INSCOPER

# User Guide

## Inscoper I.S.

***Interface version : 9.0***

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# 1. IMAGING SOLUTIONS

From core solution to custom techniques

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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.

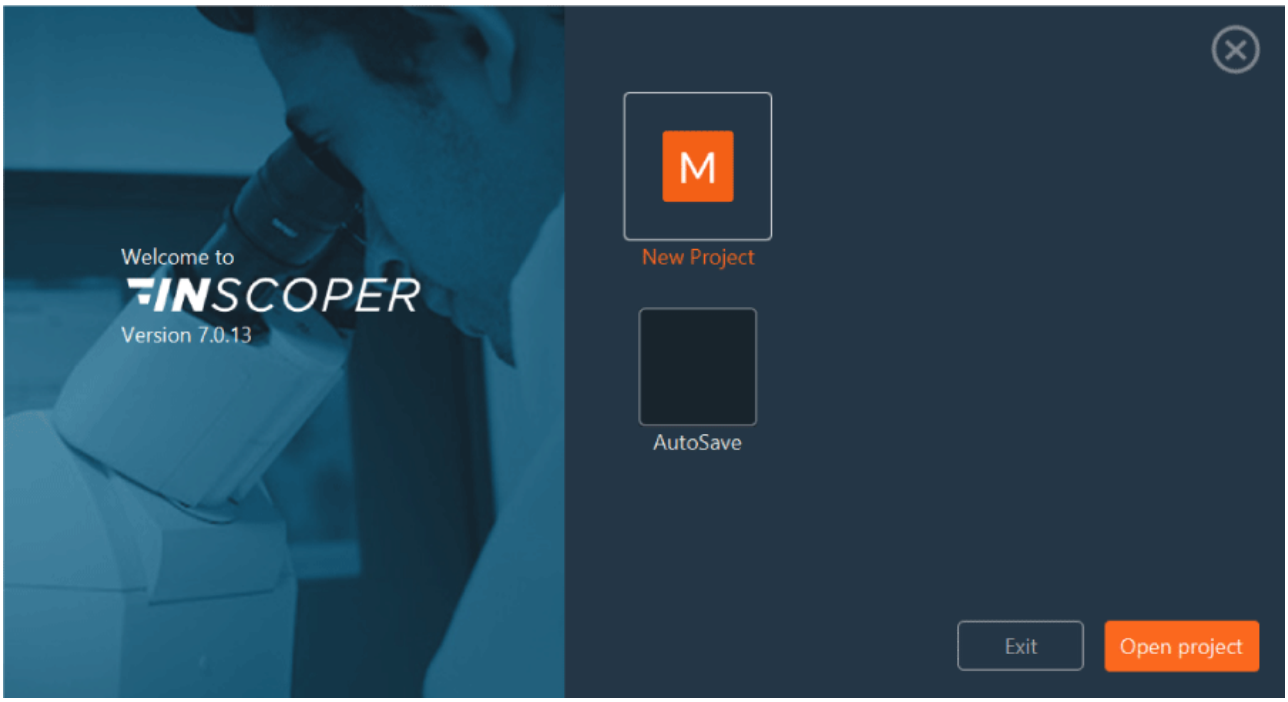
### 1.1.1. User journey

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:

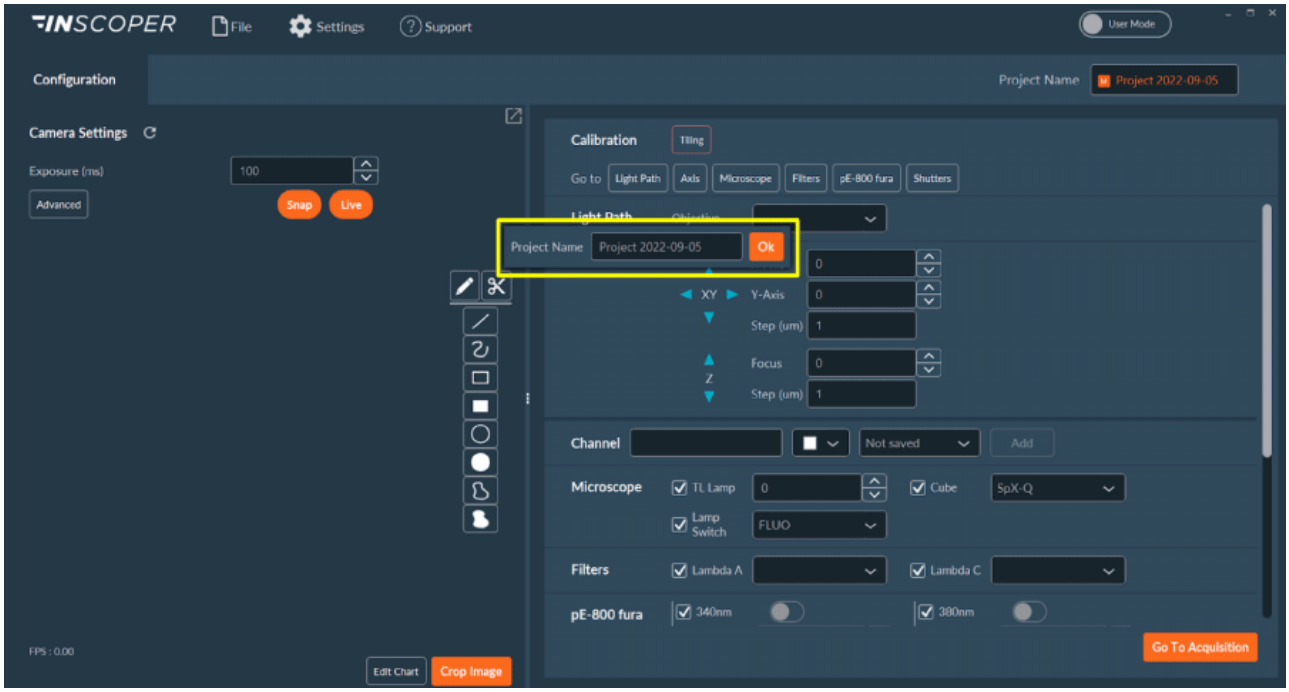
1. Configure the microscope's channels and general settings (Configuration Tab).
2. Define specific settings for the required image acquisition sequence. These settings are displayed as "dimensions" that can be combined and interlinked. Run the image acquisition sequence. Stop/Pause is possible.
3. View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

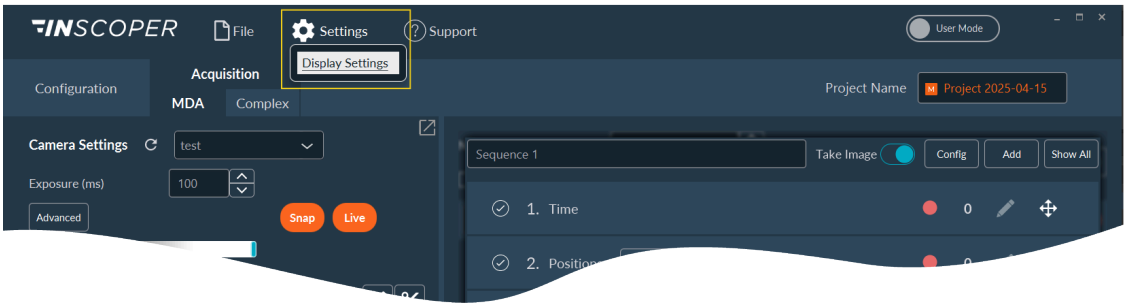
| | |
|---|---|
| **New Project** | To create a new acquisition sequence, click on **New Project**. A loading screen will appear while the interface recognizes the devices of the microscope. Wait until it disappears before starting your project. |
| **AutoSave** | The **AutoSave** button opens the most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

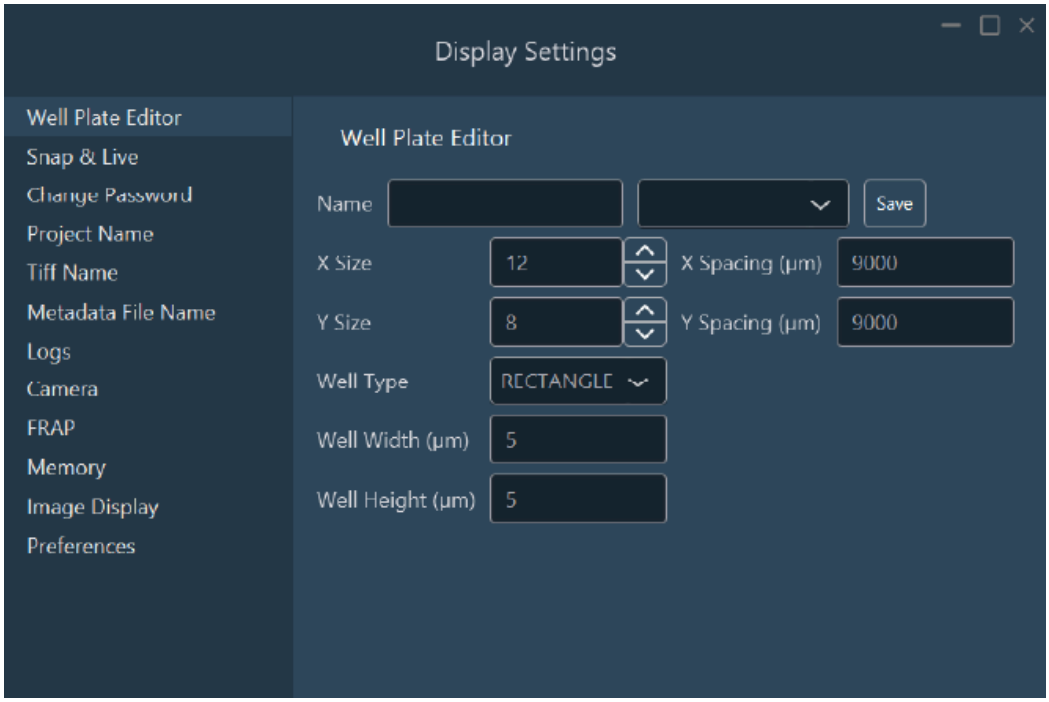
## 1.1.3. Display settings

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




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

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.

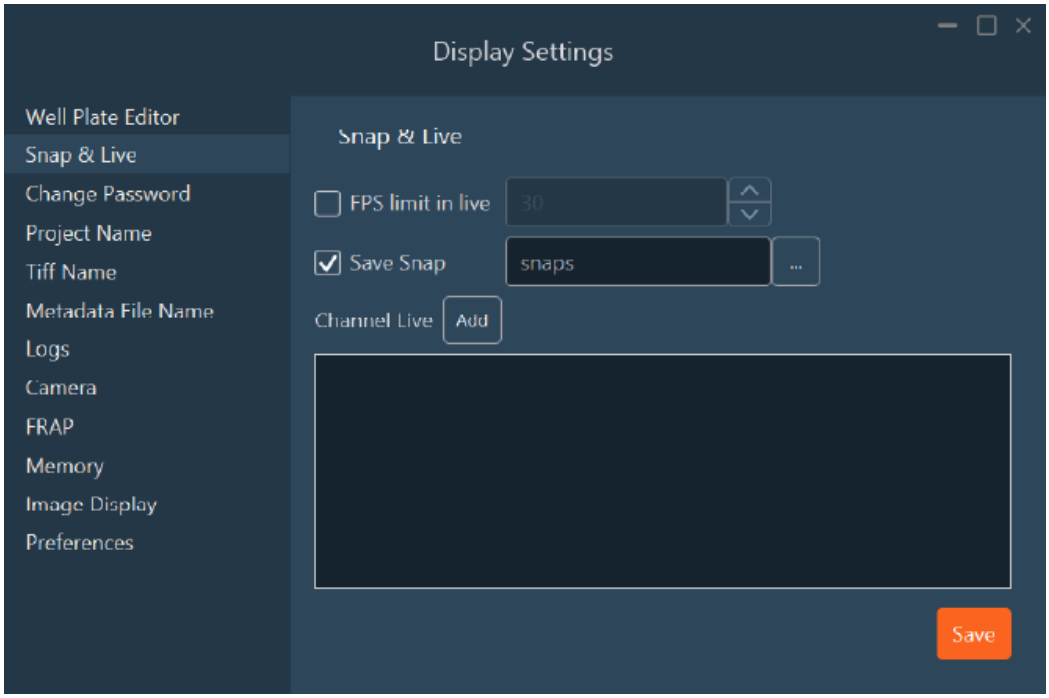
   e. Enter the well type (circle, square, rectangle, ...).

   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.

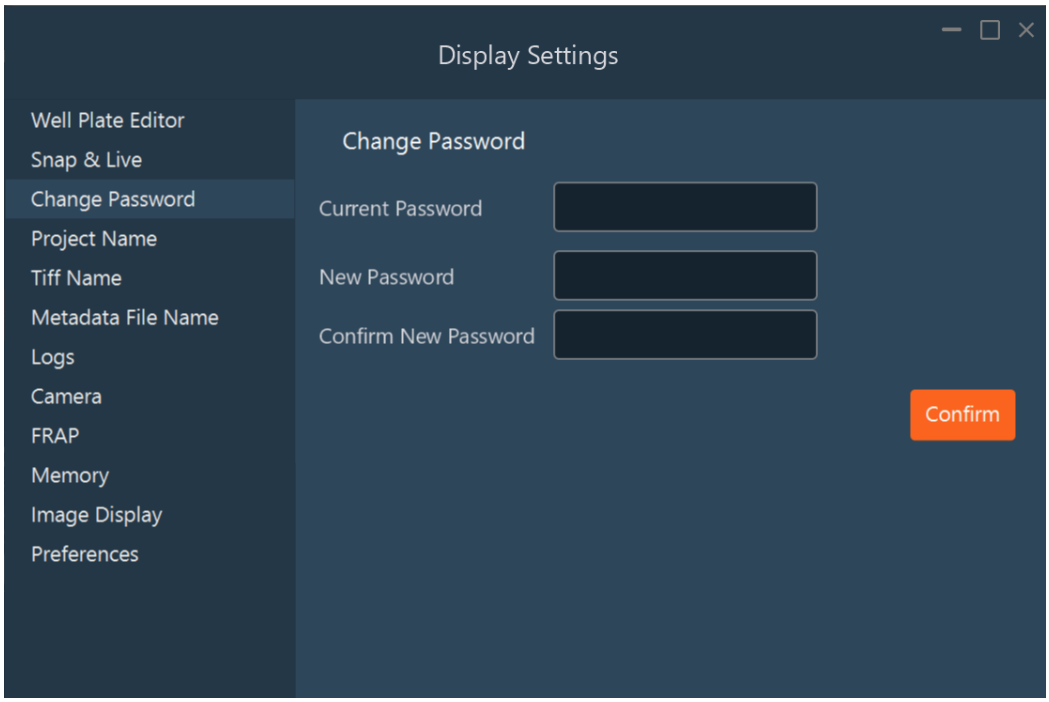
2. **Snap & Live:** Set options for the snap and live.



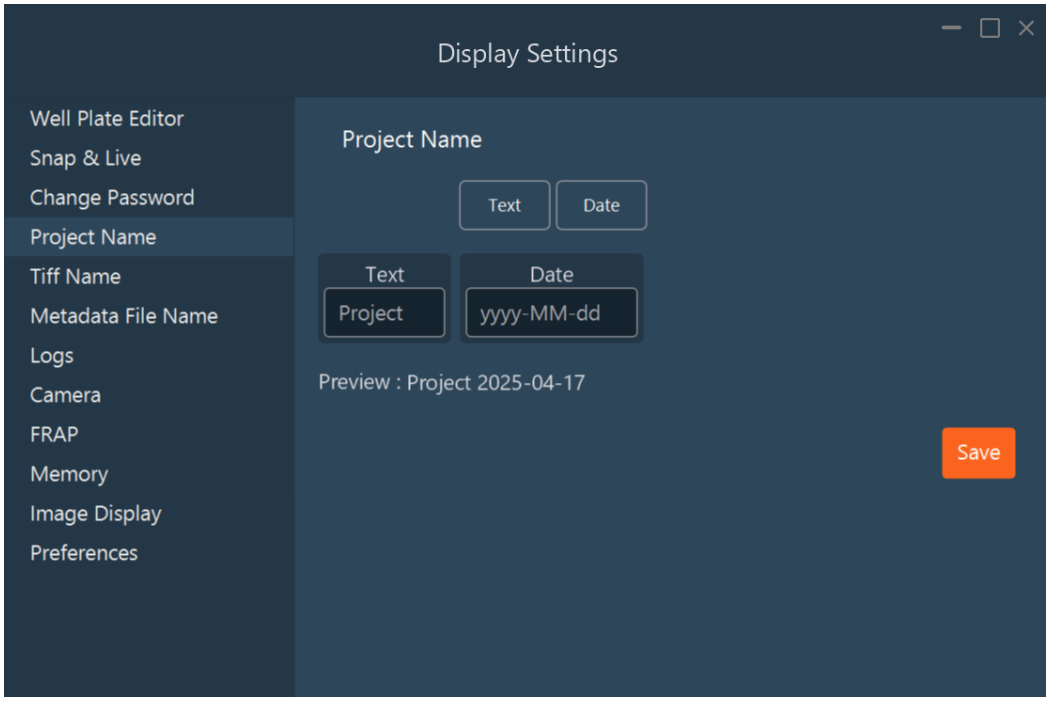

In this tab, you can:

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.

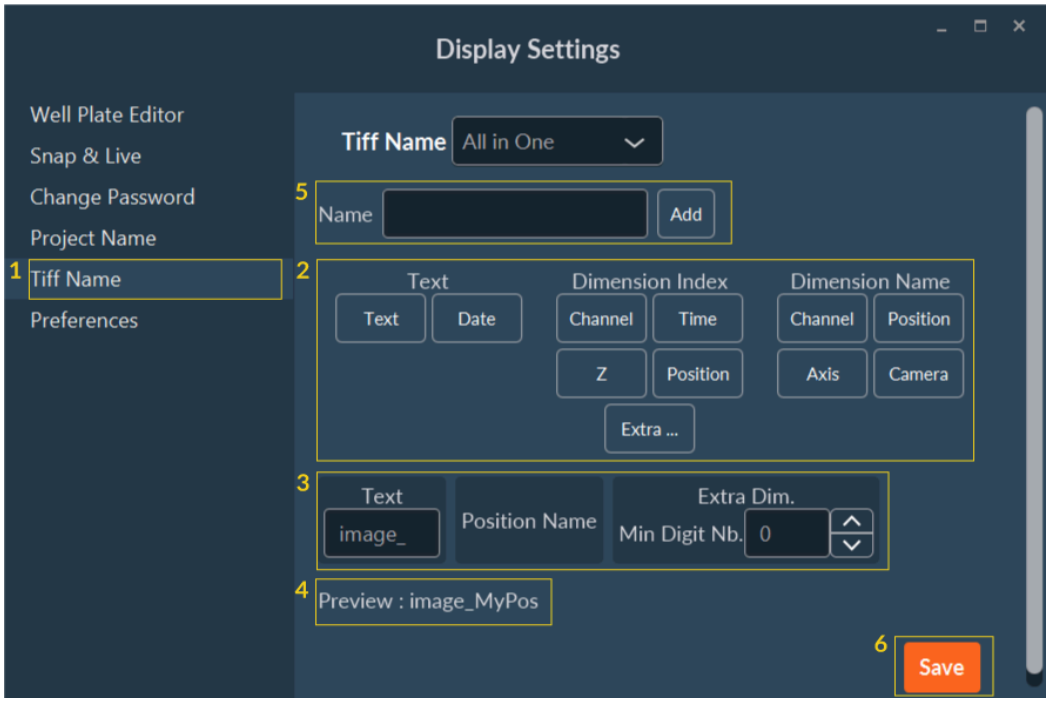
3. **Change Password**: Set or change the password to switch from user mode to expert mode.




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 to the name construction tools below. Generally the name composition is the text + dimension name + dimension

index. For example: if you have 3 positions, the name of your files will be image_pos1, image_pos2, image_pos3.

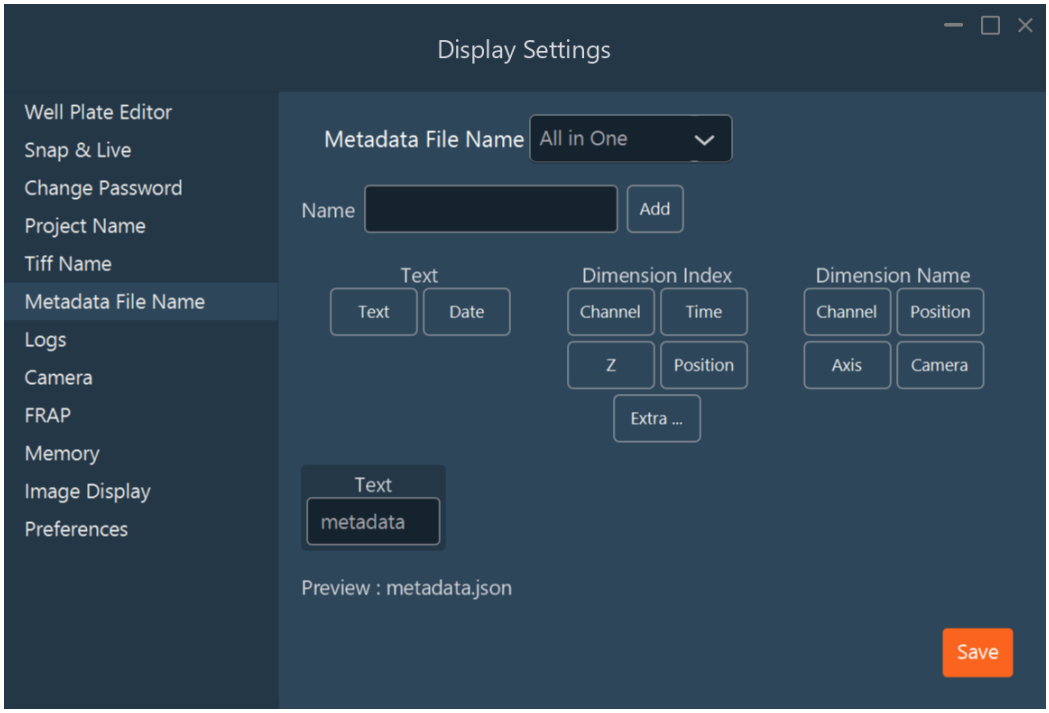
c. Name construction tool that shows schematically the name of the future saved file.

d. Name preview.

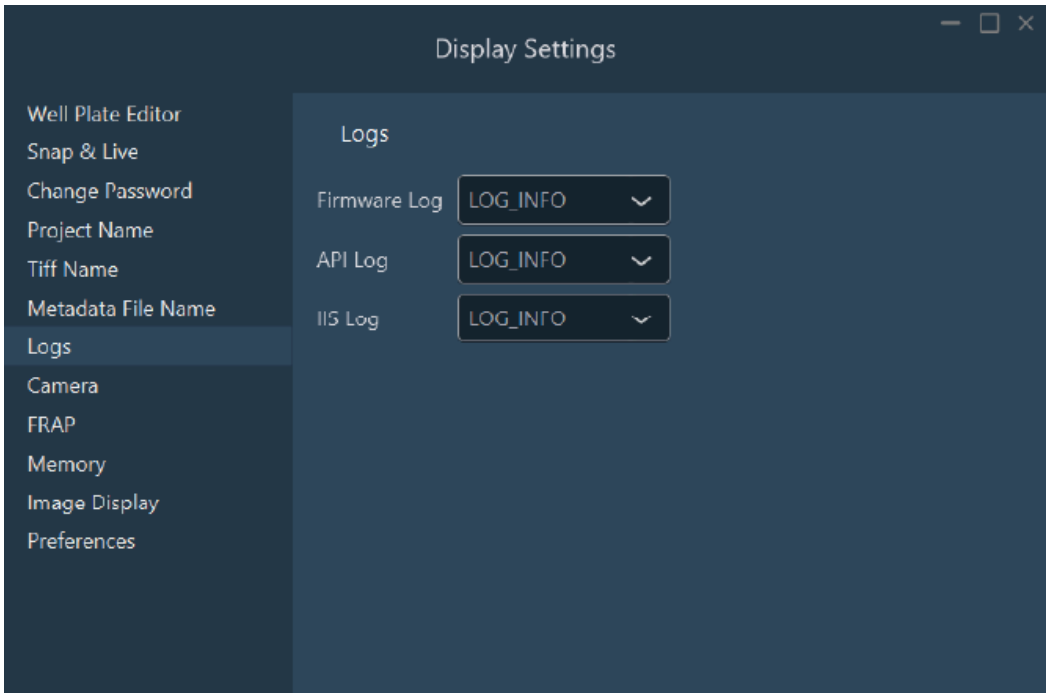
e. Enter a name and click on the **Add** to save this new default name.

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

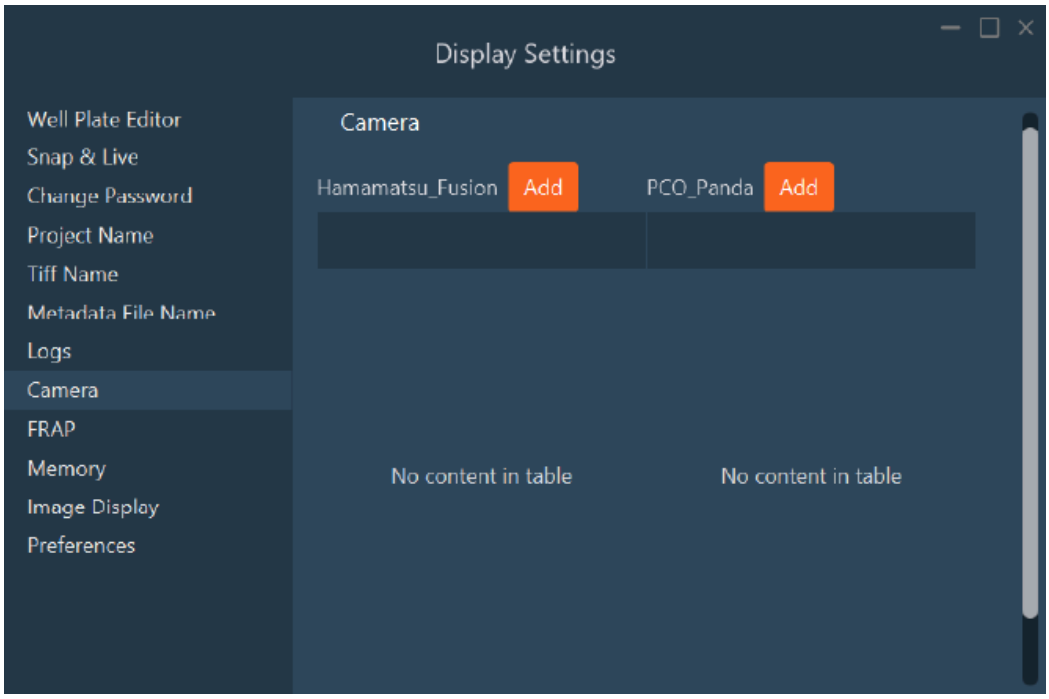
6. **Metadata File Name:** Change the format of metadata file saved.



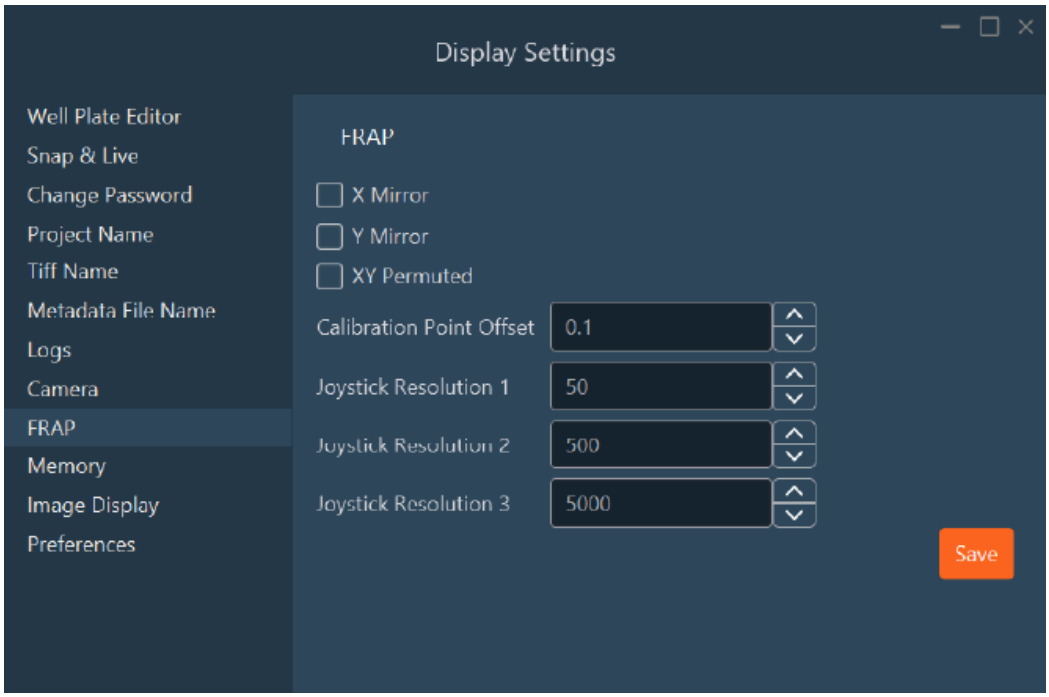

7. **Logs**: Modify logs level.



