

# User Guide

## Imaging

## Software

v9.4

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# 1. Imaging Software

## 1.1 Overview

The **Inscoper Imaging Software** (Inscoper I.S.), powered by a proprietary hardware controller, provides an integrated interface for managing optical imaging workflows.

Inscoper I.S. offers research microscopists a unified interface to manage their optical imaging systems:

- Orchestrate multi-dimensional acquisition sequences.
- Control multiple cameras and manage data streams.
- Monitor hardware status in real time.
- Manage experimental configurations via reusable projects.
- Perform basic image processing and volumetric visualization.

### 1.1.1 Prerequisites

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.

### 1.1.2 User Journey

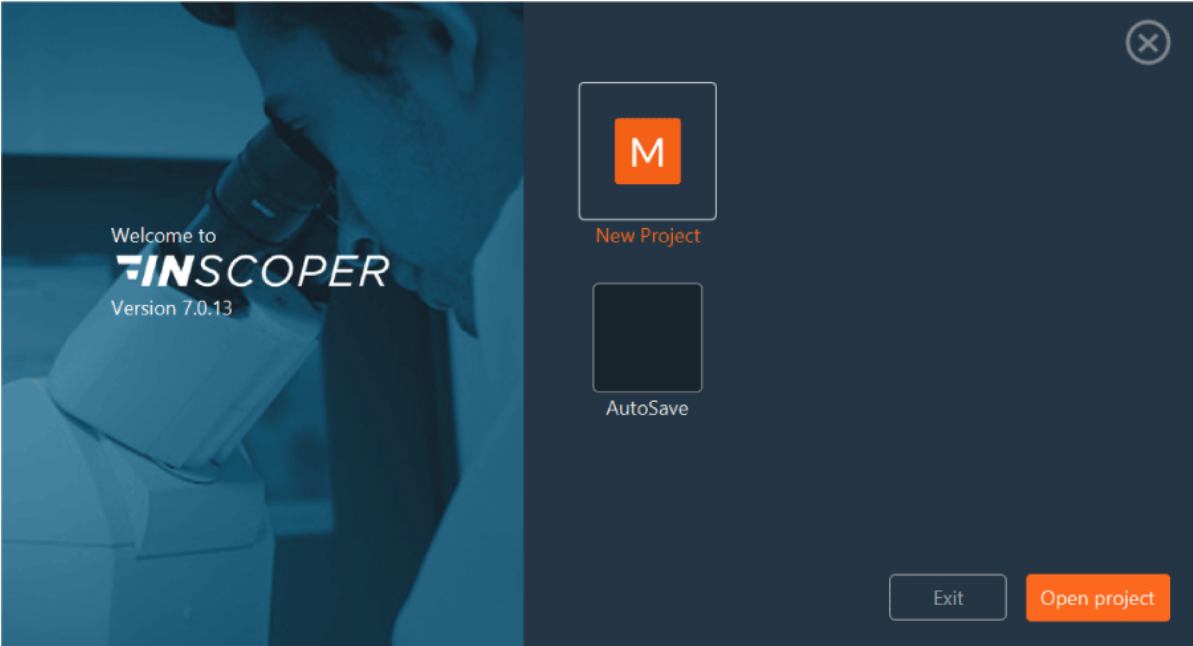
The Inscoper I.S. interface follows a guided workflow that helps new users get started quickly, while remaining efficient and enjoyable for experienced users.



This journey follows three distinct phases:

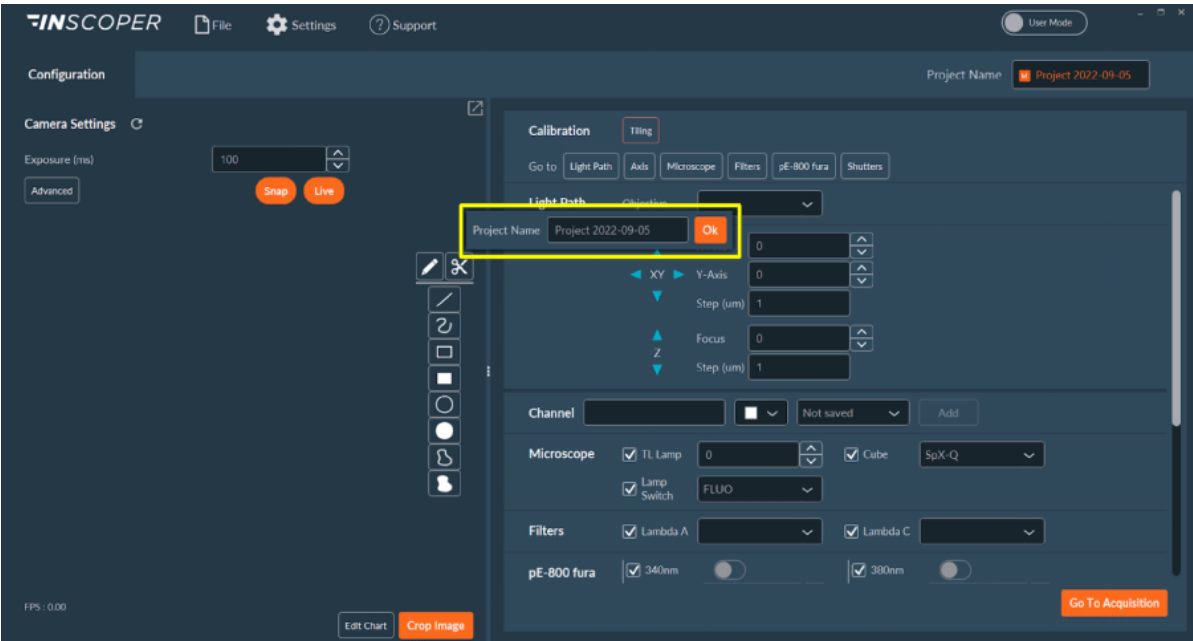
1. Establish optical pathways, configure connected hardware devices, and define parameters ([Configuration](#)).
2. Construct an acquisition protocol by configuring programmable dimensions, or design a custom pattern ([Multi-dimensional Acquisition](#) / [Designer](#)).
3. Explore the resulting images using built-in visualization tools ([Visualization](#)).

1.1.3 Getting Started



When you launch Inscooper I.S., the start window appears. The software version number is displayed, and you can choose from the following options to begin:

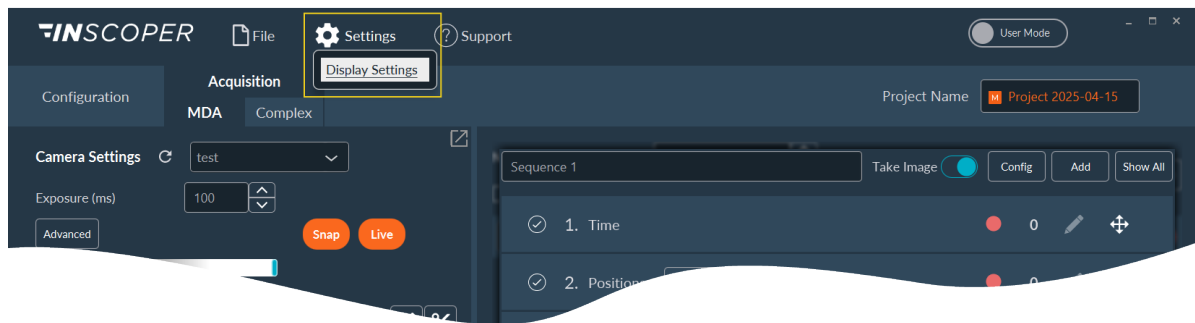
| Command Button | Operational Description                                                                                |
|----------------|--------------------------------------------------------------------------------------------------------|
| New Project    | Initializes a new working session.                                                                     |
| Auto save      | Recovers the most recent session state, including unsaved sequence parameters.                         |
| Open Project   | Opens an existing project. Select your file ( .cbf ) from the directory tree, then click <b>Open</b> . |



After you select the appropriate session setup, the software probes and initializes the connected hardware. You are then prompted to name the new project. If the name field is left blank, the system defaults to the current date, as shown in the image above.

## 1.2 Display Settings

Access interface preferences by navigating to **Settings > Display Settings**.



Use this window to configure software behavior, storage paths, and interface elements. Each tab in the left-hand list is documented below under the name shown in the application.

- [Well Plate Editor](#)
- [Snap & Live](#)
- [Change Password](#)
- [Project Name](#)
- [Tiff Name](#)
- [Metadata File Name](#)
- [Logs](#)
- [Crash Dump](#)
- [Camera](#)
- [FRAP](#)
- [Memory](#)
- [Image Display](#)
- [Objectives](#)
- [Wavelength](#)
- [Preferences](#)

### 1.2.1 Well Plate Editor

Construct and edit multi-well plate configurations used during acquisition:

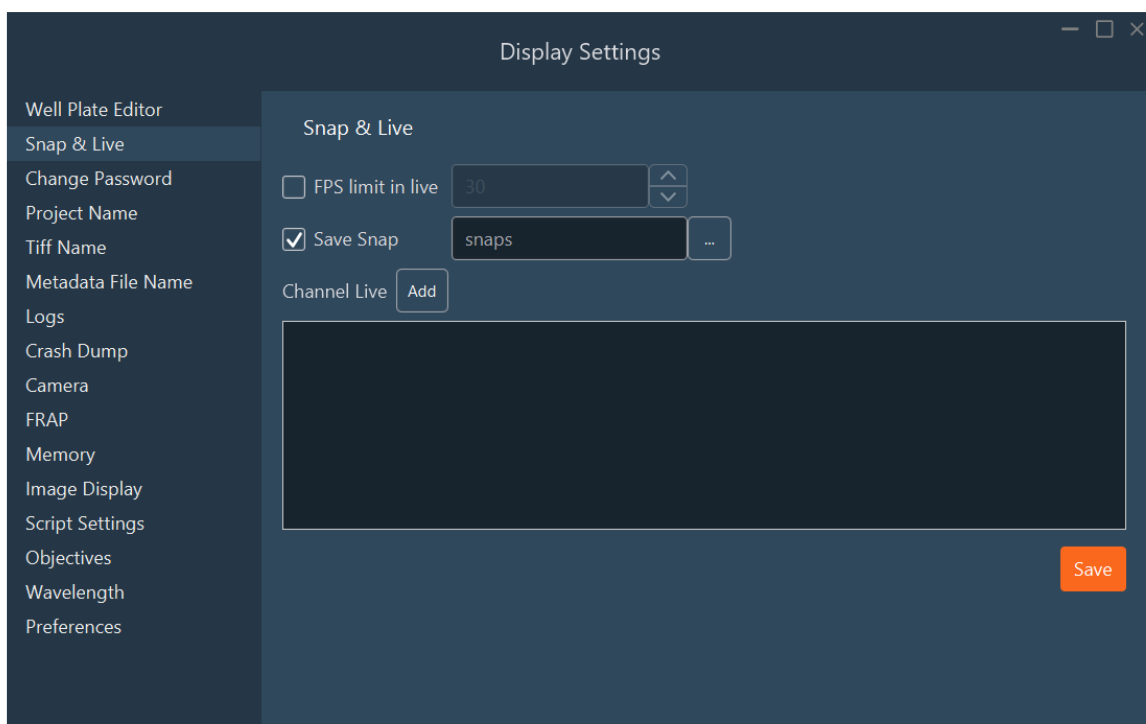
- **Name:** Assign a name to the template.
- **Edit:** Select an existing well plate configuration from the drop-down menu to modify and save its parameters.
- **Grid:** Define the number of horizontal (columns) and vertical (rows) wells, labeled X Size and Y Size respectively.
- **Spacing:** Define the distance (pitch) between the wells (in  $\mu\text{m}$ ).
- **Type:** Select the well shape from the drop-down menu (e.g., circle, square, or rectangle).
- **Well Width/Height:** Define the internal dimensions of the individual wells (in  $\mu\text{m}$ ).
- **Save:** Save this well plate configuration to the database.

#### Multi-slide holders

Define a custom multi-slide holder by describing each glass slide as a large rectangular well in a 1×4 or 1×8 grid.

## 1.2.2 Snap & Live

Configure default parameters for the Snap and Live imaging modes:

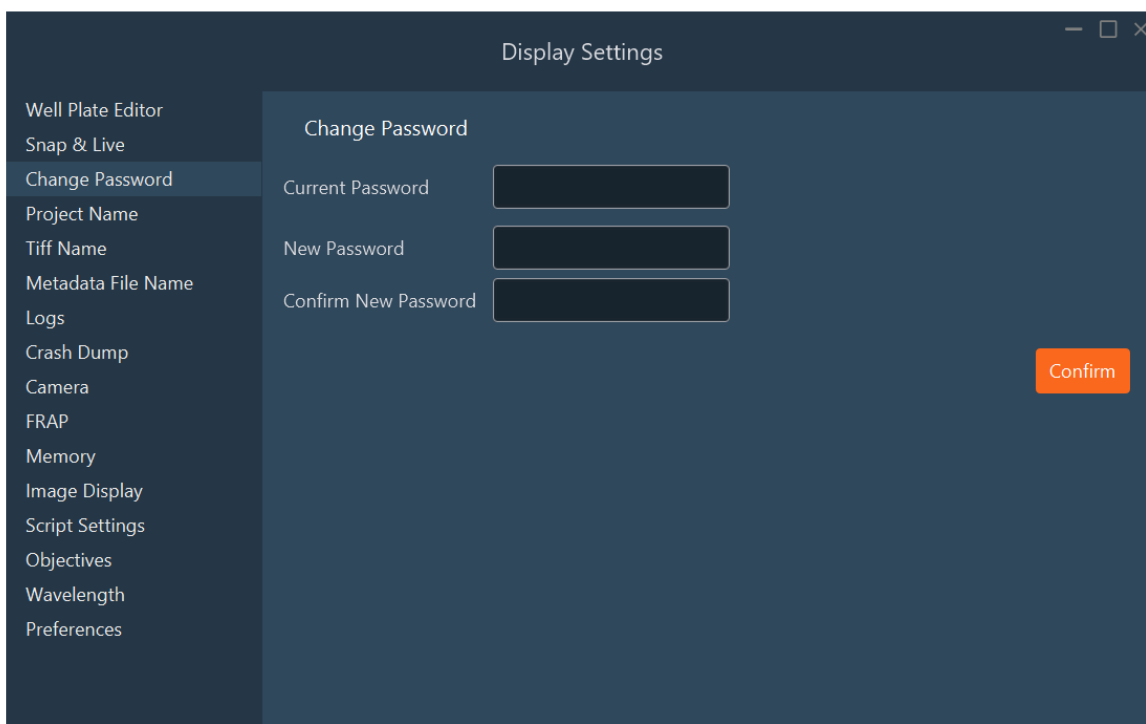


Available parameters:

- **Maximum frame rate (FPS):** Sets an upper limit on the frame rate during Live streaming.
- **Default save directory:** Specifies the output directory for Snapshots.
- **Default startup channel:** Selects the channel that automatically engages when Live mode is toggled.

