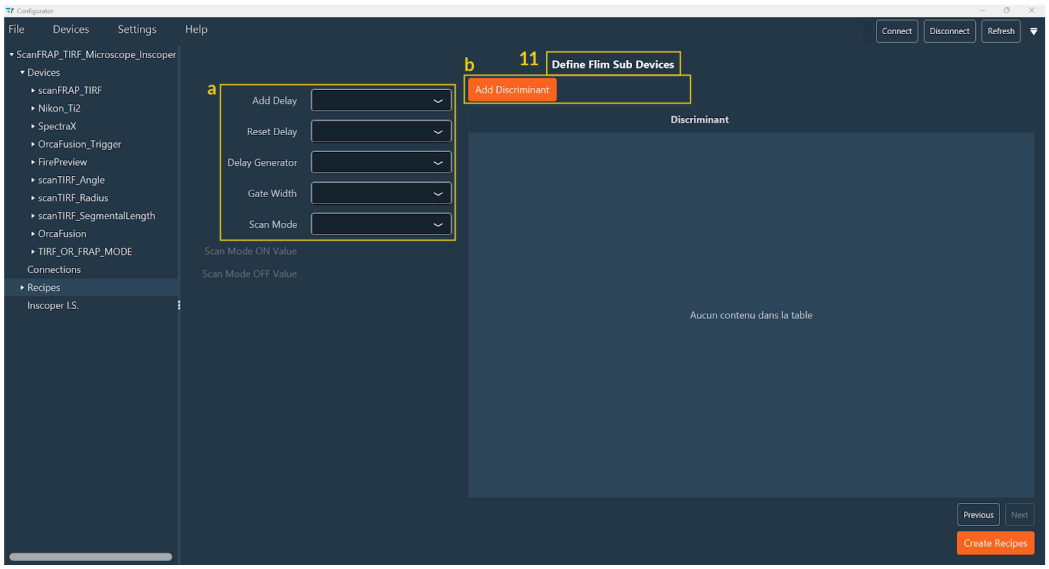
10. The **Illumination Mode** is used to add a virtual device to select the illumination mode (e.g. if you have a multimodal system with FRAP, TIRF, Spinning Disk).
    a. Select **Active Illumination Mode** to access to this option in the interface.
    b. As with FRAP, select the Sub Device that determines the density and the scanRate.
    c. Specify the density index which must to be the same number than in the Sub Device frap diameter in the Device tab.
    d. Add active channel elements.
    e. Add discriminant.



11. Click on **Next** to proceed to the last step, which is the **FLIM configuration**.

> NB: If you don't have the FLIM module, you can directly click on **Create Recipes**.

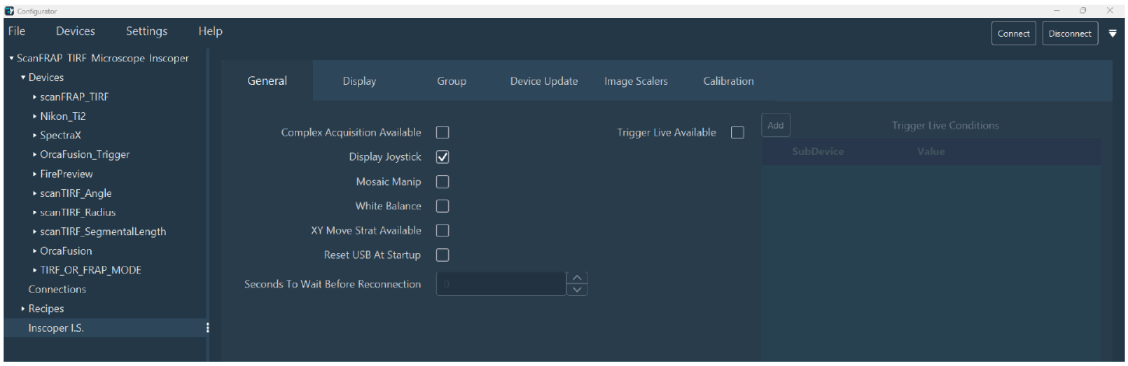
    a. Add all devices responsible for the Delay.
    b. Add discriminant.



12. Click on **Create Recipes**. Your recipe is created. You can now finalize your I.S. configuration.

2.1.7. Inscoper I.S. configuration

When the recipe is generated, the last action is to design the interface that you will use to control your system.



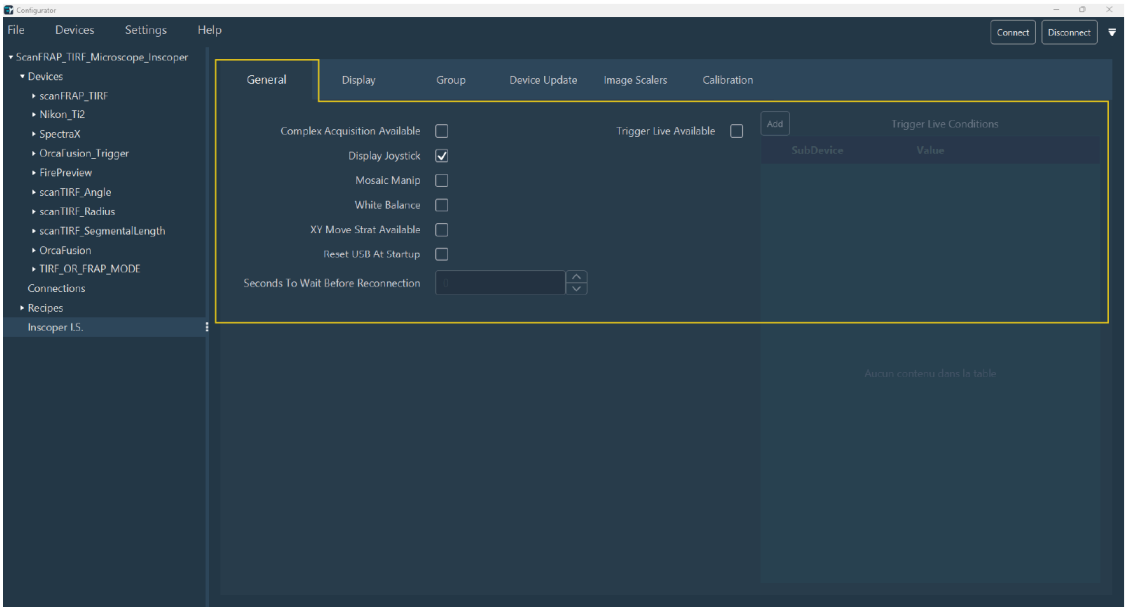
To configure Inscoper I.S. you have 6 steps to review:

- General
- Display
- Group
- Device Update
- Image Scalers
- Calibration

2.1.7.1. General

General tab allows you to select what kind of elements/options you want to have on your interface by checking boxes:

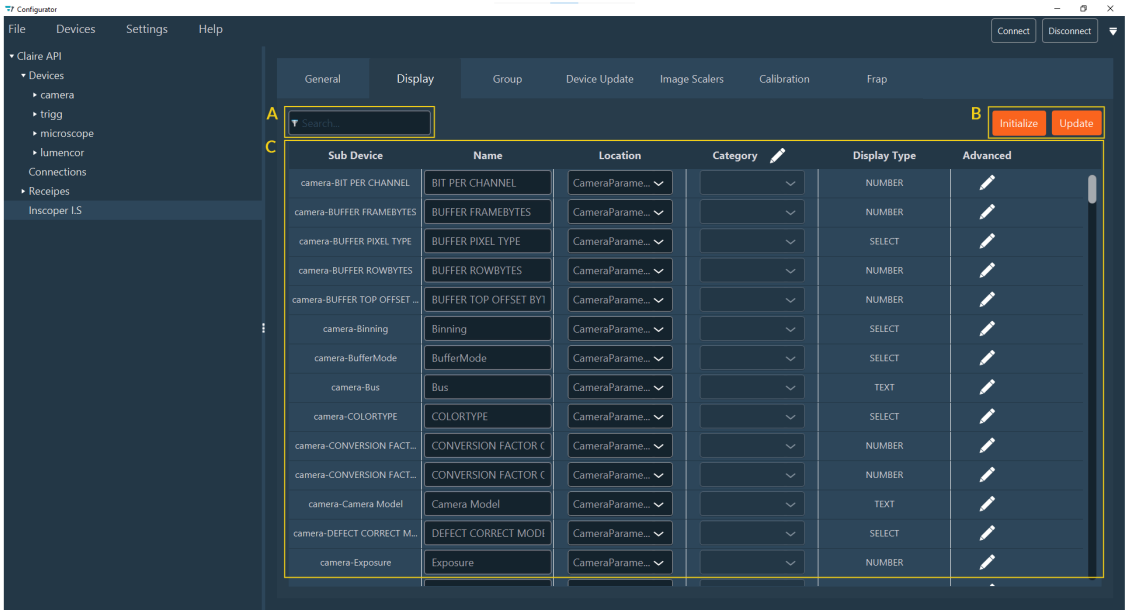
- **Complex Acquisition Available:** Option to create several acquisition sequences depending on some dimensions
- **Display Joystick**: Virtual joystick with blue arrows
- **Mosaic Manip:** Tiling calibration and experiment option
- **White Balance:** If you have color camera in your system
- **XY Move Strat Available:** Stage moving options when there is a significant distance between two positions
- **Reset USB At Startup:** Some devices need you to scan all the USB devices connected to the box, otherwise they won't be found
- **Seconds To Wait Before Reconnection:** waiting time between rescanning and reconnection (some devices may take a little longer)
- **Trigger Live Available:** allows to synchronize the Live with another device via a small sequence in the DC. You need this option if you are constrained in image capture. If you check this box you can add **Trigger Live Conditions**