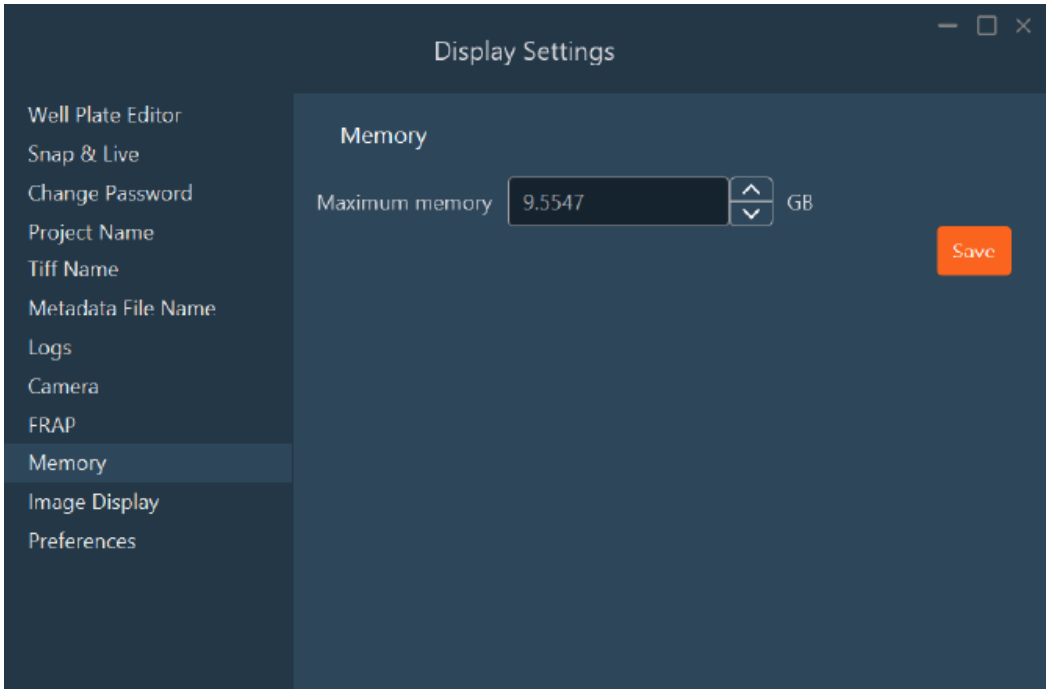
8. **Camera:** Find the camera installed on your system and add another camera by clicking on the **Add** button



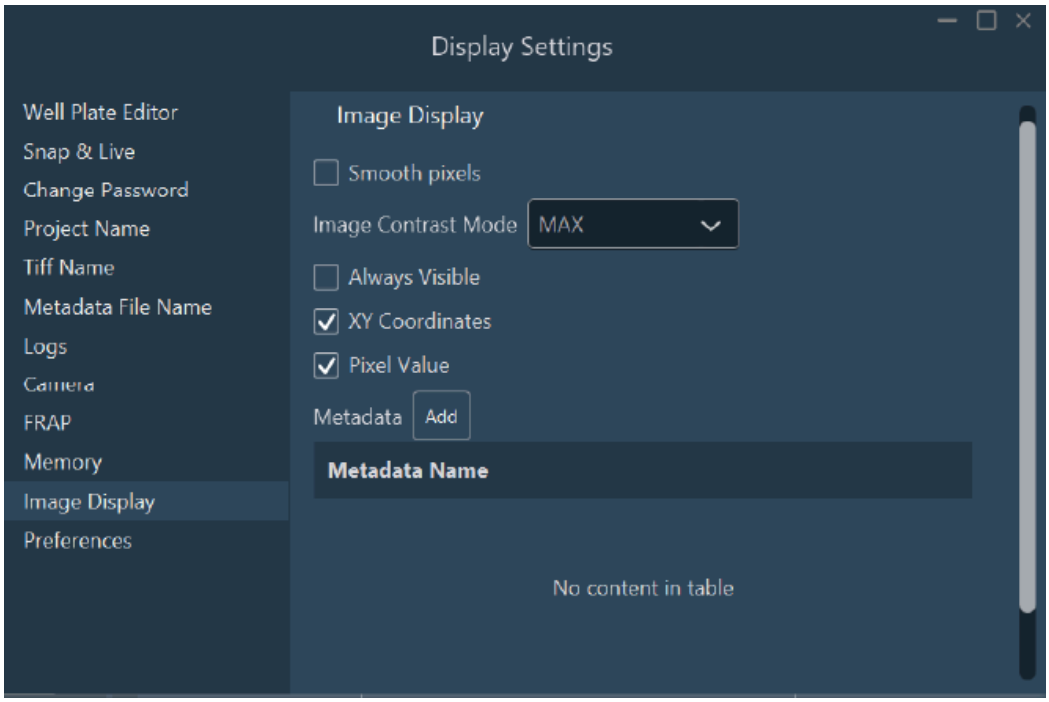

9. **FRAP:** Find the FRAP parameters and modify the sensitivity of the virtual joystick during the FRAP calibration.



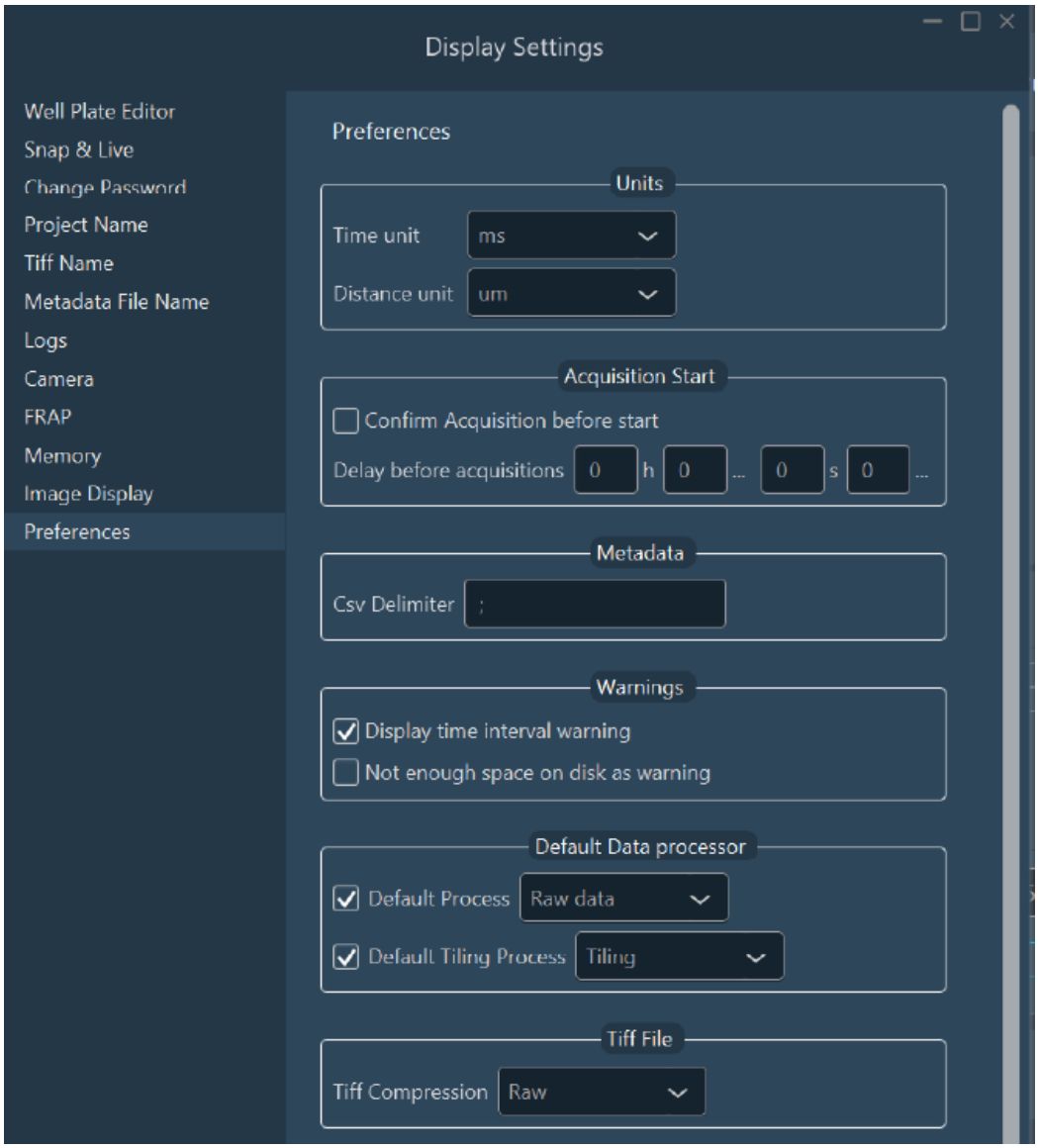

10. **Memory**: assign the memory for the Inscoper Software.




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

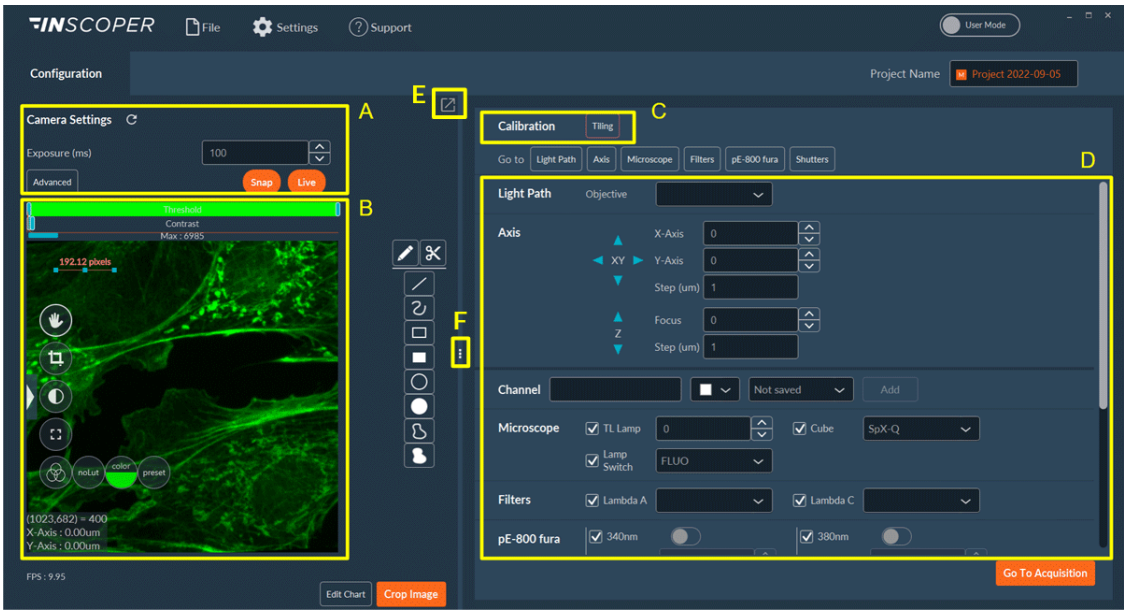


12. **Preferencies**: select and set up some other parameters (measuring units, acquisition delay, warnings, etc.).




## 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 4 parts:

- A - camera settings,
- B - live image viewing,
- C - calibration protocols,
- D - access to all the motorized devices of the microscope and the creation of channels and the saving of them for all your acquisitions.
- E - If you have two screens, you can split the interface in two by clicking on this button. This way you can display the camera view on one of the screens (the image will be much larger), while the parameters will remain on another screen.
- F - If necessary, you can move the dividing bar between sections to enlarge one or other section (camera view or settings area).

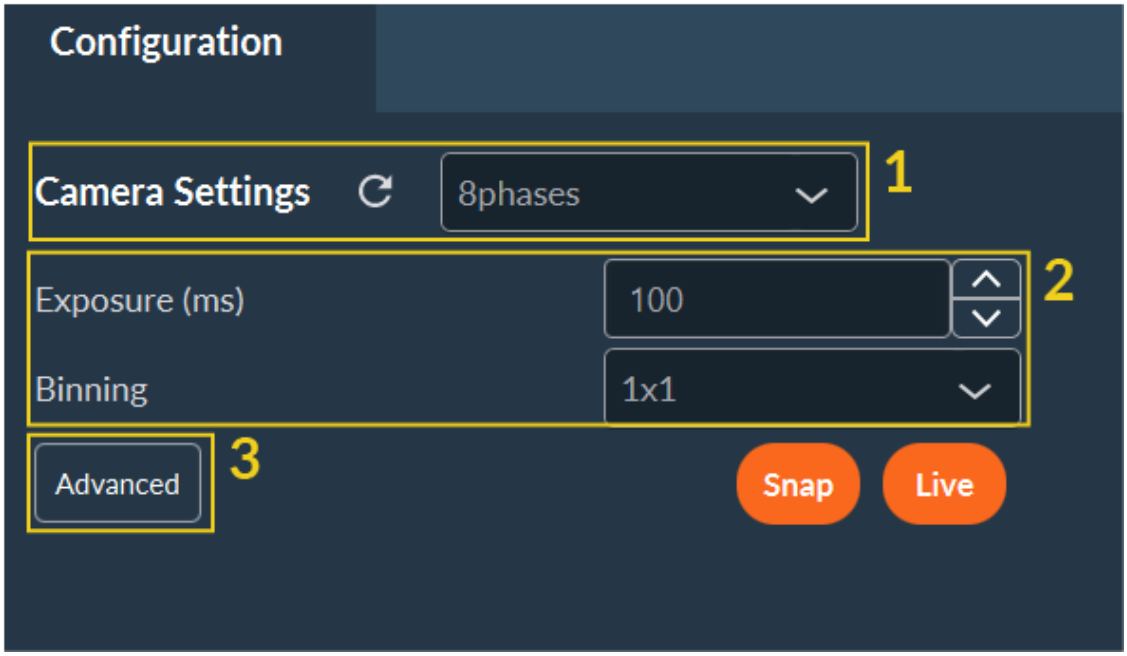
You can modify the name of the project in the top-right corner of the screen. The default file name can be set later in the settings.

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

## 1.1.4.1. Process images

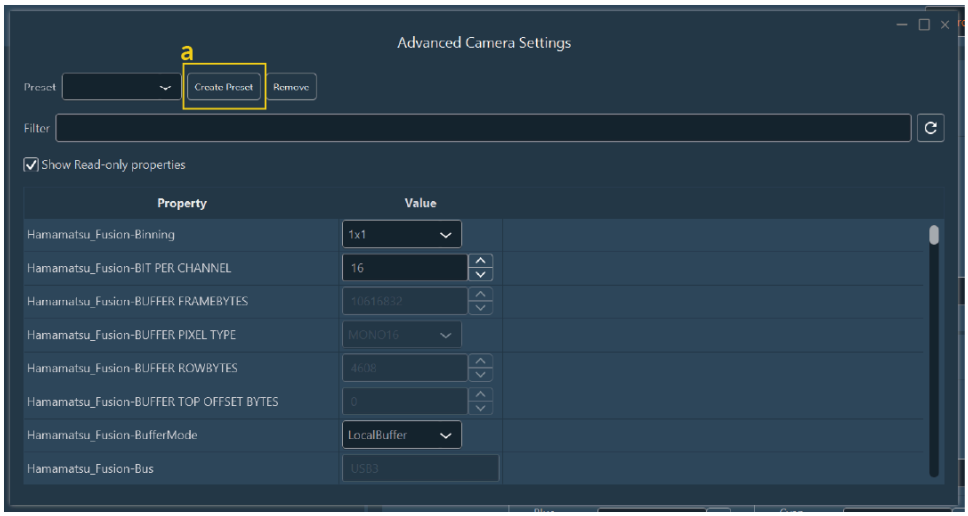
### *1.1.4.1.1. Camera settings*

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



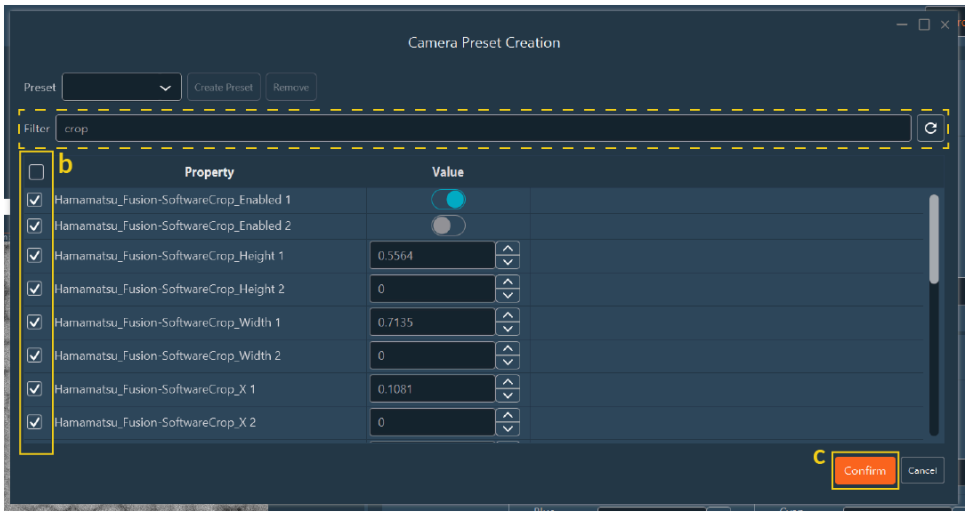

1. If you want to use the camera preset, you can select it in the drop-down list.
2. Set the exposure time and binning.
3. You can also have access to all **advanced settings** of the camera and create a camera preset:

    a. Click on **Create Preset** :

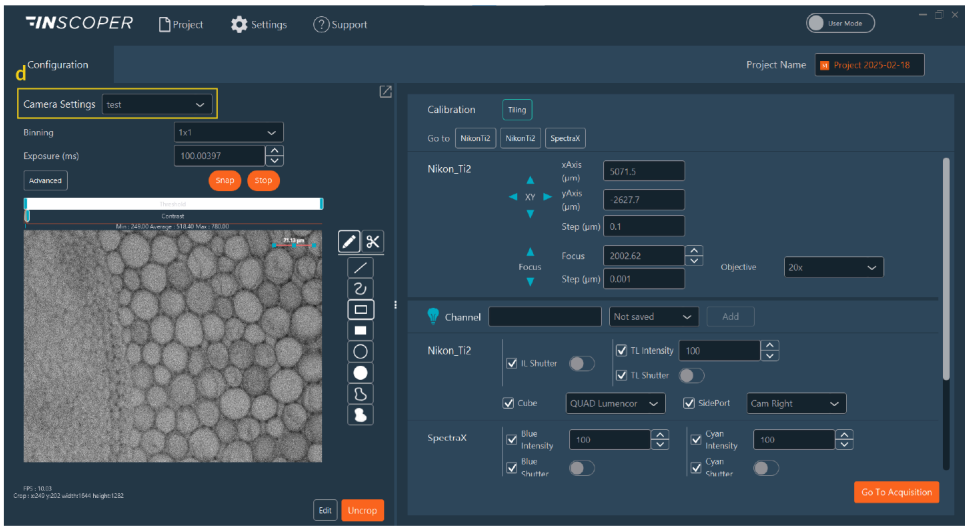



    b. Select the parameters which will be saved in the camera preset by checking boxes. You can filter the parameters by writing in the search bar.

    c. Then **Confirm**:

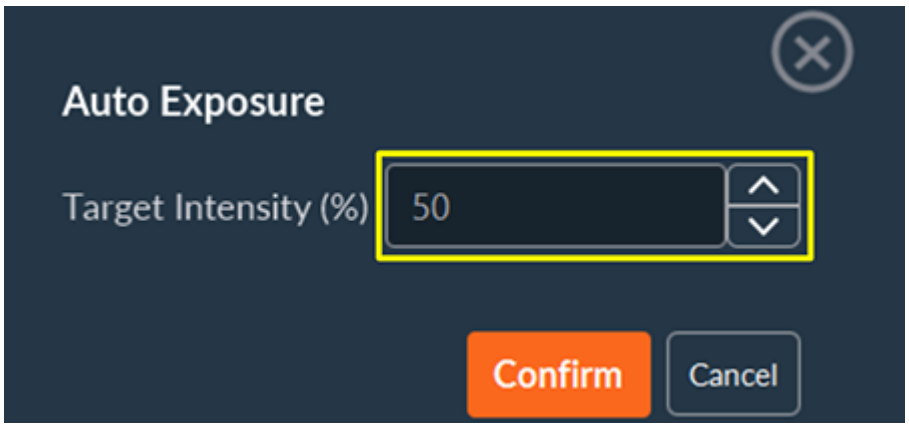



**d.** Choose the name and location for your camera preset:

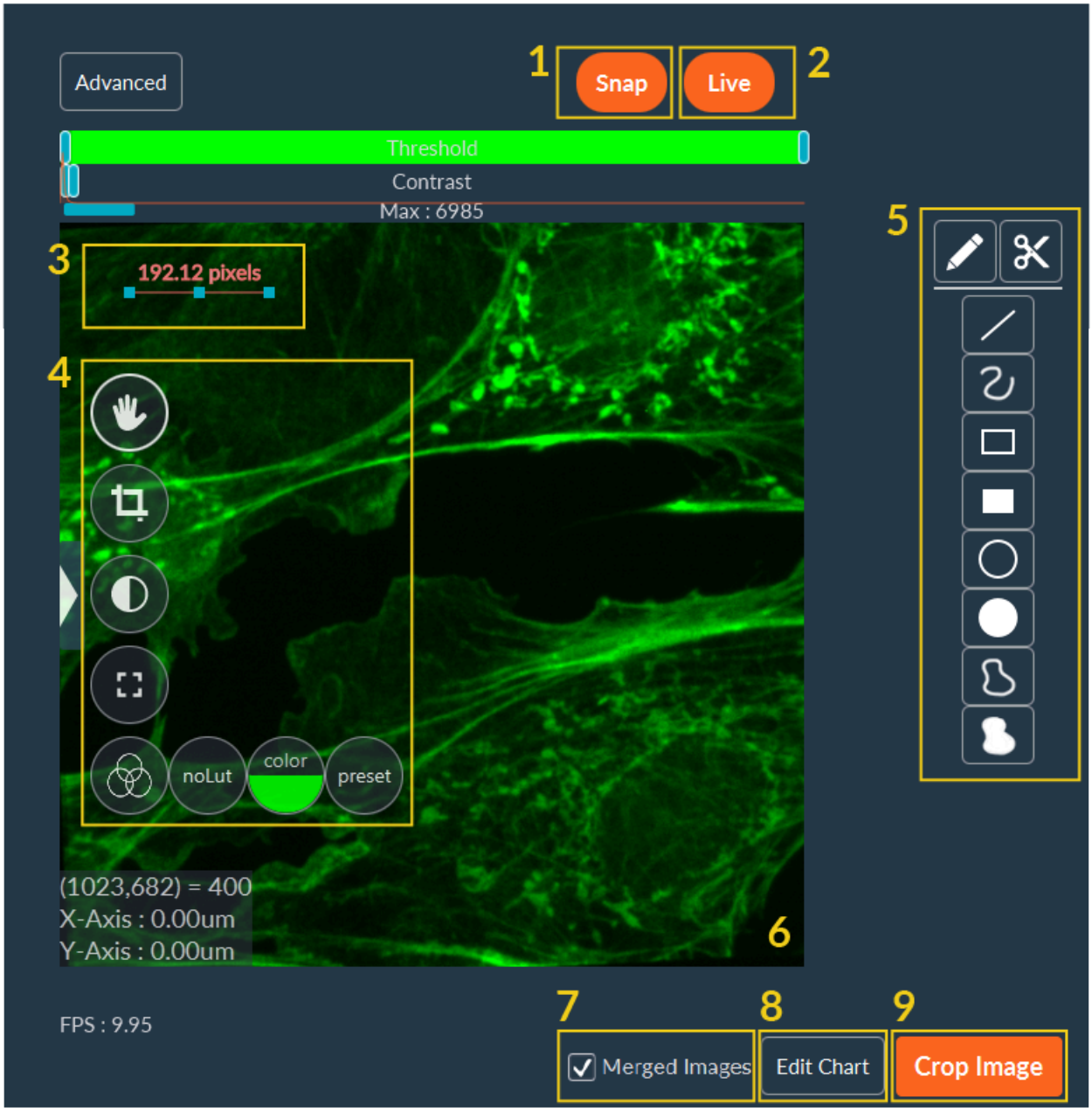



NB: If you have two or more cameras, you can view all cameras simultaneously in **Live**.

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




### *1.1.4.1.2. Interaction with the live image*



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

### *1.1.4.1.2.1. Tools to interact with the image*

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

### *1.1.4.1.2.2. ROI creation*

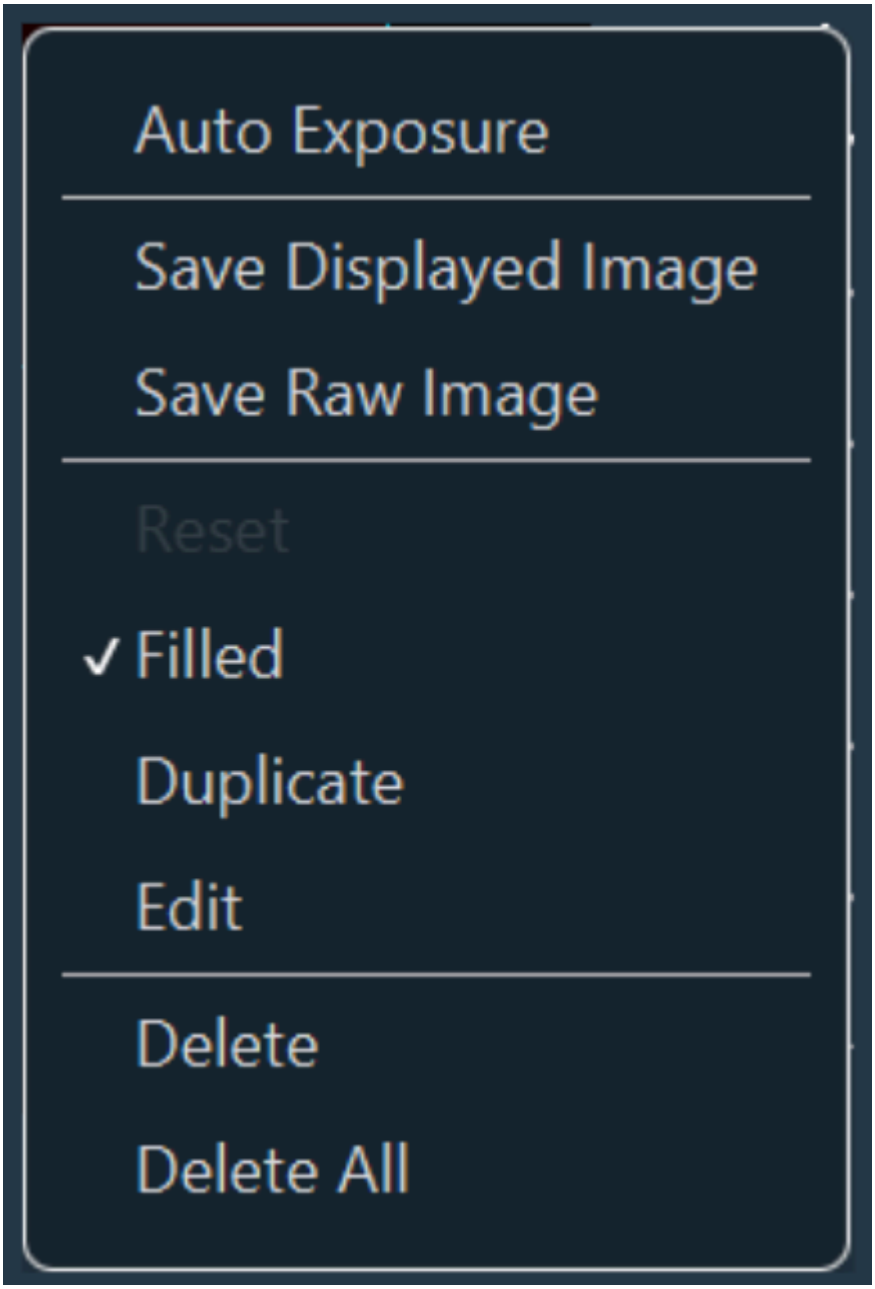
To create a ROI on the image, you can choose between two modes:

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

Then, use the shape tools to draw desired shapes:

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

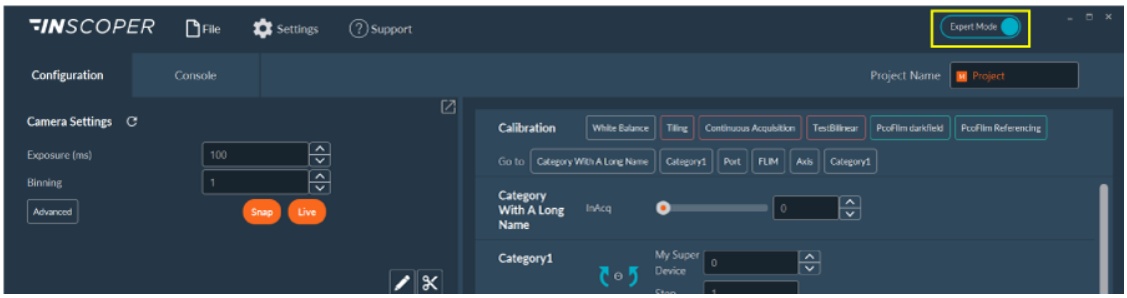
#### *1.1.4.1.2.3. ROI manipulation*




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

### 1.1.4.2. User/Expert display modes

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

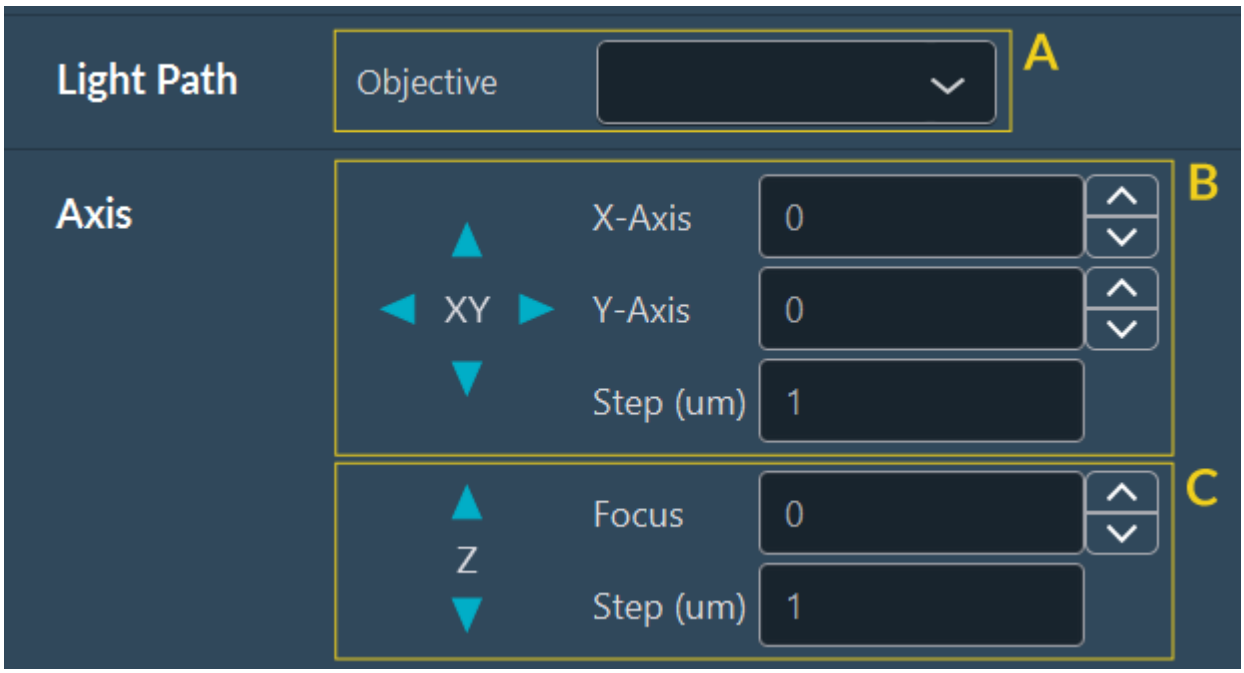



| | |
|---|---|
| Expert Mode | **Expert Mode** allows access to all settings and parameters of the system, without any restriction. This mode is basically dedicated to microscope facility managers or researchers familiar with microscopy. The “Expert” users have to prepare and save some protocol that 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.