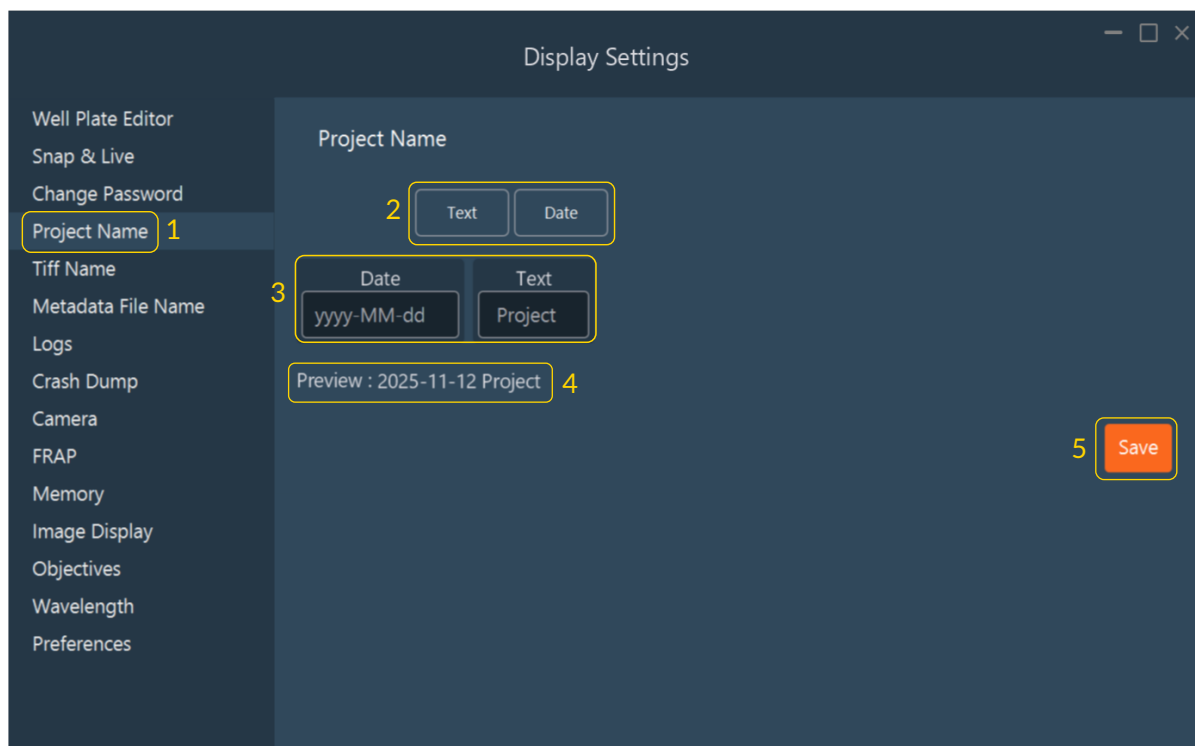
### 1.2.3 Change Password

Define or update the password required to access **Expert Mode**.



### 1.2.4 Project Name

Establish a standardized naming pattern for new projects.

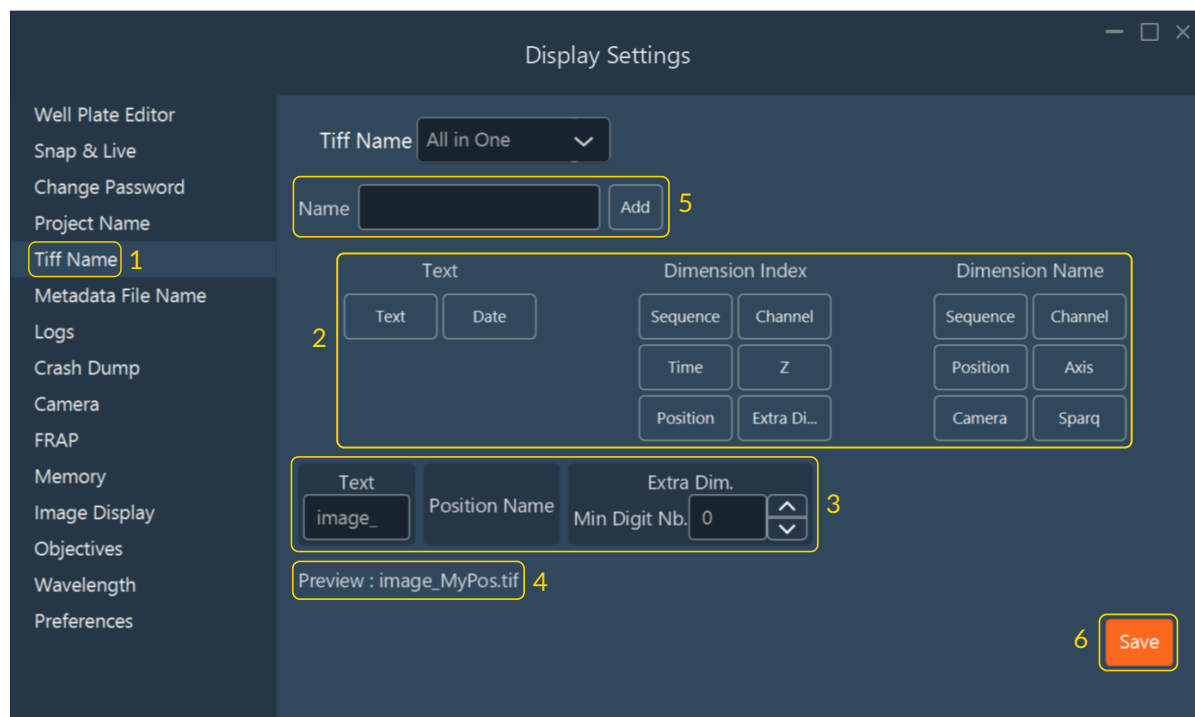


1. Select the **Project Name** tab.
2. Click the tags to add.
3. Modify the text and format of the selected tags in the line below. Organize the tags through drag and drop, or remove them with a right-click.
4. Verify in the **Preview** that the result matches the desired format.
5. Click **Save** to apply the settings.

### 1.2.5 Tiff Name

Customize how TIFF image files are named and exported.





By default, the software bundles all channels for a given position into a single multidimensional OME-TIFF file. Override this behavior to save each channel as a separate file.

1. Select the **Tiff Name** tab.
2. Drag and drop metadata tags into the central construction area to build your naming convention (e.g., **text block + dimension name + dimension index**).

Index Zero-Padding

The **Min Digit Nb.** field determines the zero-padding length for the dimension index value (e.g., a value of 3 yields `001`, `002`).

Text  
image\_

Position Name

Extra Dim.  
Min Digit Nb. 0

Position Index  
Min Digit Nb. 3

Preview : image\_MyPos000.tif

1. **Name Construction Tool**: Displays a visual schematic of the filename structure. Move tags with drag and drop, or remove them with a right-click.
2. **Name Preview**: Displays an example of the generated filename.
3. Enter a preset name and click **Add** to save the convention.
4. Click **Save** to apply the settings.

### 1.2.6 Metadata File Name

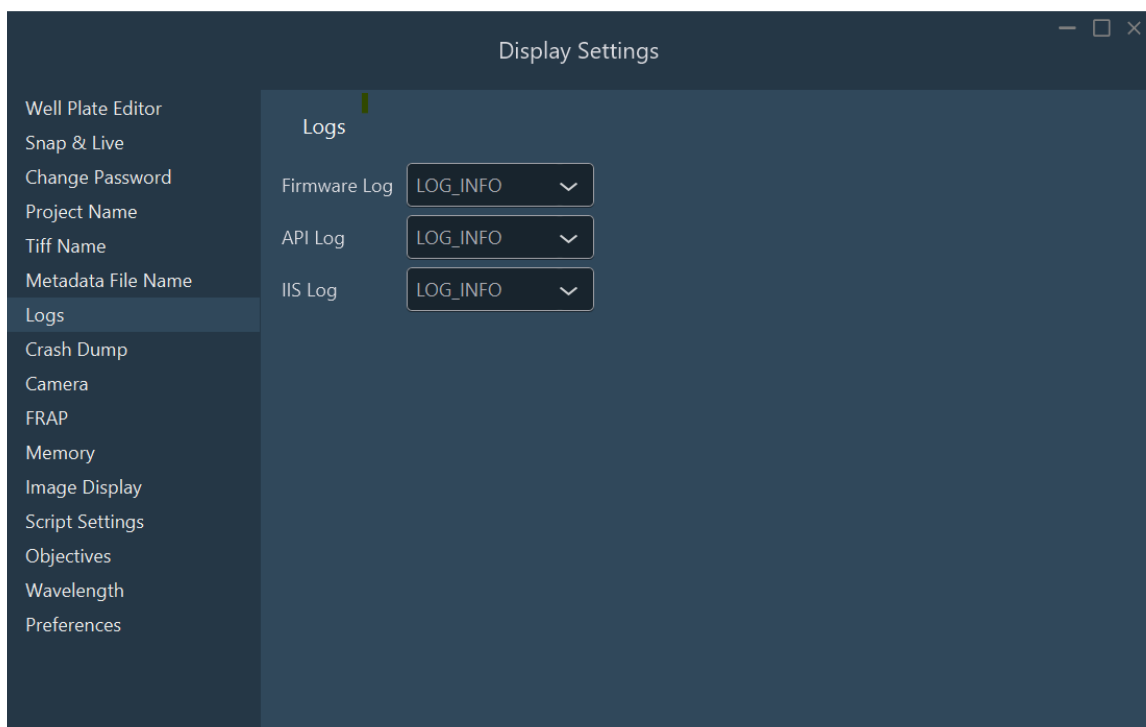
Customize how metadata files are named and exported.



1. Select the **Metadata File Name** tab.
2. Click the tags to add.
3. Modify the text and format of the selected tags in the line below. Organize the tags through drag and drop, or remove them with a right-click.
4. Verify in the **Preview** that the result matches the desired format.
5. Enter a preset name and click **Add** to save the convention.
6. Click **Save** to apply the settings.

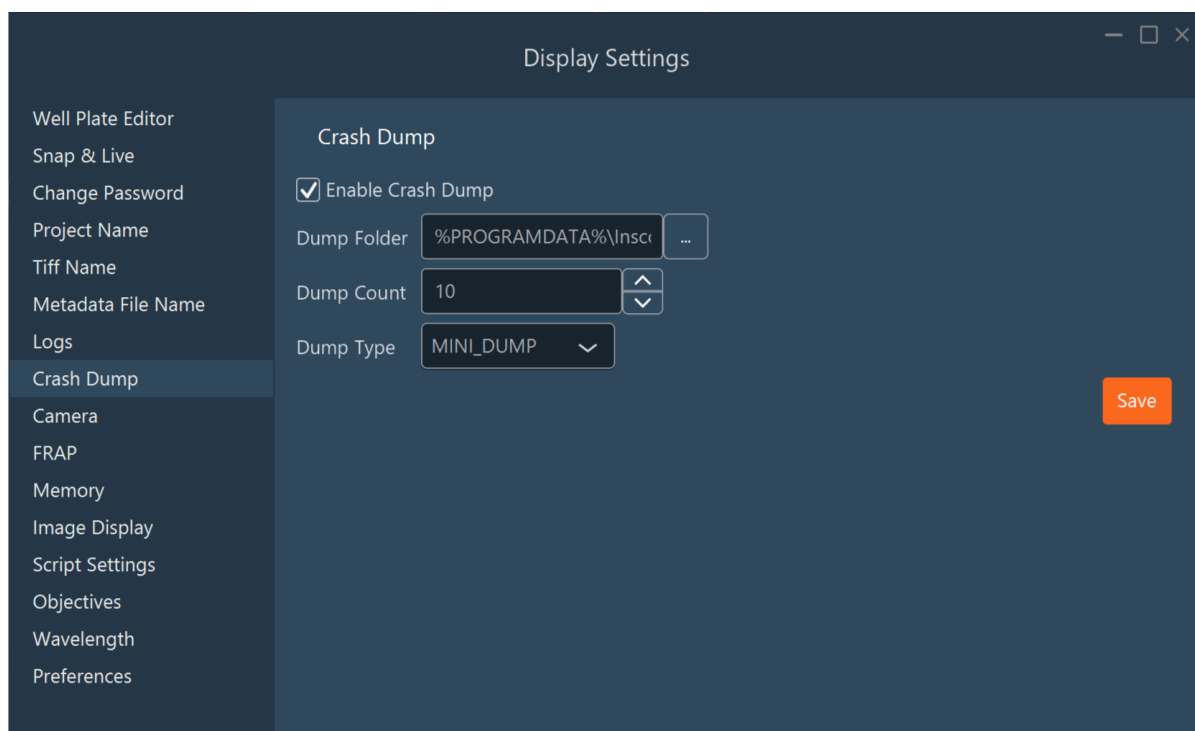
### 1.2.7 Logs

Configure the log verbosity level (e.g., Error, Warning, Info, or Debug).