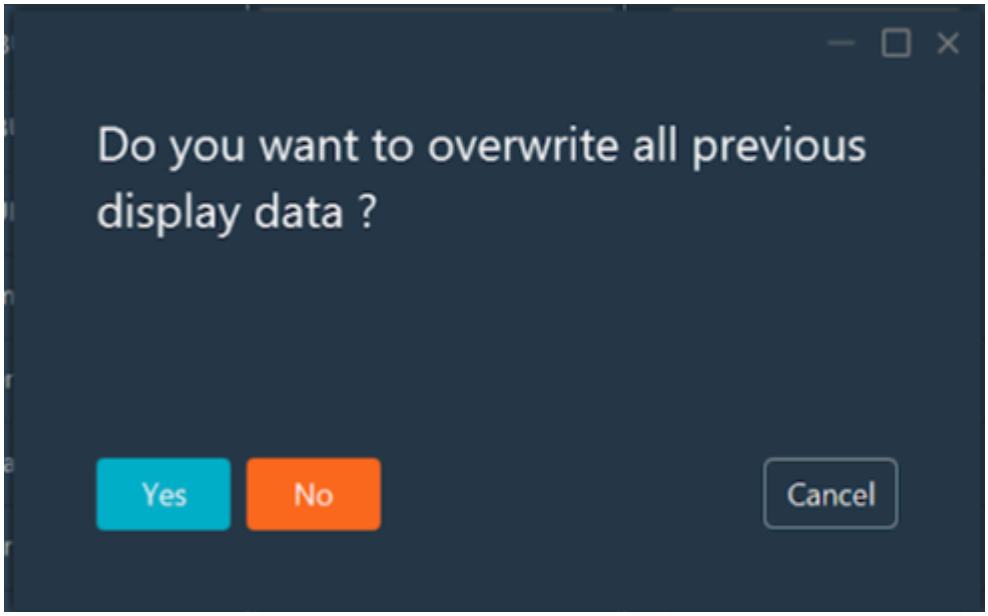
2.1.7.2. Display

Display: This tab allows you to configure the layout (**Display**) of your interface. It can be divided into different sections (**Location**) and different categories in the location (**Category**). There are 3 important points in this tab :

- A- Sub Device search field (lets you perform a quick search among items in the list)
- B- Buttons to interact with the Sub Devices
- C- The display setting table



1. Click on **Initialize** to create all Display Data (if this step has already been done but you want to add another device, click on **Update**). If you click on **Initialize** when you have already initialized your devices, you will get a message to know if you want to overwrite your current display or not.



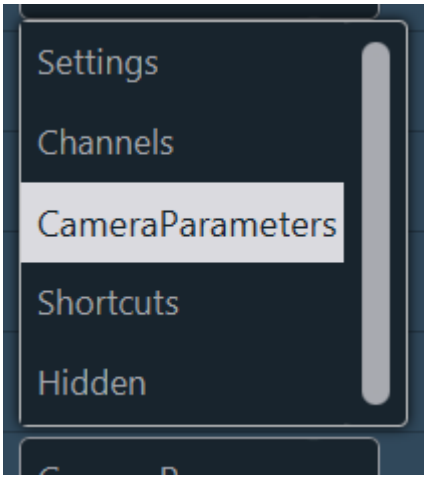
NB: After the initialization, you will get a table with all sub devices and their Location, Category, Name, Display type by default and advanced settings.

Sub Device: Sub Device bound to the display Data

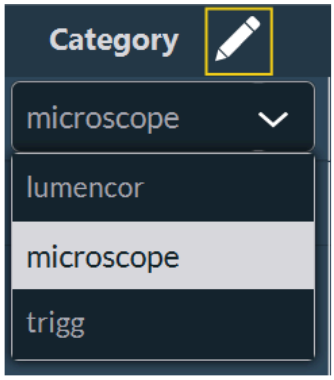
Name: name by default in the interface

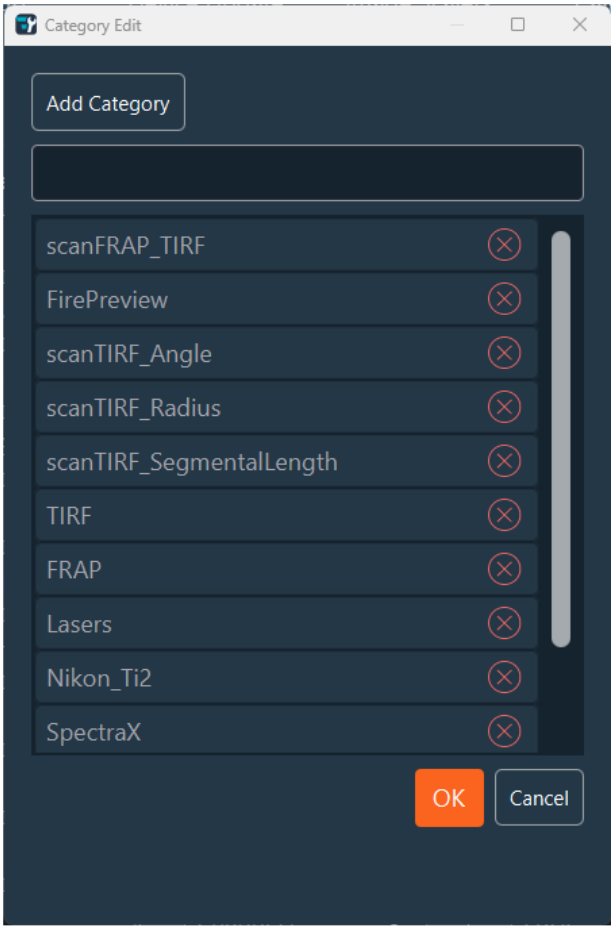
Location: in which section you want to place and display the Graphic Field.
Category: in the location you can organize sub devices by category
Display type: which kind of UI-elements it will be (for example, switcher will be a on/off button). Generated by default but you can change it by clicking on Advanced parameters.
Advanced: go to advanced parameters.

2. You can modify all the parameters directly by clicking on each column (or you can go to **Advanced**)
   # **Name**: You can change the name by editing the field.
   # **Location**: by clicking on the drop down menu you can choose another location. Depending on your system, you will find : Setting, Channel, Shortcut, CameraParameters, Hidden.



   # **Category**: You can add a category by clicking on **Edit** (pen). Write the name and click on **Add Category**, then click on **OK** to close the window. You will find the new category on the drop-down menu.