## 1.1.4.3. Control of motorized devices

### *1.1.4.3.1. Interaction with motorized devices*

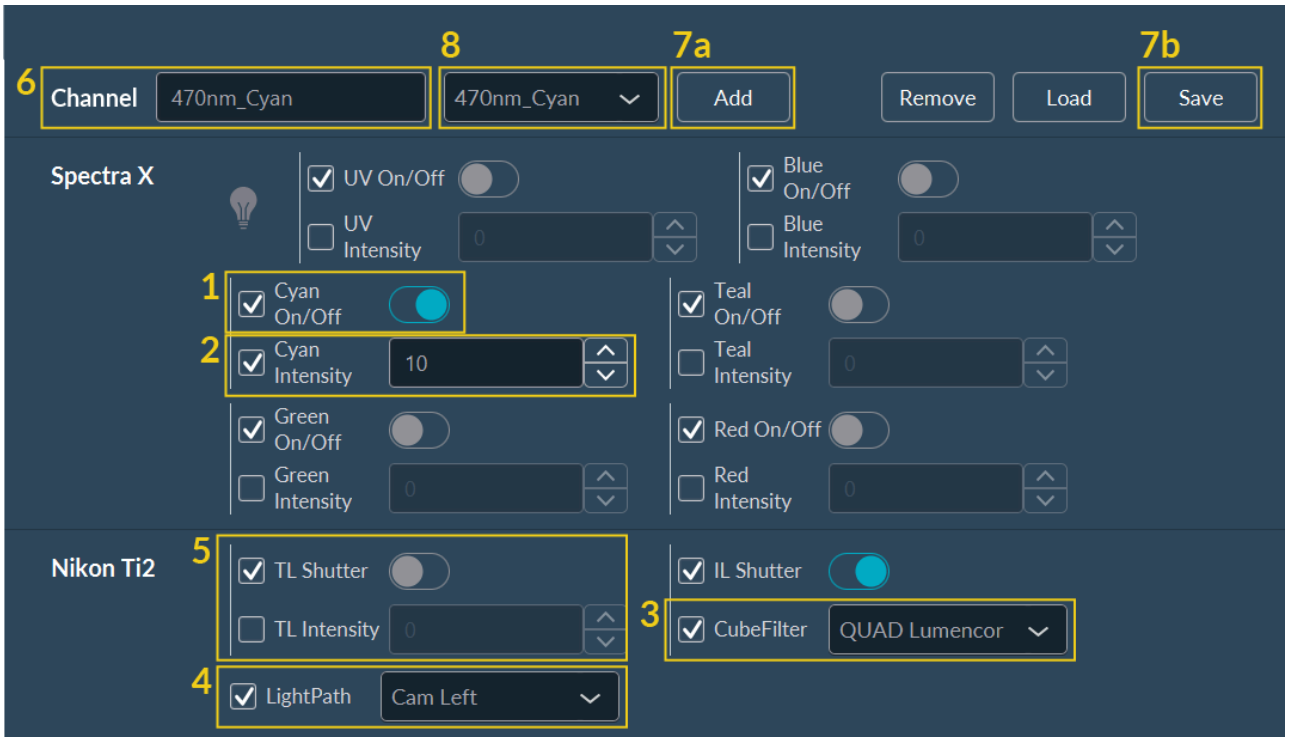


You have access to all devices controlled by Inscoper I.S., including microscope, stages, shutter, light source, wheel filters, piezo, microfluidic element, …

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

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

### *1.1.4.3.2. Create/Load a channel*




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

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

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

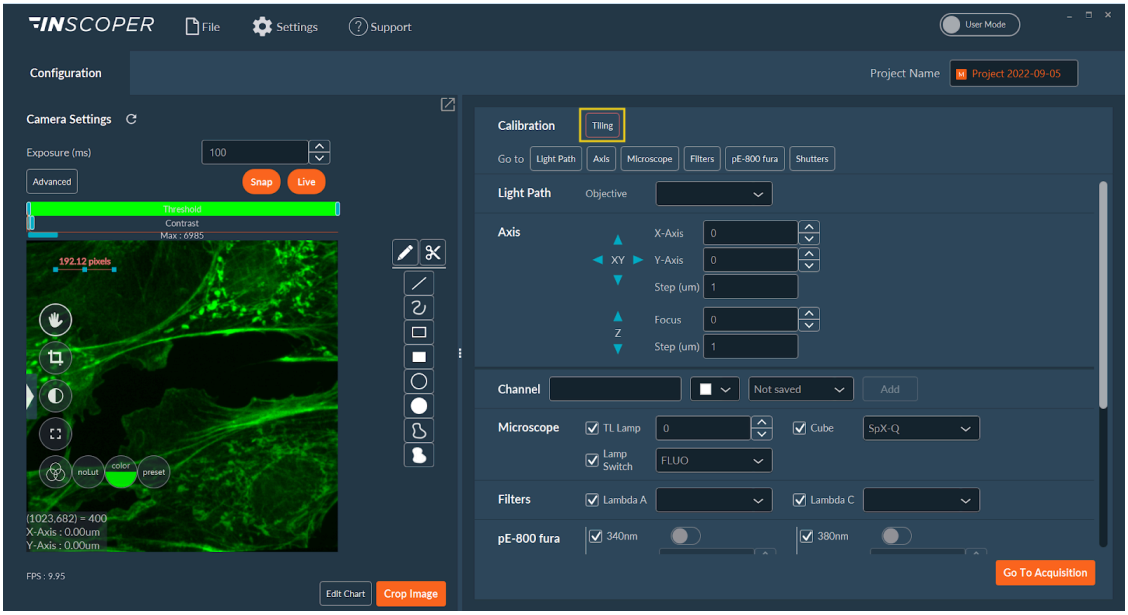
## 1.1.4.4. Calibration protocols

### *1.1.4.4.1. Tiling calibration*

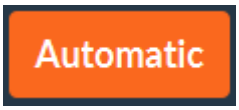
The tiling tool is used to image large samples. The whole final image is divided into several images, called "tiles", acquired one by one and "stitched" afterwards to constitute the whole sample.

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.

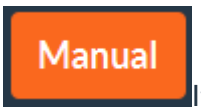
Click on **Tiling** in the Calibration section (ff not yet calibrated, the button will be colored orange).



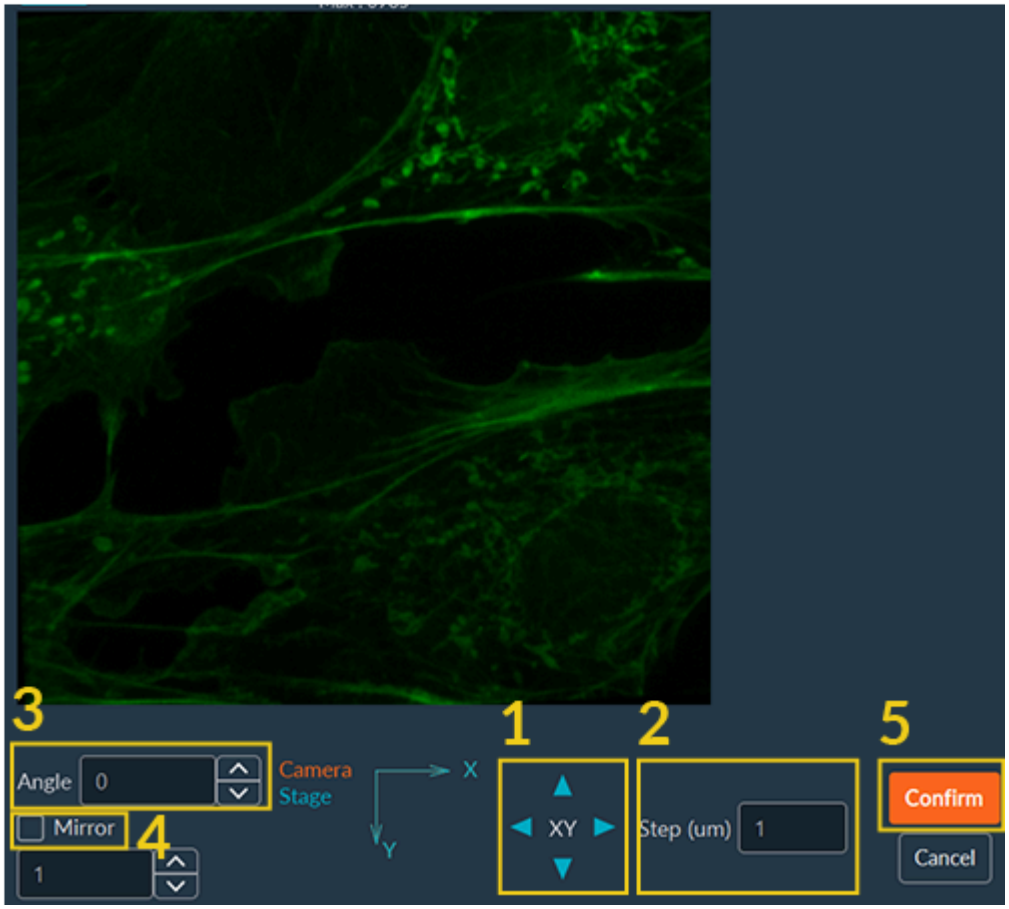

Calibration protocol can be performed according to two different approaches: **Automatic** or **Manual**.




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




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

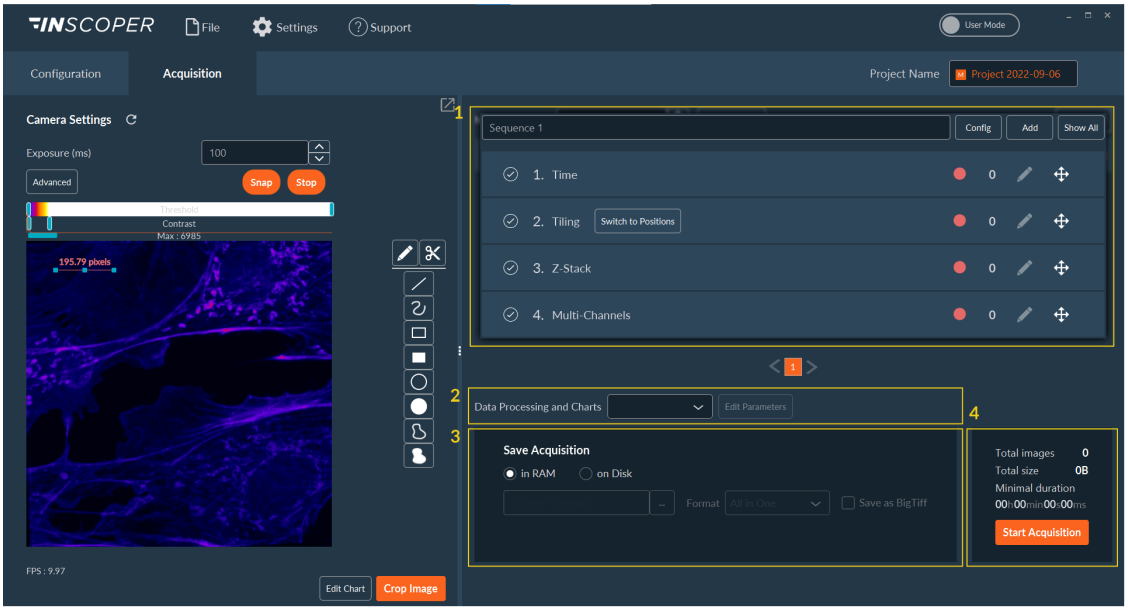



NB: First, be sure that the camera is switched on **Live**.

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

## 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.

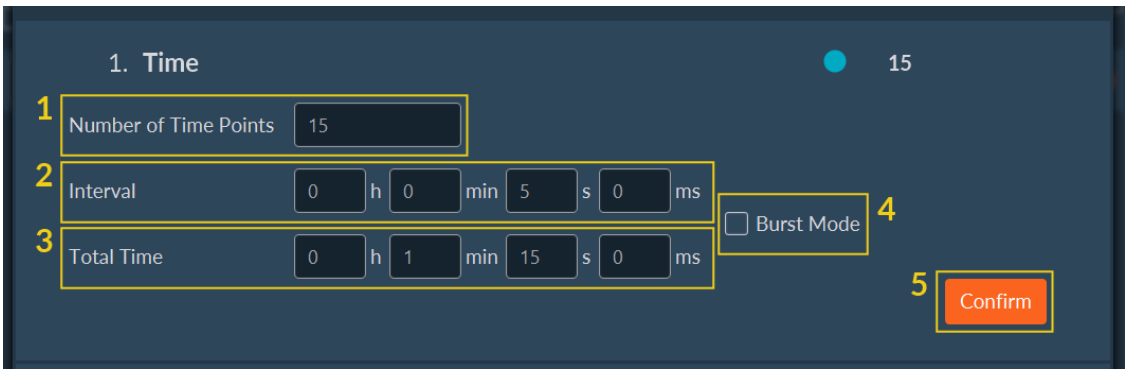
## 1.1.5.1. Dimensions

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

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

### *1.1.5.1.1. Time*

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




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

### *1.1.5.1.2. Multichannels*

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

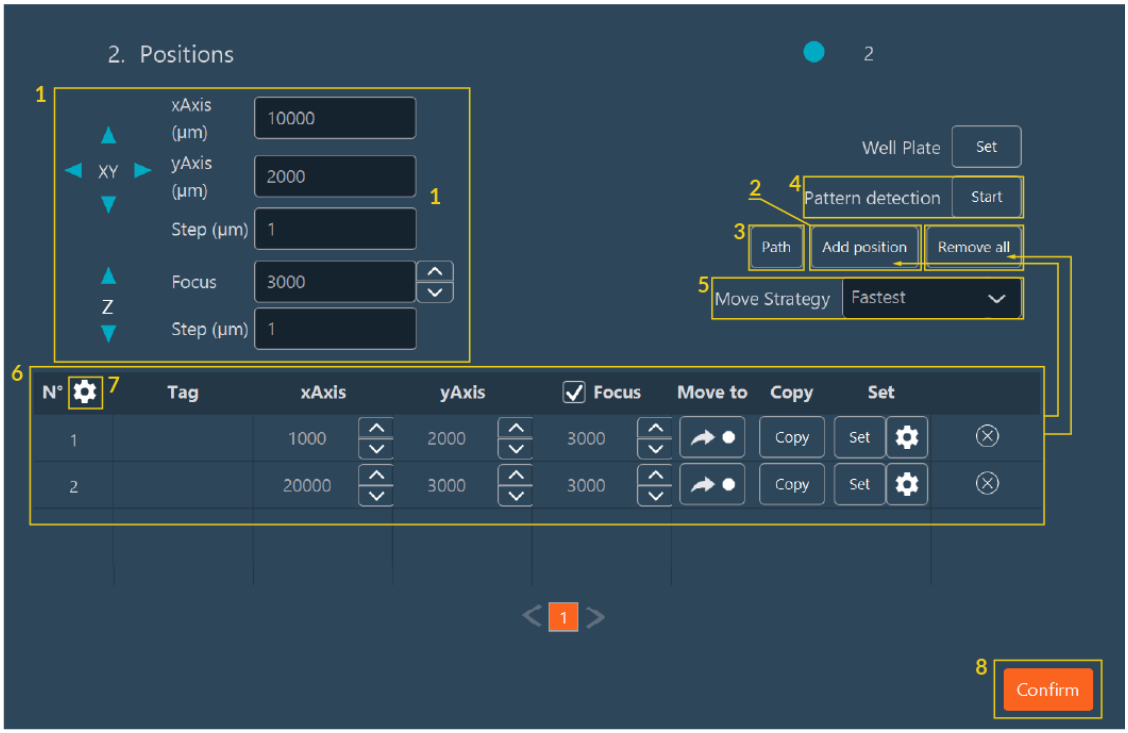

1. Click on **Add Channel**.
2. Select it in the list of the pre-configured wanted channel and customize their settings according to the sample.
3. For each channel, you can apply some additional parameters:
    a. **Z-stack**: apply z-stack dimension;
    b. **Z-Offset**: apply a different offset if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

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

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

### *1.1.5.1.3. Positions*

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

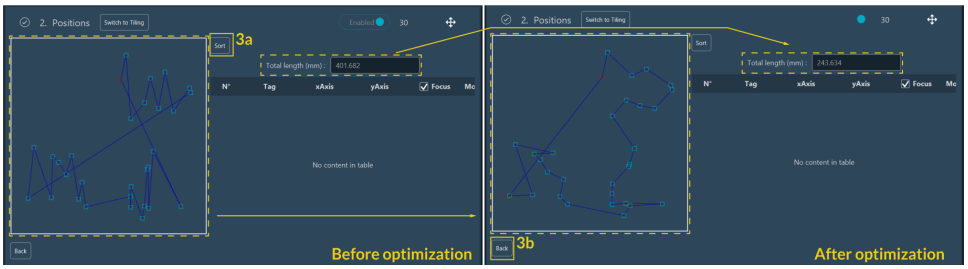



To use the **Positions** dimension:

1. Move the stage and the focus to the desired position.
2. Click on **Add position** to add the XYZ coordinates in the position list.
3. 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.




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

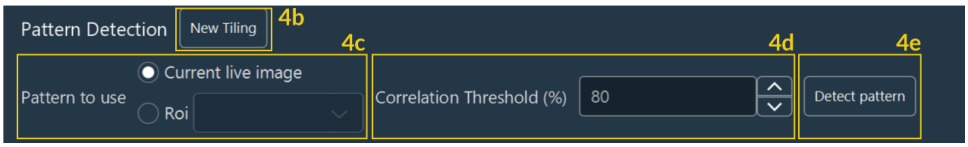
a. Click the **Start** button located in front of the Pattern Detection section.

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

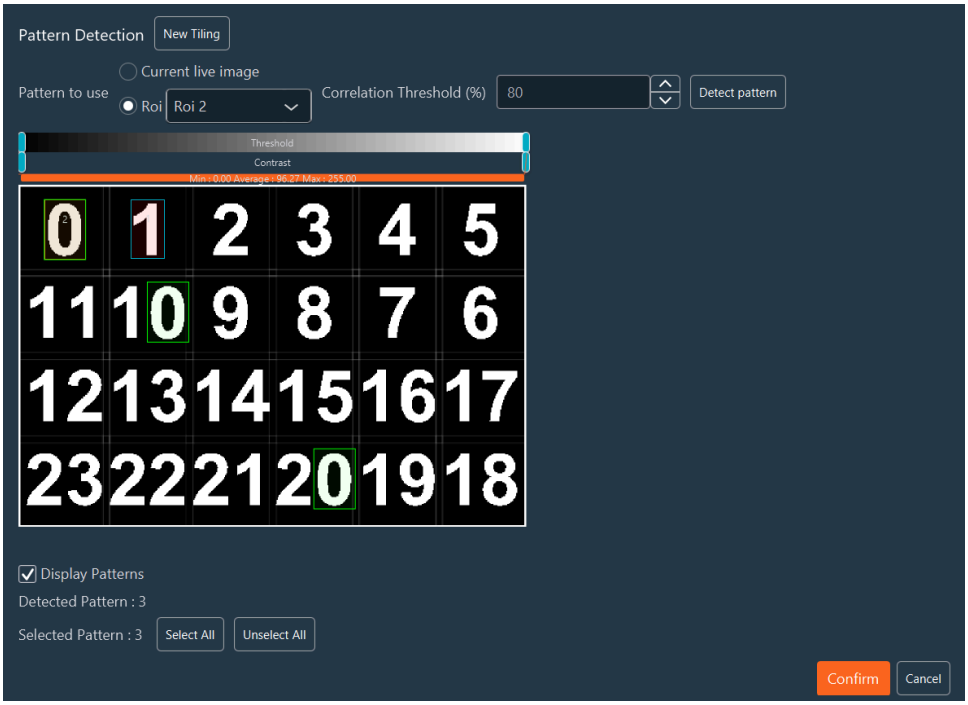
c. Select the pattern you wish to detect:
   - **Current Live Image:** Use the pattern from the live image
   - **ROI**: Draw an ROI around the specific pattern you want to detect.

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

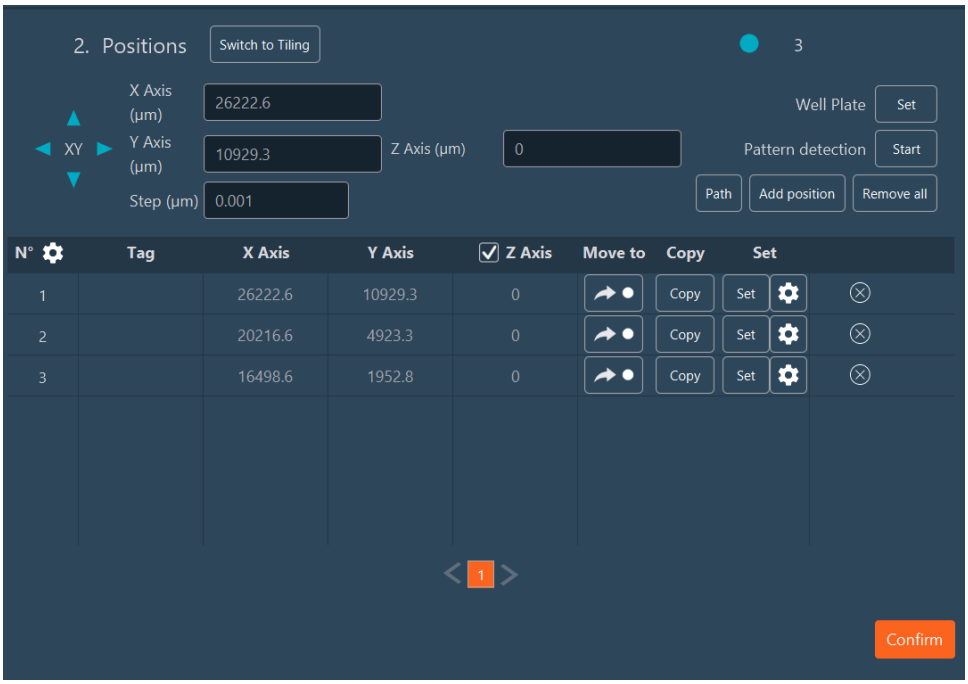
e. Click on **Detect pattern** to start the detection.




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.




5. 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);
6. Make other adjustments or manipulations if necessary:

   a. **Add position**: add a new position/s in the position list;

   b. **Remove all**: delete all position from the position list;

   c. **Tag**: Users can add names to the selected positions;

   d. **X-Axis/Y-Axis**: change/adjust position if necessary;

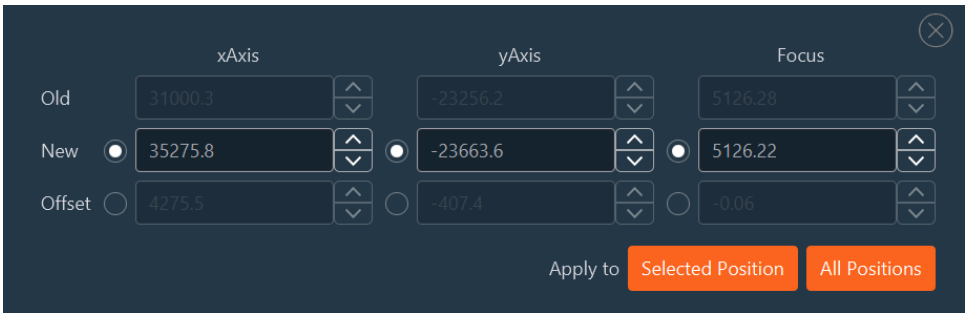
   e. **Focus**: Use the focus value used for the selection of the positions. If the checkbox is not selected, the current focus value of the microscope will be used.

   f. **Move to**: move the stage to this position;

   g. **Copy**: Copy the position you need.

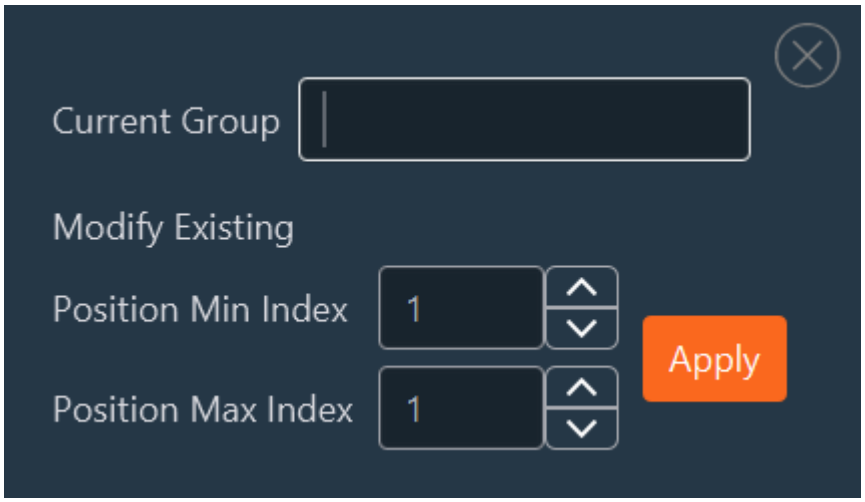
   h. **Set**: update coordinate of the position by the actual XYZ position of the system;

i. 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.




j.  : delete this position from the position list.

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

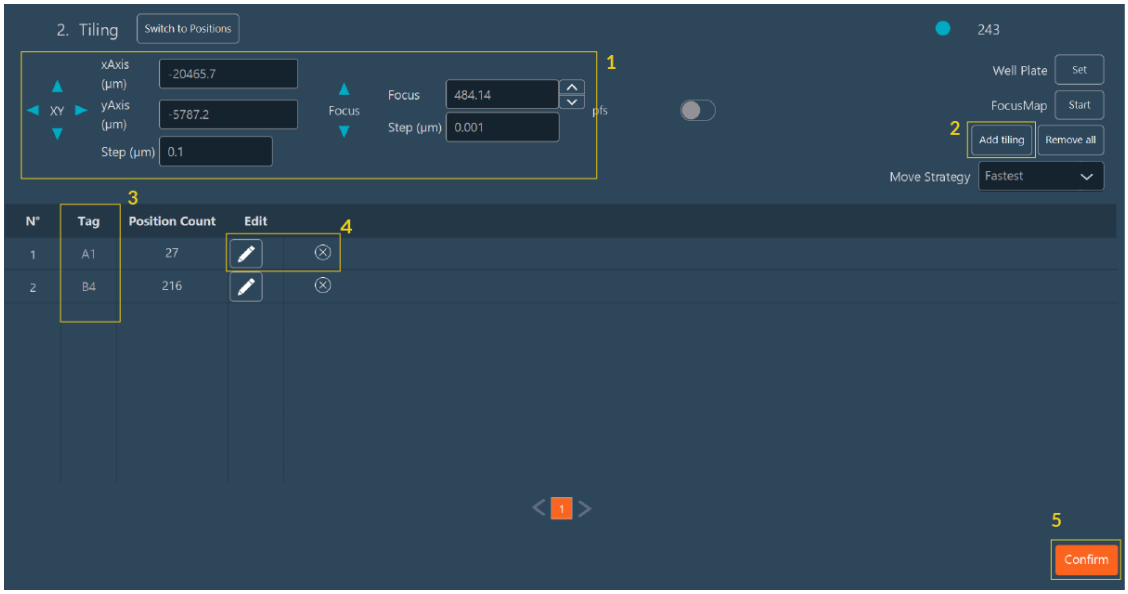



8. When all positions are set up, click on **Confirm**.

### *1.1.5.1.4. Tiling*

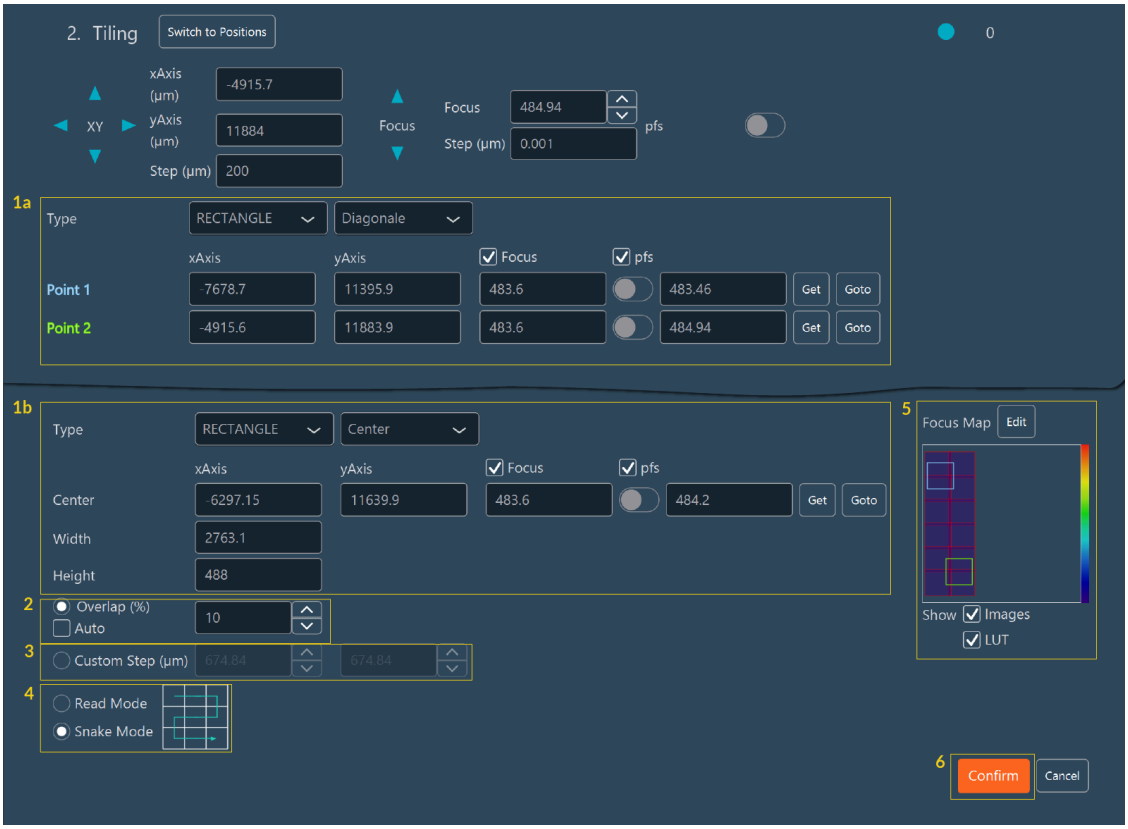
### *1.1.5.1.4.1. Tiling creation*

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

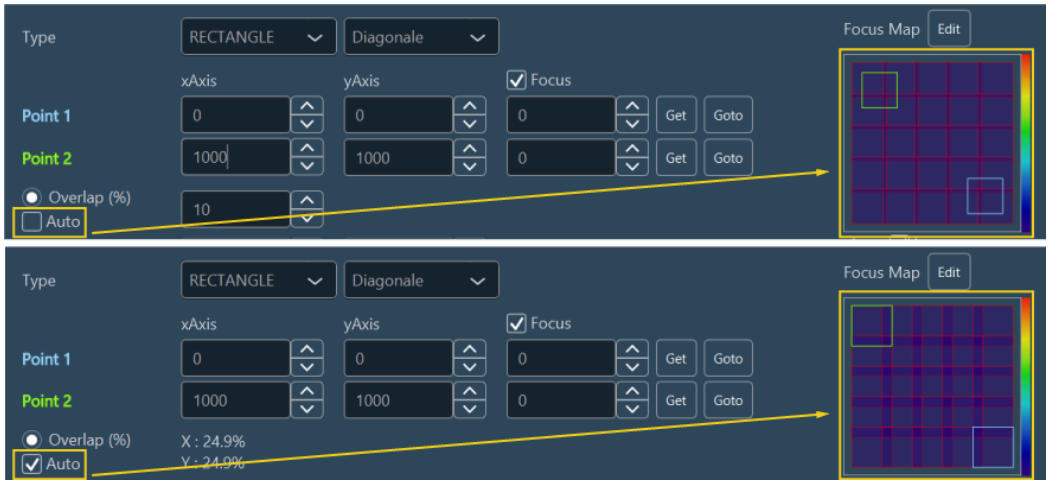



1. Move the stage and adjust the focus to the desired position.
2. Click on **Add position** to add a tiling in the tiling list.
3. You can name each tiling in the **Tag** column by simply clicking on the **Tag** field.
4. To edit/customize a tiling clicking the **Pen** icon (see the Tiling edititon)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

### *1.1.5.1.4.2. Tiling edition*




1. Choose the way to generate your tiling and set its specific parameters:
    a. For the **Diagonale** setup:
        - Move the stage to the top left corner of the structure of interest and click on **Get** for the first position.
        - Move the stage to the bottom right corner of the structure of interest and click on **Get** for the second position.
    b. For the **Center** setup:
        - Set the position of the center of your tiling.
        - Set the width and the height of your tiling.
2. Select an appropriate **overlap** pourcentage (10% is usually enough for final stitching for example) OR check **Auto**mode which allows to calculate the exact overlap between tiles to respect the 2 coordinates chosen for the tiling creation.