## 1.2.8 Crash Dump

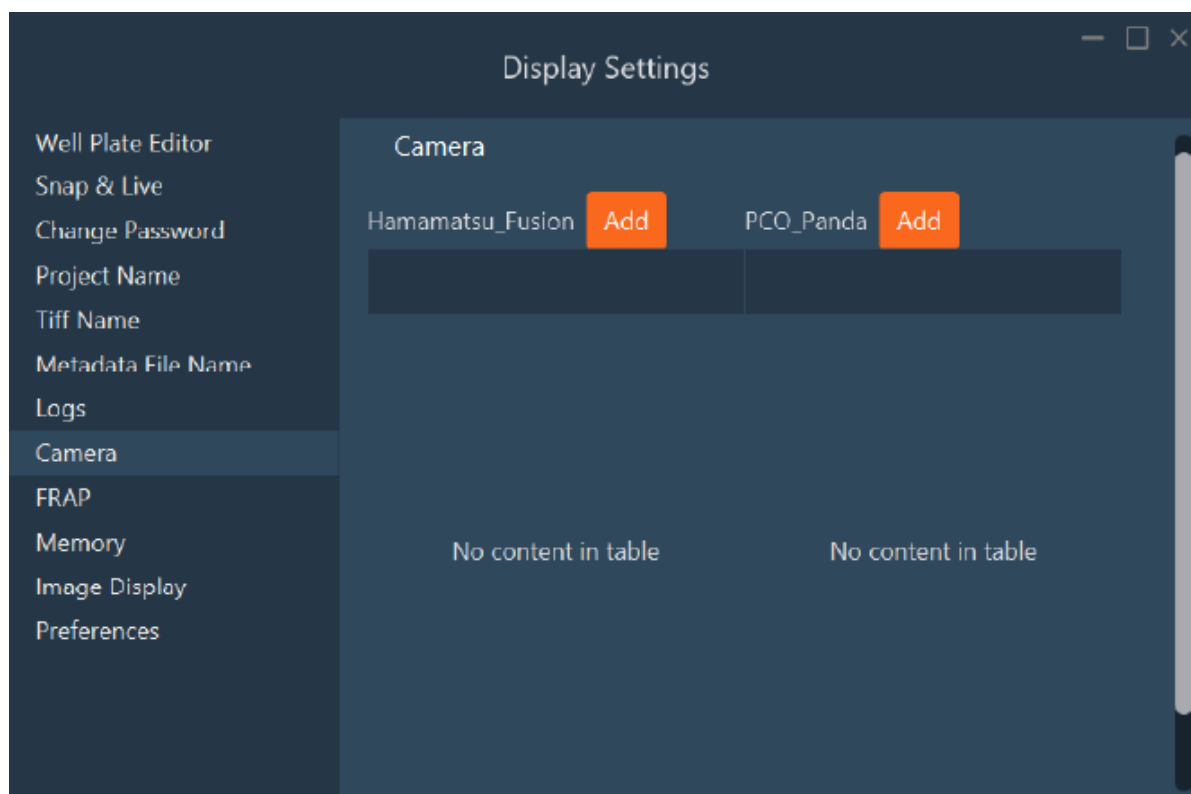
Configure crash reporting formats and retention policies.



- **Dump folder:** Define the directory path where the system saves crash reports.
- **Dump count:** Set the maximum number of reports retained (defaults to 10; the system deletes older files automatically).
- **Dump Type:**
  - **MINI\_DUMP** (Standard Default): Saves essential crash stack information, sufficient for most debugging.
  - **FULL\_DUMP** : Saves the complete memory state. Takes longer and consumes significant disk space.

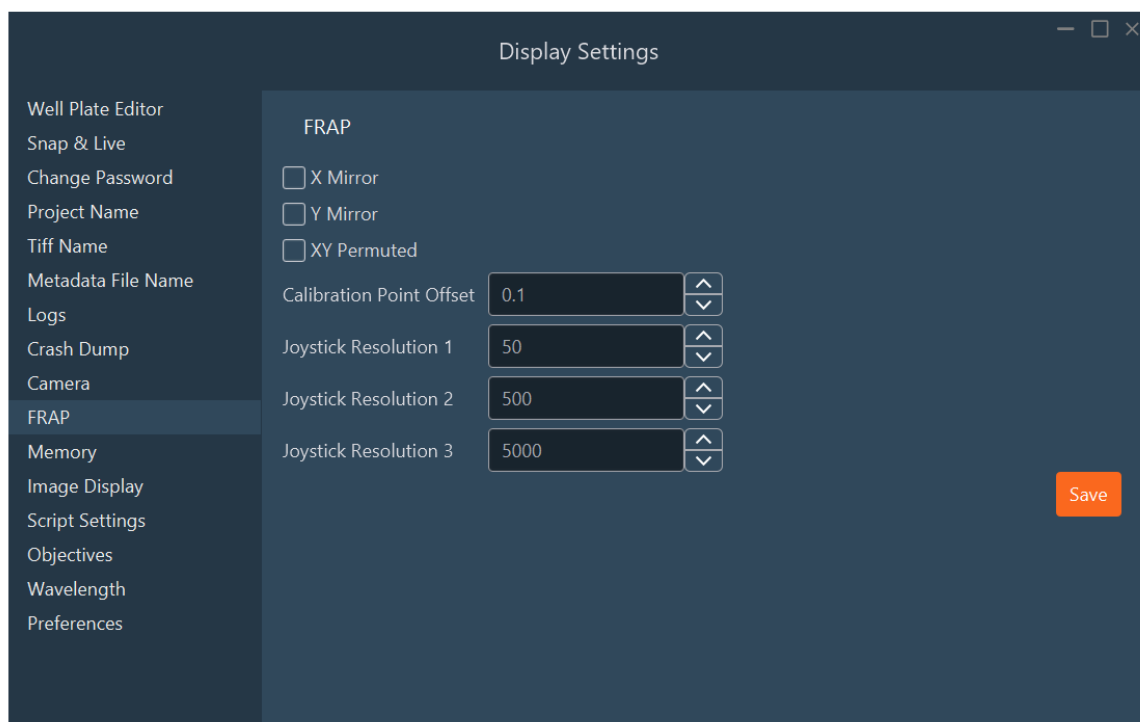
### 1.2.9 Camera

Manage connected cameras. For multi-camera setups that require field-of-view alignment, click **Add** to apply an image rotation to a camera.



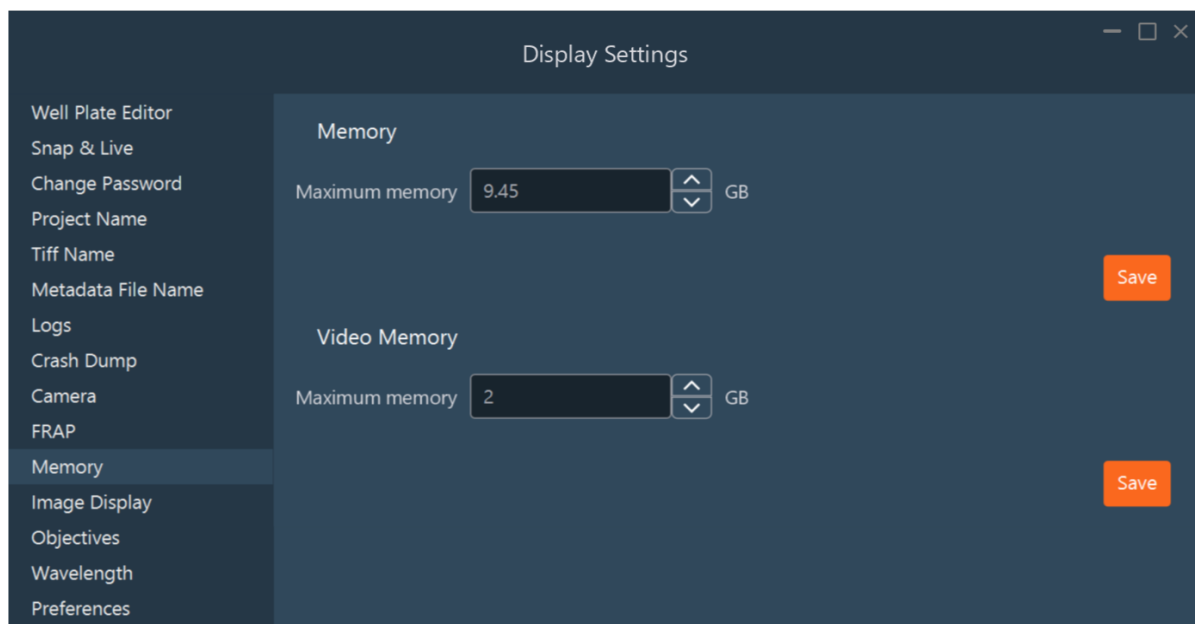
### 1.2.10 FRAP

Review the FRAP hardware limits and set the virtual joystick sensitivity used during FRAP calibration.



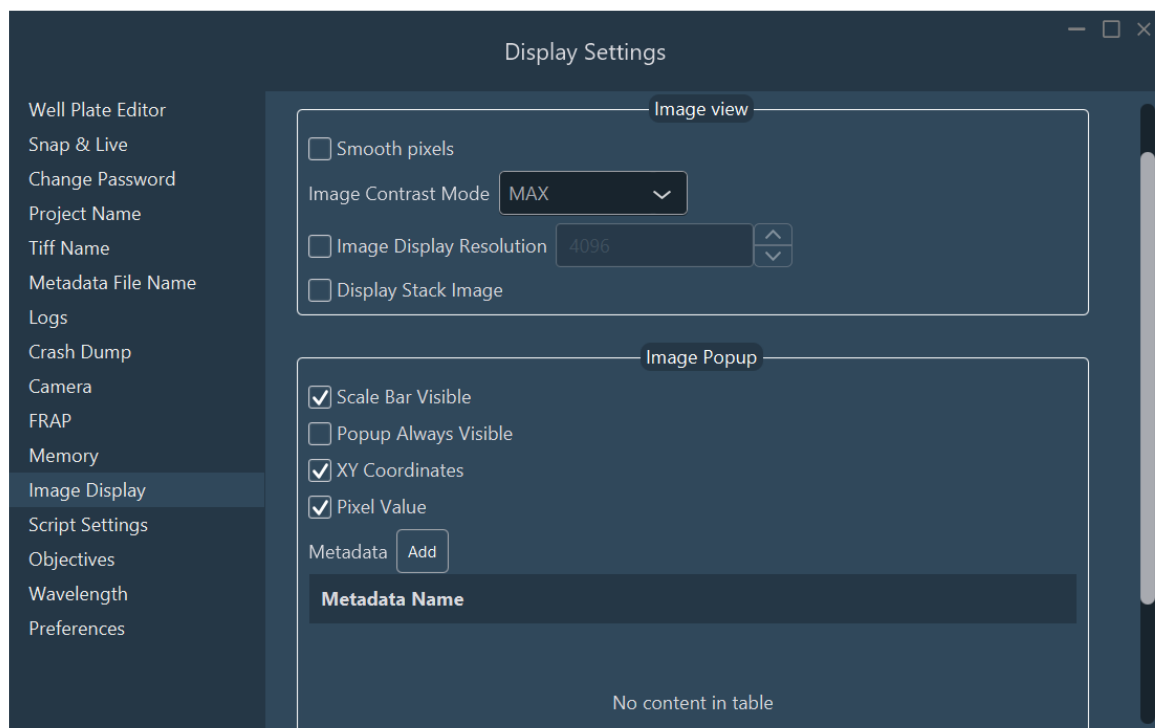
### 1.2.11 Memory

Set the memory limits for Inscoper I.S. **Memory** caps the system RAM available to the application, which defaults to 60% of the installed RAM. **Video Memory** caps the video buffer, which defaults to 2 GB.



### 1.2.12 Image Display

Configure the display preferences applied to the image viewers.



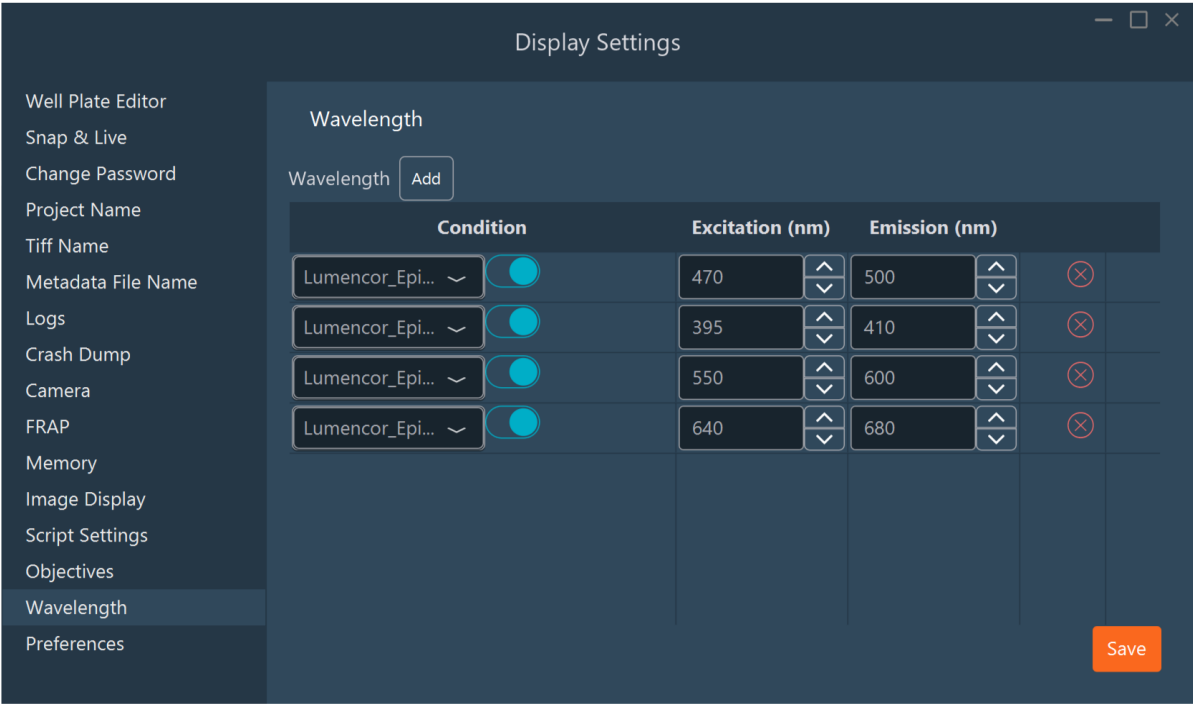
### 1.2.13 Objectives

Define the **Numerical Aperture (NA)** and the immersion **Refractive Index** parameters for your objectives. The software uses these values for Nyquist sampling limit calculations and image processing.