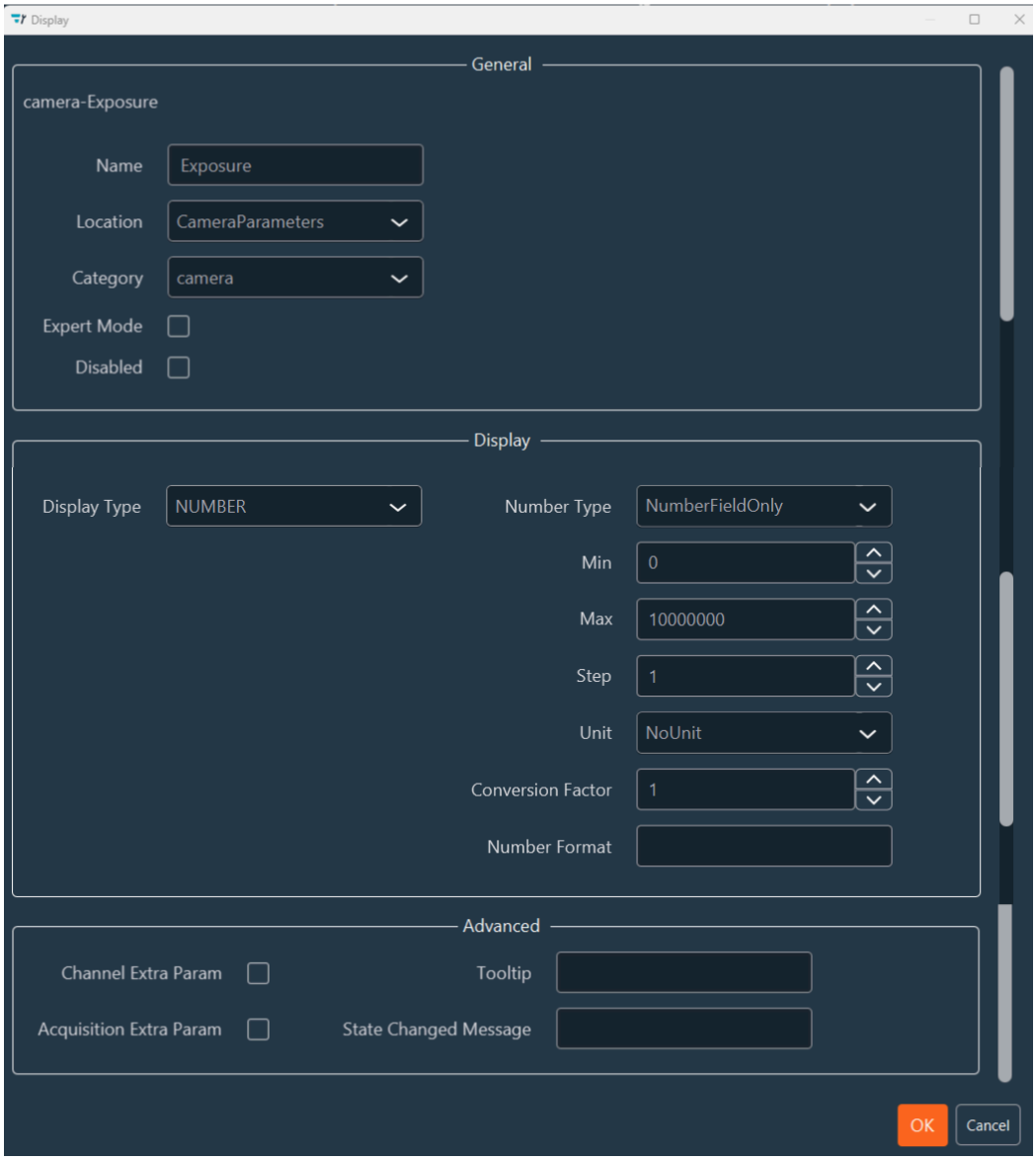




Display type: Depending on the Sub Device the display type will be by default but you can modify it by clicking on **Advanced** parameters.

Advanced Parameters.

3. **Advanced** Tab groups all previous display parameters together. Click on **Edit** (pen). You will find 3 sections to navigate by scrolling:



a. **General**:

Name: text field.

Location: drop-down list.

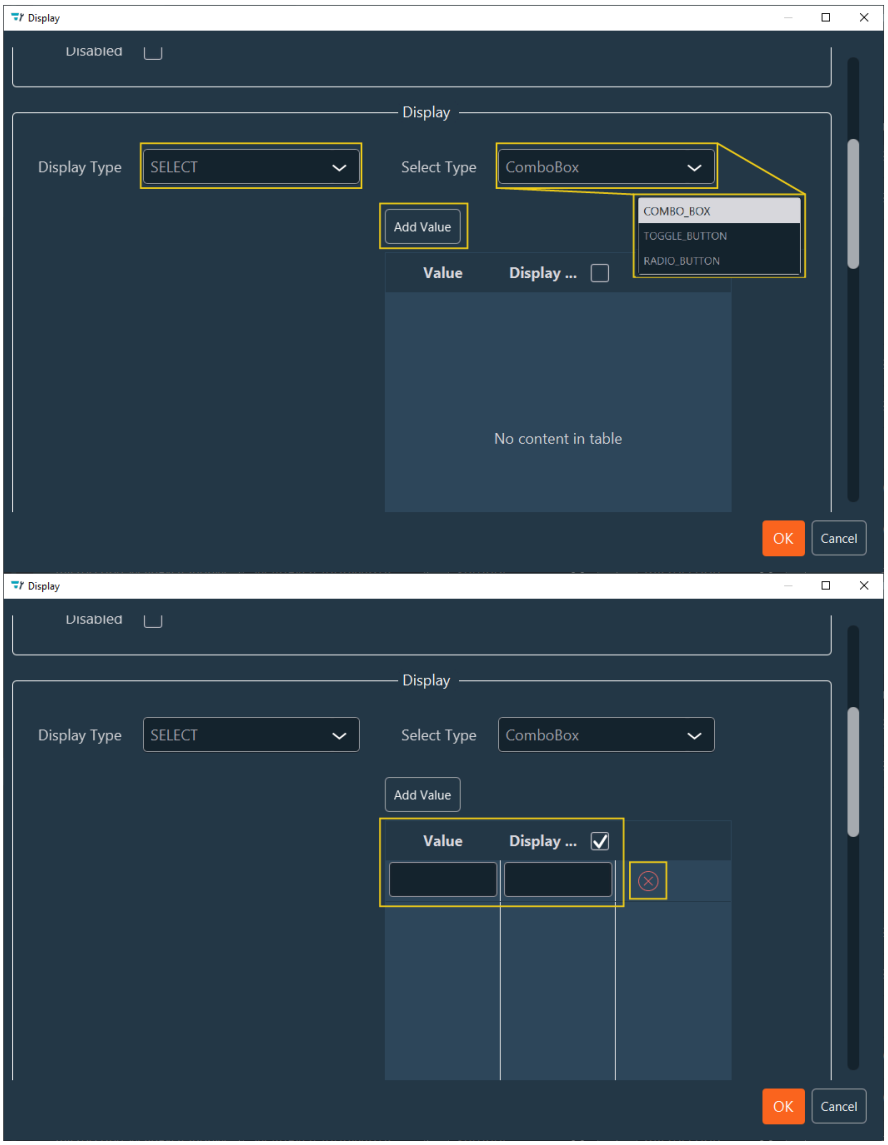
Category: drop-down list.

If you want to see this parameter in **Expert mode**, you should check the box (Expert mode allows unrestricted access to all settings and parameters of the system). If you don't check the box, the parameter will appear in **User mode**. User mode allows restricted access to some settings and parameters. The restrictions are fully customizable, from the basic channel configuration to the most advanced settings of the camera(s) or other devices.

If you check the **Disabled** box, the setting cannot be changed. It is possible to switch from User Mode to Expert Mode at any time. A password can be set to access the Expert Mode. These authorization levels are optional, depending on the use of the system.

b. Display Type:

SELECT - Select either **Combo_Box** (drop-down list), **Toggle_Button** (switching between two states) or **Radio_Button** (multiple button but one choice). Then click on **Add Value** and fill in required values. You can delete them one by one by clicking on the red cross.



NB: Example : you have 5 positions in the filter wheel (from 0 to 4 [you can find this information in the property of your Sub Device]). The display type will be SELECT and ComboBox. To configure these 5 positions you need to add 5 Values (see the example table below):

Val-ue	Dis-play
O	DAPI
1	GFP
2	YFP
3	Cy3
4	Cy5

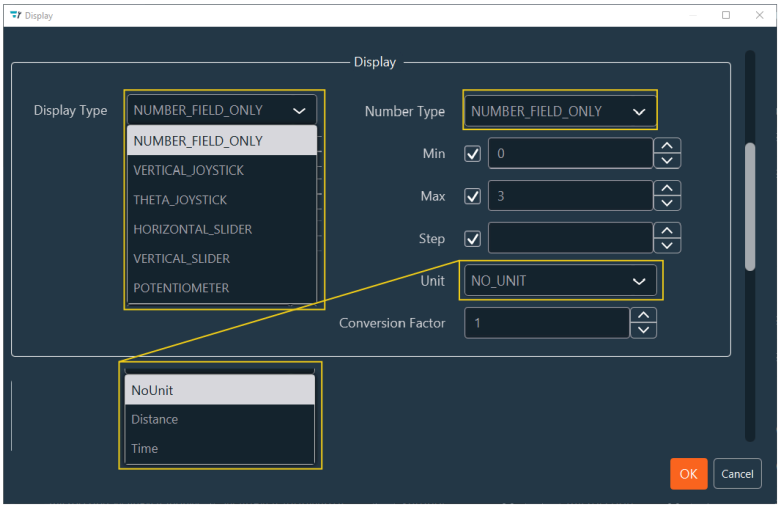
NUMBER - Select Number Type in the drop-down list:

- # Number field only
- # VerticalJoystick
- # ThetaJoystick
- # Horizontal Slider
- # Vertical Slider
- # Potentiometer

For each number type, you need to indicate:

- # Minimal and the maximal value
- # Step to change the value
- # Unit of this value: it can be **Distance**, **Time** or **No unit**
- # Number Format: decimal, the number of decimal or no decimal
- # Conversion Factor: decimal
- # For the VerticalJoystick and the ThetaJoystick, you can add a JoystickName

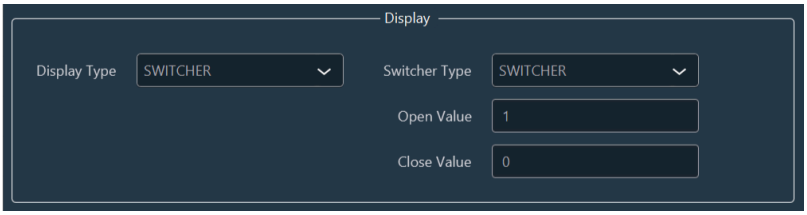
NB: Example: used for stage.