3. If needed, adjust the **Custom Step** (distance between the tiles).
4. Select the acquisition order of tiles.

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

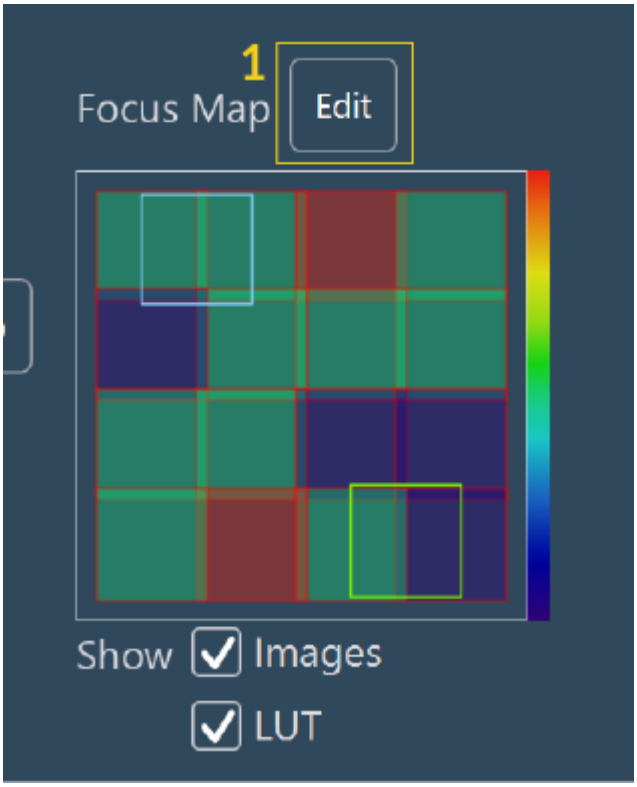
5. Edit the **Focus map** if you need to use it.
6. Click on **Confirm**.

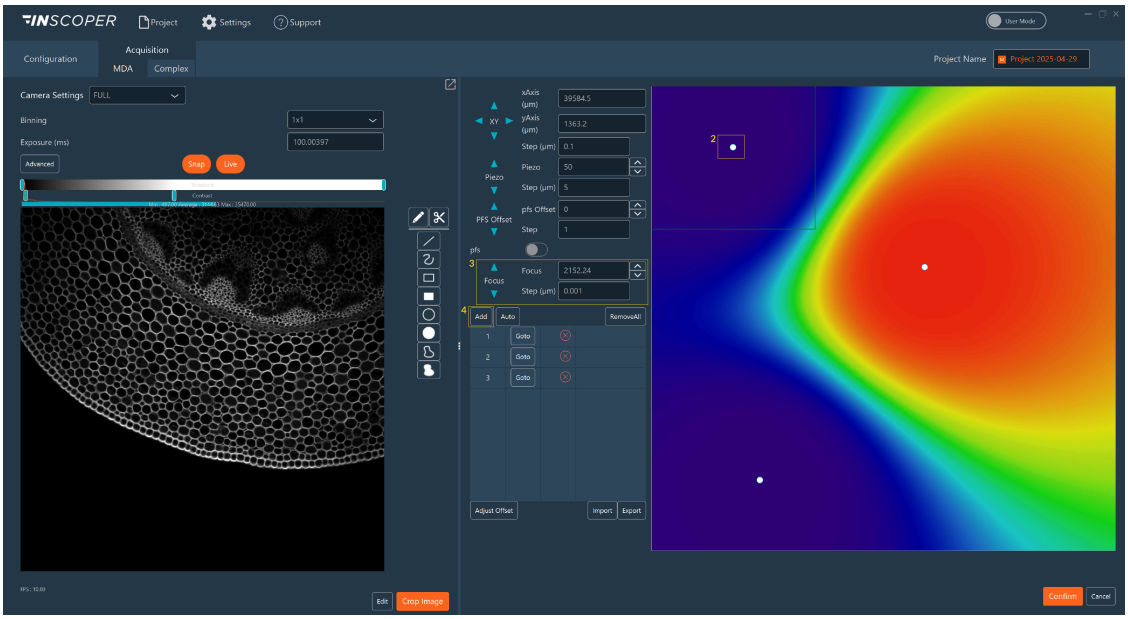
> NB: Users can remove the **Focus** checkbox if they don't want to consider the Z coordinate used for the tiling creation.

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

### *1.1.5.1.4.3. Focus Map manual setup*

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




To use the **Focus Map**:

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

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

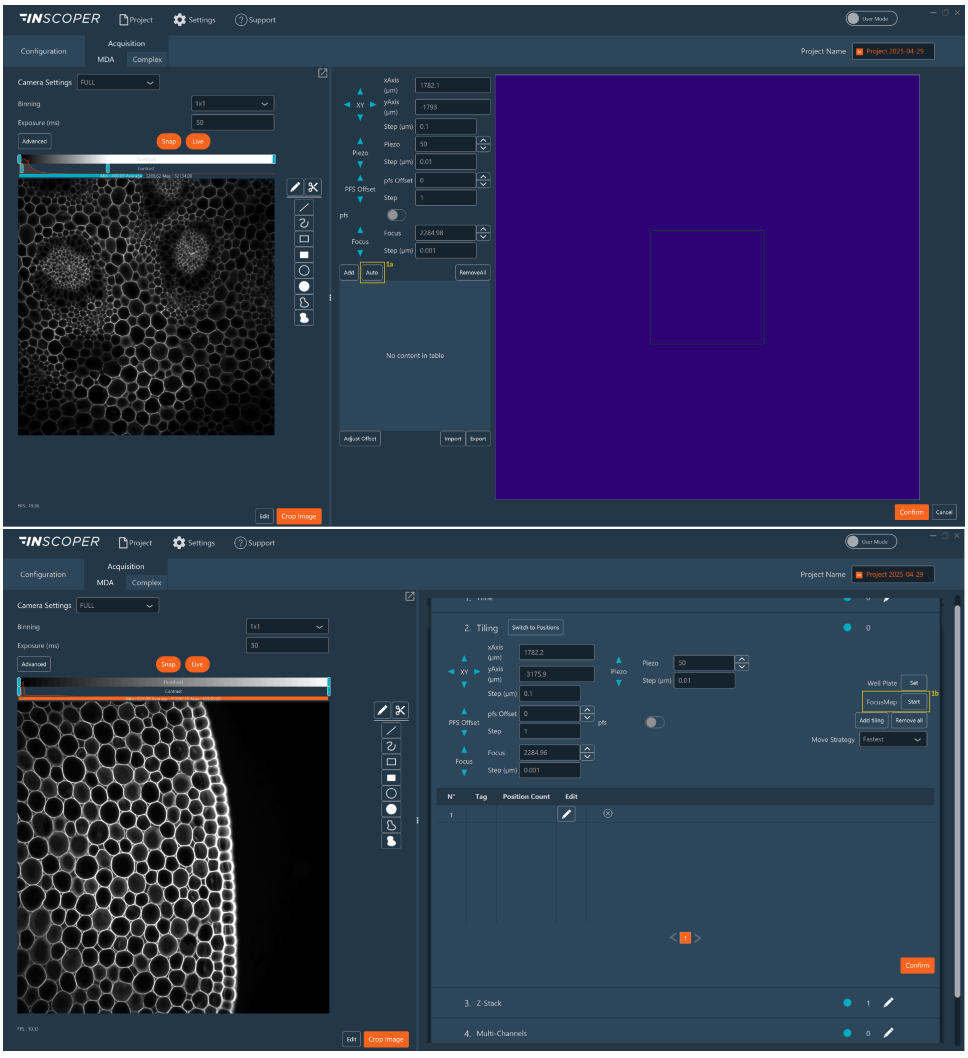
NB: The button **Remove All** can be used to remove all points on the Focus Map.

### *1.1.5.1.4.4. Focus Map automated setup for simple and sultiple siling*

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. There are 2 types of the Automated Focus Map ：

- Simple tiling;
- Multiple tiling.

1. To start configuring:
    a. simple tiling: click on **Auto** in **Tiling Dimension**.
    b. multiple tiling: click on **Focus Map Start** button in **Tiling Dimension**.



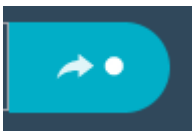
2. The following parameters must be set for both simple and multiple tiling:
    a. Choose the **step** and **size** of the stack.
    b. Select an existing pattern or click **Edit** to create a new one.
    c. If **Edit** is selected:
        - **Grid:** set number of images (horizontal and vertical), gap, offset X and Y. The offset X and Y allows you to move your pattern in the well.
        - **Random:** set number of positions and minimum distance between them.
    d. Click **Add** to save the pattern.
    e. Return to the Dimension via **Switch to Tiling**.
    f. Select channel to acquire the stack.
    g. Optional: Check **Filter unsure position** and set parameters:
        - **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:** ROI for auto-calculating background values.
        - **Background:** Mean background value.
        - **Background Delta:** Noise amplitude.
        - **Valid Pixels:** Percentage needed to validate position.
3. If using multiple tiling: select the tiling area for which you want to create a Focus Map
4. Click on **Start**.
5. Click **Confirm** to validate and proceed. You can continue to configure your acquisition sequence.

#### *1.1.5.1.5. Multiwell plate mode*

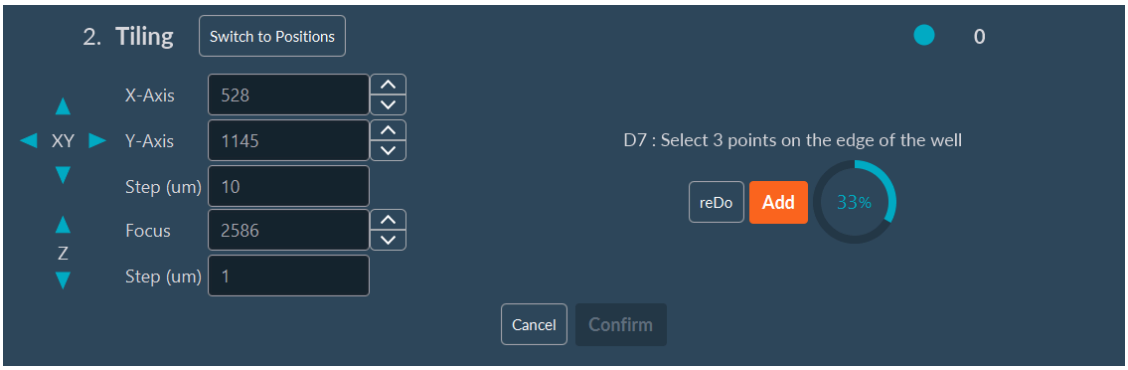
The Inscoper software has a strong feature for imaging multiwell plates and for some applications including High Content Imaging.The Multiwell plate mode of the Inscoper I.S. is compatible with all commercial or homemade supports (multi-well, multi-slide, etc.). An option **Well Plate Editor** allows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

##### *1.1.5.1.5.1. 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 |

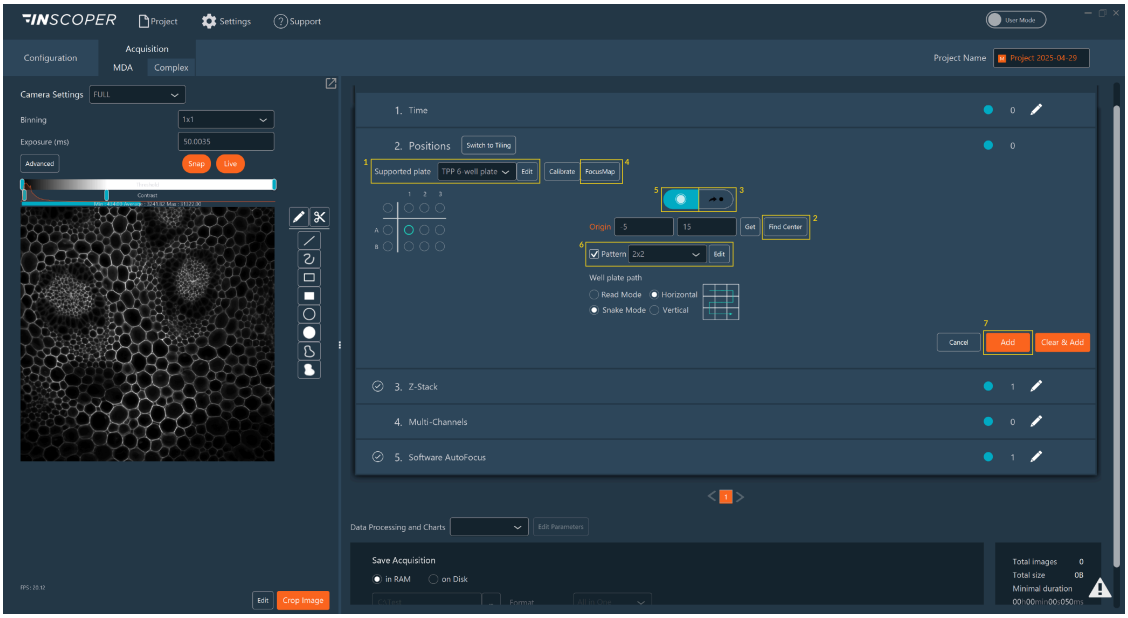
### *1.1.5.1.5.2. Multiwell plate calibration*




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.

### *1.1.5.1.5.3. Multiwell plate mode in Positions dimension*

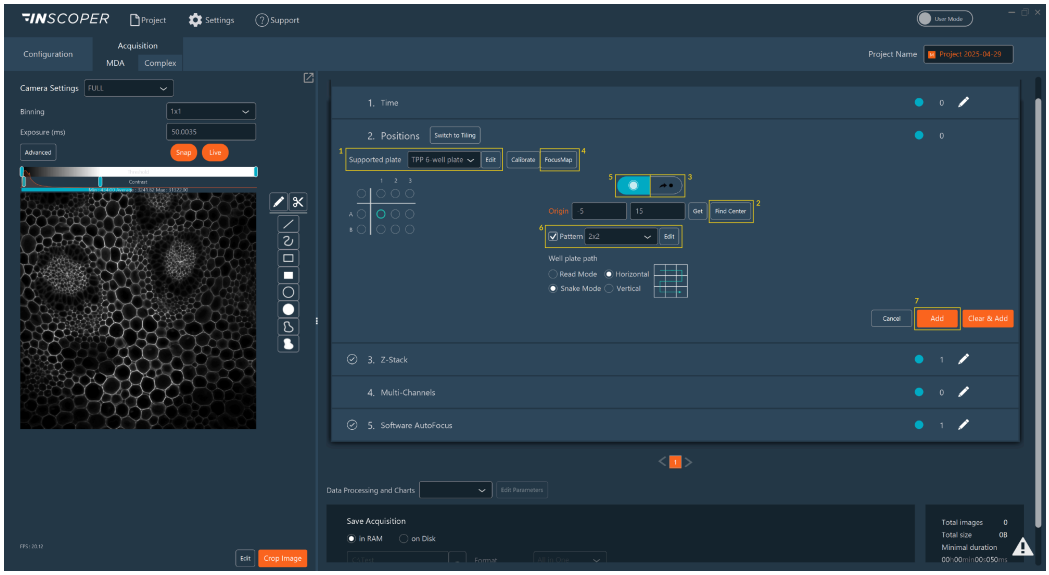



1. Use the **Supported plate** dropdown list to select the support you need.
2. Detect the well plate using the Find Center.
3. Automatically move the stage to some wells.
4. Create a Focus Map for some wells.
5. Select the wells that you want to image.
6. By checking the box **Pattern**, you can generate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

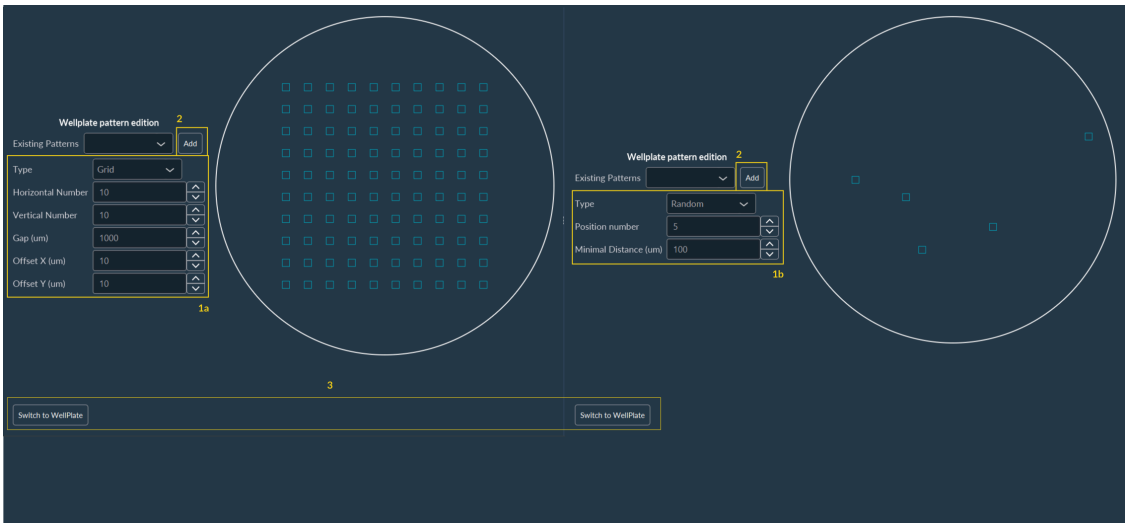
> NB: You can select multiple wells at once by holding down the mouse and moving the mouse over different wells.

You will find all your positions inside the dimension.

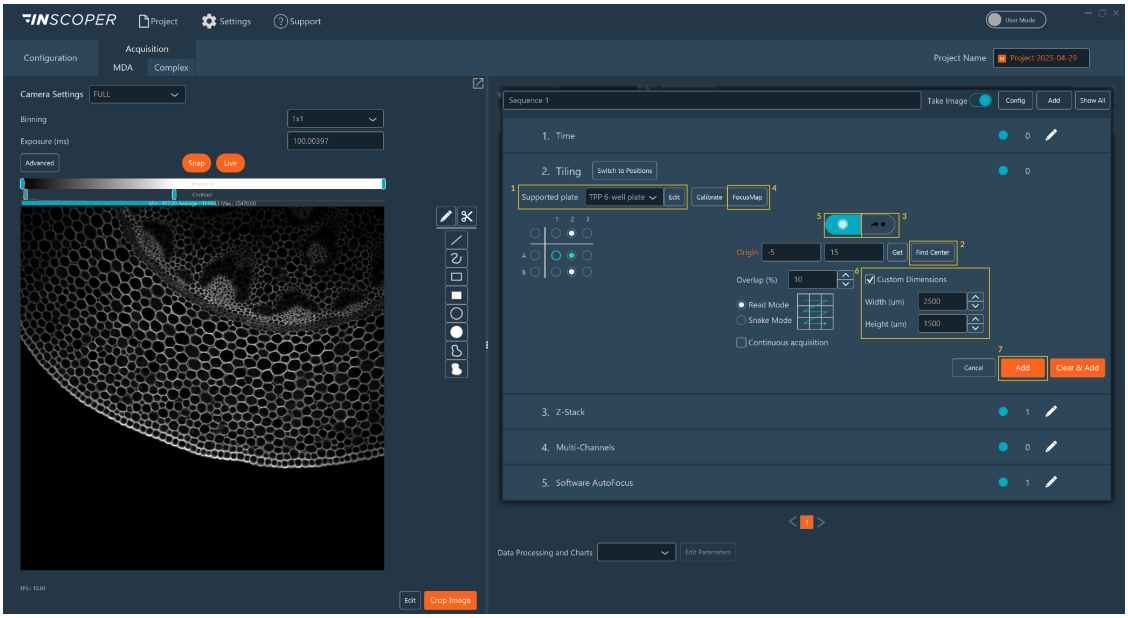



To edit pattern, click on **Edit**.

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**.



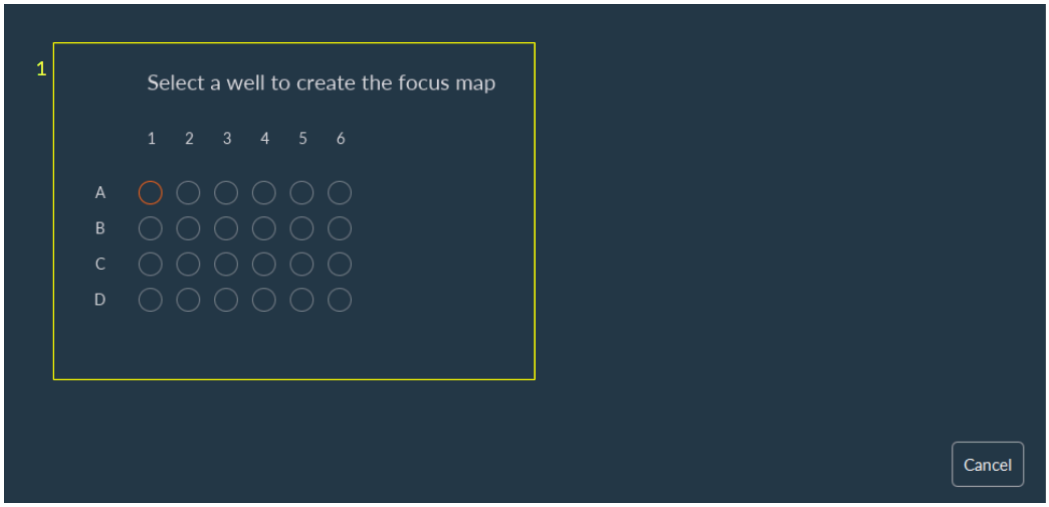

## *1.1.5.1.5.4. Multiwell plate mode in Tiling dimension*




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**.

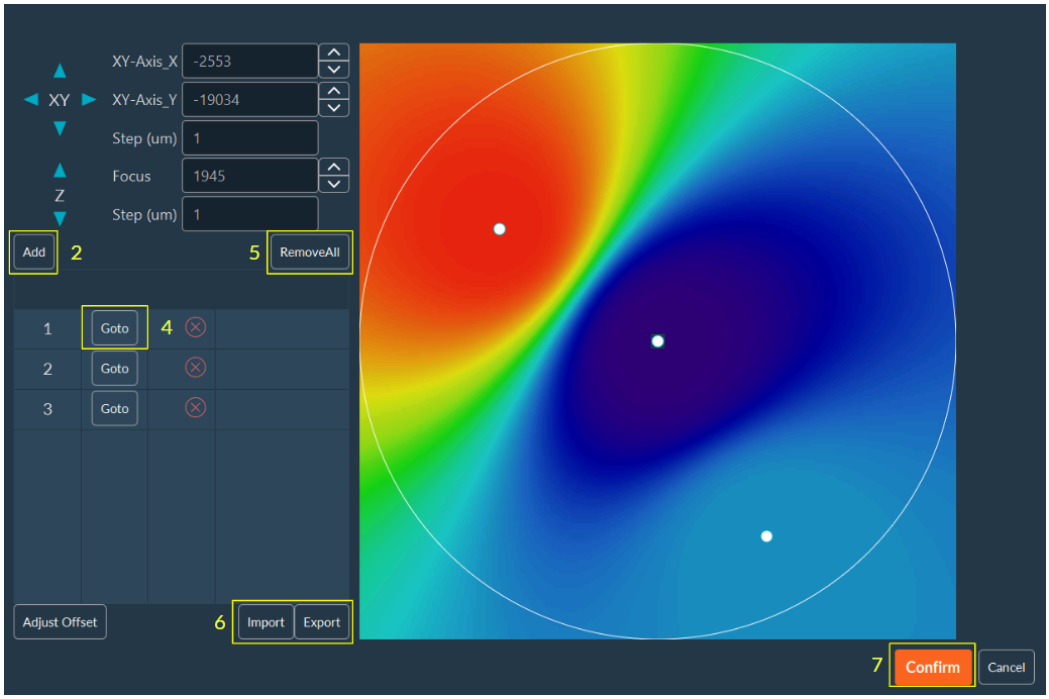
### *1.1.5.1.5.5. Focus Map manual setup*

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.

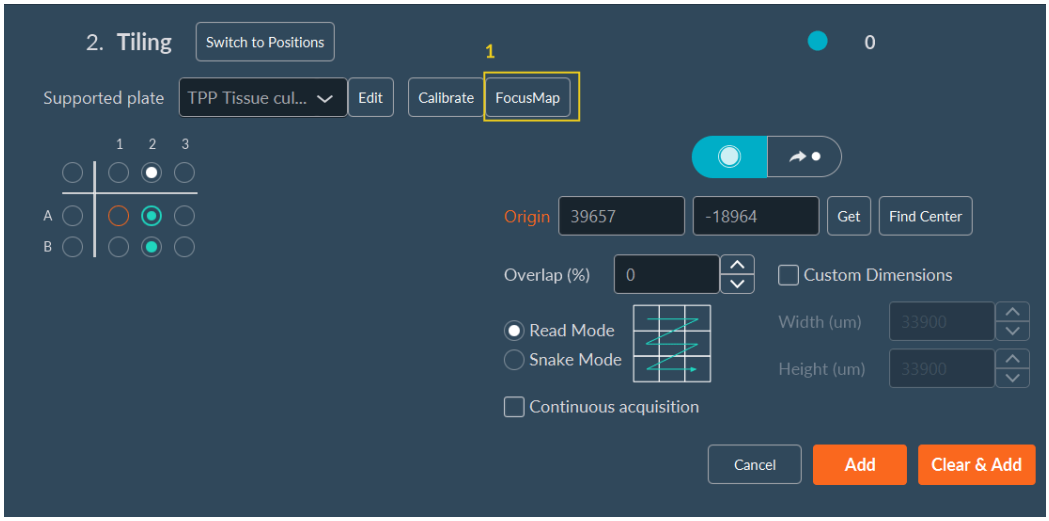
4. Click on **Go to**, to move to the position.
5. Click on **Remove all** to delete all the points of your focus map if necessary.
6. You can import or export a Focus Map.
7. Click on **Confirm** to validate your Focus Map.



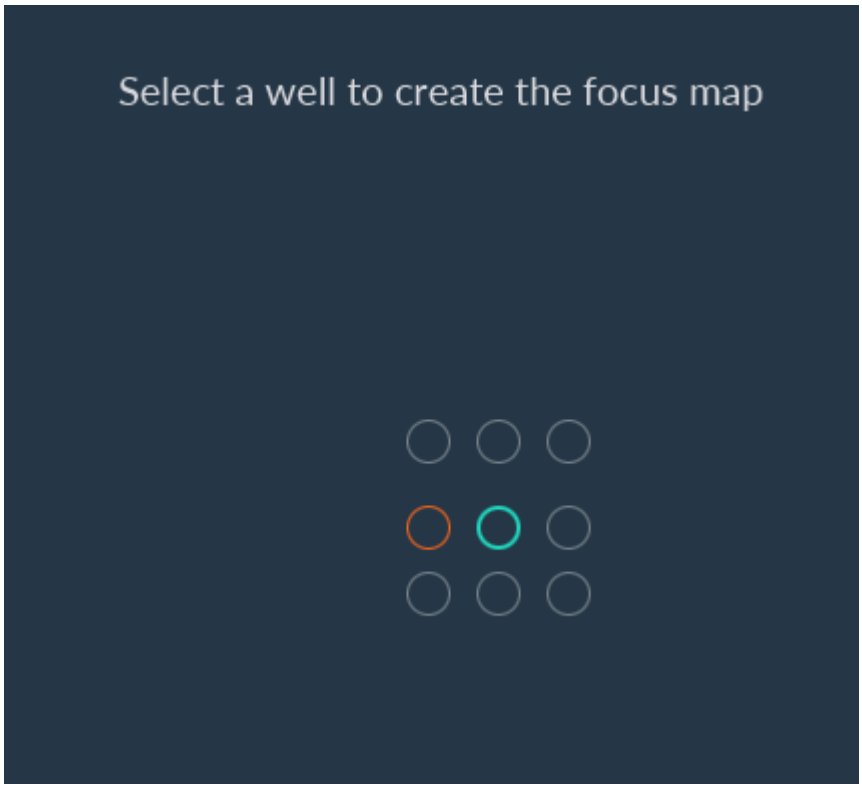

### *1.1.5.1.5.6. Focus Map automated setup*

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

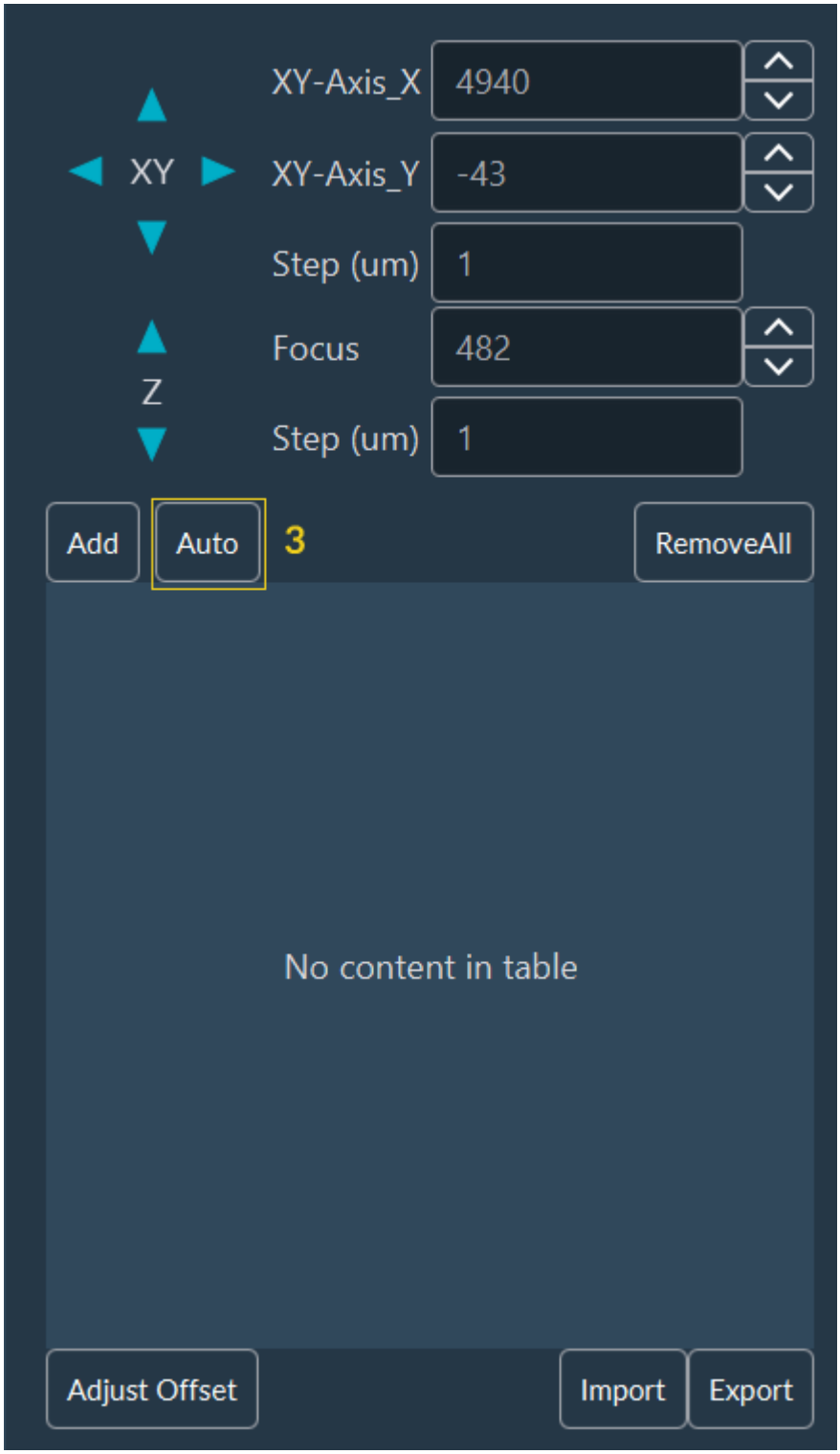
1. Click on **Focus Map** to configure it.