1.2.14 Wavelength

Define the **excitation and emission wavelengths** for your connected light sources, lasers, and filter cubes. The software uses this data for [shading corrections](#), Nyquist sampling limits, and processing algorithms, and embeds it into the image OME-TIFF metadata.



1.2.15 Preferences

Configure general preferences, including measurement units, timing delays, and warning-prompt behavior.

Display Settings

Well Plate Editor  
Snap & Live  
Change Password  
Project Name  
Tiff Name  
Metadata File Name  
Logs  
Crash Dump  
Camera  
FRAP  
Memory  
Image Display  
Script Settings  
Objectives  
Wavelength  
Preferences

**Preferences**

**Units**

Time unit: ms

Distance unit: um

**Acquisition Start**

☒ Confirm Acquisition before start

Delay before acquisitions: 0 h 0 min 0 s 0 ms

**Metadata**

Csv Delimiter: ;

**Warnings**

☐ Display time interval warning

☐ Not enough space on disk as warning

**Default Data processor**

☒ Default Process: RawData

☒ Default Tiling Process: Tiling

**Tiff File**

Tiff Compression: Raw

**Feature**

☐ Auto Exposure

☐ Force Camera in Channels

☐ Custom Tiling Step

**Temporary Datas**

Temporary Datas Folder: C:\Users\Claire\AppData\ ...

Save

- **Auto exposure:** Enables the auto-exposure UI button on the primary dashboard.
- **Force camera in channels:** Associates specific cameras with specific channels, recommended for multi-camera or MAICO configurations.
- **Custom tiling step:** Define a translational overlap distance (in  $\mu\text{m}$ ) rather than relying on a relative percentage for sequential tiling acquisitions.

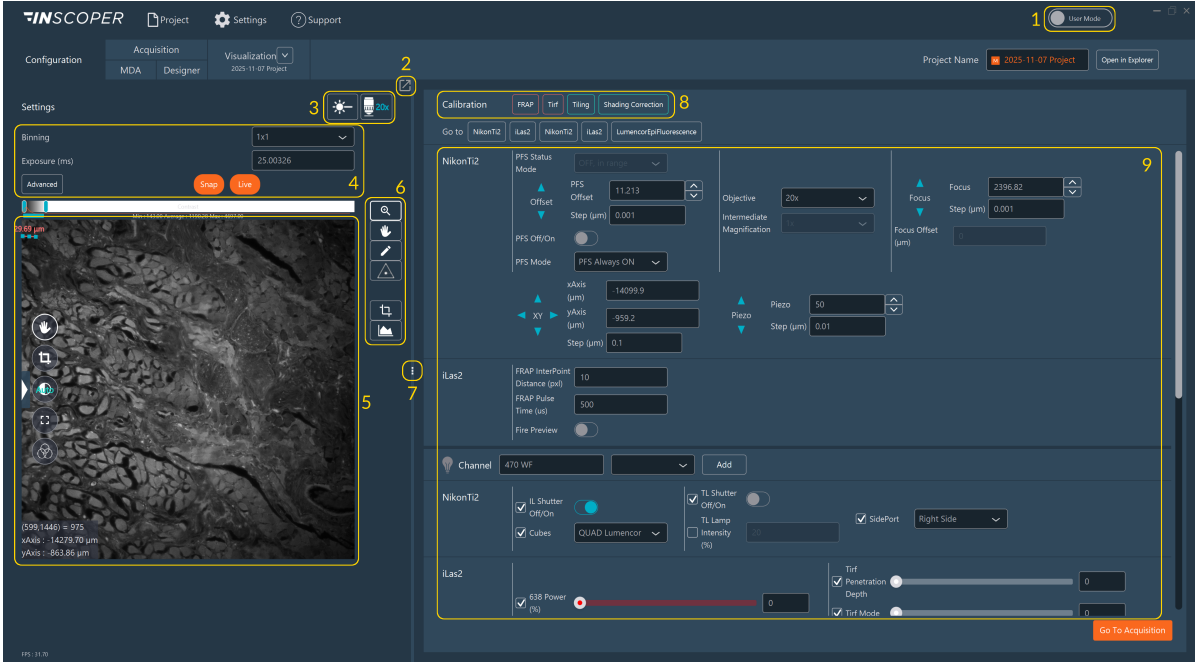
## 1.3 Configuration

Use the **Configuration** tab to control each motorized device on the microscope, find the correct focus plane, select the optimal camera settings, and set up the channels.

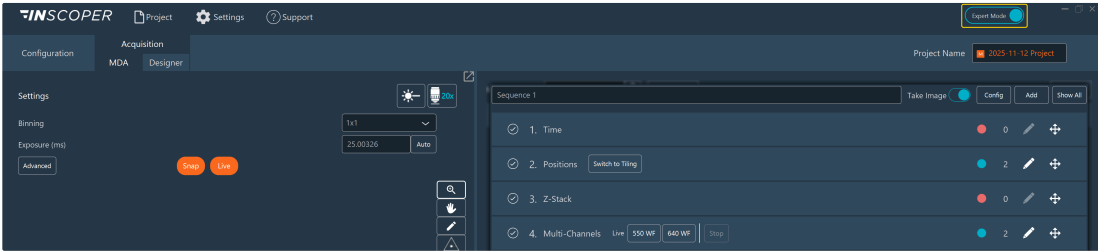
### 1.3.1 Overview

The configuration interface is divided into several sections:





1. **User Modes:** Two access levels are available in Inscope I.S., based on the user's level of expertise in microscopy:



| Mode | Description                                                                                                                                                                                                                                                                                           |
|------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|      | <b>Expert Mode</b> provides unrestricted access to all system settings and parameters. This mode is intended for microscope facility managers and researchers experienced in microscopy.                                                                                                              |
|      | <b>User Mode</b> provides restricted access to selected settings and parameters. The restrictions are fully customizable, ranging from basic channel configuration to the most advanced camera and device settings. This mode is intended for biologists who are not yet experienced with microscopy. |

**Note**

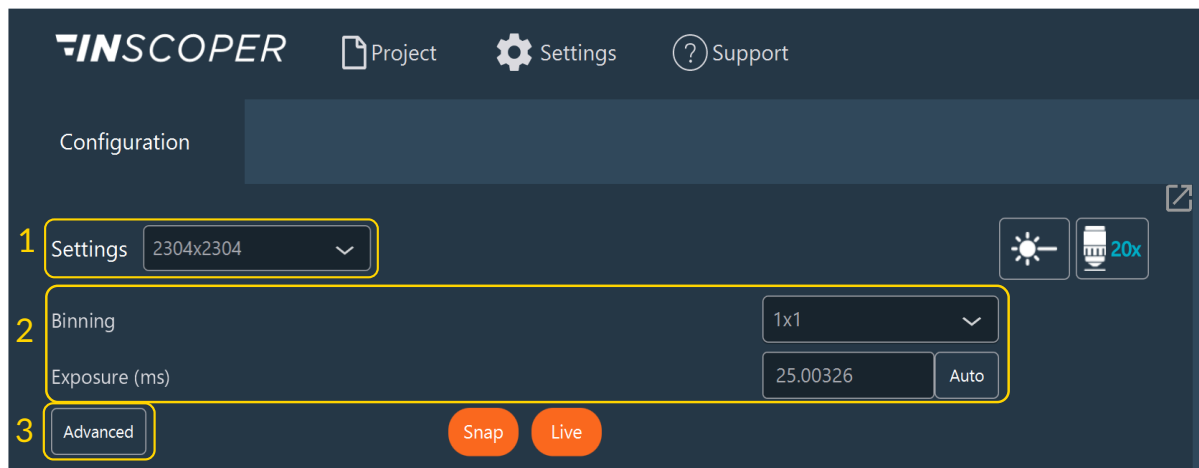
Users can switch from User Mode to Expert Mode at any time. Facility managers can set a password to restrict access to Expert Mode.

- 2. **Split Screen:** For dual monitors, detach the live camera feed view to a separate screen to decouple it from the settings panel.
- 3. **Channel/Objective Access:** Switch between defined channels and objectives with a single click.
- 4. **Camera Settings:** Configure camera parameters.
- 5. **Live Viewing:** View the real-time image feed.
- 6. **Image Tools:** Interact with the image display.
- 7. **Divider:** Adjust the screen size of the central camera view and the side settings panel.
- 8. **Calibration Protocols:** Access optical and stage calibration tools (see [Tiling Alignment](#) and [Shading Correction](#)).
- 9. **Motorized Devices:** Access connected motorized hardware, create new channels, and save them to the project.

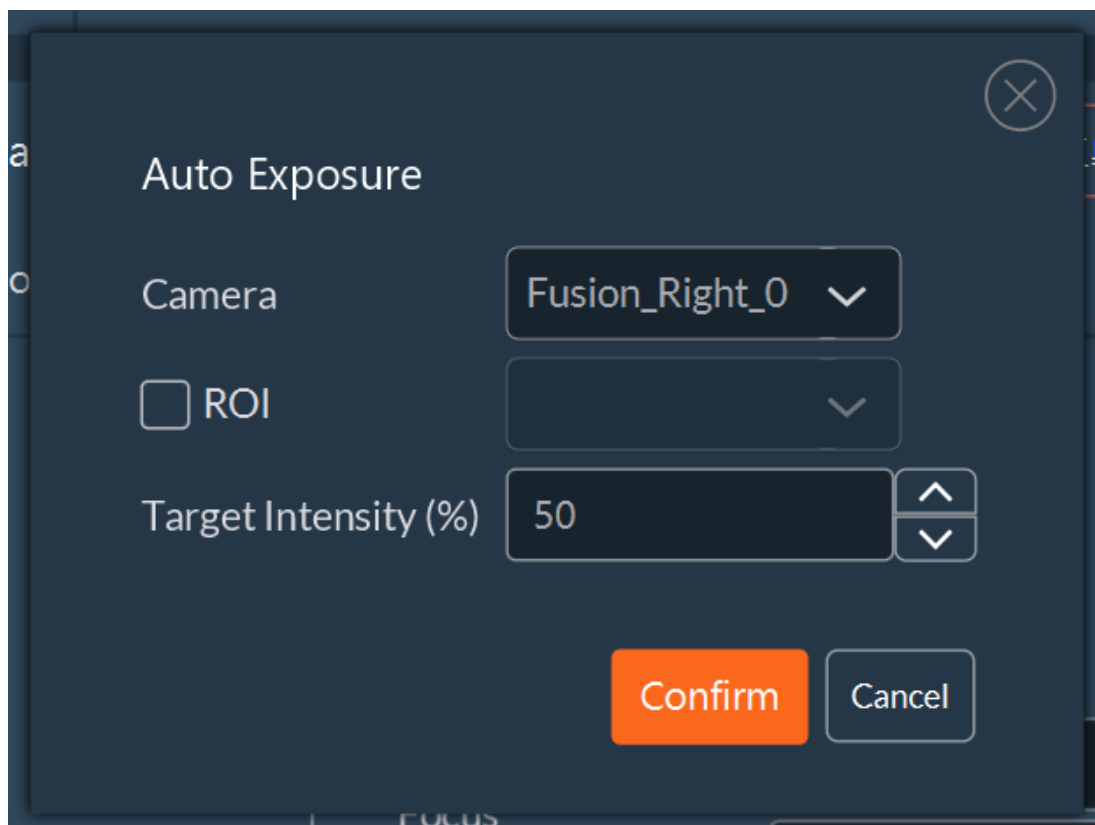
Rename the current project using the text field in the top-right corner.

1.3.2 Camera Settings

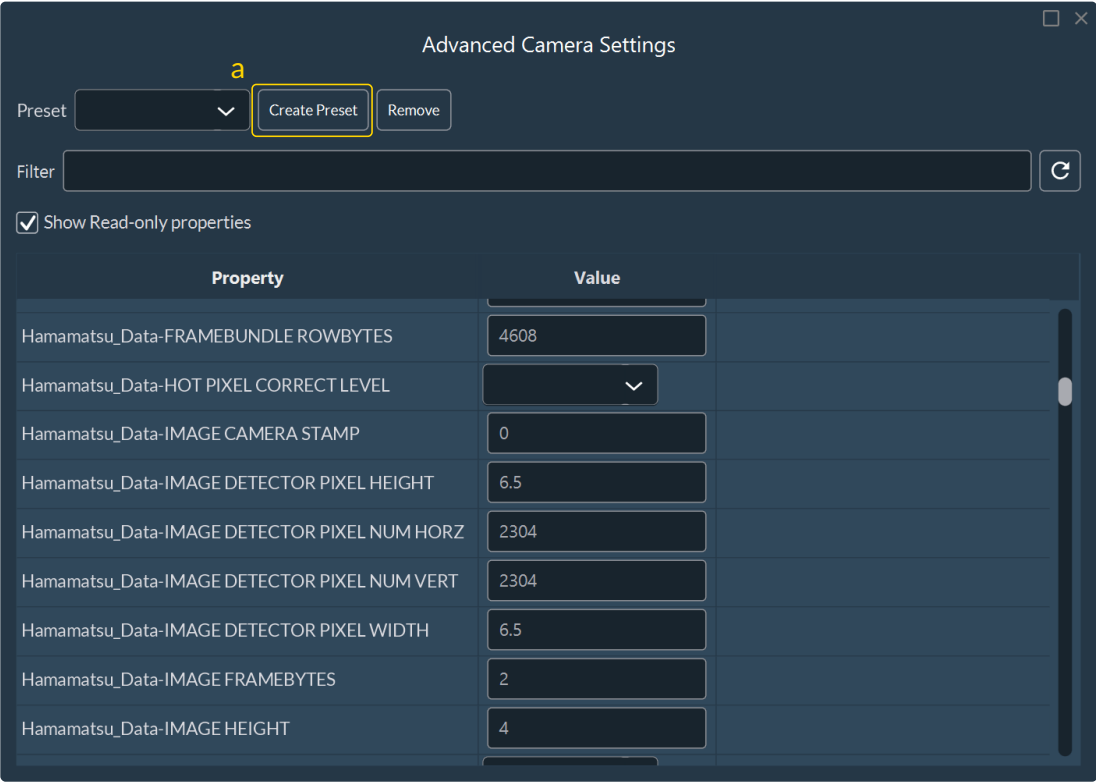
The software supports up to 4 cameras simultaneously. Create and save parameter presets for each camera using this section.