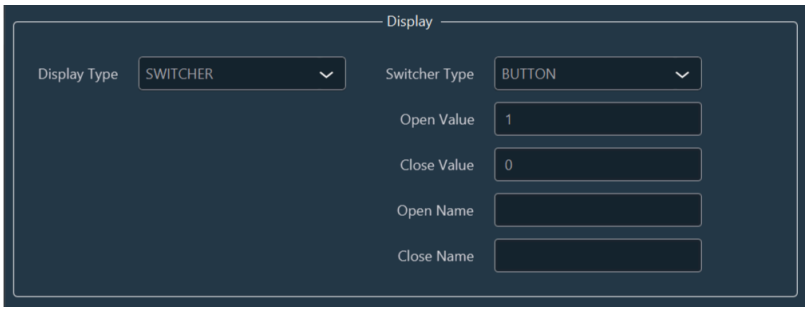
SWITCHER - Select **Switcher Type** between **Switcher** or **Button** in the drop-down menu:

If **Switcher**, indicate the **open** and **close** value.

> NB: For Inscoper, 0 is for close value and 1 is for open value.



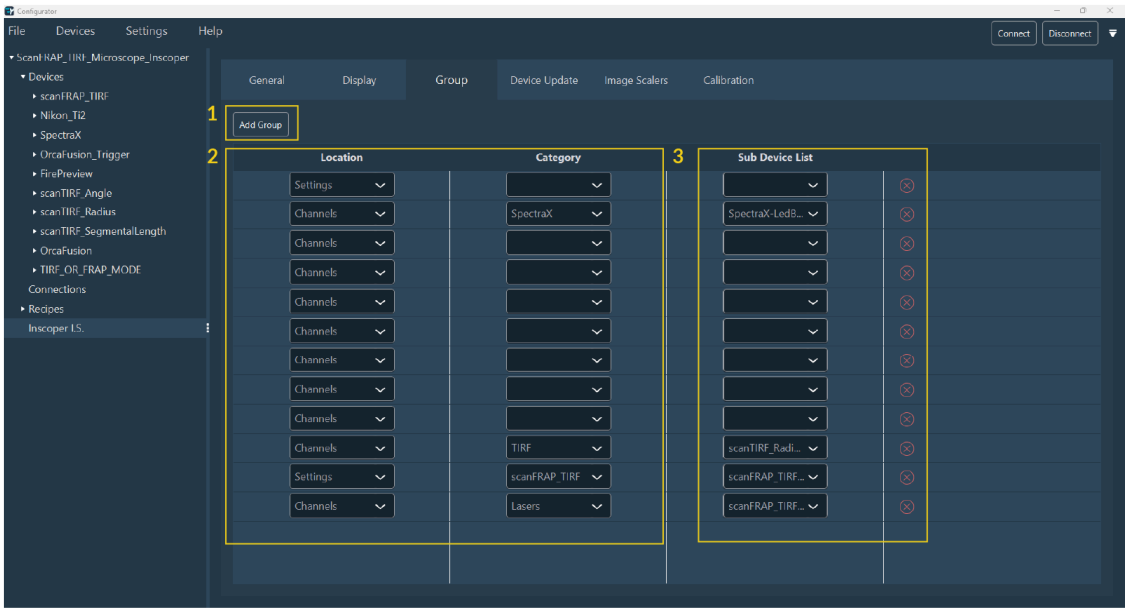
If **Button**, indicate the open and close value, open and close name.



TEXT - Text display type requires no action from your part.

2.1.7.3. Group

This tab allows you to group the display of several settings.

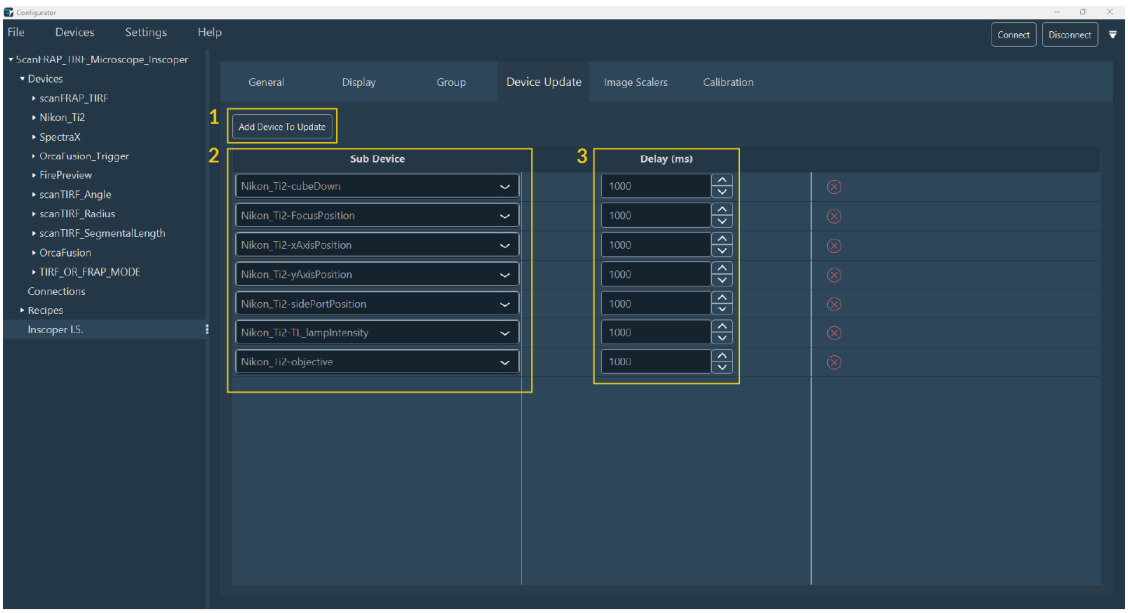


1. Click on **Add group**.
2. Find the sub devices you need to group by filtering by **Location** and **Category**.
3. Select them in the **Sub Devices** drop-down list.
4. Repeat the previous steps if you need to group more items.
   If necessary, you can delete the group by clicking on **Delete** (red cross).

2.1.7.4. Device Update

This tab allows you to select the devices whose values are to be updated automatically. The interface will query the drivers (DC, custom and Micromanager) to update the device value.

Example: it is important to update the values because the stage can be moved manually with the joystick.

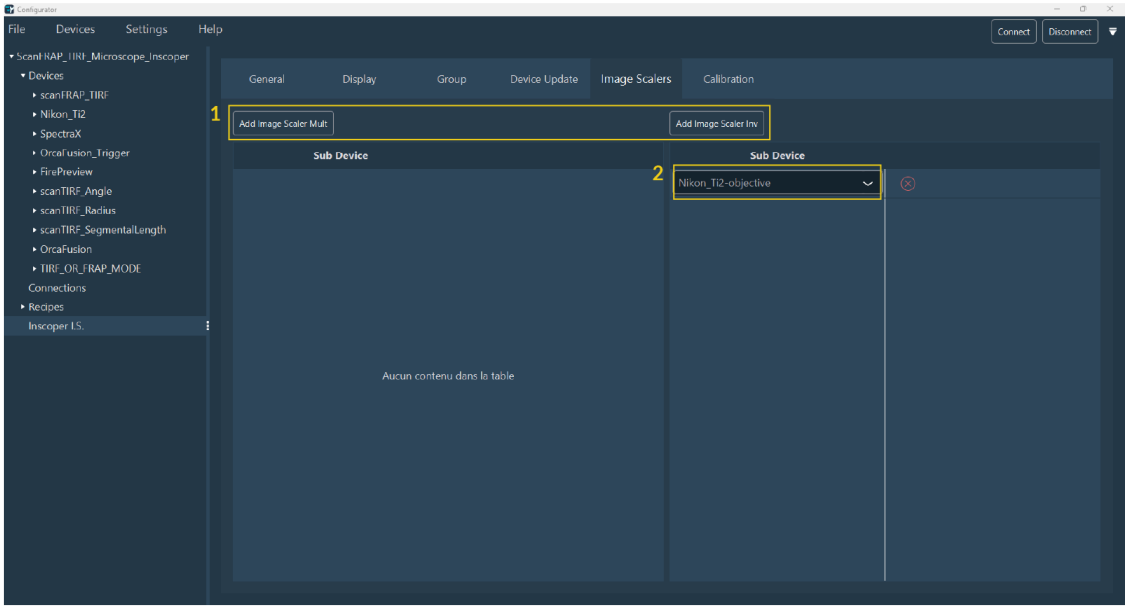


1. Click on **Add Device to Update**.
2. Use the search form to select the devices you need.
3. Indicate the delay of the update.
4. Repeat these steps if needed.
   If necessary, you can delete the device by clicking on **Delete** (red cross).

2.1.7.5. Image Scalers

All devices that can change the pixel size of the image should be specified in this tab. Example: objective.

This is very important for tile calibration and experiment, scale bar, and metadata.