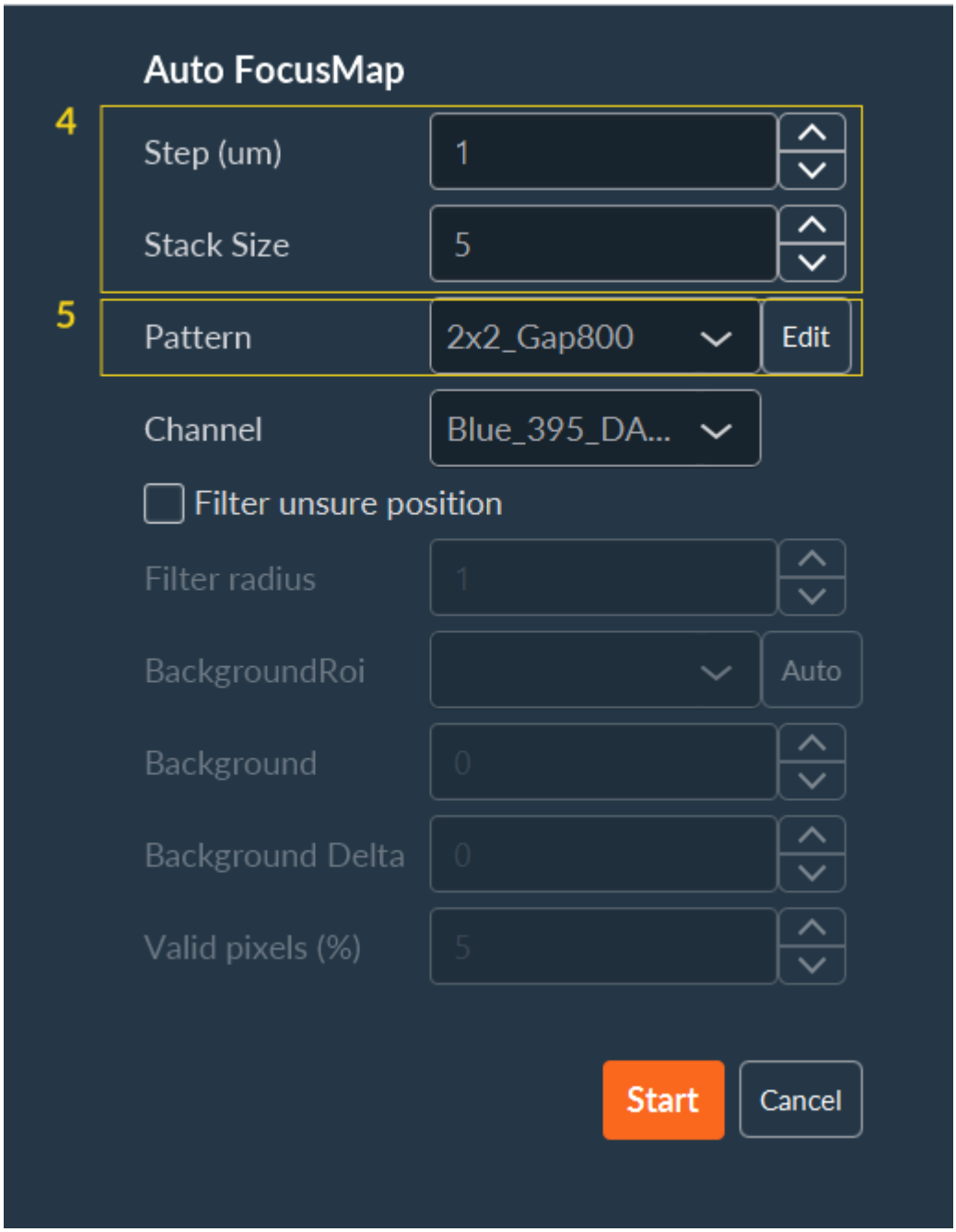

2. Select your well where you would like to create your **Focus Map** then click on **Confirm**.




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**.

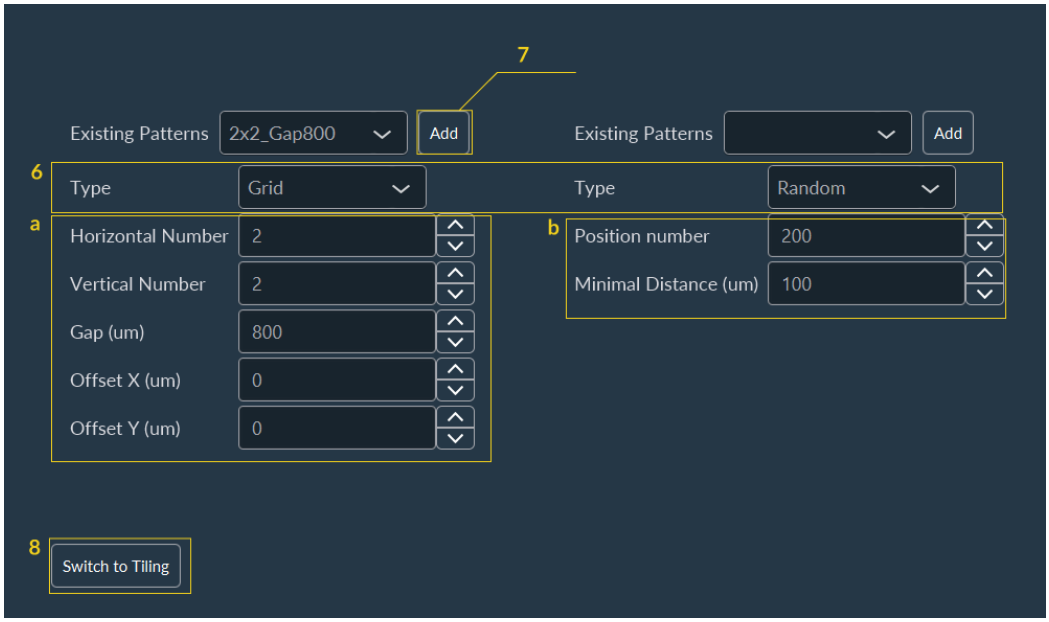



6. Select the pattern you want: **Grid** or **Random**:

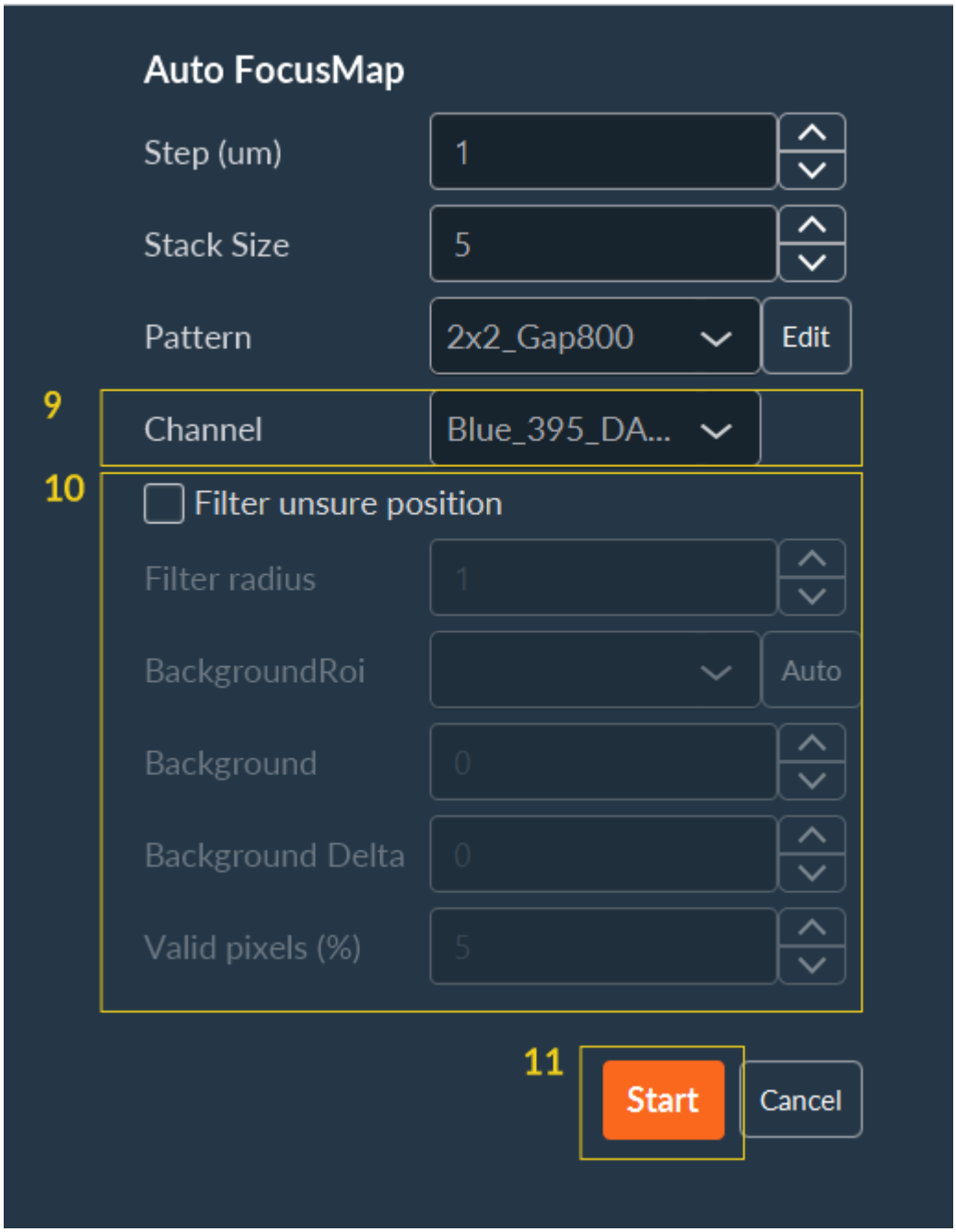
    a. For **Grid**, indicate the number of image you want to acquire horizontally and vertically, and the Gap (distance between 2 images).
    The offset X and Y allows you to move all your patterns in the well.

    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**.

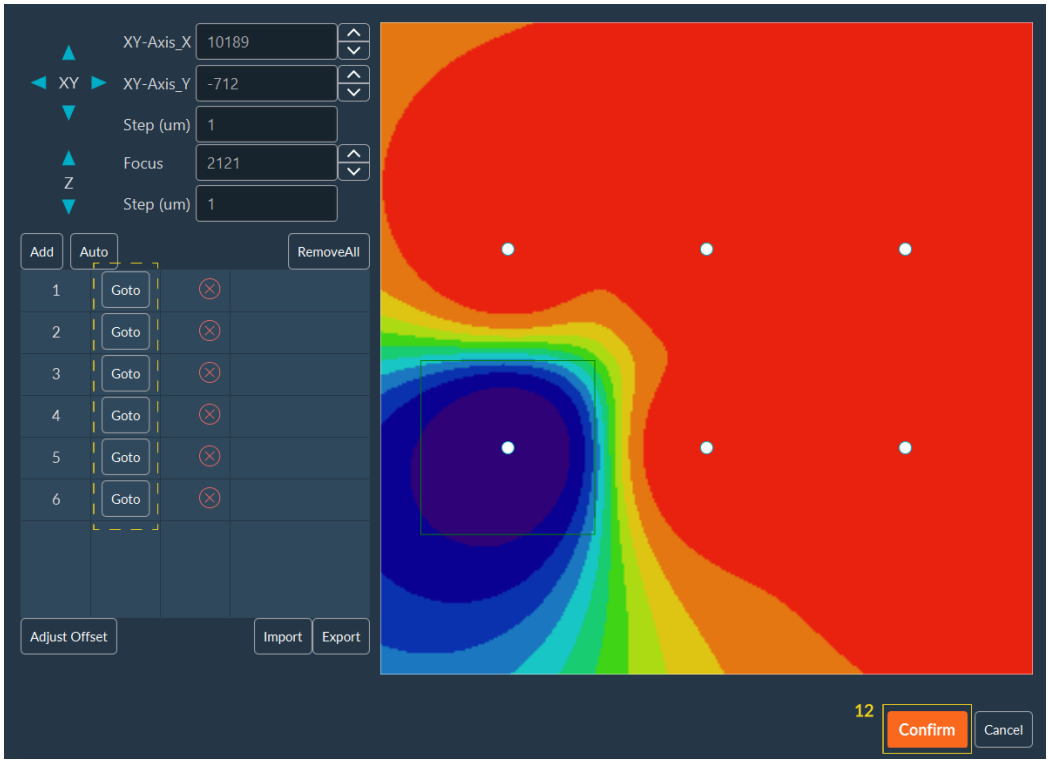



9. Choose the **channel** to acquire the stack.
10. You can apply some filters by checking the box **Filter unsure position**:
    - Filter Radius: filter to smooth the noise by replacing each pixel by the median of the neighbor (0= no smoothing, 1= median on 3x3 square around the pixel, 2= median on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

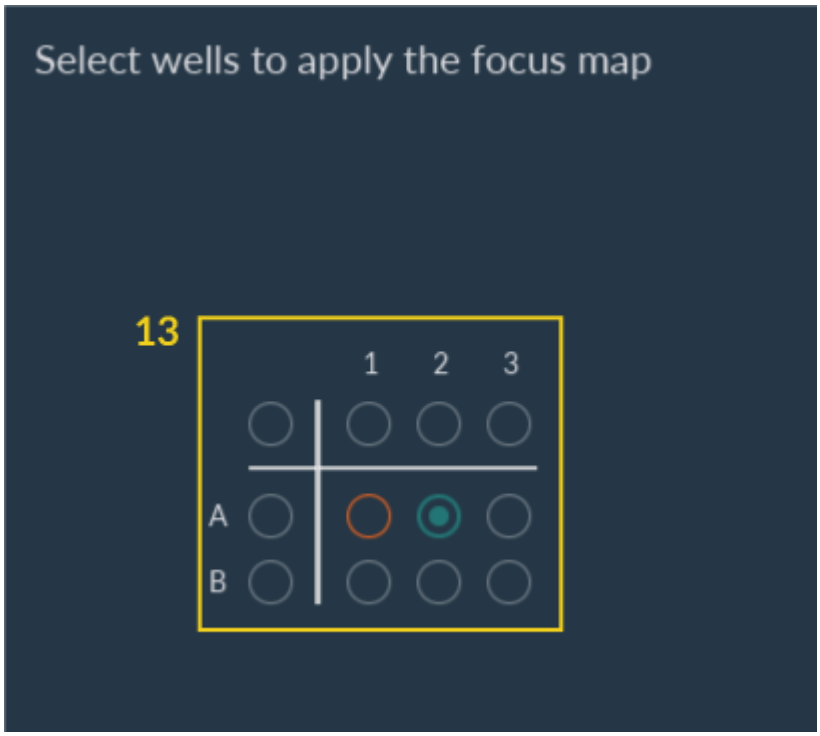



System will perform all points to create the Focus Map and you will obtain a list of points. By clicking on **Goto** or on the image, you can check the focus.

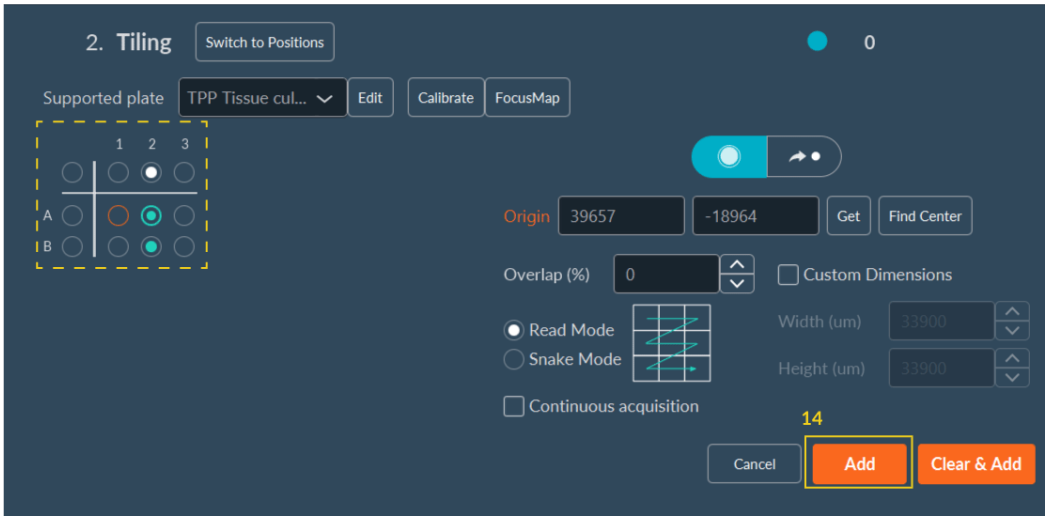
12. Click on the **Confirm**, the **Focus Map** is ready to use.



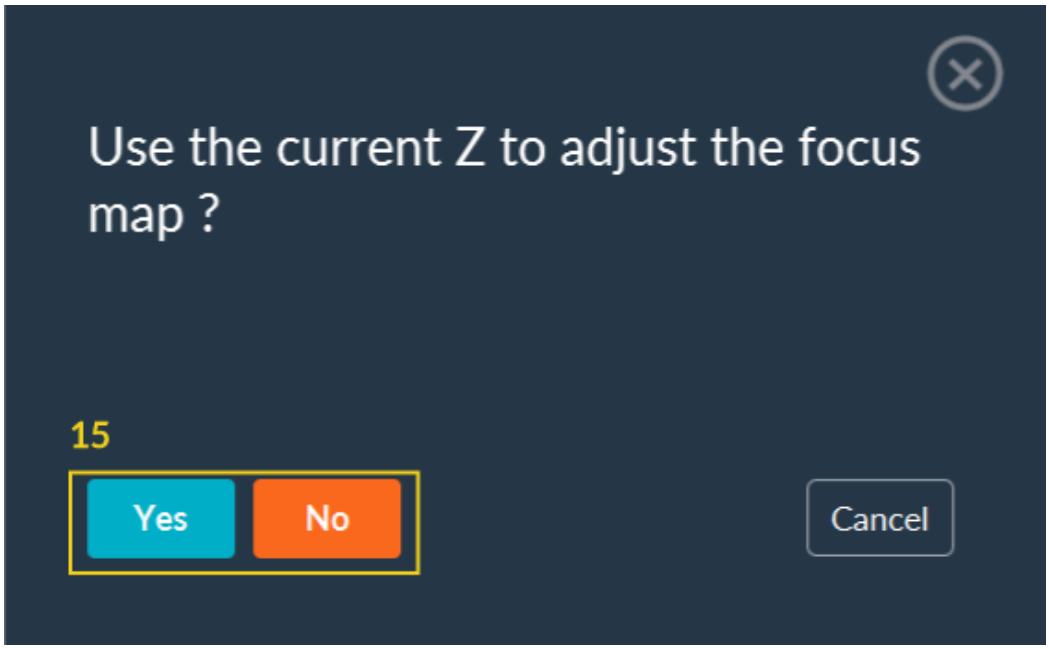

13. Select the wells where you want to apply the Focus Map then click on **Confirm**.




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



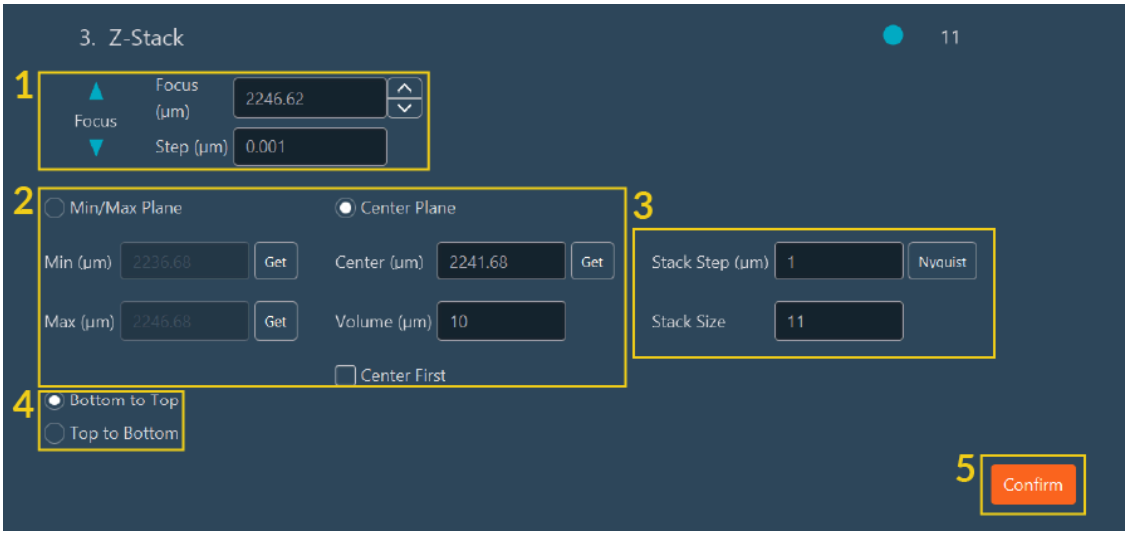

15. Select if you want to use or not the current Z to adjust the FocusMap.




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

### *1.1.5.1.6. Z-stack*

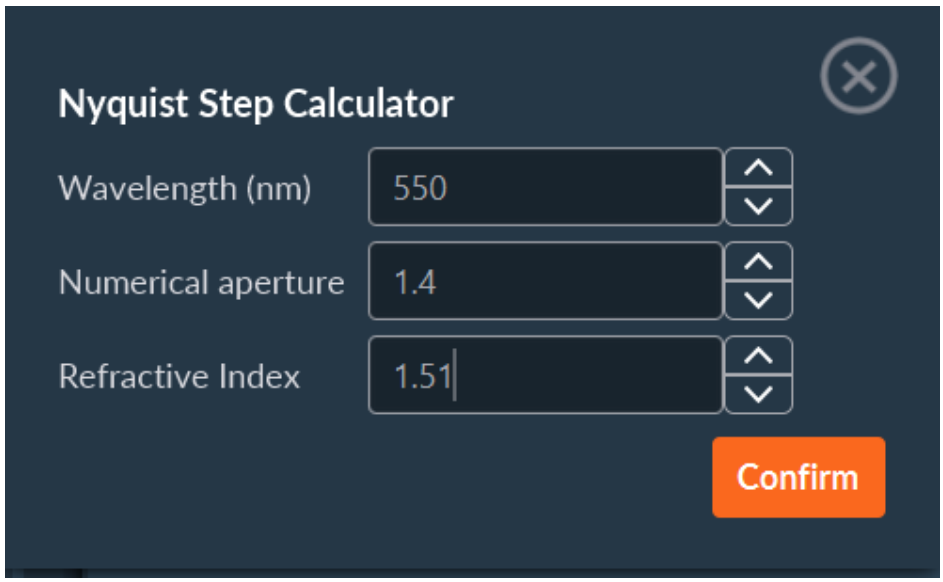
The volumetric imaging with Inscoper I.S. can be performed using the Z-Stack dimension.




1. If necessary adjust the focus and the step.
2. Select the mode you want to use:
   - Set the **Min/Max Plane** (limits of your stack) by clicking on the **Get** button.
   - Set the **Center Plane** (middle of your stack) by clicking on the **Get** button, setting up the **Volume** of your ZStack and, if necessary, checking the option **Center First**.
3. Indicate the **Stack Step** (between 2 focal plans) and the **Stack Size** (number of plans).

NB: If you set your ZStack with the center plan, the Volume, Step and Size are linked.

NB: For the step size you can help you with the Nyquist Step Calculator: Indicate the emission wavelength, the numerical aperture of your objective and the refractive index of your objective immersion medium. Once all information is set up, click on **Confirm** to validate your settings. The step size will be automatically calculated.

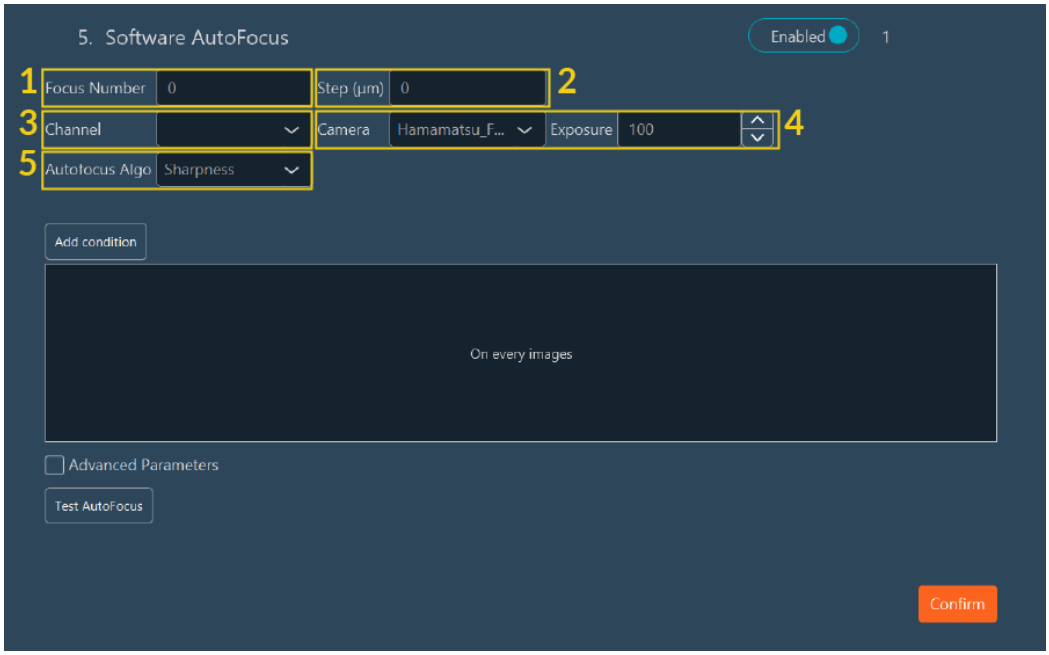



4. Specify the desired scanning direction: **Bottom to Top** or **Top to Bottom**.
5. Once you have finished editing the dimension, click on the **Confirm**.

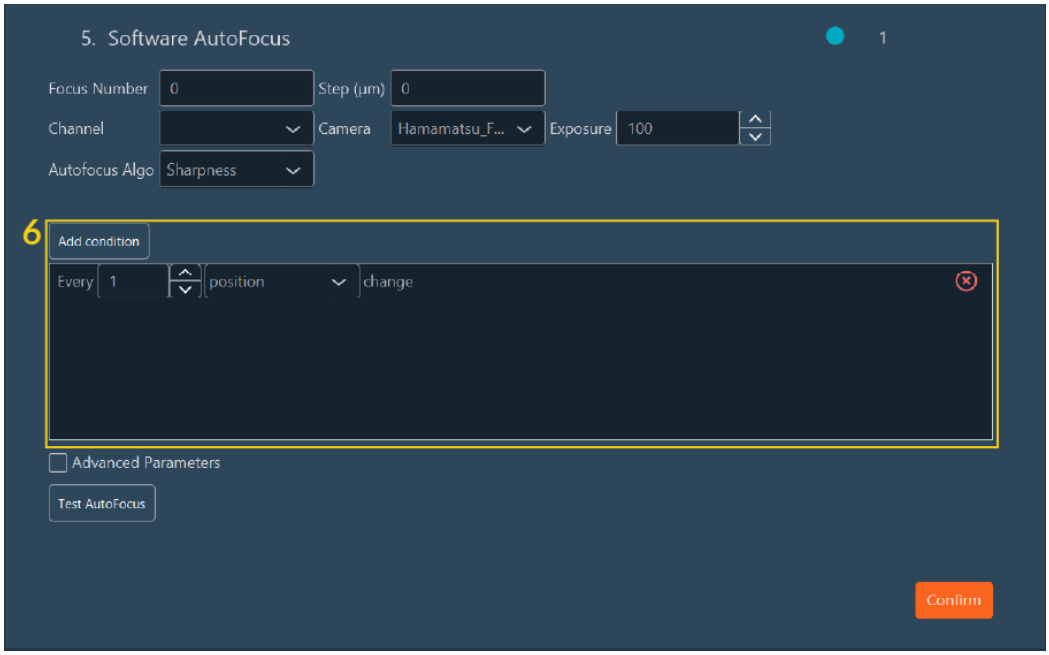
#### *1.1.5.1.7. Software autofocus*

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

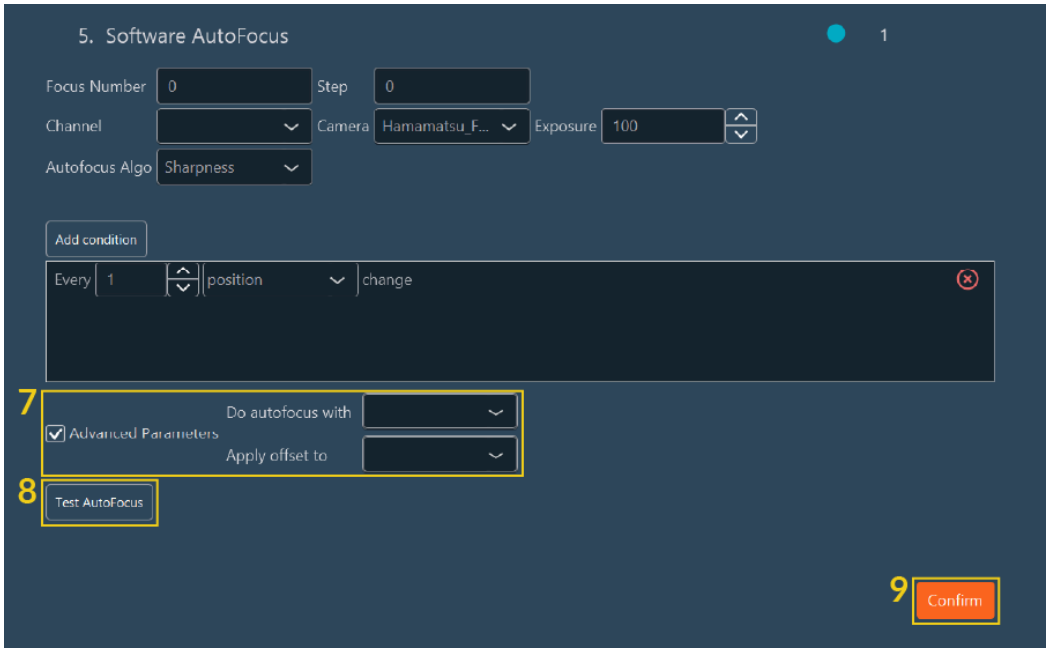
1. Select the number of focus to perform to each position.
2. Select the step (µm) between each focus.
3. Select the channel to use.
4. Select the camera to use and its exposure time.
5. Select the autofocus algorithms to be applied: maximal intesity or sharpness (the best focus plane will be the sharpest).