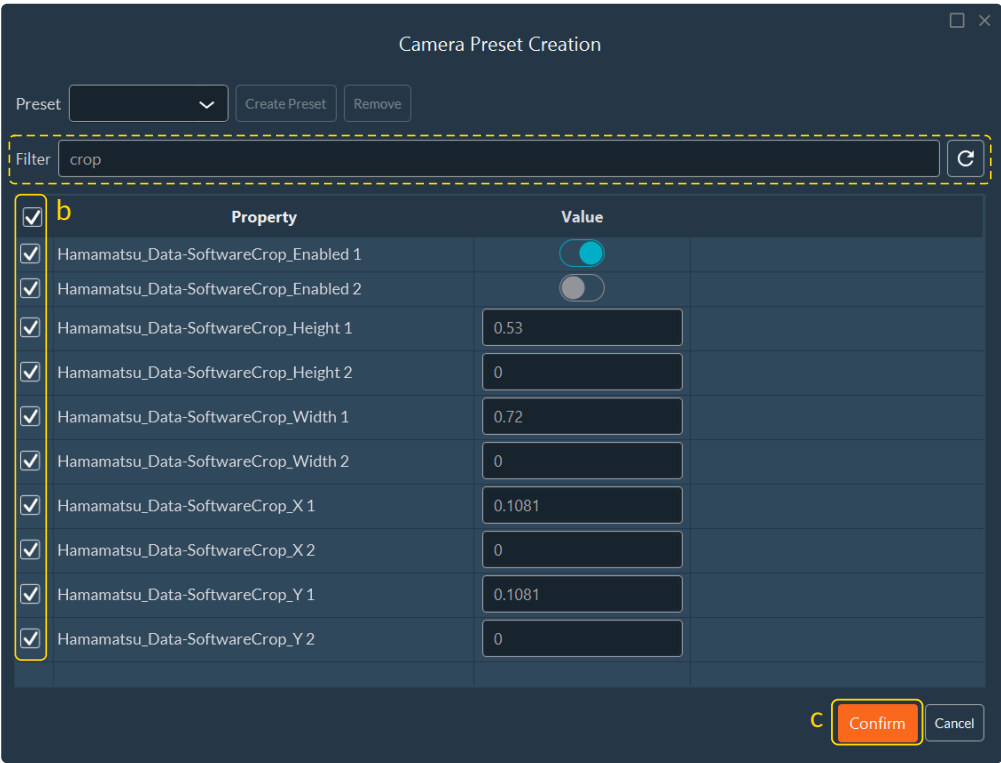
1. Select a camera preset from the drop-down list.
2. Adjust the exposure time and pixel binning. If **Auto Exposure** is enabled in the [Display Settings](#), an **Auto** button is available. Auto Exposure adjusts the exposure time to prevent pixel saturation by analyzing the real-time pixel histogram.



3. Access **advanced camera settings** to create custom presets:

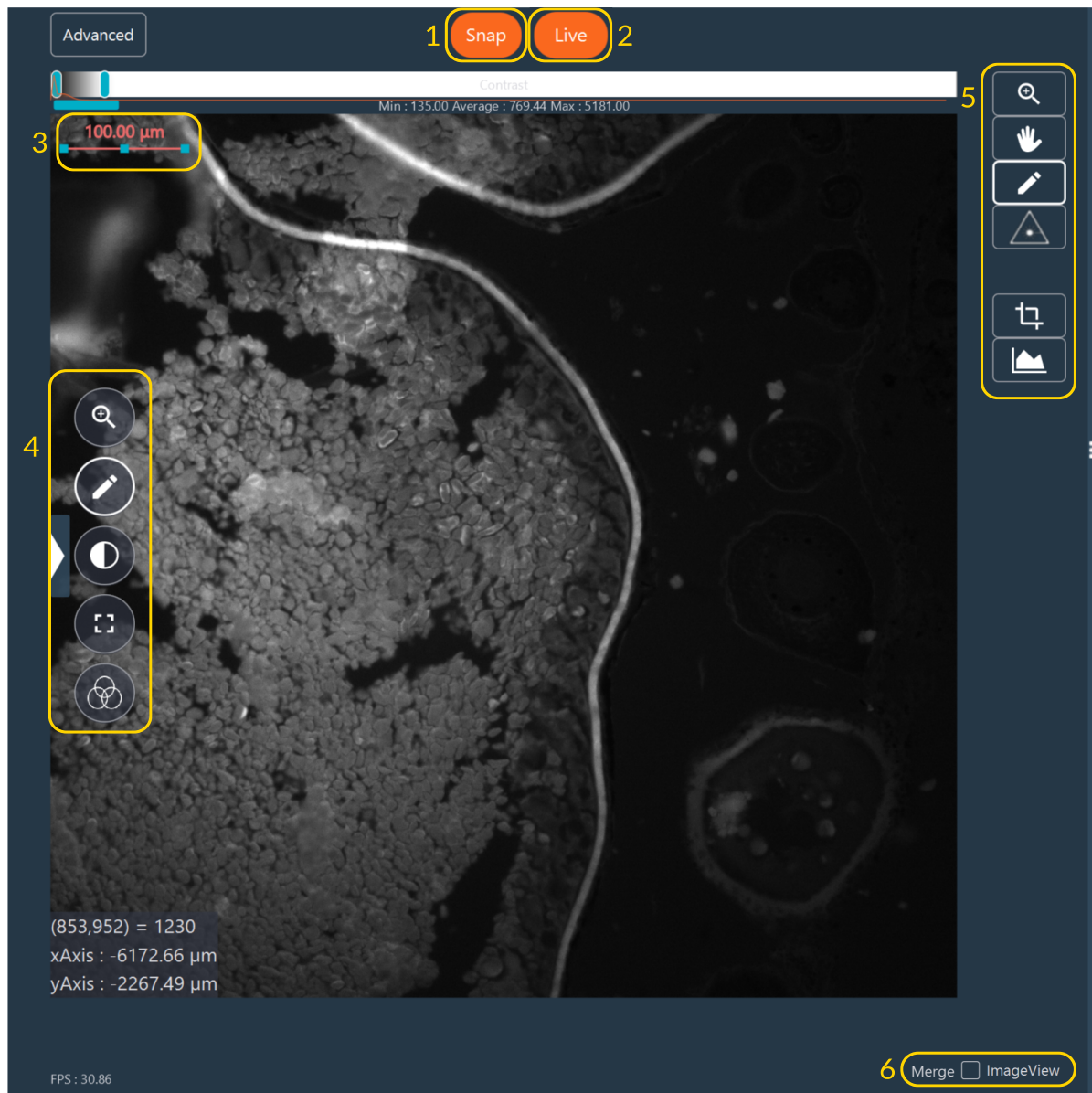


1. Click **Create Preset**:



2. Select the parameters to include in the new preset using the checkboxes. Use the search bar to filter the parameter list.
3. Click **Confirm**, then assign a name and save location for the preset.

### 1.3.3 Live Image Interaction



Use the toolbars to interact with the live image stream:

- 1. **Snap**: Capture a single image frame.
- 2. **Live**: Toggle the real-time camera stream.
- 3. **Scale Bar**: Double-click to modify orientation and physical length.
- 4. Tools to interact with the image:

| Tool                                                                              | Description                                                                                                                               |
|-----------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------|
|  | Toggle automatic or manual contrast adjustments. Use the intensity sliders above the camera view to establish manual contrast boundaries. |
|  | Toggle full-screen mode. Exit by pressing the button again or clicking the 'X'.                                                           |
|  | Apply LookUp Tables (LUTs) to the image display in real time. Options include no LUT, single-color LUT, or a preset LUT.                  |

 **LookUp Tables (LUTs)**

Applying a LookUp Table maps raw grayscale intensity values to a specific colored gradient. This is critical for drawing ROIs accurately during quantitative assays, as pseudo-colored "heatmaps" (like the fire LUT) visually exaggerate subtle intensity differences that remain indistinguishable to the human eye in standard grayscale.

Available preset LUTs include:

- **Pixel indicator**: Highlights saturated (overloaded) pixels in red.
- **Inscooper ratiometric**: Designed for visualizing ratiometric images.
- **Multicolor LUTs**: Standard palettes such as "fire", "physics", etc.

5. **Advanced Image Tools** (a secondary toolbar with specialized functions):

| Tool                                                                                | Description                                                                                                   |
|-------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|
|  | Pan across the field of view by dragging and dropping. Scroll the mouse wheel to move along the Z-axis.       |
|  | Zoom digitally by scrolling the mouse wheel over the image.                                                   |
|  | Draw Regions of Interest (ROIs) or crop the live image. Configure as a hardware sensor crop or software crop. |



Create and manipulate ROIs to control processing, cropping, and photo-manipulation.

Creation Modes

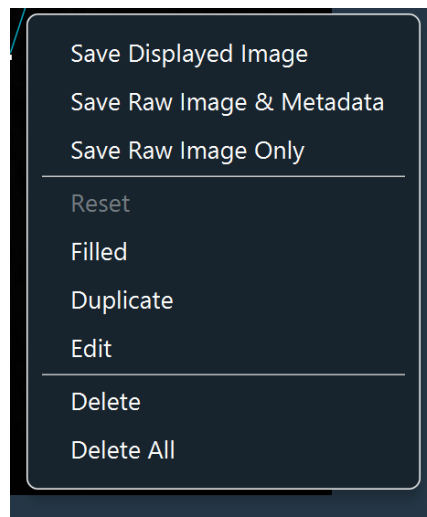
| Tool                                                                              | Mode     | Description                                                                     |
|-----------------------------------------------------------------------------------|----------|---------------------------------------------------------------------------------|
|  | Pen      | Add one or multiple discrete ROIs to the view.                                  |
|  | Scissors | Remove a drawn sub-shape from an existing ROI, retaining the external boundary. |

Drawing Geometries

Select the desired geometry tool to draw the ROI boundaries:

| Tool                                                                                | Shape            | Description                           |
|-------------------------------------------------------------------------------------|------------------|---------------------------------------|
|    | Line             | Draw a 1D straight line.              |
|    | Curve            | Draw a 1D freehand line.              |
|    | Rectangle Edge   | Draw the 2D perimeter of a rectangle. |
|   | Filled Rectangle | Draw a solid 2D rectangular area.     |
|  | Circle Edge      | Draw the 2D perimeter of a circle.    |
|  | Filled Circle    | Draw a solid 2D circular area.        |
|  | Free Form Edge   | Draw a custom closed 2D perimeter.    |
|  | Filled Free Form | Draw a solid, custom closed 2D area.  |

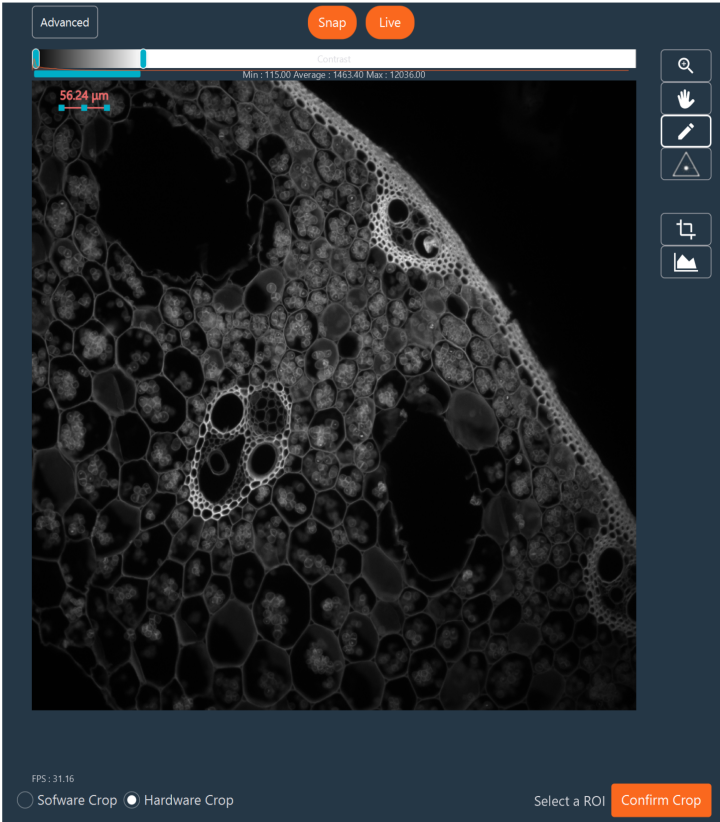
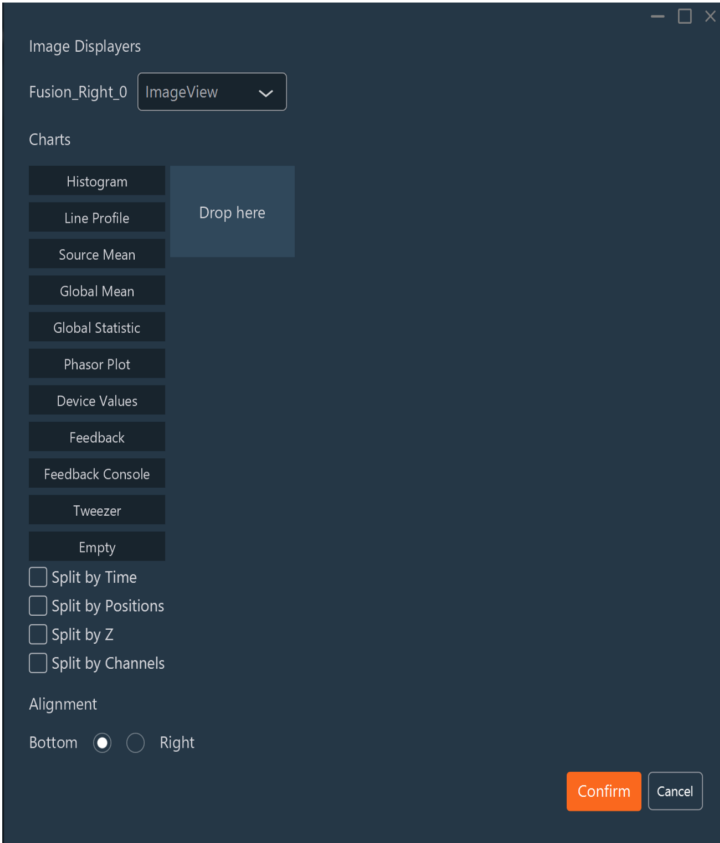
ROI Management