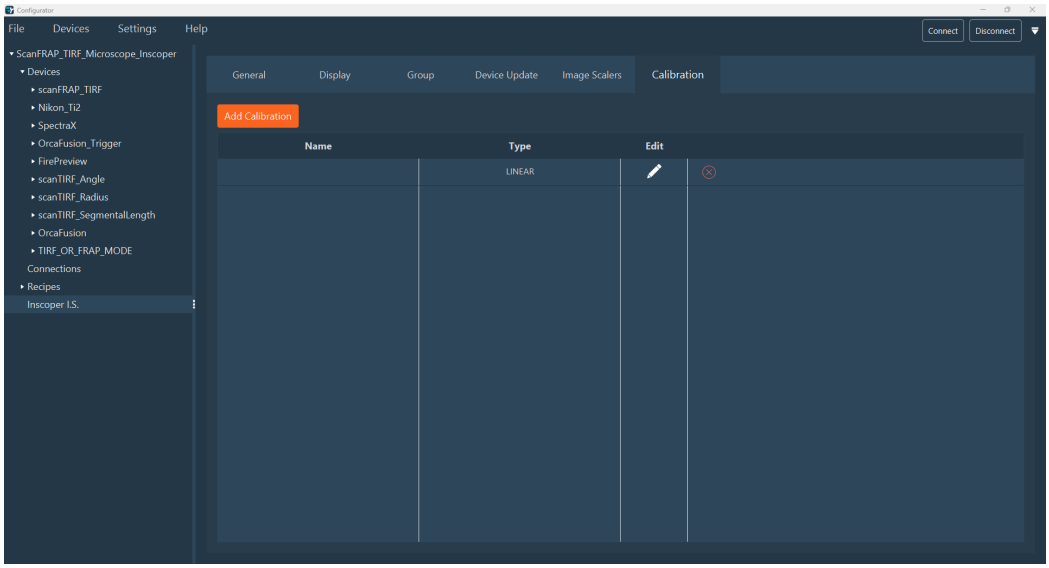


1. Click **Add Image Scaler Mult** or **Add Image Scaler Inv** to specify the Sub Device that enlarges or reduces the image size respectively.
2. Select the Sub Device of interest from the drop-down menu.
3. Repeat these steps if needed.
   Click on **Delete** (red cross) to delete the Sub Device.

2.1.7.6. Calibration

Calibration means putting a dependency link between 2 sub devices. Allows to set up different calibrations for your application.

1. Click on **Add Calibration** to create a new one.
2. Once you add it, you can edit it by clicking on the **pen**. You can delete it by clicking on the red cross.



3. In the Calibration Edit window, you can:

    a. Indicate the **name** of your calibration.

    b. Select the **type** of calibration (which formula you want to use to move the device). You can find several type like :

        # **Linear:** Linear formula (ax+b);
        # **Linear delta:**
        # **Bilinear:** representing a 3D plane (ax+by+c);
        # **Linear piece wise:** allows a curve approximation;
        # **Constant:** applying a fixed parameter between 2 devices;
        # **Formula:** you can enter a formula that you need

    c. **Auto Update:** If you check this box, you will get a button in your interface to deactivate the calibration. If this box is checked, you will have to specify the name of the button and its location (Category).

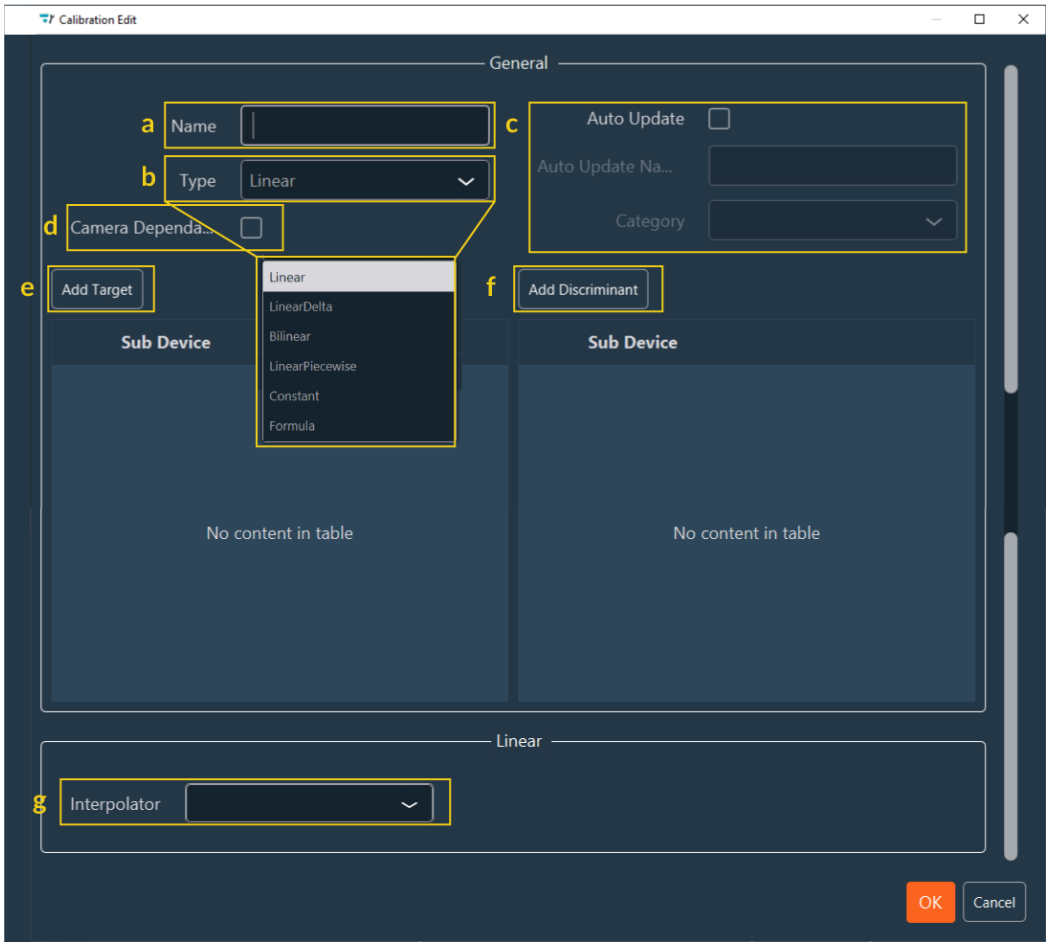
    d. **Camera Dependant:** Check this box if your calibration depends on the camera (example: Tiling and FRAP).

    e. **Add Target:** Add the target device, i.e. the device to be modified.

    f. **Add discriminant:** The discriminant is a Sub Device or device if you modify one of these Sub Devices, you must make the calibration again. For example, for FRAP calibration, if you change your objective or filter, you need to do another calibration.

NB: The discriminants will be filters and objectives.

g. **Interpolator:** select the Sub Device that is not the objective. For example, with the bilinear formula, you will have 2 interpolators.



4. Click **OK** to save the calibration.

2.2. Glossary

2.2.1. References

2.2.2. Devices

A device refers to a peripheral that can be either physical (hardware) or virtual. Each device is controlled by a driver and includes a sub-device responsible for its initialization (Init) and shutdown (Finish). A device may contain multiple sub-devices that perform various functions or actions. These

can be either mechanical (e.g., moving a motor) or informational (e.g., retrieving values or version data). A hardware device can be connected either to the Inscoper Device Controller (DC) or directly to the PC (e.g., a USB camera).

2.2.2.1. Device Description

The device description is a structure summarizing all known information about a device. It is stored either in the firmware or within the driver, if applicable. The information includes:

Field	Description
Driver	The type of driver used to control the device (see Driver Type).
Brand	The hardware manufacturer of the device. Example: Nikon for a Nikon microscope.
Model	The model(s) compatible with this device description. Example: Ti2 for a Nikon Ti2 microscope. Example: All models for Hamamatsu cameras that can be operated using the same driver.
Description	A short text describing the driver.
Author	The author of the device description.
Changelog	A manually maintained log of changes (no specific content convention).
Release Date	The date and time when the driver version was tagged.
Version	The version number of the driver.

2.2.2.2. Detect

The Detect function scans all hardware connected to the computer that are compatible with the selected driver and retrieves their serial numbers. It allows the user to select the serial number corresponding to the device being added, ensuring the correct module is identified. This is particularly critical when multiple devices using the same driver (e.g., two Hamamatsu cameras) are present on the system. The Detect function is available only for custom drivers .

2.2.2.3. Diagnostic

The Diagnostic function performs two main tasks:

1. It verifies that the device is properly connected to the device controller.
2. It retrieves all sub-devices associated with the device, indicating whether each is available or unavailable.

Each sub-device can have one of three possible statuses:

Status	Meaning
Available	The sub-device is detected and operational.
Unavail-able	The sub-device is detected but not functional.
Unknown	The system cannot automatically determine the presence or absence of the sub-device.

For custom drivers and Micro-Manager drivers, the function returns the list of device properties (equivalent to the list of available sub-devices).

For all drivers, the Diagnostic function updates parameter constraints such as minimum, maximum, and allowed values (list).

2.2.2.4. Camera

A Camera is a specialized device responsible for acquiring image data. In the Inscoper API, cameras are treated as high-level devices that manage image buffers, acquisition parameters, and triggering modes.

Trigger Device

A Trigger Device is used to provide precise control of a camera through triggering (TRIG) with the device controller. When installing a camera, two devices may be required:

1. A Camera Device, which retrieves all camera functions and properties.
2. A Trigger Device, which enables fine control and synchronization of the camera through hardware triggering by the DC.