6. Add Condition: interval of autofocus depending on dimension (for example, every 3 time points).



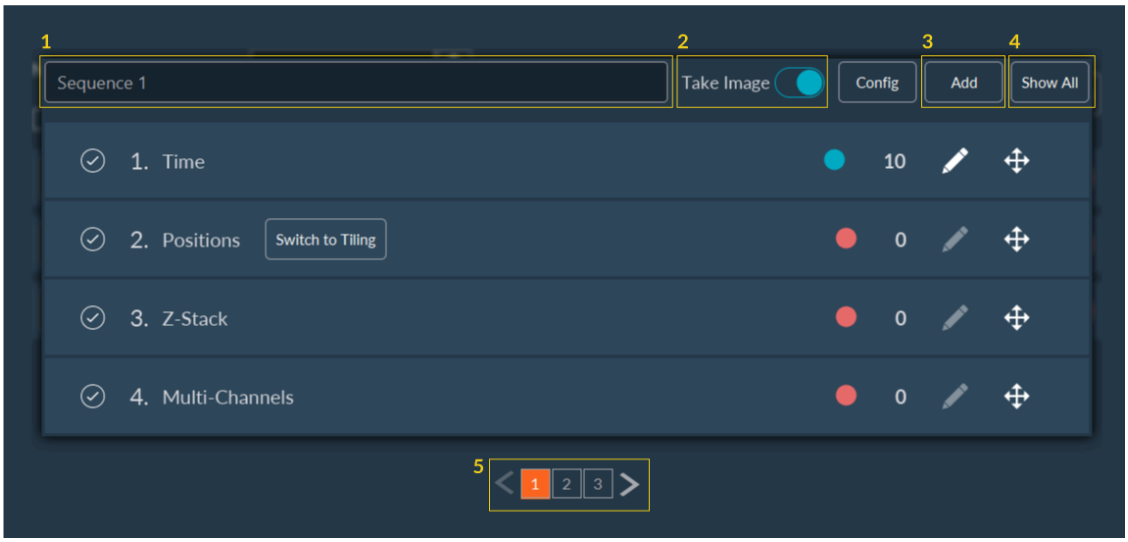

7. Check **Advanced Parameters** to set up the device to do the autofocus (for example, the piezo or the focus of the microscope) and if you need to apply offset to another device.
8. Test the autofocus protocol.
9. Click on **Confirm**.




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

### *1.1.5.1.8. Multi-sequence acquisition*

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




Each sequence has several options for setting up:

1. You can assign a name to a sequence to make it easier to identify.
2. Disable the image acquisition for a specific sequence by deactivating the "Take Image" button.
3. Add a new sequence to the multi-sequence acquisition.

4. You can have a synthetic view of the different sequences already prepared.
5. You can seamlessly navigate between sequences. The selected sequence is highlighted in orange.

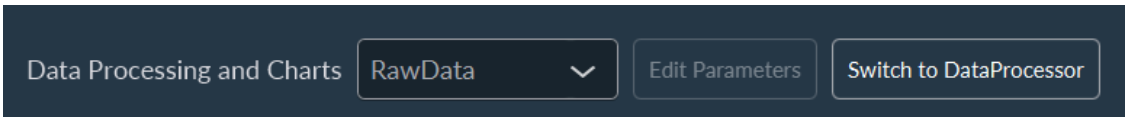
In the summary tab below you have the following options:

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

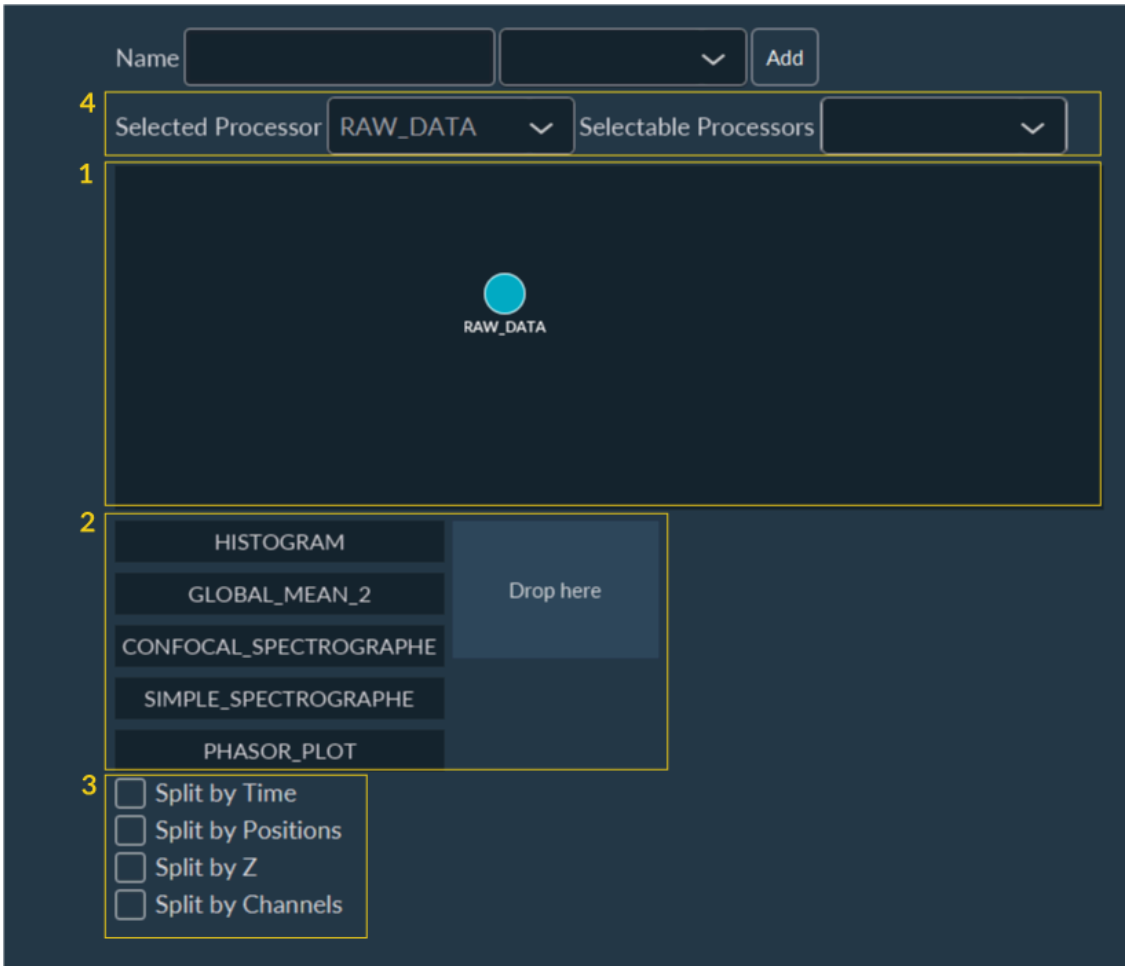

### *1.1.5.1.9. Data Processor*

#### *1.1.5.1.9.1. 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.



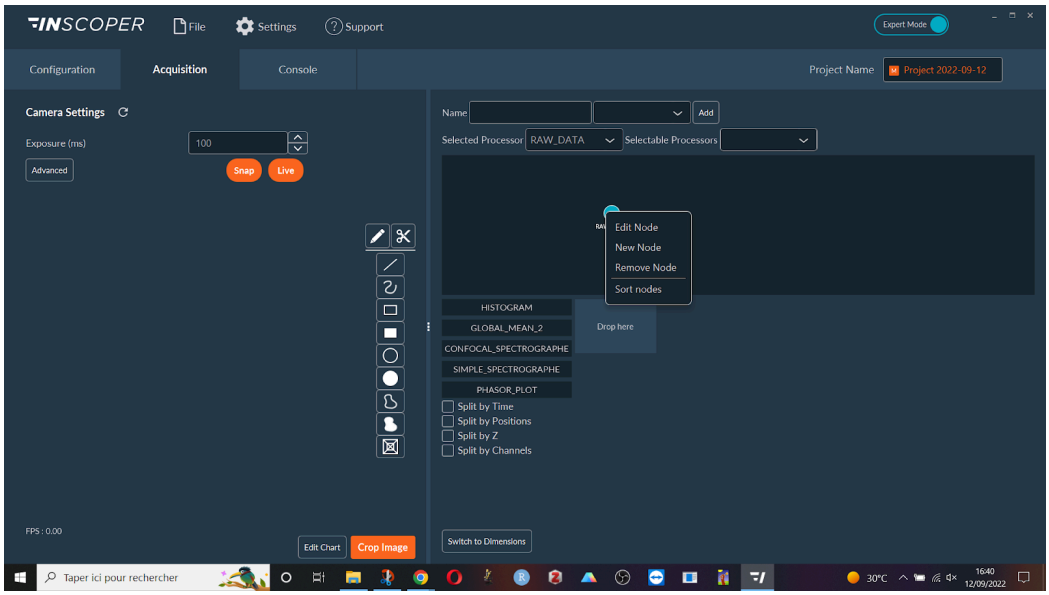

1. Space for creating data processing workflow.
2. Data visualization customizing tools.
3. Tools for analyzing data after splitting by dimension.
4. Tools to customize the image visualization after pre-treatments:

> 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.

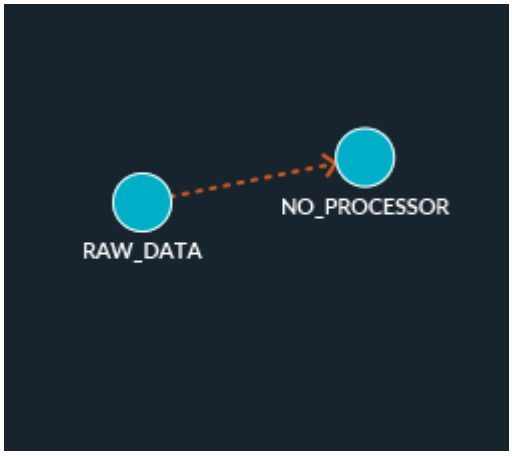
### *1.1.5.1.9.2. Create a new data processing workflow*

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



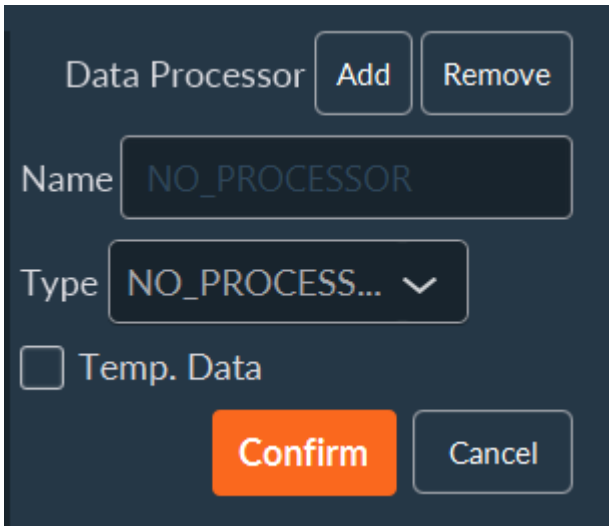

2. A new node will appear on the screen. At the moment there is no treatment associated with this node. To add one, double click on it or right click and select **Edit Node**.

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




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

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




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

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

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

### *1.1.5.1.9.3. Data visualization personnalization*

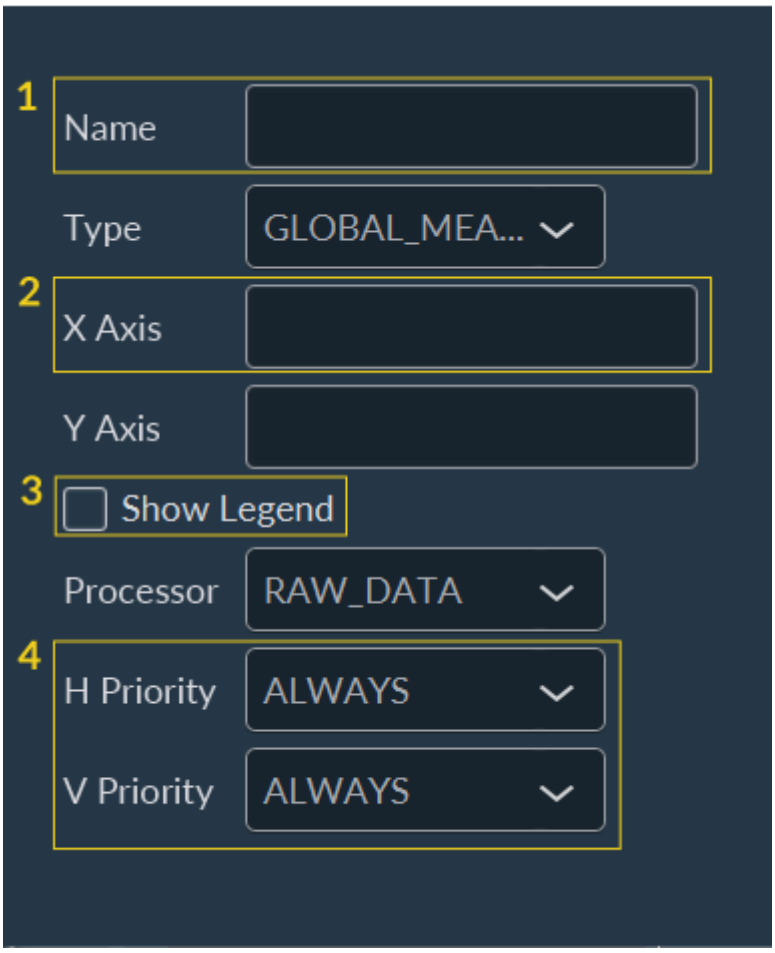
You can customize the display of the data during the acquisition, such as the evolution of the fluorescence intensity for example.


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.

### *1.1.5.1.10. Saving images and metadata*

#### *1.1.5.1.10.1. Interface presentation*


To save the acquired images and metadata:

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

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

## 1.1.5.2. Data export

### *1.1.5.2.1. Video exportation*

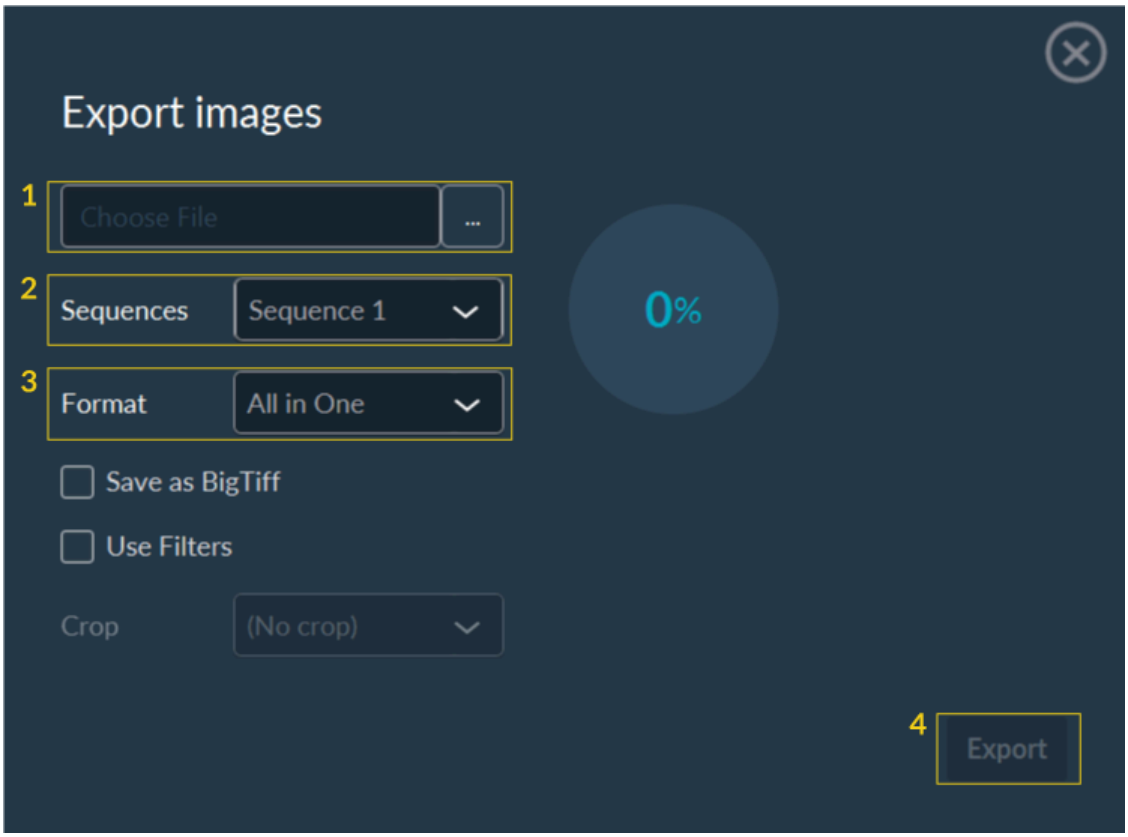
It is possible to export a video from the Inscoper I.S.. Select with filters all images to put together and click on **Video** of the "Export" list.

To export a video:

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

### *1.1.5.2.2. Stack exportation*

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

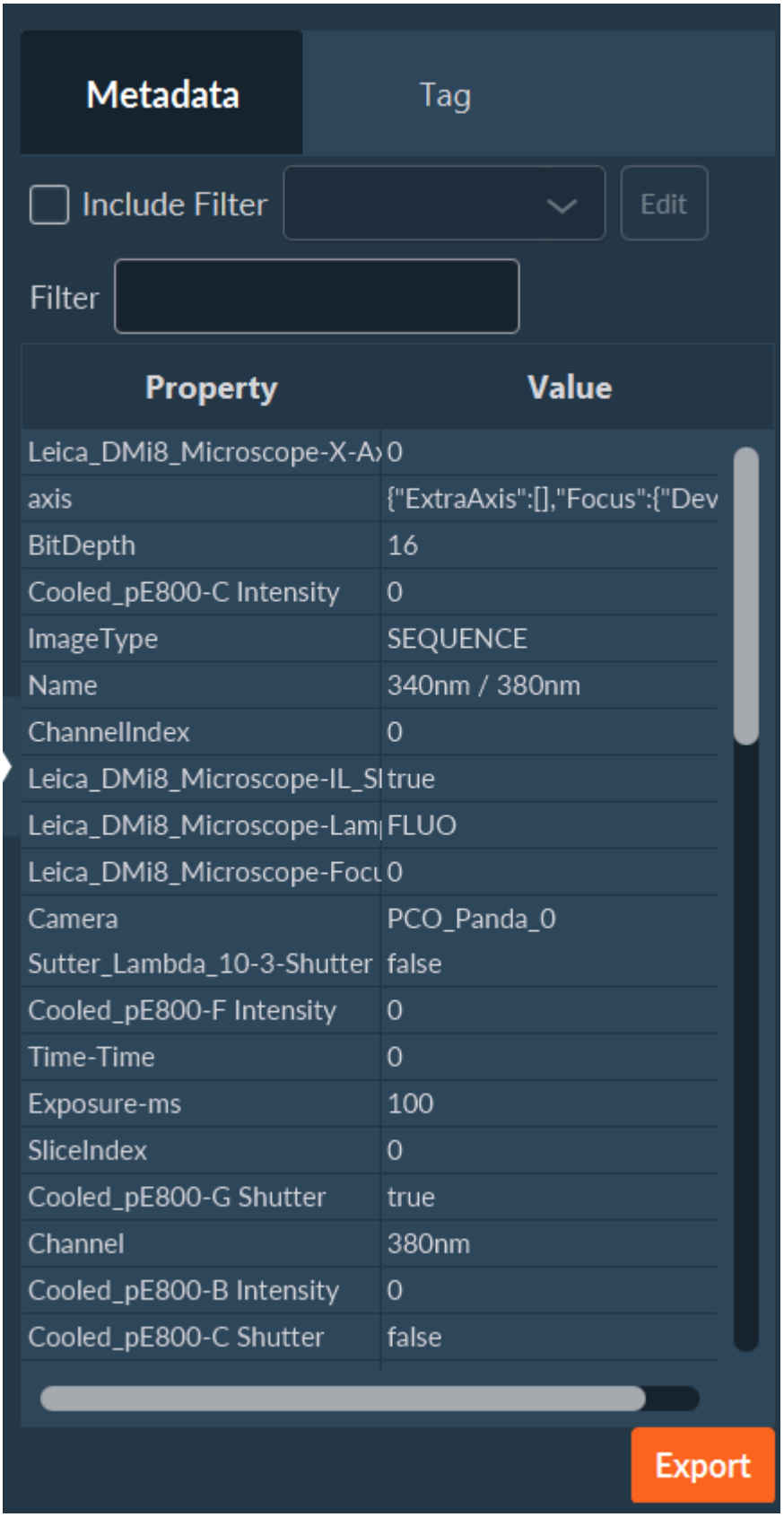



To export a stack:

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

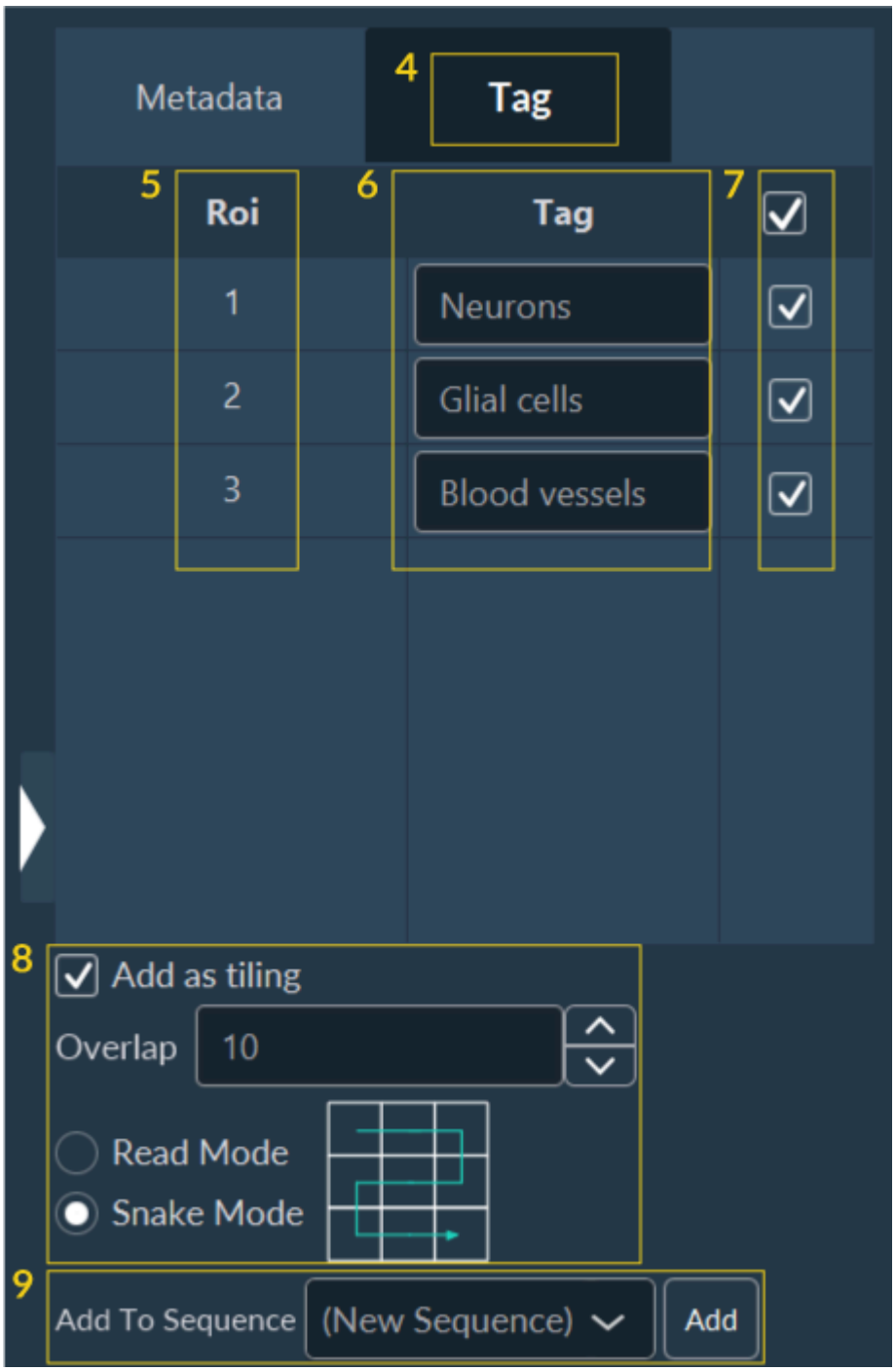
#### *1.1.5.2.3. Metadata access*

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


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

### 1.1.5.3. Semi-automated feedback microscopy feature

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




To use this feature:

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

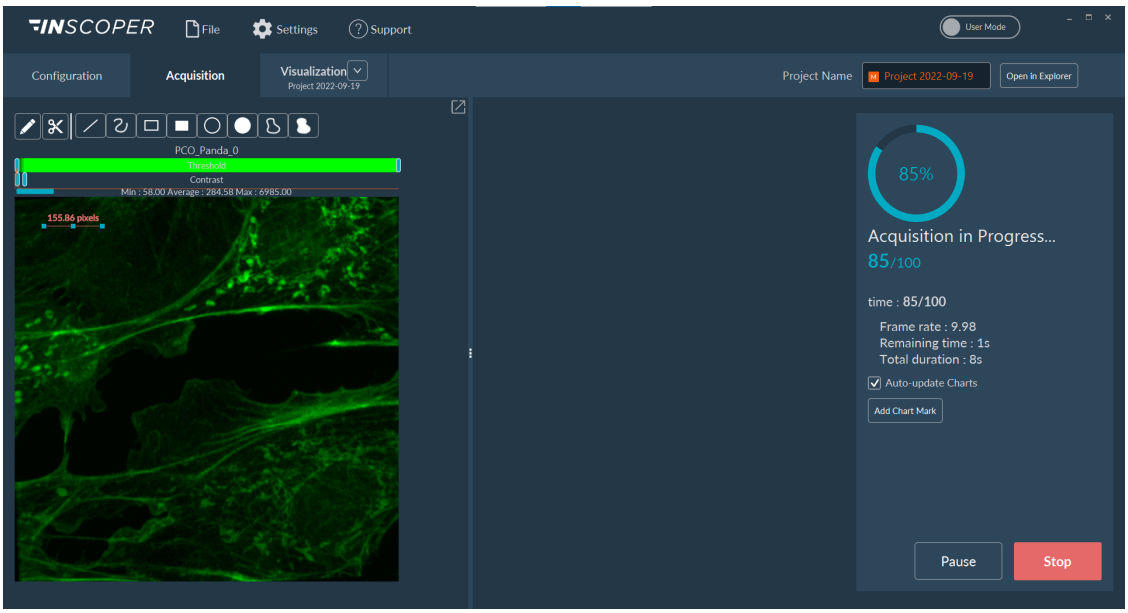
3. In the **Visualization** tab, click on the white triangle on the right of the window to open the **Metadata** tab.
4. Select the **Tag** tab.
5. Here all ROI are presented. You can add, edit or remove ROI according to your needs.
6. Add some tag name to each ROI if necessary.
7. Select ROI of interest.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

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

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

## 1.1.6. Visualization

### 1.1.6.1. Visualization during acquisition

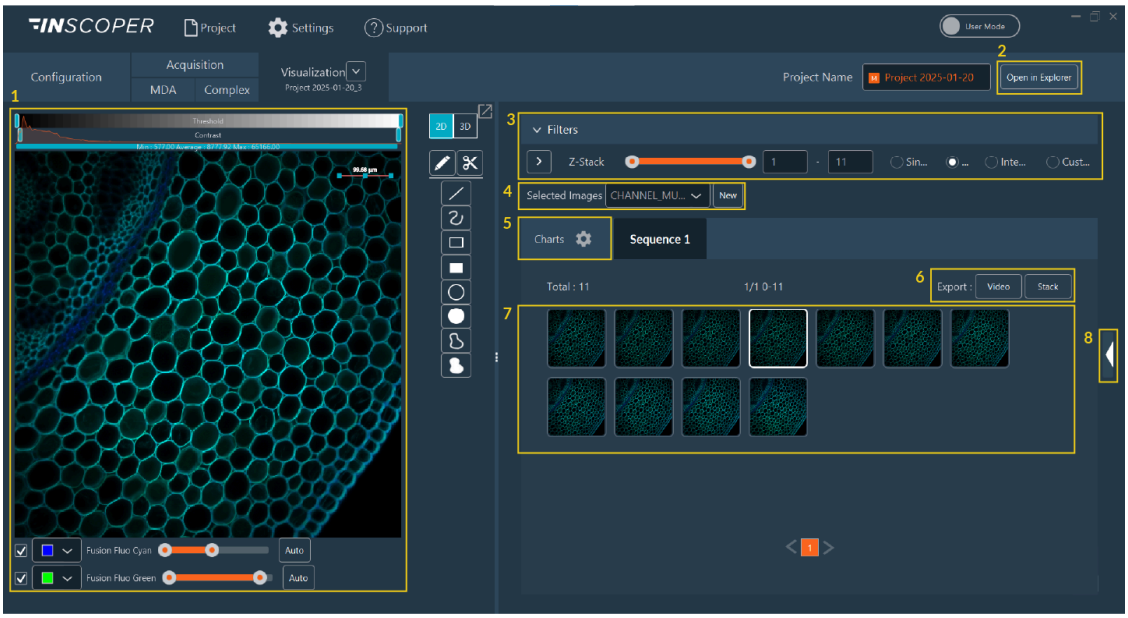



You can monitor the acquisition sequence while it is running. The left part of the window shows the current images of the sequence. The middle part is reserved for graphics if needed (not here). And in the right part of the window, you can follow the progress of the acquisition, pause or stop the sequence.