Right-click an existing ROI on the image canvas to access the spatial management window:

- Fill internal areas.
- Duplicate existing ROIs.
- **Edit ROI:** Enter the ROI dimensions in microns and the center coordinates within the camera field of view.
- Remove single or all drawn ROIs.



| Tool                                                                                | Plugin           | Description                                                                                                                                                                                                                                                                                          |
|-------------------------------------------------------------------------------------|------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|    | Fire on Click    | Trigger targeted photo-manipulation lasers. (Requires the FRAP module to be installed).                                                                                                                                                                                                              |
|    | Crop Access      | <p>Configure either <b>hardware</b> (sensor-level) or <b>software</b> (digital) cropping. Draw a ROI, select the target mode, and click <b>Confirm Crop</b>.</p>                                                  |
|  | Histogram Access | <p>Dock the quantitative histogram (or other 1D visualizations) below or to the right of the image panel. Adjust the X-axis scaling relative to camera bit-depth by hovering over the top of the histogram.</p>  |

- **Contrast Adjustment:** Hover over the contrast bar located at the top of the image view. Use the high/low threshold sliders to modify the minimum and maximum displayed values, effectively redefining the black and white points to optimize the visual dynamic range. Zoom directly within this micro-histogram tool.

Display: Auto Manual

Min: 5586

Max: 22201

Histogram Zoom: Auto Reset

Min: 0

Max: 65535

6. **Merged Images:** If the system is equipped with multiple cameras, use the **Merged images** toggle to display a combined live multiplexed superposition of all active camera streams.

### 1.3.4 Motorized Device Control

#### Interacting with Devices

NikonTi2

PFS Status Mode: [Dropdown]

Offset: [Up/Down arrows]

PFS Off/On: [Toggle]

PFS Mode: [Dropdown]

Objective: 10x [Dropdown]

Intermediate Magnification: [Dropdown]

Focus: 2200 [Up/Down arrows]

Step (nm): 1 [Up/Down arrows]

Focus Offset (nm): 0 [Text]

XY: [Up/Down arrows]

xAxis (nm): 100 [Text]

yAxis (nm): 1000 [Text]

Step (nm): 100 [Text]

Piezo: 0 [Up/Down arrows]

Step (nm): 10 [Text]

Panel (a) highlights Objective and Intermediate Magnification.

Panel (b) highlights XY stage controls.

Panel (c) highlights Focus controls.

This panel provides control over hardware connected to the Inscoper I.S., including the microscope stand, motorized XY stages, optical shutters, illumination sources, filter wheels, piezo Z-drives, and microfluidics pumps.

- Use panel **(a)** to move the objective turret.
- Panels **(b)** and **(c)** provide virtual joysticks to move the XY stage and Z-focus. Each arrow moves the stage or focus in the selected direction by the defined step.

## Creating and Loading Channels



Expert users have direct control over optical path components (filter wheels, dichroic cubes, LEDs, laser lines). To configure a new fluorescent imaging channel:

1. Select the excitation light source.
2. Set its output intensity.
3. Select the optical filter cube.
4. Select the LightPath geometry.
5. (Optional) Select brightfield LED illumination to use instead of, or simultaneously with, the fluorescence path.
6. Assign a name to the channel configuration.
7. Save the channel:
  - **a. Temporarily** (for the current session) by clicking **Add (User Mode)**.
  - **b. Permanently** (to the database) by clicking **Save (Expert Mode)**.
8. The newly created channel populates the channel list.

### Camera Port Alignment

Many microscopes use more than one camera port (e.g., left and right ports). It is critical to select the correct active port before initiating imaging. Failing to select the active port may result in blank images or hardware errors during acquisition.

### FRAP channels

To create a channel dedicated to photomanipulation, see the [FRAP Channel](#) section. FRAP channels do not appear in the channel list shortcut above the image view on the left side.

## 1.3.5 System Calibration

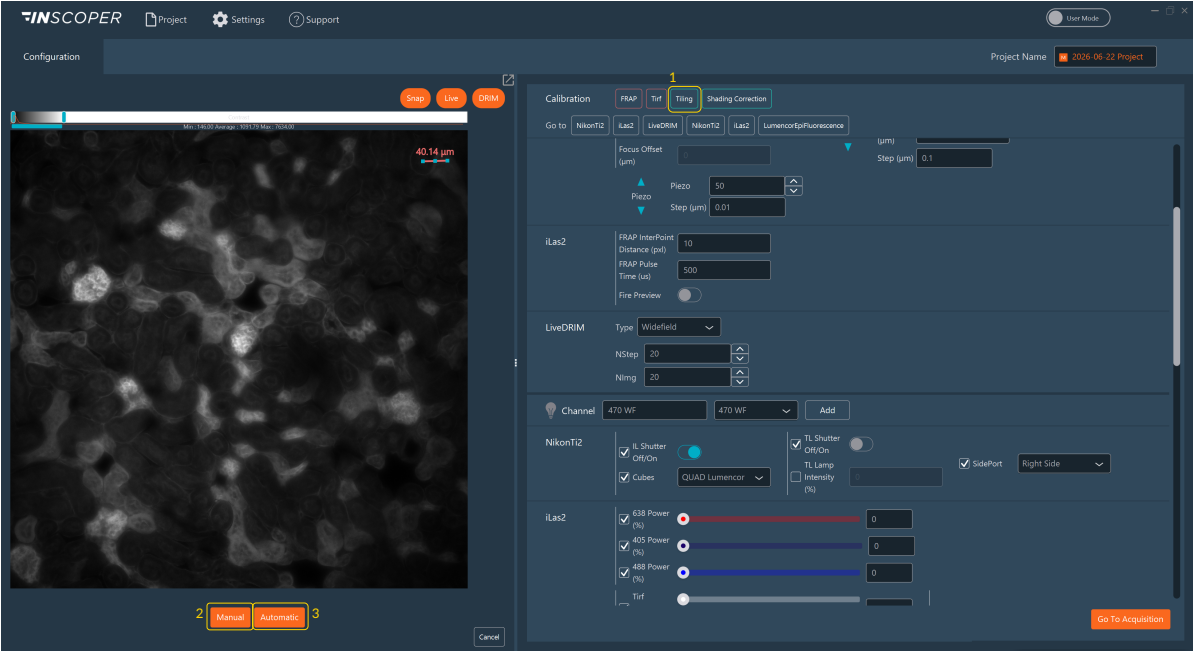
Access critical optical and stage calibration tools required for various sequences.

### Tiling Alignment Calibration

Tiling calibration aligns the camera sensor's X/Y orientation with the motorized XY stage axes. This hardware alignment is strictly required for accurate image stitching.

### Tiling Calibration

Each camera on the system requires this calibration.



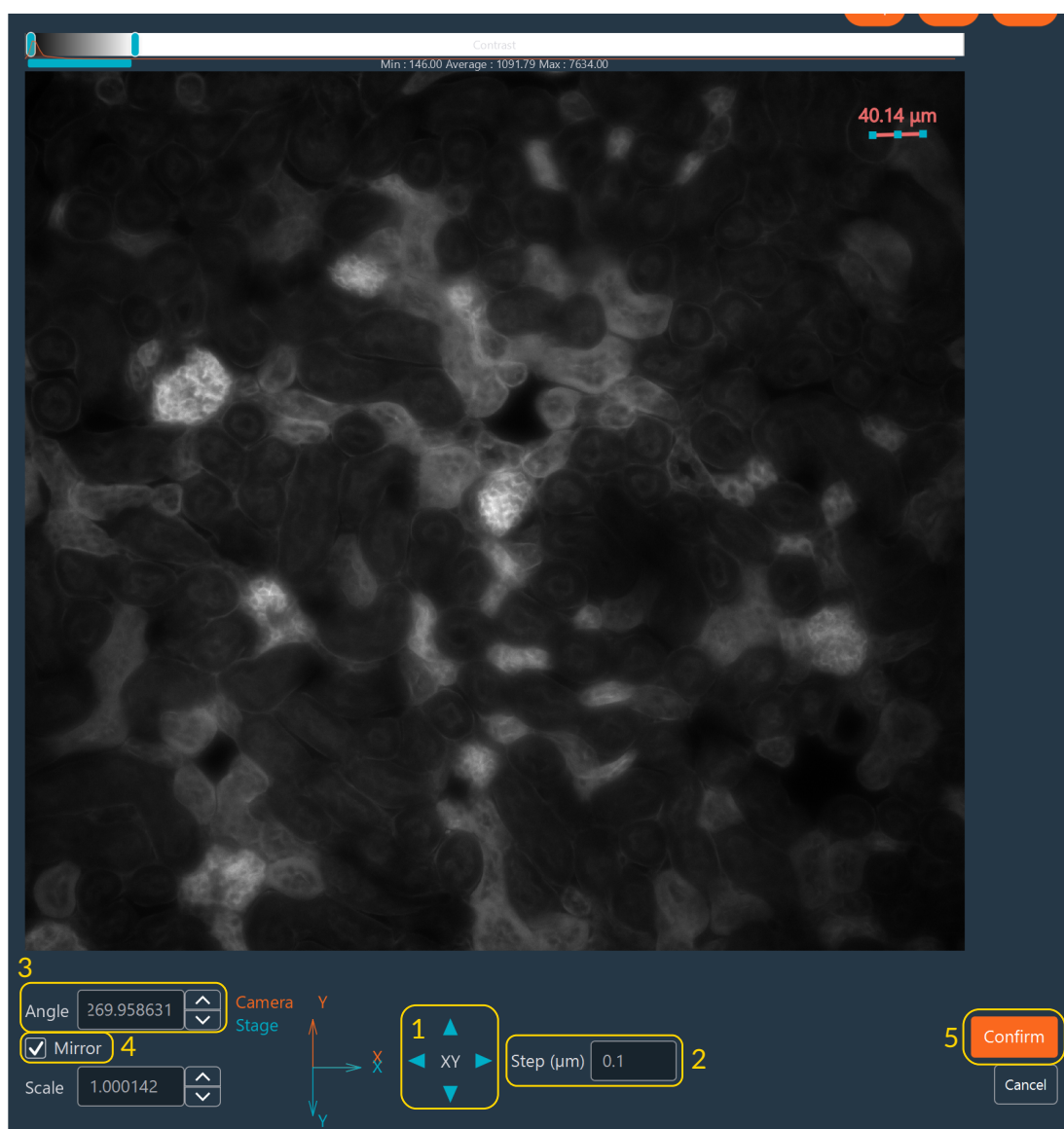
1. Click **Tiling** in the Calibration section. The indicator is orange if uncalibrated, and turns green when calibrated. Then **Select** a calibration mode.