If a camera is connected only via USB to the computer and not through the DC, only the camera device needs to be created, as trigger control is not handled by the Inscoper hardware in this case.

Camera Stream

A Camera Stream defines the method used to trigger image acquisition and control exposure timing. It determines whether the camera operates autonomously, responds to software commands, or synchronizes with external hardware signals.

Stream Mode	Description
Internal	Used for Live or Snap operations (but not triggered Live). Also used for acquisitions not requiring specific hardware driving.
Soft-ware	The camera is triggered by a software command; exposure time is handled by the computer.
Exter-nal Edge	The camera is triggered by an external TTL edge; exposure time is internal to the camera. Exposure cannot vary within a single sequence.
Exter-nal Level	The camera is triggered by an external TTL level; exposure lasts as long as the trigger is asserted. Allows multiple different exposure times within one sequence.

Start Acquisition

A function that sets the camera to image acquisition mode. Unlike "Snap," which captures a single image, this starts continuous image acquisition (Live). This is useful when a continuous stream of images is required. Note that images may not be acquired immediately, depending on the camera's trigger mode (Internal, Software, Edge, Level).

Stop Acquisition

A function that stops the current acquisition mode of the camera.

Snap

A function to capture a single image.

NB: For specific cameras like Maico or PcoFlim, a "Snap" may acquire multiple images (e.g., different selected wavelengths for Maico, or different phases for PcoFlim).

Camera Stream Definitions

Software Trigger

The sub-device used for image capture in software stream mode. The image capture signal is sent via software rather than an electronic signal.

Edge Trigger

The sub-device used for image capture in external edge stream mode. A short electronic signal is sent to start image capture.

Level Trigger

The sub-device used for image capture in external level stream mode. An electronic signal with a duration equal to the exposure time is sent to start image capture and control the exposure duration.

Check Ready

A sub-device that waits for the camera to signal it is ready to take the next picture. It waits for an electronic signal from the camera (often called "Trigger Ready") or uses a timer delay if the camera does not provide such a signal. This ensures the system waits until the camera is ready before sending the next trigger signal.

Is Controlled

A function used when the camera's external triggers are controlled by a device other than the Inscoper Controller (DC).

NB: Confocal.nl hardware.

2.2.2.5. Select For IIS

Automatically selects the subset of sub-devices/functions that are useful for controlling the camera with the Inscoper software (IIS). This is available only for custom drivers.

2.2.2.6. Shutter

A motorized element in the microscope or an external component that allows the light source beam to pass through or be blocked.

NB: The specific values (0/1, True/False) depend on the implementation.

Update Only When Imaging

When enabled for a shutter, the "Open" status is sent only while the camera is actively acquiring images, even if the shutter is set to "On" in the software. Otherwise, it remains closed to preserve the sample.

2.2.3. Device Controller

2.2.3.1. Firmware

Software operating within the Device Controller (DC) that enables the execution of sequences sent by the IIS. While the firmware offers many functionalities (providing device descriptions, restarting the box, etc.), its primary role is to execute pre-received sequences.

2.2.4. Drivers

2.2.4.1. External Resources

Path to the directory on the computer where Micro-Manager and custom drivers (DLL files) are stored.

2.2.4.2. Drivers Type

Custom

Custom driver

Drivers that have been integrated directly via their SDK and are controlled on the computer. This primarily applies to cameras.

µManager

Drivers for controlling devices using Micro-Manager.

Module Name

"Module Name" and "Device Name" are concepts specific to Micro-Manager.

Inscoper Box

Drivers that operate inside the Device Controller (DC).

Hardware ID

A unique identifier for a given hardware model.

2.2.5. Sub Devices

A sub-device is a functional component within a device that represents a controllable property, capability, or action. Sub-devices are the fundamental building blocks that allow the Inscoper system to interact with hardware at a granular level.

Core Concept:

While a device represents an entire piece of hardware (e.g., a motorized stage, a camera, or a filter wheel), a sub-device represents a specific aspect or function of that hardware. This modular approach allows complex devices to be controlled through simple, well-defined interfaces.

Categories of Sub-Devices:

1. Property Sub-Devices (State/Value Access)

Represent readable or writable properties of the hardware:

- Camera exposure time
- Stage X, Y, or Z position
- Filter wheel position
- Shutter open/closed state
- Objective turret position
- Device firmware version number

2. Action Sub-Devices (Commands/Operations)

Represent operations or commands that don't necessarily correspond to a single property:

- `MoveXY` - Triggers coordinated movement of both X and Y axes
- `StartAcquisition` - Initiates camera image capture
- `StopAcquisition` - Halts ongoing acquisition
- `Initialize` - Performs device initialization sequence

3. Virtual Sub-Devices (Software-Defined)

Exist purely in software without direct hardware correspondence:

- Calculated values derived from multiple hardware readings
- Software-managed state flags

- Version information and metadata
- Diagnostic status indicators

Examples:

A motorized XY stage device might contain these sub-devices:

- `XPosition` (property) - Current X-axis position in micrometers
- `YPosition` (property) - Current Y-axis position in micrometers
- `XResolution` (property) - Step size for X-axis movement
- `YResolution` (property) - Step size for Y-axis movement
- `MoveXY` (action) - Command to execute coordinated movement
- `Version` (virtual) - Firmware version string

A camera device might contain:

- `Exposure` (property) - Exposure time in milliseconds
- `Binning` (property) - Pixel binning factor
- `ROI` (property) - Region of interest coordinates
- `StartAcquisition` (action) - Begin capturing images
- `StopAcquisition` (action) - End image capture
- `Snap` (action) - Capture a single image

2.2.5.1. Properties

2.2.5.2. Status

Indicates whether a sub-device is present or not for a device.

Status	Meaning
Available	The sub-device is detected and operational.
Unavail-able	The sub-device is detected but not functional.
Unknown	The system cannot automatically determine the presence or absence of the sub-device (e.g., after a diagnostic check failed to verify status).
Partially Available	Only some functions of the sub-device are available.

2.2.5.3. Param

- Property Name: The name of a configurable parameter for a sub-device (e.g., "Exposure" for a camera's exposure time). This is valid only for external drivers (Custom and Micro-Manager). It is always FIXED for IIS, though not necessarily for API use.
- Property Value: The value assigned to a property (e.g., "200ms"). Valid only for external drivers. The default value is set when IIS starts but can be modified by the user.
- Example: To start a camera in "Fast Mode" upon IIS launch, set Property Name = "Read Mode" and Property Value = "3".
- Default: The default value of the parameter.
- Fixed: If checked, the sub-device's value cannot be modified.
- Example: For a 3-axis controller, three sub-devices are created. The axis number (name) is marked as Fixed for each, while the position value remains editable.
- Note: There should never be two unfixed parameters in one sub-device.
- Min: The minimum allowed value. Defined by the device creator (based on the datasheet) or provided by the driver/Micro-Manager.
- Max: The maximum allowed value. Defined by the device creator (based on the datasheet) or provided by the driver/Micro-Manager.
- Step: Minimum increment for value update.
- The type of constraint can define the step; e.g. for integer constraint type, the default step is 1 (the step is induced by value type). If no step is provided by the firmware/drivers, IIS takes either step = 1 (if integer) or step = (max - min) / 1000 (for float).
- Type: (parameter type, among:), unsigned char, double, STRING

Description

Name

Name of the sub-device.

Type

Flags/tags to characterise the nature of a subdevice.

Lifecycle / System

Type	Description
NONE	No specific category or behavior associated with this sub-device.
INIT	Sub-device that triggers the device initialization sequence.
FINISH	Sub-device that triggers the device shutdown sequence.
DIAG	Sub-device that executes the diagnostic (DIAG) procedure.
USB_CHECK	Verifies that the USB device is connected and responsive.

Motion & Axis Control

Type	Description
X_AXIS	Represents movement or positioning along the X axis.
Y_AXIS	Represents movement or positioning along the Y axis.
MOVE_-AXIS	Generic movement command for an axis (X, Y, Z, or other).
FOCUS	Controls the focus mechanism (typically Z axis).

Optical / Hardware Components

Type	Description
SHUTTER	Controls opening and closing of a shutter.
OBJECTIVE	Selects or controls a physical objective lens.
CUBE	Controls filter cubes or optical paths.
FILTER_WHEEL	Selects filter positions using a rotating wheel.
MAGNIFI-CATION	Handles magnification settings (e.g., objective changer, zoom optics).
PROPERTY	Generic hardware property used for reading/writing vendor-specific parameters.

Triggering & Synchronization

Type	Description
TRIG_EDGE	Trigger based on a digital edge (rising/falling edge).
TRIG_LEVEL	Trigger based on a digital level (high/low).

Camera Operations

Type	Description
CAM_CHECK_READY	Checks whether the camera is initialized and ready for acquisition.
CAM_START_-CONTINUOUS	Starts continuous acquisition mode (Live).
CAM_START	Starts a single-sequence acquisition.
CAM_STOP	Stops acquisition (Live or sequence).
CAM_SNAP	Camera snap sub-device: performs a single image capture.
CAM_SOFTWARE_TRIG	Software-triggered image acquisition sub-device.