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

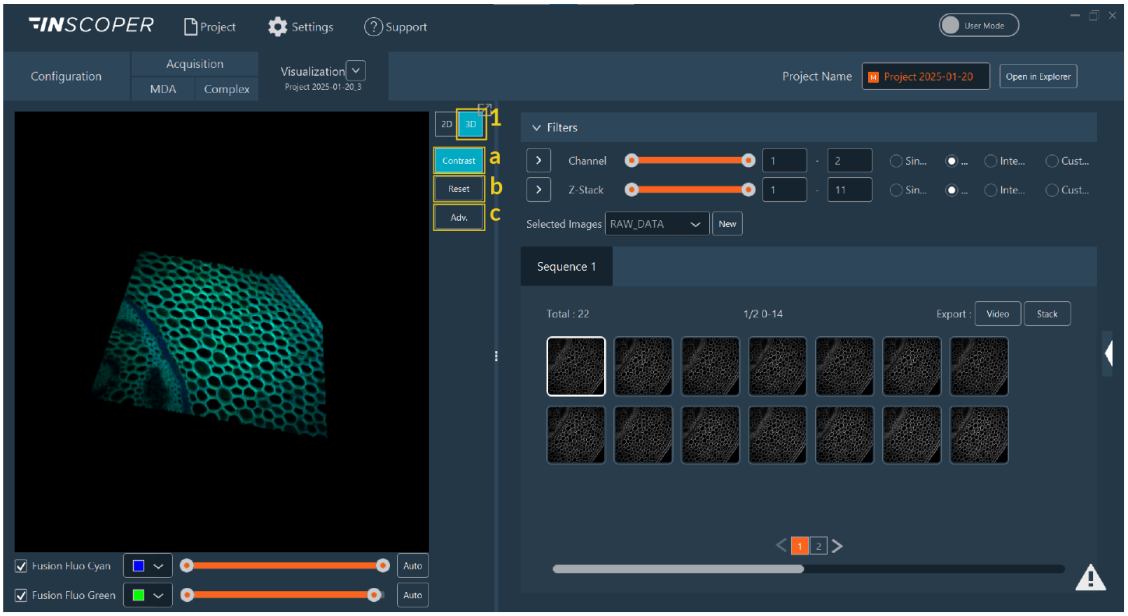
## 1.1.6.2. Visualization after acquisition

### *1.1.6.2.1. Interface presentation*

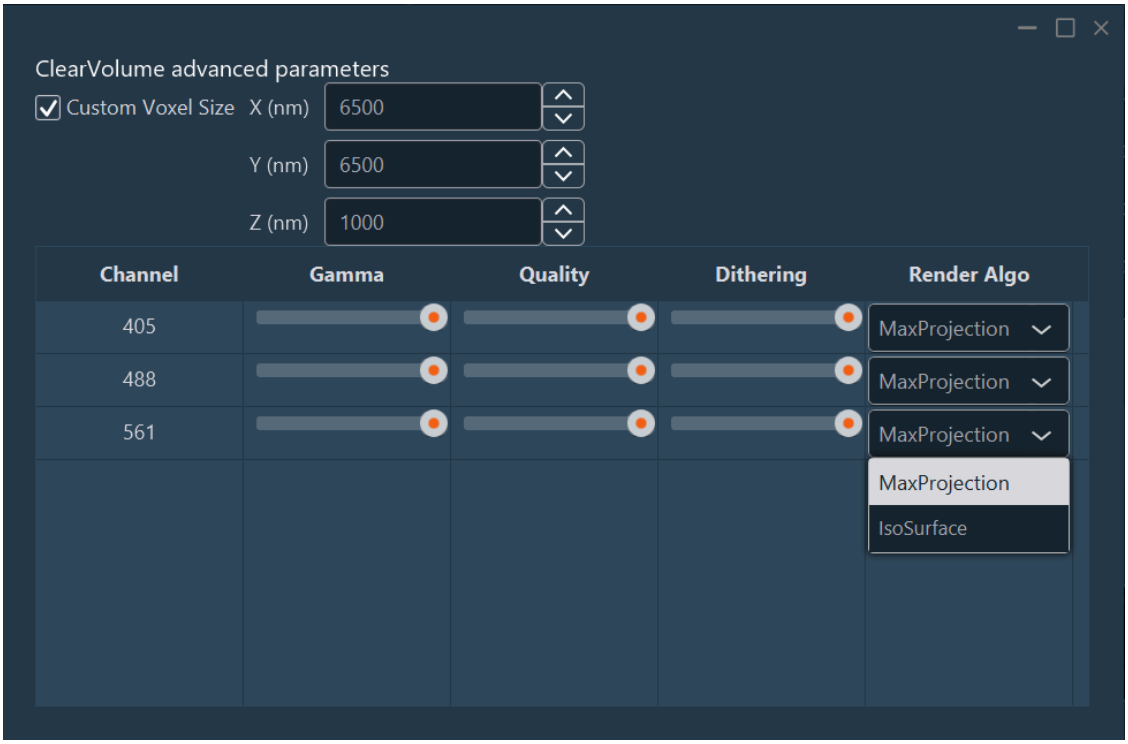



1. As in the rest of the software, this part of the screen is reserved for viewing images.
2. Opens the location where images are stored. Only works if you save your data to disk.
3. Filters to select images by dimensions.
4. Data processor access.
5. Button to switch to graphics visualization.
6. Options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

### *1.1.6.2.2. 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.

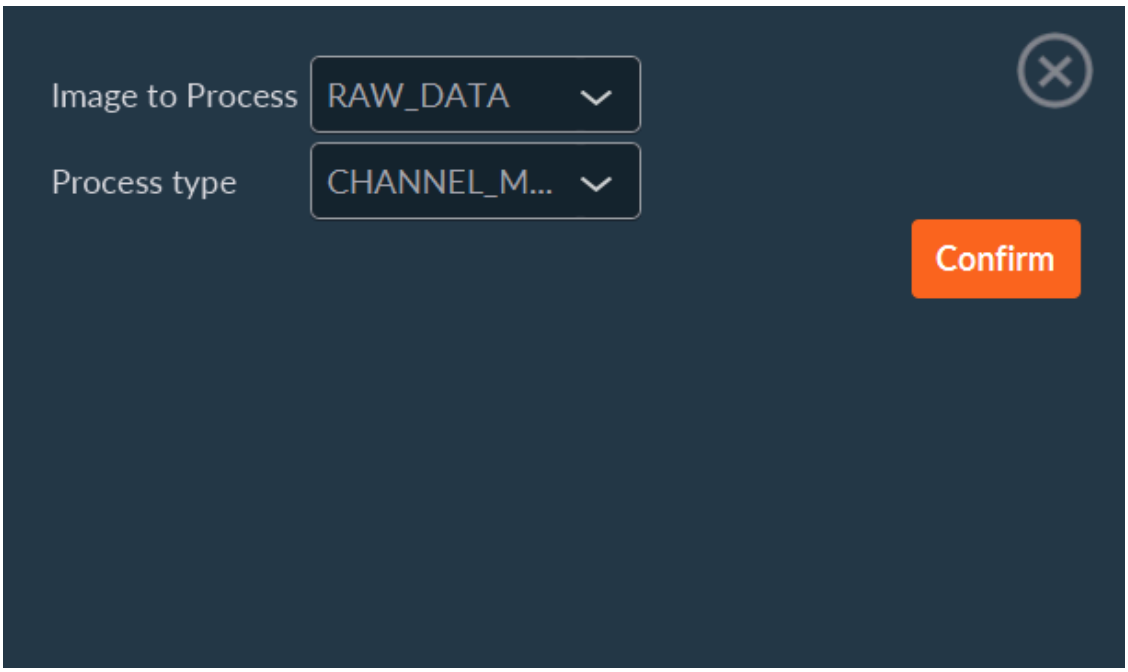



### *1.1.6.2.3. Interacting with filters*


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

### *1.1.6.2.4. Image Processing*

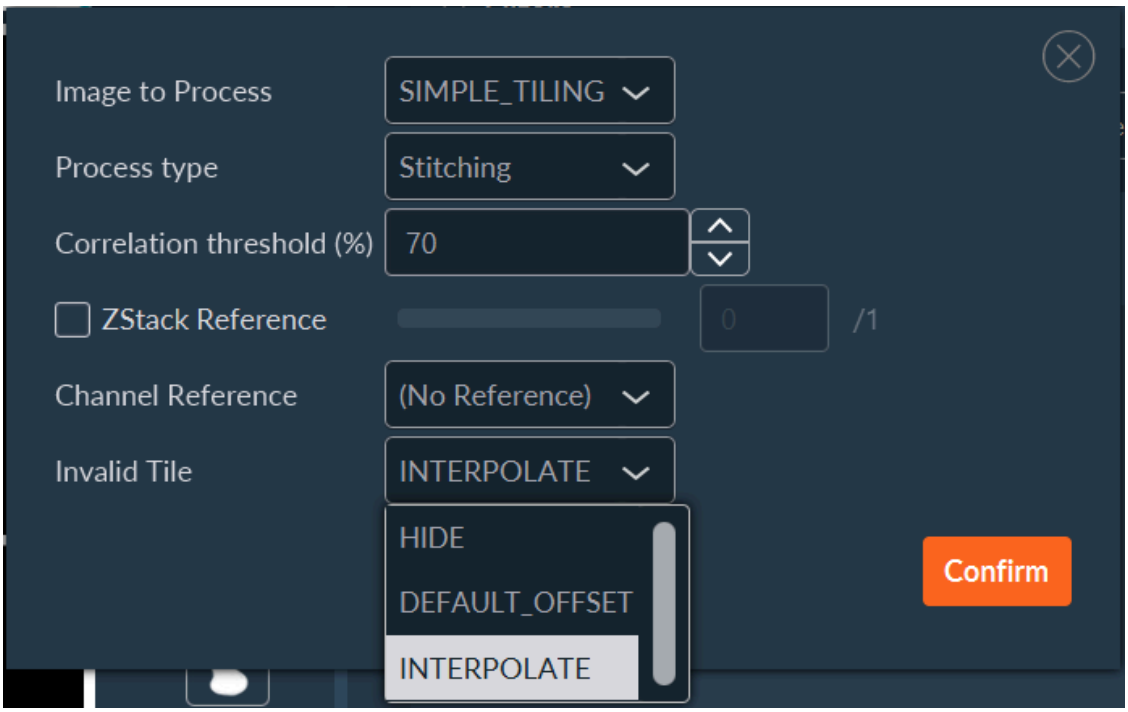
In this window you can process images acquired with processors of your choice.




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:



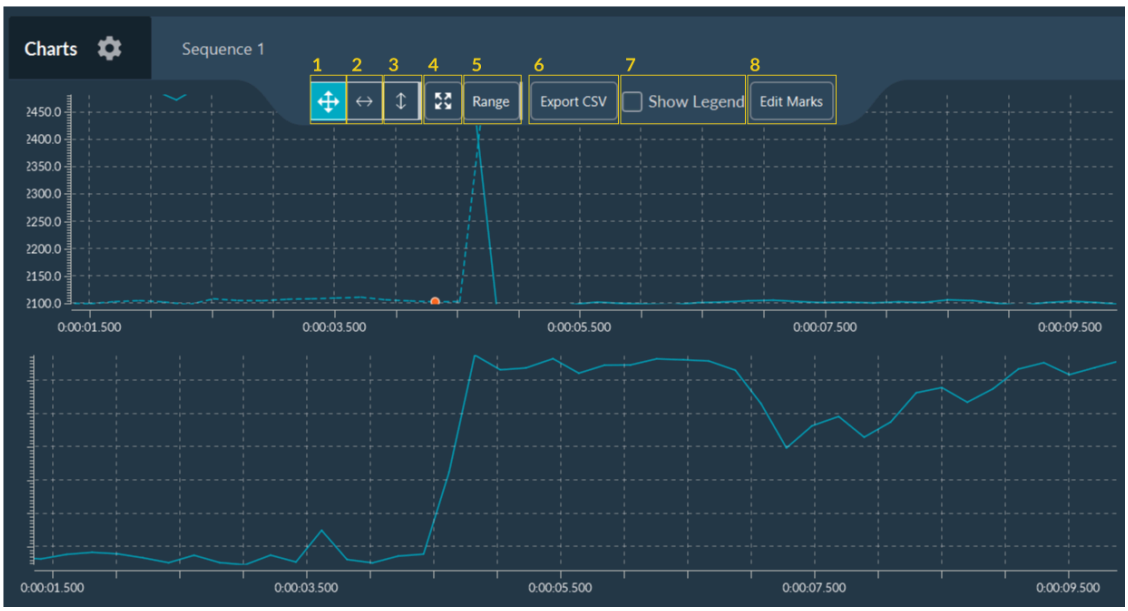
1. Select the image to process.
2. Select the process type.
3. Indicate the correlation threshold (%).
4. Check if you want to apply ZStack reference and select the good plane to calculate the stitching.
5. Select the Channel Reference if needed.
6. Select the action to apply to the invalid tile:
  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

### *1.1.6.2.5. 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.

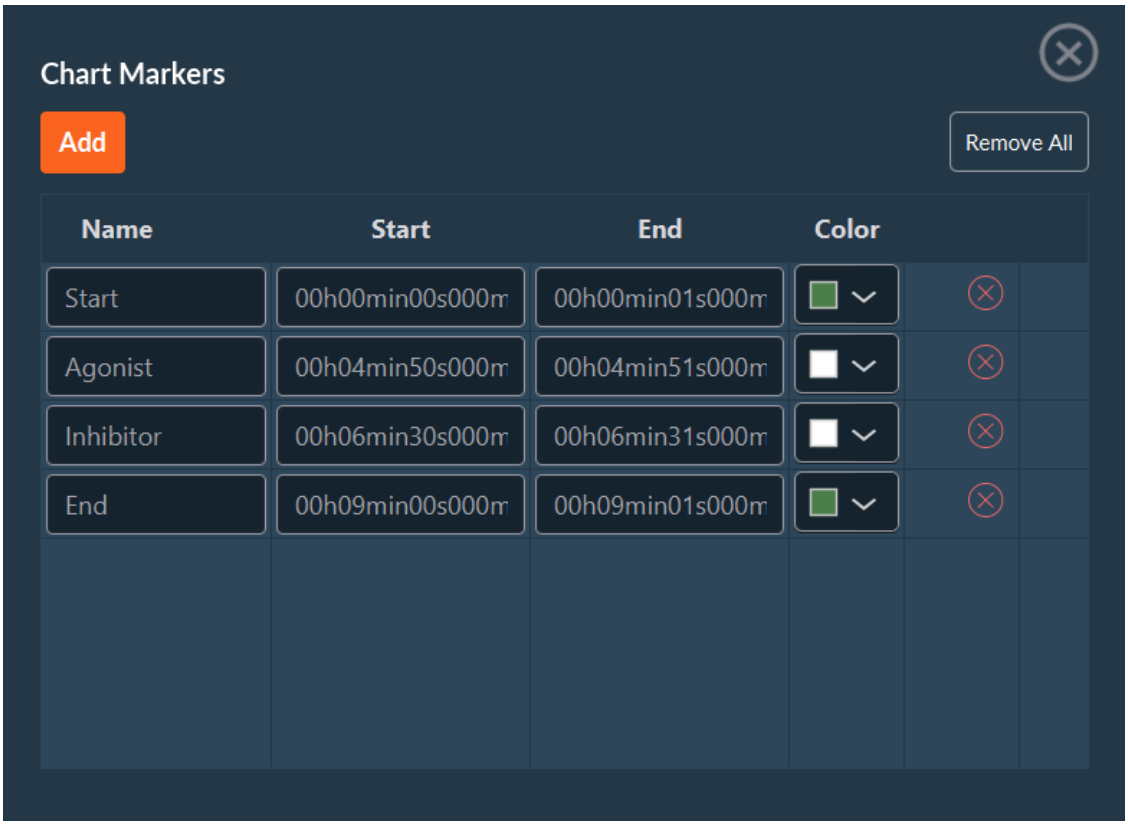
It is possible to:

- 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.

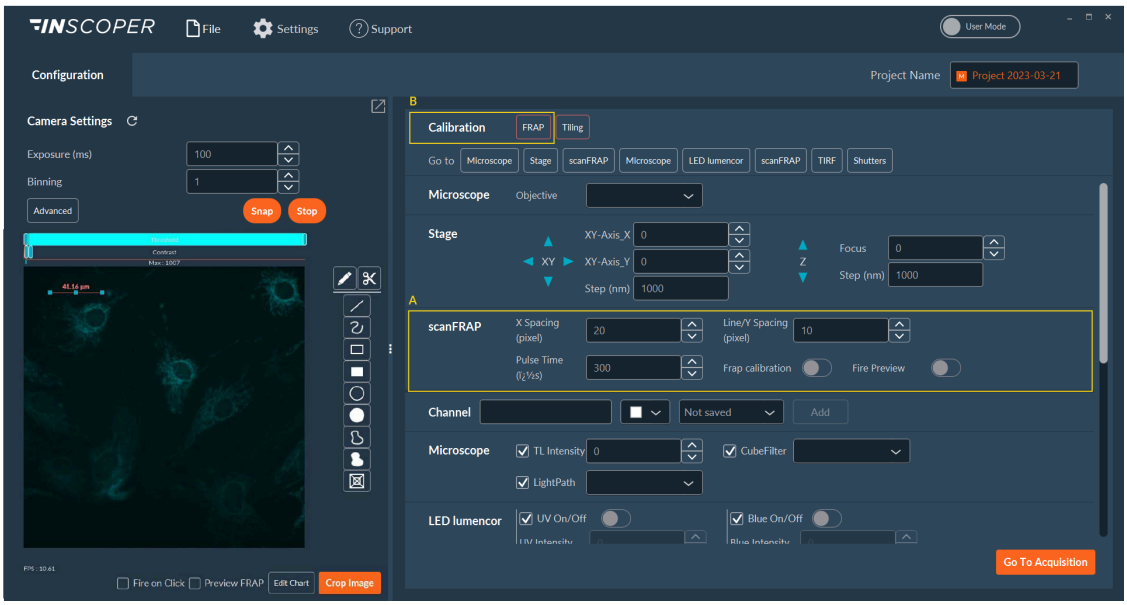



In this window you can add information about your experiment as markers. These events, which are fully customizable, can be associated with the acquisition itself (start, pause, end), external events (addition of an inhibitor, medium supplementation), or others. These markers can be saved in a .csv file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

## 1.2. scanFRAP

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

## 1.2.1. 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.

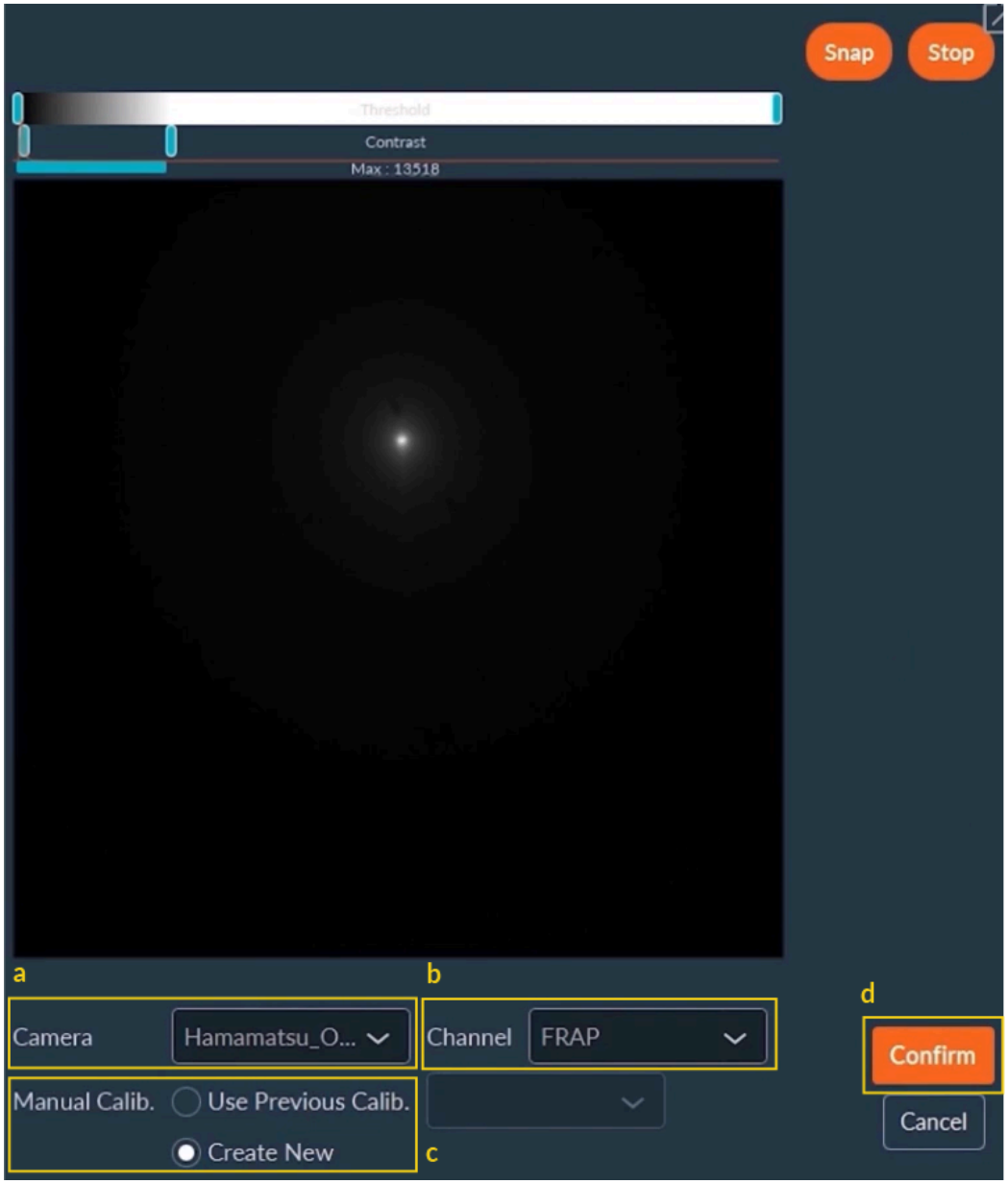
NB: The **Fire Preview** option requires (i) some ROI to be drawn on the image and (ii) the correct channel to be selected.

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.

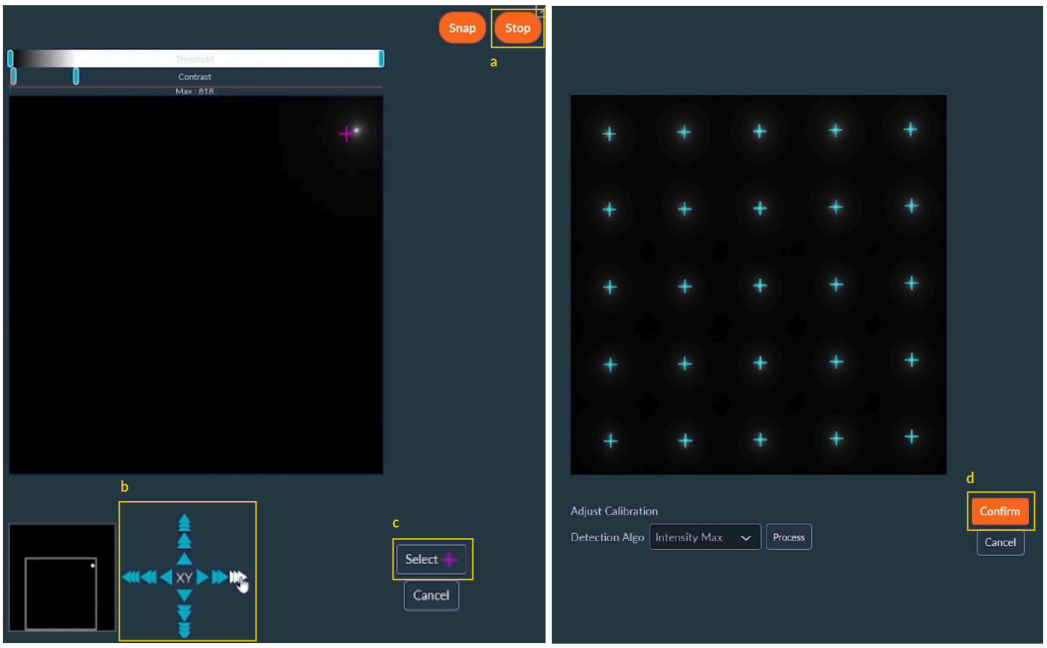
a. Click on **Live** if it is not already done (then you see **Stop**).

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.



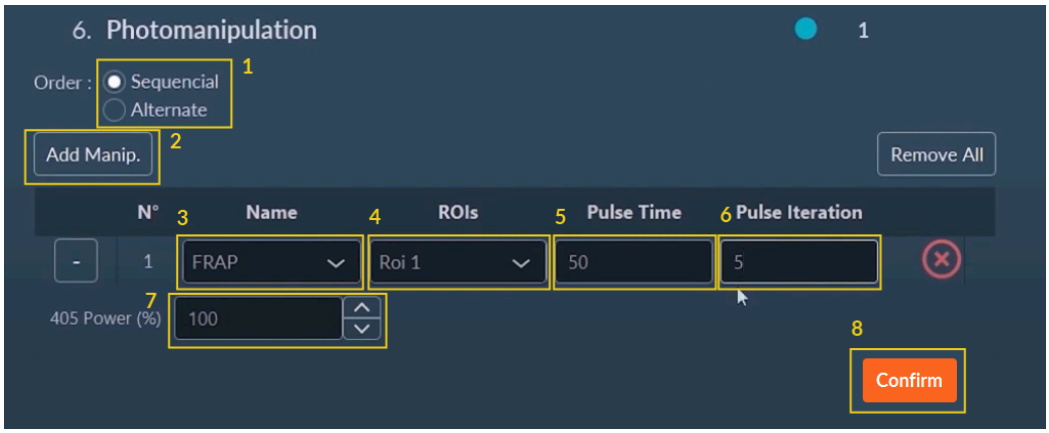

NB: To validate the calibration, do not hesitate to do some tests. For this, place some ROI on the live image and click on **Fire Preview**.

## 1.2.2. 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.

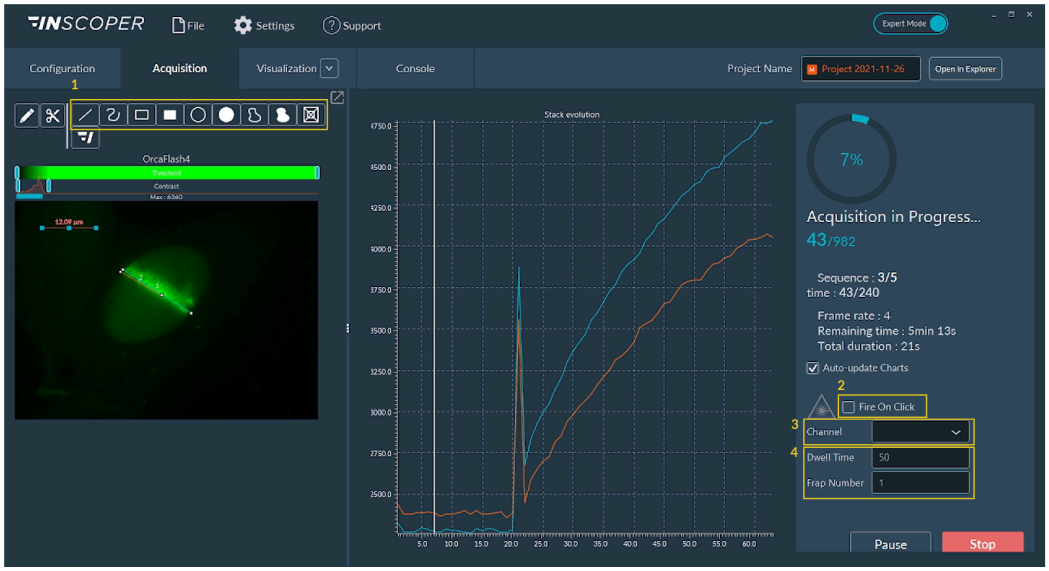



NB: Before starting a photomanipulation experiment, be sure that the scanFRAP is well calibrated.

## 1.2.3. Acquisition in progress

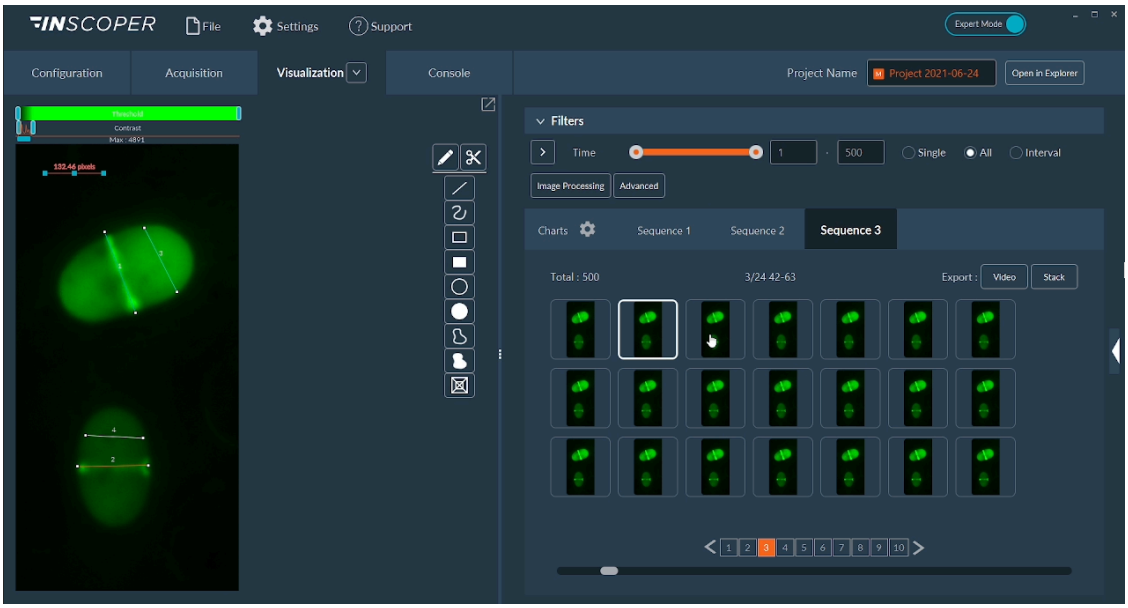
During acquisition, you can monitor the progress of your acquisition in real time. You will see the current images and graphs showing the raw data of each ROI. You can also add new area to photomanipulate during the sequence with the **Fire On Click** feature. To use the **Fire On Click**:

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.




## 1.2.4. Visualisation

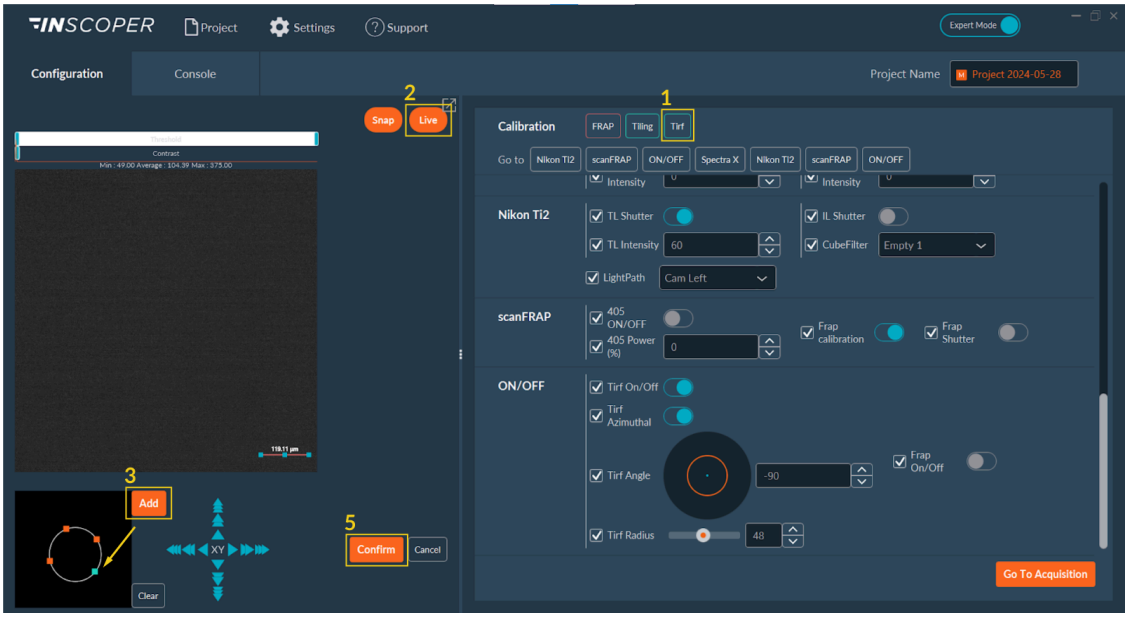
In the **Visualisation tab**, you will see the result of all sequences and the graph to follow your fluorescence intensity in the ROI.