#### Streaming Requirement

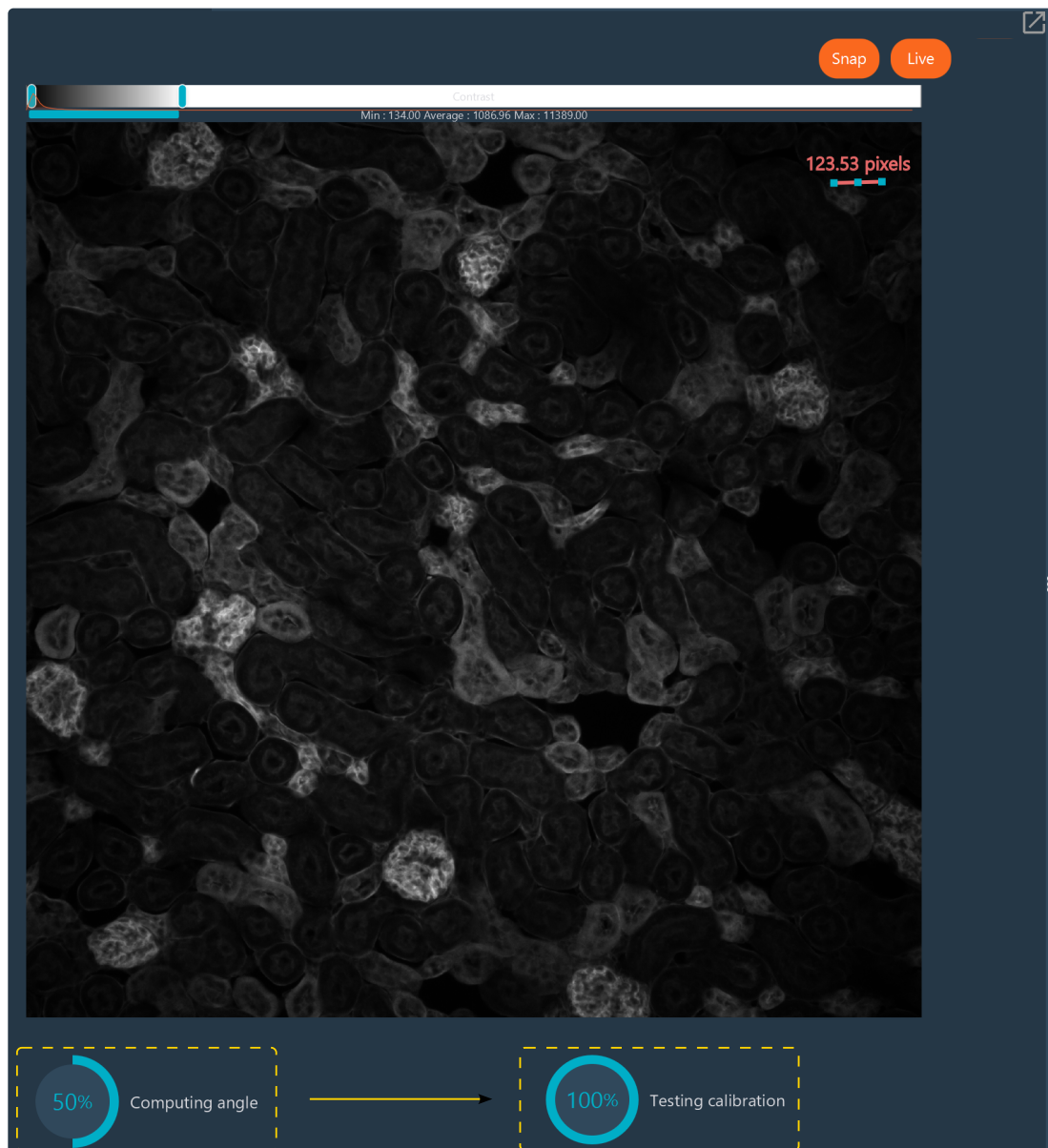
Stream camera data in **Live** mode and bring a sample into focus before initiating either calibration.

## 2. Manual Calibration Protocol:

1. Move the XY stage using the virtual joystick. Confirm that the stage movement matches the image's orientation on the screen.
2. Adjust the movement step size for finer control.
3. Apply rotation to the live camera feed until it aligns with the stage movement axes.
4. Apply mirroring to the live camera feed until it aligns with the stage movement axes.
5. Click **Confirm** to save the calibration.

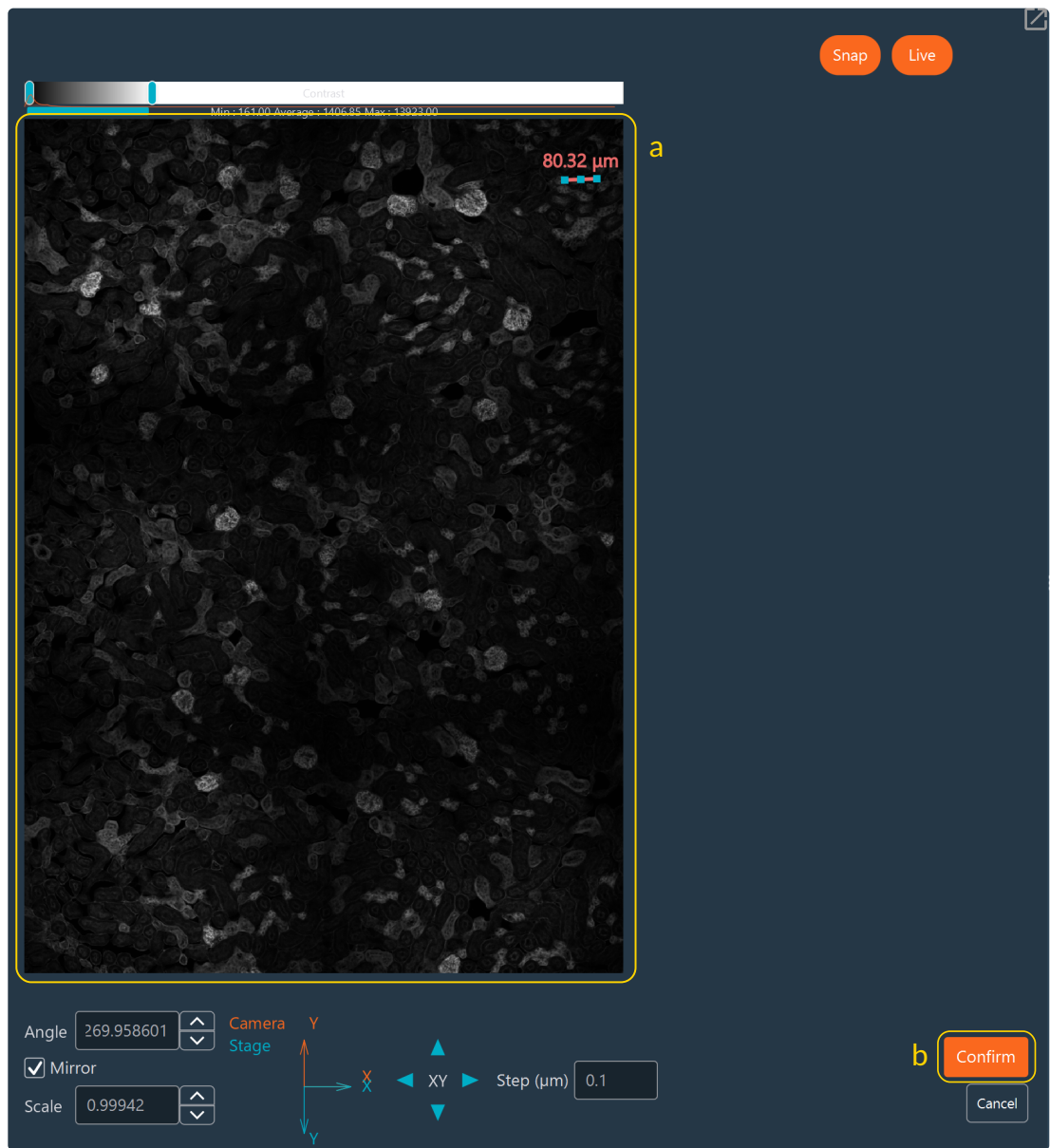


## 3. Automatic Calibration Protocol:



The automatic tiling calibration runs independently. Track its progress using the **circular indicator**, which fills completely with blue upon completion.

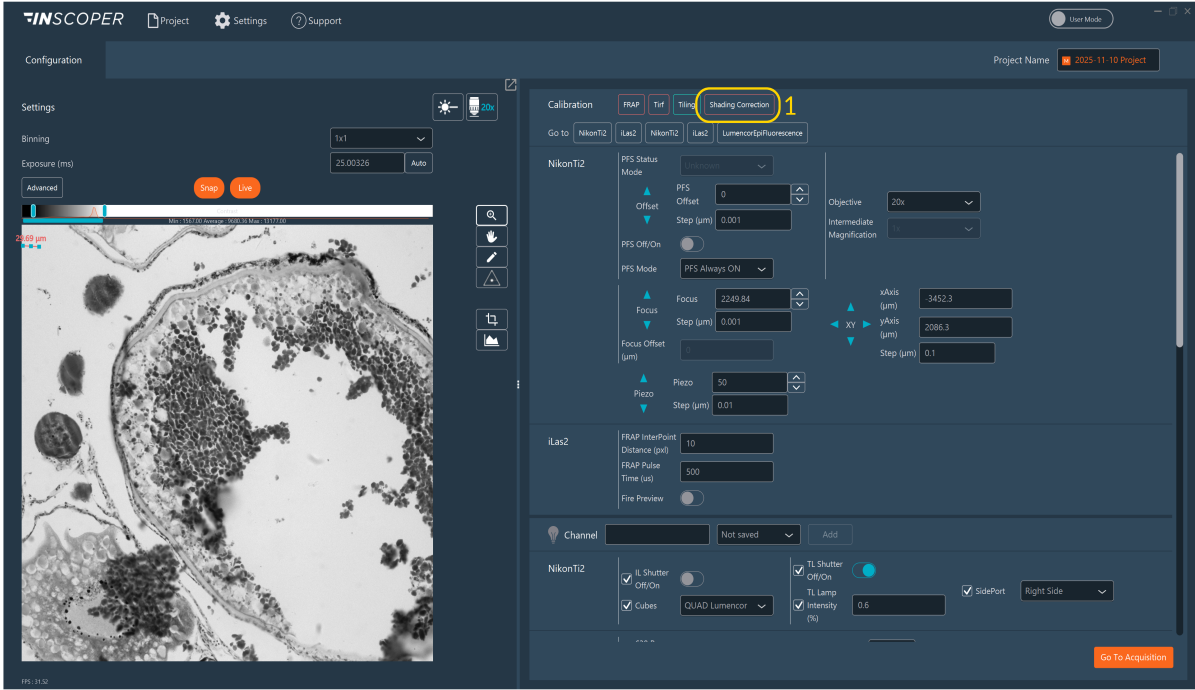
Review the resulting tiling image **(a)** to verify the calibration, then click **Confirm (b)** to validate.



### Shading Correction Calibration

**Shading Correction** (flat-field correction) ensures uniform illumination intensity across the camera field of view, compensating for light source vignetting or unevenness. Perform this calibration using a uniform reference sample, such as an auto-fluorescent slide (e.g., Chroma).