2.2.5.4. Functions (Set/Get/Check)

Functions

Func-tion	Description
Set	Assigns a new value to the device (e.g., moves an objective lens, opens a shutter).Example: Sending 0 or 1 to a shutter sub-device to close or open it.
Get	Retrieves the current value from the device.
Check	Verifies whether a SET function completed successfully by repeatedly calling GET until the retrieved value matches the expected target.Example: Waiting until a stage's X-axis position matches the requested position.

For each function, there are:

- Input Params: Parameters sent to the function.
- Output Params: Parameters returned by the function.

Connections

A Connection defines the physical or logical communication channel used to control a device and exchange commands with it. Connections establish the link between a device and the Inscoper Device Controller (DC), enabling the system to send control signals and receive status information.

Key Concepts:

- Each device is configured with one or more connections that specify how it communicates with the DC
- Sub-device functions (SET, GET, CHECK) are executed through these defined connections

- The connection type determines the protocol, signal characteristics, and data flow direction
- Different connection types support different capabilities (digital signals, serial communication, analog values, etc.)

NB: The "Connection" configuration field for sub-devices is only available when using INSCOPER_BOX drivers. Custom drivers and Micro-Manager drivers handle connections differently through their respective APIs. Connection Types:

Type	Signal Type	Direction	Description
TTL_IN_GEN	Digital (0-5V)	Input	Digital signal read by the Device Controller from external hardware. Used for reading trigger signals, limit switches, or sensor states.
TTL_OUT_GEN	Digital (0-5V)	Output	Digital signal sent by the Device Controller to external hardware. Used for triggering cameras, controlling shutters, or activating relays.
SERIAL	Serial Data	Bidirectional	RS232 or USB serial connection for command-based communication. Supports both sending commands and receiving responses.
USB_GENERIC	USB Protocol	Bidirectional	Standard USB connection requiring manufacturer-specific drivers. Used for devices with proprietary USB protocols.
USB_SERIAL	USB Serial	Bidirectional	USB connection emulating a serial port (Virtual COM Port). Combines USB convenience with serial protocol compatibility.
USB_HID	USB HID	Bidirectional	USB Human Interface Device protocol. Used for devices that present themselves as HID-class peripherals.
AIN	Analog	Input	Analog voltage input to the Device Controller. Used for reading sensor values, potentiometer positions, or other analog signals.
AOUT	Analog	Output	Analog voltage output from the Device Controller. Used for controlling variable-intensity light sources, motor speeds, or other analog-controlled devices.
UART	Serial Data	Bidirectional	Universal Asynchronous Receiver-Transmitter (RS232) connection. Low-level serial communication protocol.

Usage Example:

A motorized filter wheel might use:

- SERIAL connection for sending position commands and receiving status
- TTL_IN_GEN connection for reading a home position sensor
- TTL_OUT_GEN connection for triggering movement completion signals

2.2.6. Inscoper Imaging Software

This tab allows you to set up Inscoper Imaging Software (IIS). You can:

- Define and modify graphical elements.
- Activate modules such as tiling options or virtual joysticks.
- Configure advanced calibrations linking sub-devices.
- Set up the graphical representation of sub-devices in IIS (e.g., relabeling objectives, adding side ports/filter cubes, representing laser power with sliders).

This section is divided into: Display, Group, Devices Update, Image Scaler, and Calibration.

2.2.6.1. Display

The Display tab allows you to add, modify, and organize the graphical representations of sub-devices in IIS.

2.2.6.2. Initialize

The Initialize button adds all available sub-devices defined in the configuration to the device display list. This allows you to modify how a function is displayed in the software. Note: Upon configuration creation, the display data list is empty and must be initialized.

2.2.6.3. Update

If new devices are added to an existing configuration, the Update button automatically adds their associated sub-devices to the device display list.

2.2.6.4. Image Scaler

A sub-device that enlarges or reduces the image size.

- Image Scaler Mult: A sub-device that enlarges the image size (e.g., lenses or objectives that produce image reduction).
- Inv: A sub-device that reduces the image size (e.g., lenses or objectives that produce image magnification).

2.2.6.5. Calibration

A model used to link two or more sub-devices.

Type

Type	Description
Linear	`sd1 = a * sd2` or `sd1 = a * sd2 + b` (Linear formula).
Linear Delta	Same as Linear, but presented differently to expose the alpha parameter in IIS (used only for SoSPIM).
Bilinear	Represents a 3D plane (`ax + by + c`).
Linear Piecewise	Allows for curve approximation.
Constant	Applies a fixed parameter between two devices.
Formula	Allows entry of a custom formula.

Interpolator

Interpolators are the sub-devices used in formulas to compute new values (the sub-device that is not the objective). Example: In $sd1 = a * sd2 + b$:

- `sd1` is the target.
- `sd2` is the interpolator.
- `a` and `b` are computed during calibration.

Discriminant

A discriminant is a sub-device that, if modified, invalidates the current calibration. Each calibration is saved for a specific combination of discriminant values. Example: In a FRAP calibration, changing the objective or filter requires a new calibration.

Target

The sub-device that will be modified by the calibration. It is linked to an interpolator.

2.2.6.6. Complex Acquisition Available

Enables the "Complex Acquisition" / "Acquisition Designer" module if the customer has purchased the option. This module allows for advanced imaging workflows not possible with basic MDA. The module will not appear if the license does not include it, regardless of this checkbox.

2.2.6.7. Mosaic Manip

Activates Tiling options in IIS. The Tiling Dimension will appear in the graphical interface. A 5D license is required.

2.2.6.8. XY Move Strat Available

Adds an option to move the stage gradually during large movements. This reduces potential drift and prevents autofocus systems from losing track during motion.

2.2.6.9. Trigger Live Conditions

Associates a sub-device value with a condition, allowing other devices to be controlled during the camera's live trigger sequence. Example: Trigger live acquisition only if the fluorescence shutter is open, or apply a condition based on the side-port position.

2.2.6.10. Reset USB at Startup

If checked, USB connections are reset each time IIS is opened.

2.2.6.11. Device Update

This tab allows you to select devices to be updated automatically. The interface will query the drivers (DC, Custom, Micro-Manager) to update the device value by calling the GET function at the interval specified in the "Delay (ms)" column.

2.2.6.12. Connections

Link your device to the Device Controller. Available connections for a selected device are highlighted in green.

USB View

- Serial/Digital/Analog
- PID/VID: Product ID and Vendor ID. Any USB device provides these values, along with Manufacturer, Product, and Serial Number. They are used to identify and distinguish USB peripherals.

2.2.7. Recipe

A structured set of instructions defining system operation, including action order, execution conditions, and sequence optimization. It defines how statuses are applied.

2.2.7.1. Recipe Element

A single unit within a recipe that associates a sub-device with one or more actions.

Action

Defines which sub-device function to execute or which recipe ID to set.

- Sub-device / Recipe Id: The target sub-device or group.
- Function: The operation to perform (SET, GET, CHECK).
- Default value: Relevant only for SET. Usually left empty for user input, but can be set for specific recipes (e.g., "Close shutter" = 0).

Type

- Simple: Contains only one element.
- Group: Calls a combination of recipes.
- Event: A Recipe Element that allows for sequence interruptions. It handles user events such as Pause, Stop, or Restart during a sequence.

Call ID

An identifier used in `callConditions` to determine if a recipe has been executed.

Example (Motion Stages): `Recipe Set X -> callID = setX Recipe Set Y -> callID = setY Recipe MoveAxis -> callCondition = setX || setY`

Example (Shared Call ID): `Recipe Set X -> callID = axisMoved Recipe Set Y -> callID = axisMoved Recipe MoveAxis -> callCondition = axisMoved`

Recipe ID

An identifier assigned to one or more sub-devices. Historically used to group devices (e.g., all shutters), it is now mostly used for sub-devices created dynamically when IIS launches (e.g., timing devices, abstract sub-devices for scripting).

Tag

A label or identifier used to categorize and filter recipe execution based on the current system state or configuration. Tags are added to statuses and used in tag conditions to ensure that only relevant recipes are executed for the active devices or modes. Example: When using a specific camera model, its tag (e.g., "Fusion" or "Panda") is added to the status, ensuring only recipes with matching tag conditions are executed.

Condition

A graphical section grouping condition options for calling recipes.

Tag Condition

Ensures an action is executed only if a specific tag is present in the status. Example: If a sequence uses the "Fusion" camera, the tag "Fusion" is added to the status. Recipes requiring the "Panda" tag will not be executed, ensuring only the correct camera devices are used.

Call Condition

Ensures an action is executed only if a specific previous action has occurred.

Value Condition

Determines whether an action is executed based on a sub-device's value. Example: Change light source intensity only if the shutter is open. If the shutter is closed, the intensity change is skipped.

Optimization

Prevents reapplying a recipe if the sub-device value has not changed between two statuses. Example: Do not send XY coordinates again if they are identical to the previous position.

2.2.7.2. ILDA

Controls the Ilas and Starscan modules.