# 1.3. TIRF

Total Internal Reflection Fluorescence

## 1.3.1. 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.

## 1.3.2. 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.

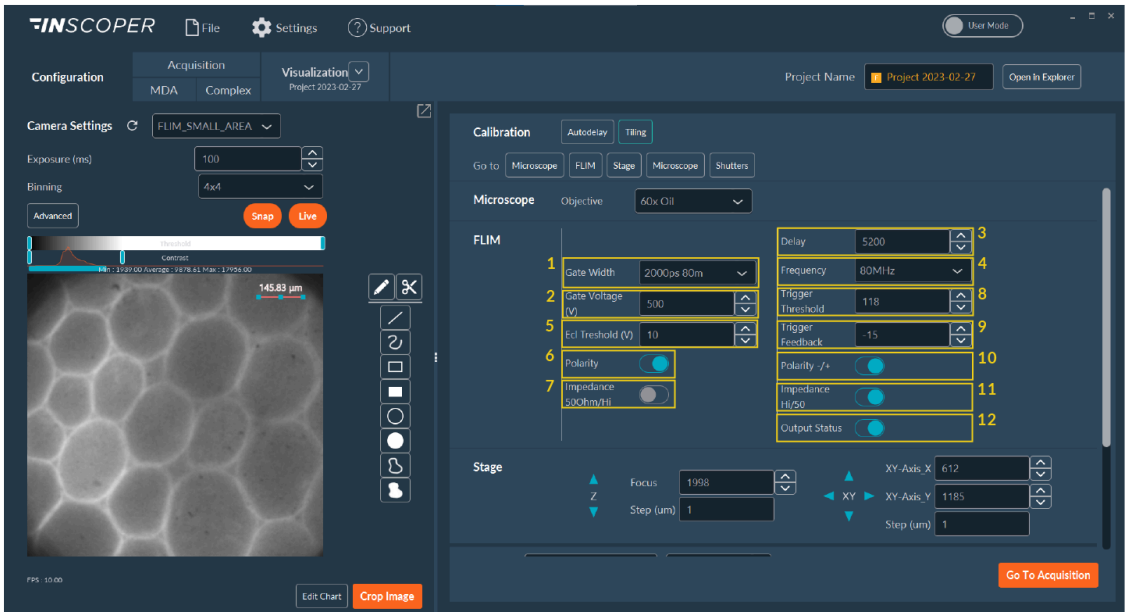
NB: Those parameters (Tirf Azimuthal or point, Angle and Radius) need to be saved in the Tirf channel for Tirf Imaging

## 1.4. 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.

## 1.4.1. Configuration




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

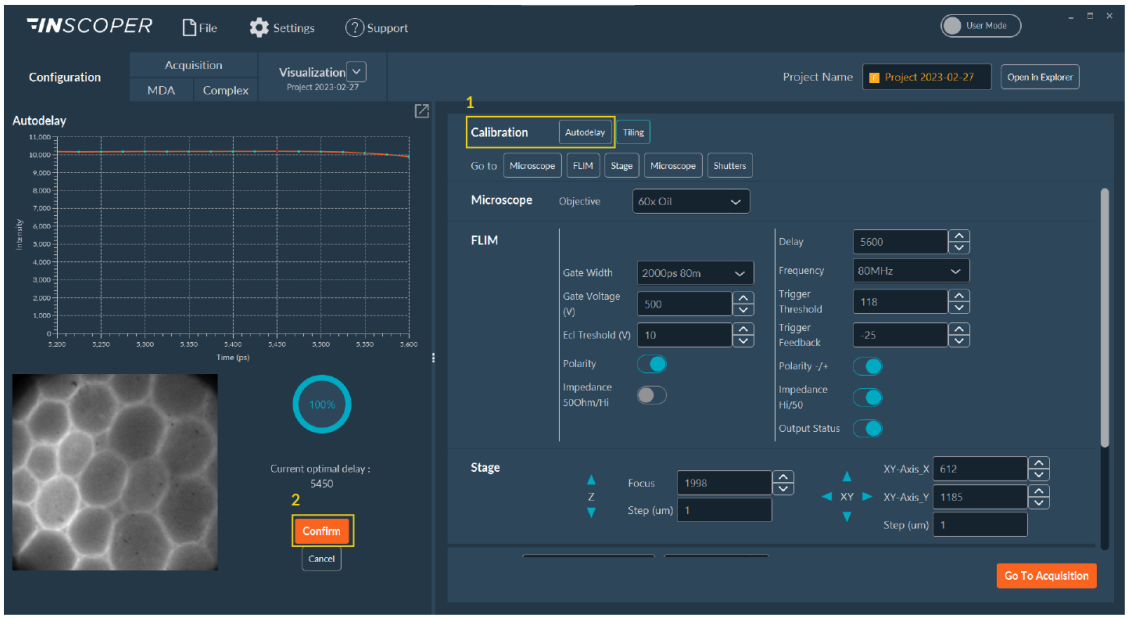
1. **Gate Width:** Width of the temporal gate.
2. **Gate Voltage:** Multichannel Plate (MCP) Voltage to amplify the input signal.
3. **Delay:** Time in picoseconds which corresponds to the delay generated regarding the synchronization signal of the pulsed laser.
4. **Frequency:** Frequency of the delay 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.

### 1.4.1.1. 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**.

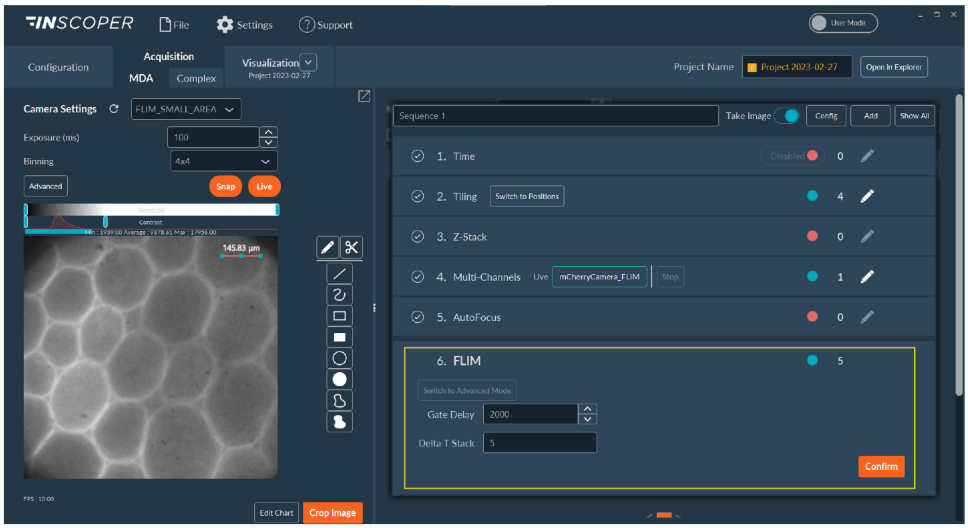
## 1.4.2. Acquisition

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

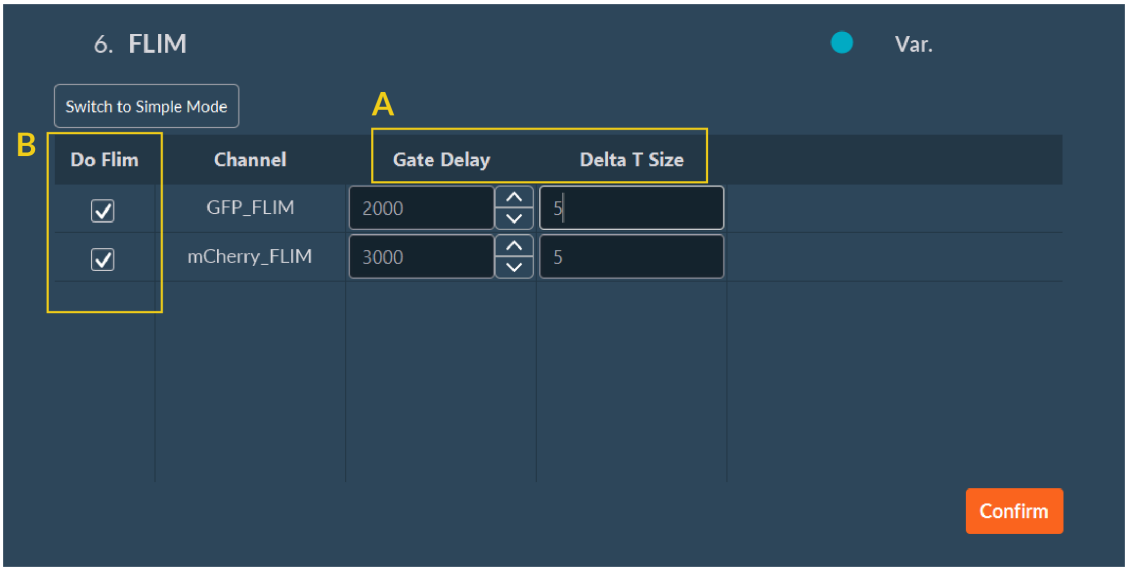
You will need to set your channel using the Multi-channel dimension. Thus you can set the FLIM and fluorescence channels.

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).

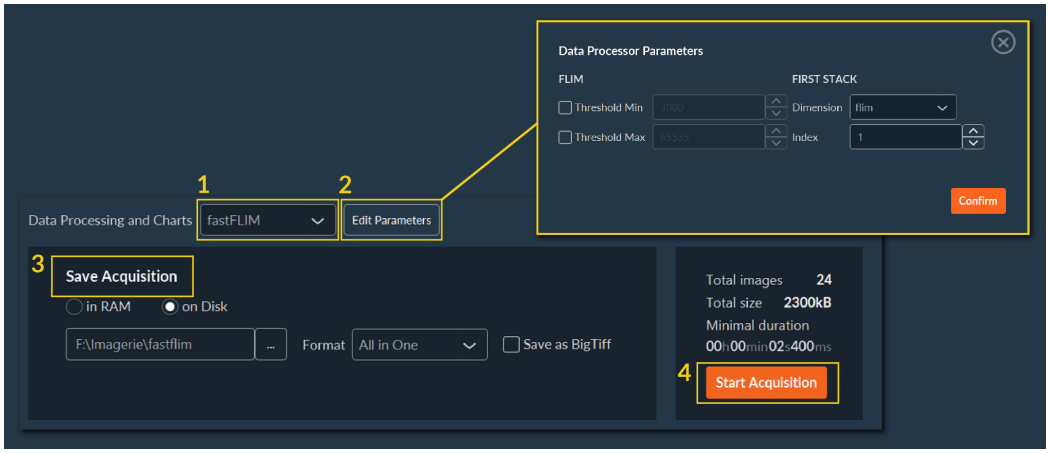



- **Advanced mode:** if you have more than one FLIM channel during acquisition. In this mode you can set:
  A. a specific **Gate Delay** and **Delta T Size** for each channel.
  B. Select the channel on which you wish to perform the FLIM measurement by checking the **Do Flim** box.




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**.




## 1.4.3. 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.

## 1.5. 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.

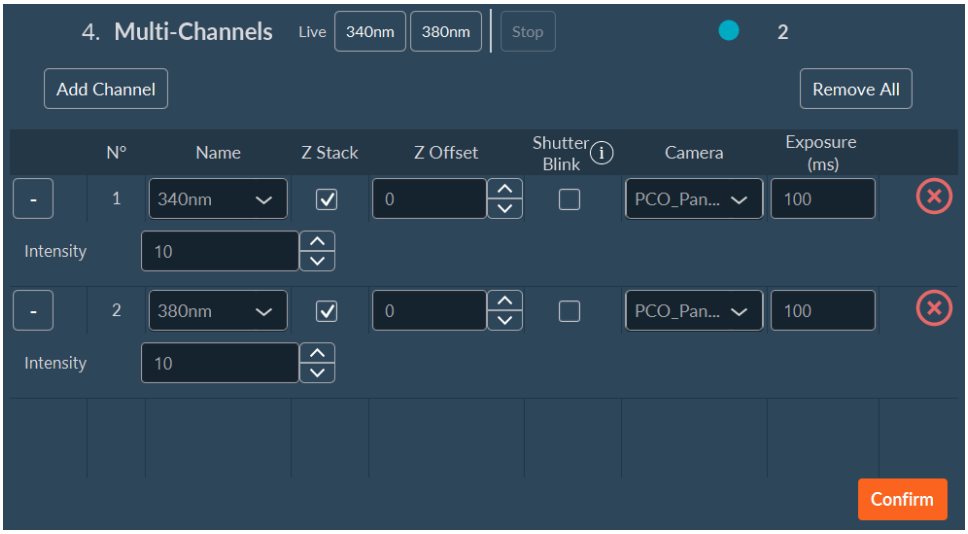
### 1.5.1. Configuration

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

### 1.5.2. Acquisition

1. Wavelengths set up

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

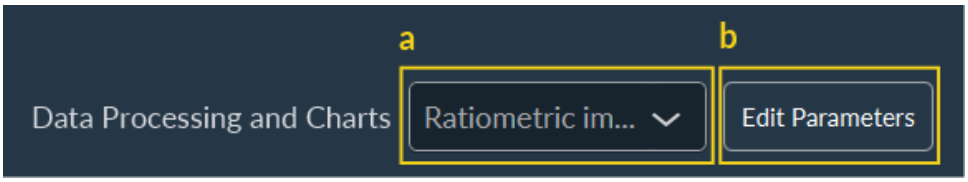



    b. Select two or more wavelengths.

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.

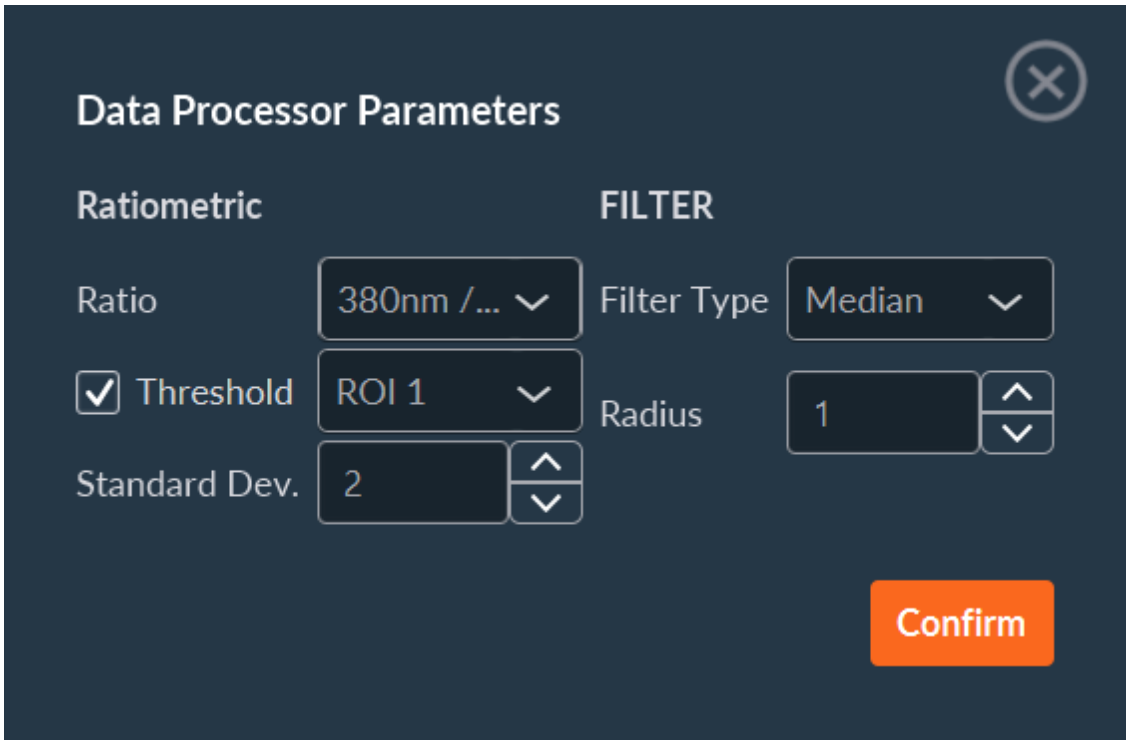
e. If checked, select a ROI without any signal.

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).

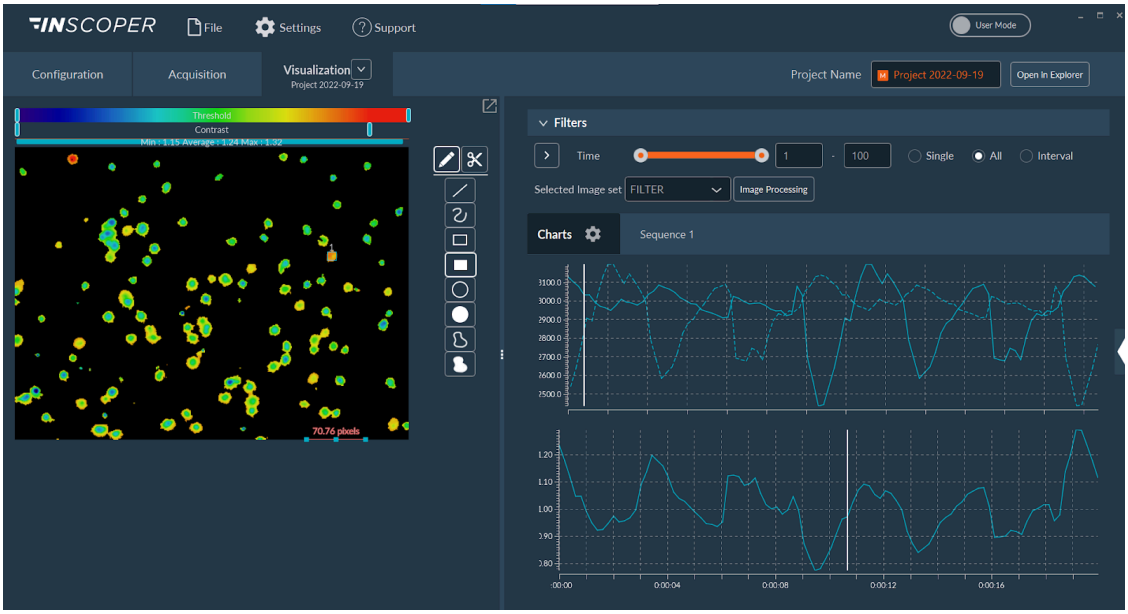
h. Select the radius to consider for this filter.

i. Click on **Confirm**.



## 1.5.3. 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.




## 1.6. 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.

## 1.6.1. MAICO I.S.: before to start

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.

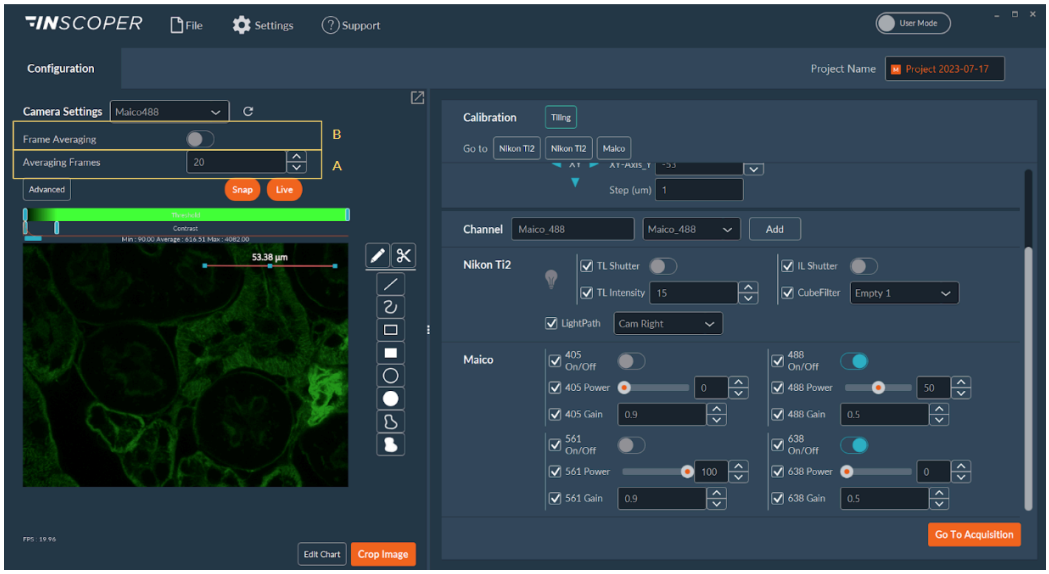
> NB: the shutter needs to be open for this step.

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.

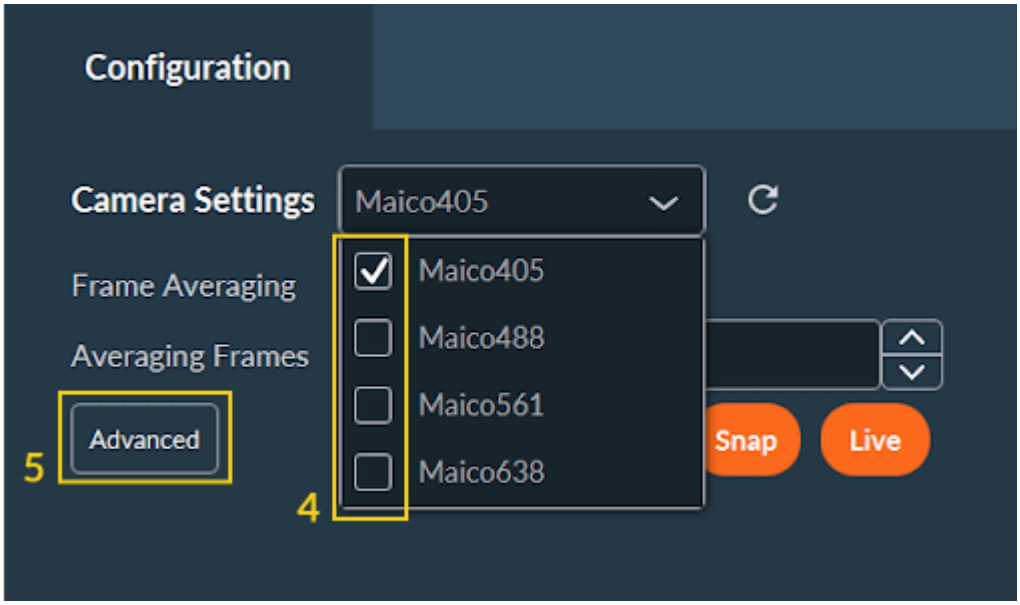
## 1.6.2. Configuration

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).

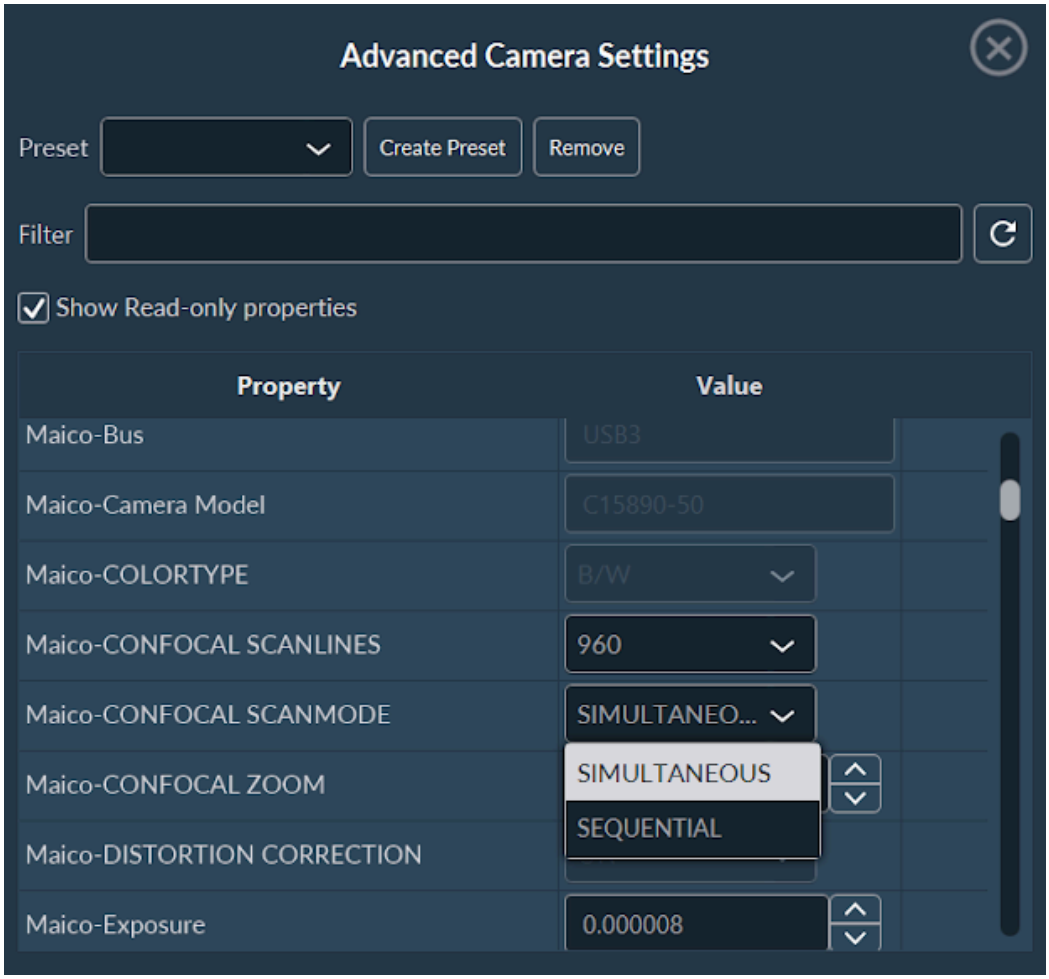
1. To do multicolor imaging, you need to create ONE channel with ALL laser lines that you will use during your acquisition even if you do sequential acquisition.
2. You can set the intensity and detector gain for each wavelength.
3. You can choose the frame averaging improve the quality of your acquisition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

## 1.6.3. Acquisition

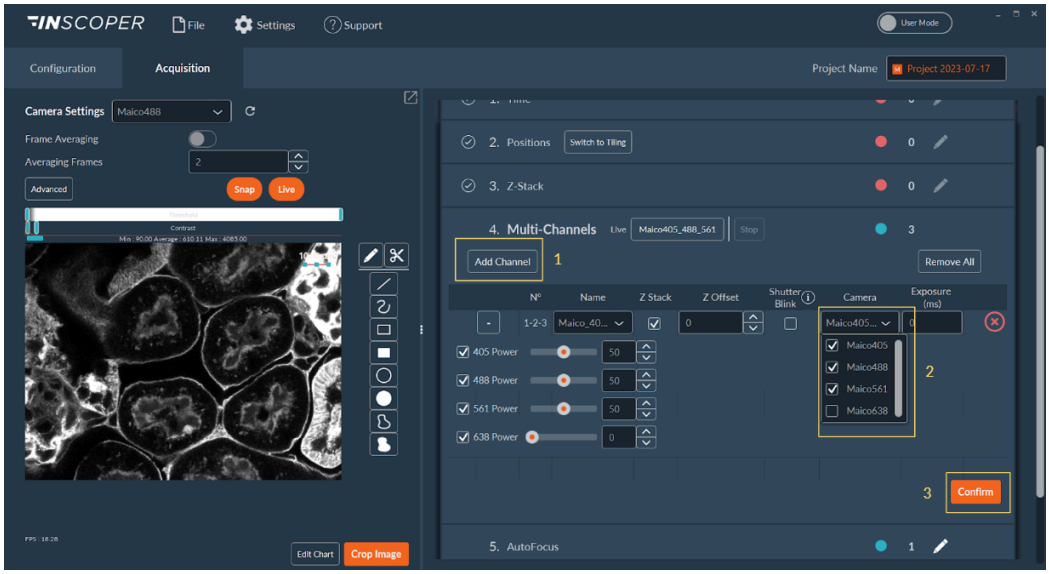
In the Acquisition tab, you can set up all your acquisition sequences as explained in the I.S. User Guide.

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

On the **Multi-channels** dimension:

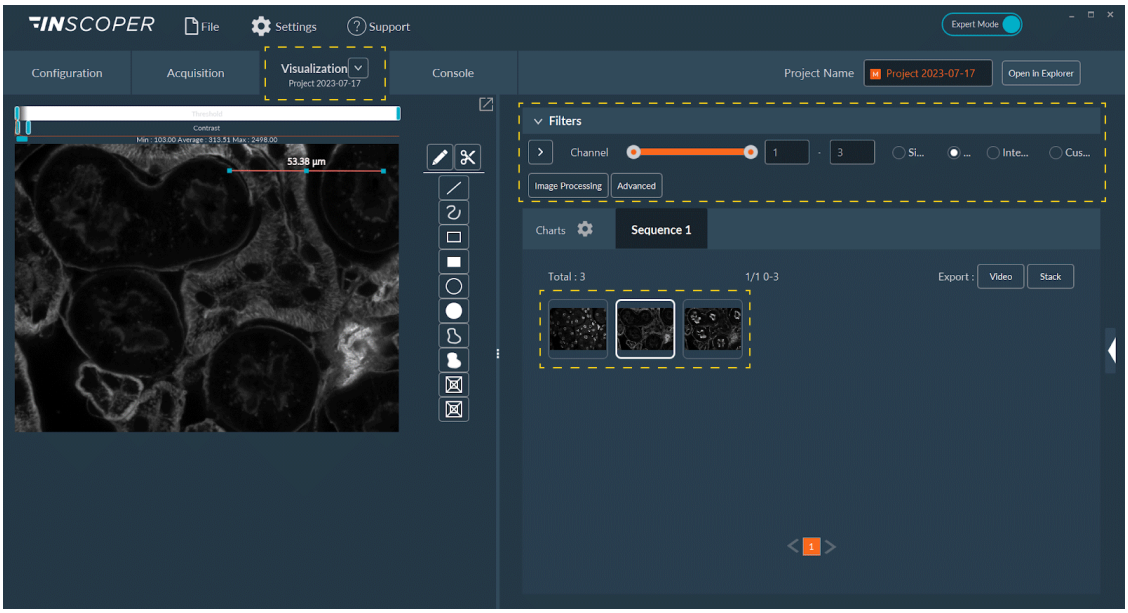
1. You can add your channel by clicking on **Add Channel**(Add your channel including all laser lines that you will use).
2. You need to check the box of the detection you want to use.

3. Click on **Confirm** to validate your multi-channel dimension.




## 1.6.4. 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).