Mode

Describes the different illumination modes available in the system.

Illumination Mode

Adds a virtual device to select the illumination mode (e.g., FRAP, TIRF, Spinning Disk) in a multimodal system.

FRAP

- Scanrate: Controls the time between two points (galvo instructions).
- Diameter: Controls the distance between two points during a scan.
- Density Index: An index indicating the mode (FRAP, TIRF, WF, Spinning Disk) when sharing the scan rate sub-device. Must match the FRAP diameter value in the Device tab.
- Fire Preview: Enables the fire preview function (continuous scanning of an ROI).
- Active Channel Element: Indicates which channel is the FRAP channel. Must be activated for each illumination mode.

TIRF

- Angle (formerly Tirf Angle): The position of the point (or arc) on the circle traversed by the galvos.
- Diameter (formerly Interpoint Distance): Controls the distance between two points during a scan.
- Scanrate (formerly Pulse Time): Controls the time between two points.
- Density Index: (Obsolete after 9.3).
- Radius (formerly Penetration Depth): Corresponds to the laser angle at the lens outlet.
- Angle Length (formerly Tirf Mode): The length in degrees of the circle made by the galvos.
- Active Channel Element: Indicates which channel is the TIRF channel.

2.2.8. API

The Inscoper API (Application Programming Interface) is a comprehensive C++ library that enables external software to interact with and control the Inscoper system. It acts as a bridge between high-level applications (such as the Inscoper Configurator or third-party software) and the underlying hardware drivers.

Key Architectural Concepts:

- Bridge Pattern: The API exposes a single main entry point, the `Inscoper::Bridge` class, which simplifies interaction by abstracting the complexity of internal subsystems.
- Manager-Based: Core functionalities are handled by specialized singleton managers (e.g., `ConfigManager` for settings, `DeviceManager` for hardware, `SequenceManager` for acquisition workflows).
- Device Abstraction: The API provides a unified interface (`AbstractSystem`) for all devices, regardless of whether they are controlled by Inscoper hardware, Micro-Manager, or custom drivers.

Core Capabilities:

- Configuration: Loading and saving system states via XML files.
- Control: Direct manipulation of device parameters (SET/GET/CHECK).
- Acquisition: Execution of complex, multi-dimensional acquisition sequences and recipes.
- Imaging: Management of image buffers, metadata, and pixel formats.
- Events: A listener system (`SequenceListener`, `ImageListener`, `ErrorListener`) for real-time feedback and synchronization.

2.2.8.1. Log Level

Indicates the importance of a log message. Log levels are inclusive (e.g., enabling DEBUG also enables INFO, WARN, ERROR, FATAL).

Log Level	Description
LOG_-ALL	Enables all log levels.
LOG_-TRACE	The most fine-grained information, used only for deep debugging (e.g., annotating algorithm steps or individual queries).
LOG_-DE-BUG	Less granular than TRACE but more detailed than INFO. Useful for diagnosing issues and troubleshooting in test environments.
LOG_-INFO	Standard log level indicating normal application events (e.g., "Authorization successful"). Purely informative; missing these logs should not hide critical issues.
LOG_-WARN	Indicates an unexpected situation that does not stop the application (e.g., "Parsing error, document skipped"). The process continues, but the event is noteworthy.
LOG_-ER-ROR	Indicates an issue preventing specific functionalities from working (e.g., "Payment system unavailable"). The application may still run, but features are broken.
LOG_-FATAL	Indicates a critical failure where core functionality is lost (e.g., "Database connection failed"). The application cannot perform its primary business functions.
LOG_-OFF	Disables all logging.

2.3. Inscoper API

This page is under construction.

3. TECH SPECIFICATIONS

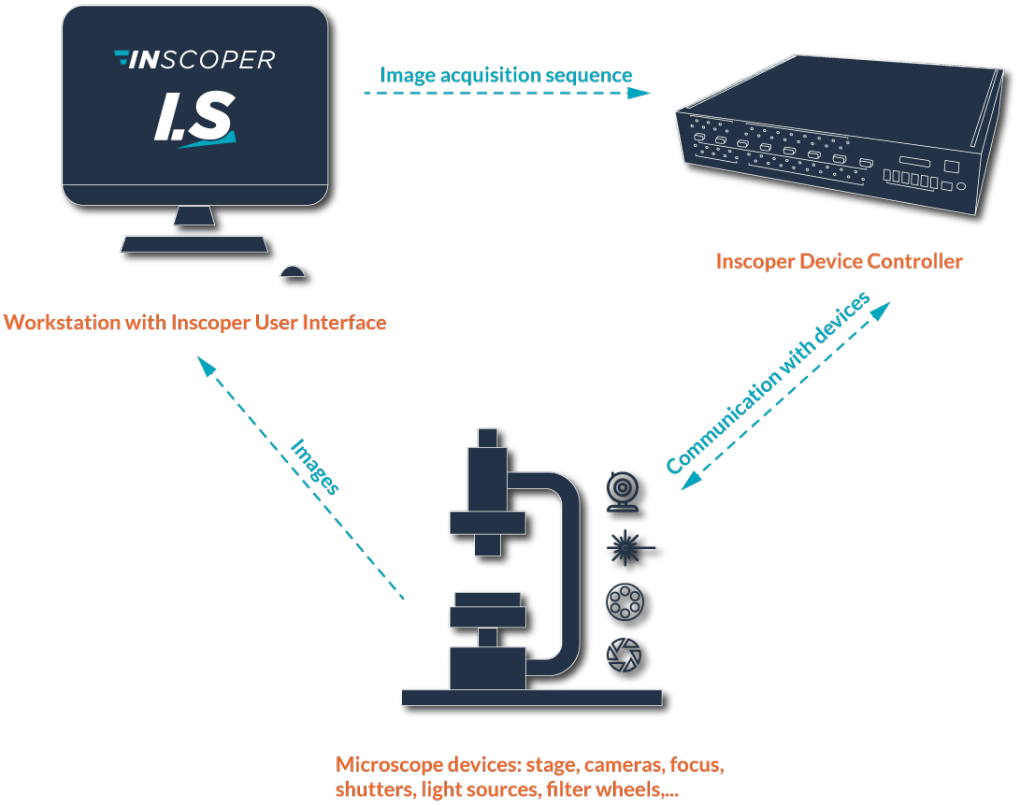
Operating specifications and parameters, input/output connexions, system requirements

3.1. 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.

3.2. Inscoper Device Controller

3.2.1. Warnings and cautions

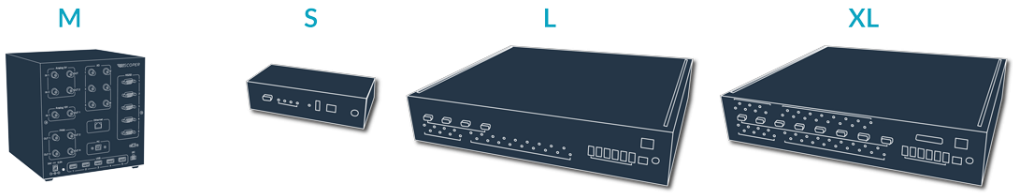
1. Always check that the Inscoper Device Controller is powered up before starting the computer. If in doubt, restart the computer.
2. The equipment can only be powered through Safety Extra Low Voltage that also complies with the limits of 6.3.1/6.3.2 of IEC 61010-1:2010.
3. Never use cables longer than 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.

3.2.2. 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.

3.2.3. 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>
<tr><td>ADC resolution 12 bits
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>

3.3. 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

3.4. 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.

4. 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.

4.1. 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.

4.2. Copyright

INSCOPER products include technology protected by the following patents:

- US Patent No. US10330911
- EP Patent No. EP3123149
- FR Patent No. FR3019324

INSCOPER will update the product periodically, and such changes will be included in future editions of this user guide.

4.3. Disclaimer

The information in this manual is provided “as is”, without any warranties, conditions, or representations of any kind, whether express, implied, statutory, or otherwise. This includes, without limitation, any implied warranties of merchantability, non-infringement, or fitness for a particular purpose.

To the fullest extent permitted by law, INSCOPER shall not be liable for any loss or for any direct, indirect, special, incidental, consequential, or other damages arising from the use of this manual or the information contained in it, whether based on contract, tort, or any other legal theory.

4.4. 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 installed by a qualified professional.

5. RELEASES

Here are the available previous and current release versions in PDF format.

Table 1.

Name	Released Version	Link to download	Comments
User Guide Imaging Software	9.3 Current version	Download the PDF file	What's new: Ergonomic • Responsiveness • Icon set & interaction • Mouse navigation & stage control Exploration • Tiling preview /scan mode / stiching • Focus Map • Shading correction • TIRF calibration • 3D rendering • Z-Stack definition • Standalone viewer Expanded modalities • LiveDRIM • Custom Scripting • Emulation mode • New drivers
User Guide Configurator	1.1.9 Current version	Download the PDF file	Glossary included
User Guide Imaging Software	Version 9.1 & 9.2	Download the PDF file	What's new: • TIRF • 3D viewer • Auto mode in Tiling • Positions: Pattern detection Path New Z/Offset