EXECUTING THE SHADING CALIBRATION SEQUENCE



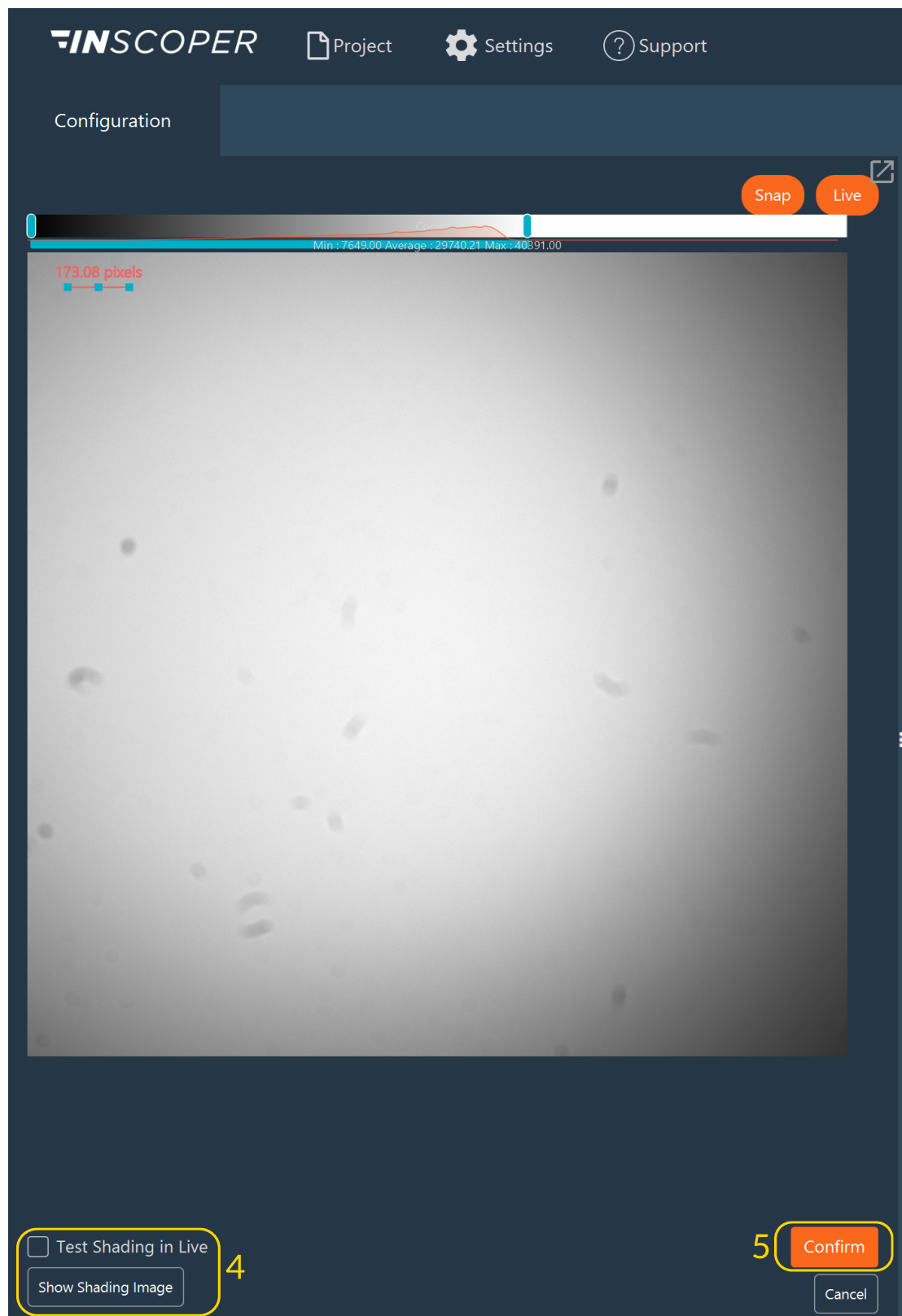


1. Click **Shading Correction**.



2. Specify the number of acquisitions. The software averages the captured frames to reduce noise.

3. Click **Confirm** to start the calibration routine.



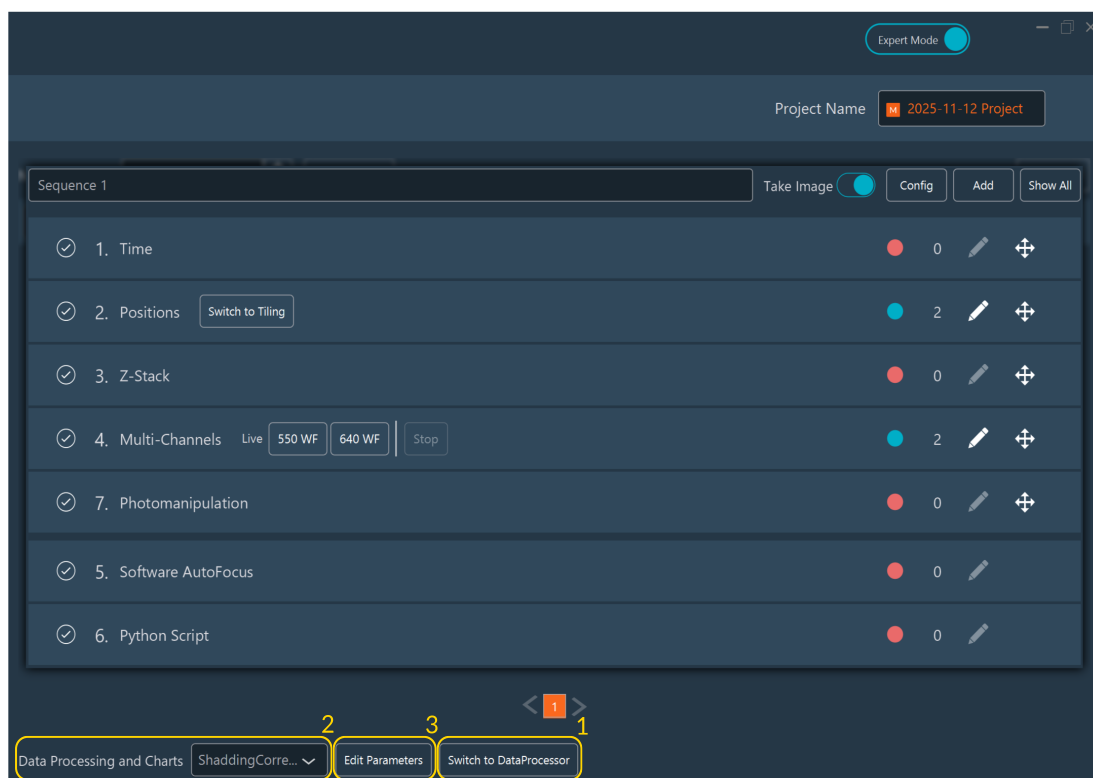
4. Once the calibration is complete, validate it:
  - Verify the result by clicking **Test Shading** in **Live** mode.
  - View the generated shading image map by clicking **Show Shading Image**.
5. Click **Confirm** to save the calibration.

Once calibrated, the **Shading Correction** indicator turns **green**.

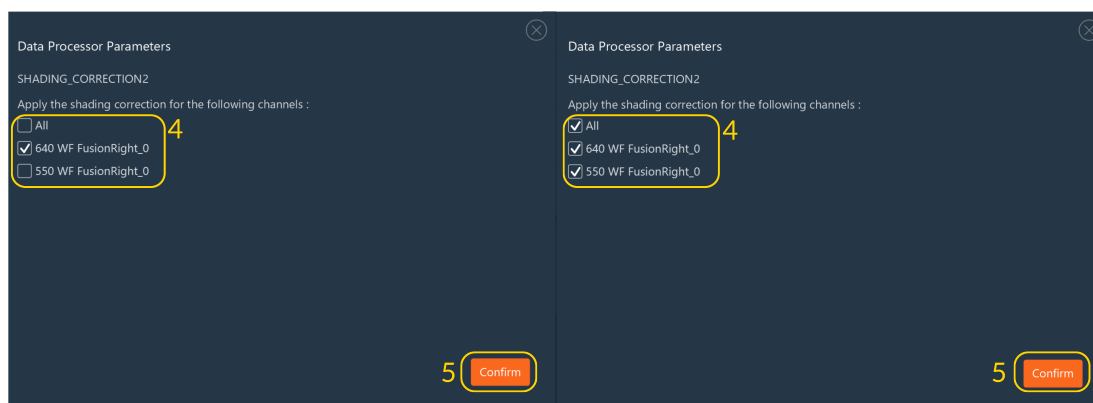
## APPLYING SHADING CORRECTION VIA THE DATA PROCESSOR

Once calibrated, shading correction is not applied automatically; it must be explicitly invoked within the data processing pipeline.

1. In **Expert Mode**, construct a data processing pipeline containing the **Raw Data**, **Shading Correction**, and **Tiling** nodes.
2. Assign this pipeline to your sequence during the Acquisition setup.
3. Click **Edit Parameters** on the **Shading** node.



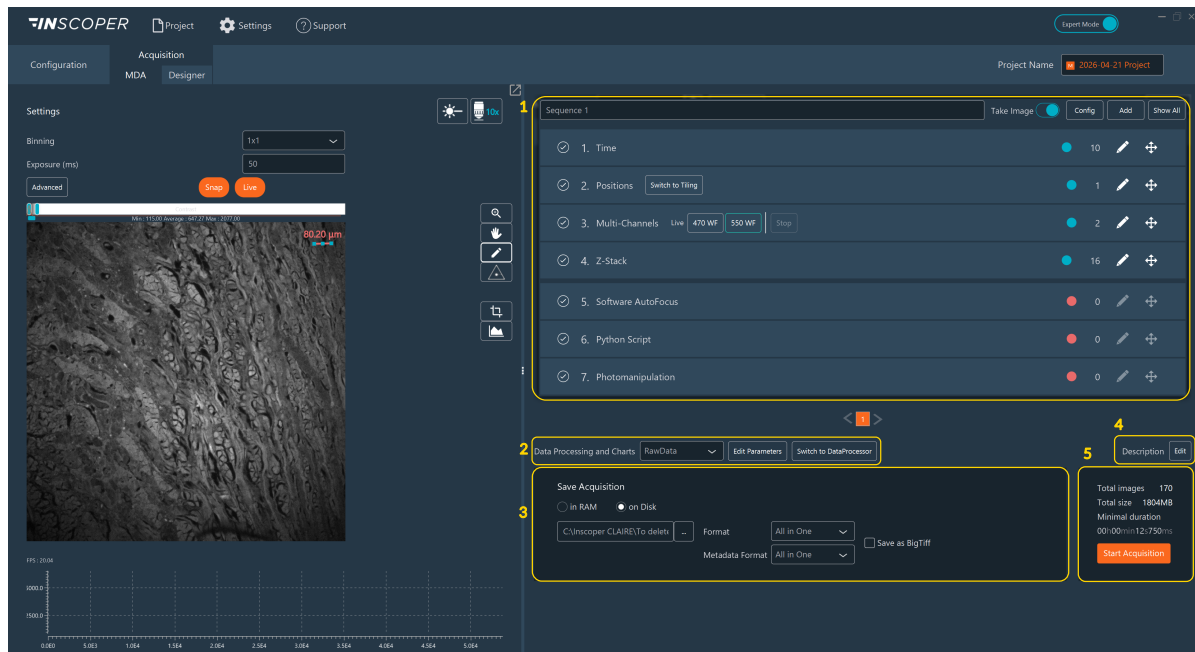
4. Select the channel(s) that require shading correction.
5. Click **Confirm** to validate your selection.



## 1.4 Acquisition

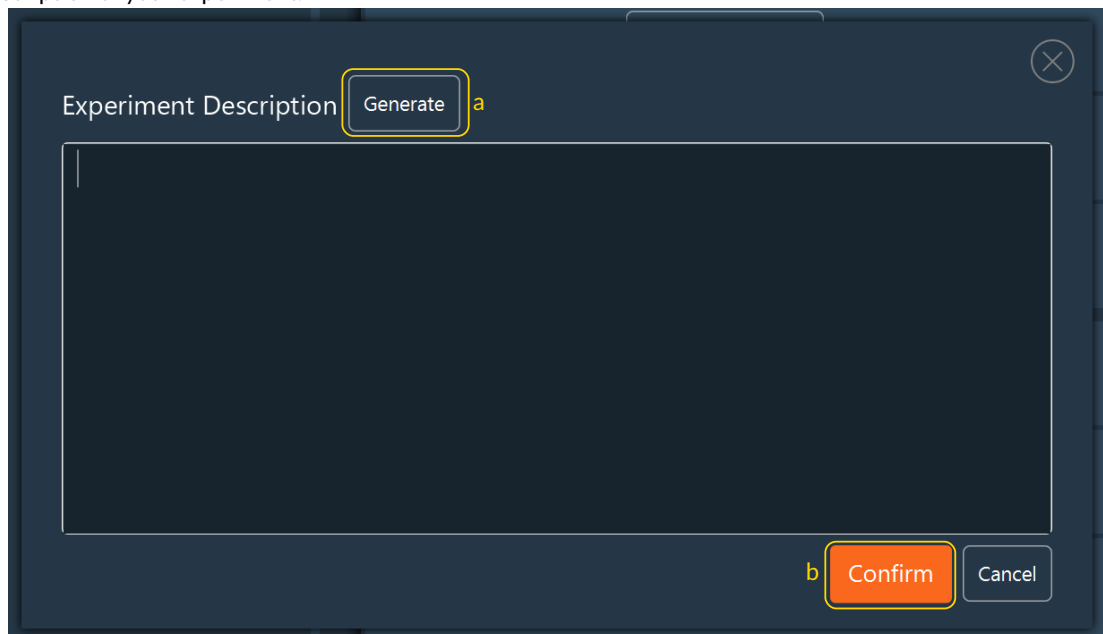
### 1.4.1 Acquisition

Use this tab to configure complete multi-dimensional acquisition sequences across all available dimension modules.



To set up a multi-dimensional acquisition:

1. Select the required dimension(s) from the panel.
2. Choose the appropriate [data processor](#).
3. Specify the save directory for the output files.
4. Edit a description of your experiment.



- a. Click **Generate** to add acquisition sequence description and/or Write your own experiment description.
  - b. Confirm to close the window.
5. Launch the acquisition.

 **Note**

The interface varies depending on the microscope system hardware, particularly the available dimensions.

All dimensions share a consistent interface. The UI controls for configuring dimensions include:

| Icon                                                                                | Description                                                                                                                                                        |
|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|    | <b>Inactive:</b> The sequence excludes the dimension. Click to activate.                                                                                           |
|    | <b>Active:</b> The sequence includes the dimension. Click to deactivate.                                                                                           |
|    | <b>Configured:</b> The dimension's parameters are set. Here, 11 images will be acquired for this dimension.                                                        |
|   | <b>Edit:</b> Click to configure the dimension settings.                                                                                                            |
|  | <b>Reorder:</b> Drag and drop to change the dimension execution order. The dimension order defines the nesting loop of the sequence, executing from top to bottom. |

Dimension Execution Order

The order of the active dimensions dictates the nested loop structure of the acquisition sequence. Use the **Reorder** handle to drag and drop dimensions to change their execution order.

Dimensions at the top of the list act as the outer loops of the sequence, progressing more slowly. Dimensions positioned lower in the list act as the inner loops, executing more frequently. For example, if **Time** is placed above **Channels**, the system acquires all channels for a single time point before progressing to the next time point. Conversely, if **Channels** is placed above **Time**, the system acquires the entire time-lapse sequence for the first channel before switching to the next channel. Permuting these dimensions correctly is critical, as it directly determines how the hardware coordinates the image acquisition.

## 1.4.2 Time

Use the **Time** dimension to configure time-lapse acquisitions to observe dynamic biological processes.

### Configuring a time-lapse

1. Specify the **total number of time points** (frames) required in the acquisition.
2. Define the **interval duration** between consecutive time points (minimum time to wait between frames).

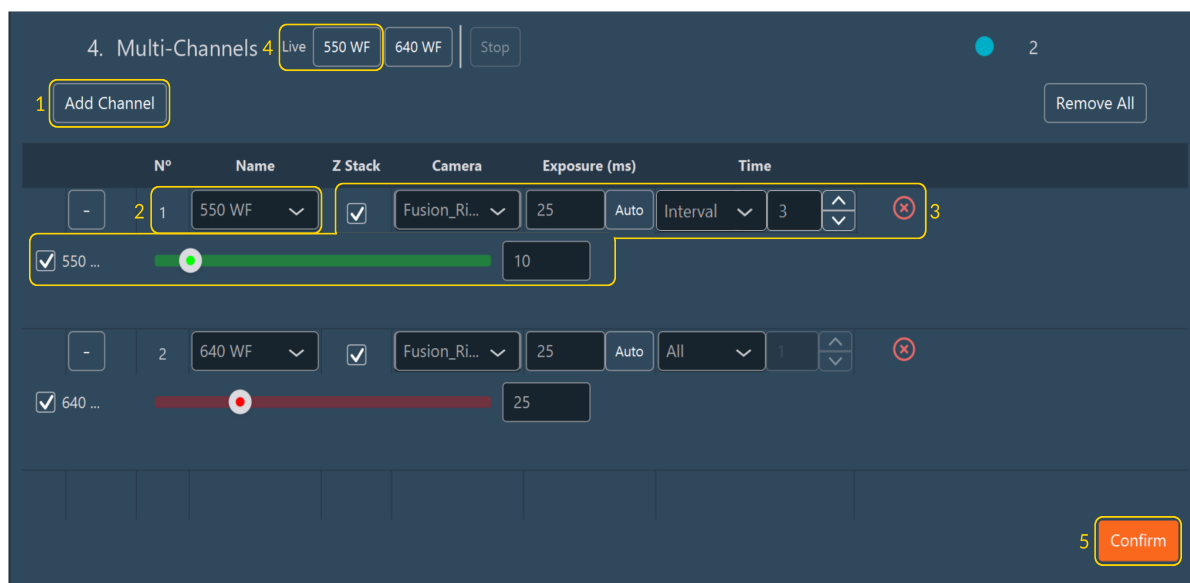
#### Note

The software adapts the **Total Time** automatically. You may also set the total time manually and infer the interval duration for a fixed number of time points.

1. Enable **Burst Mode** to acquire images continuously at the maximum hardware frame rate, ignoring the specified interval. Use this to capture rapid kinetics.
2. Click **Confirm** to save the time dimension settings.

### 1.4.3 Multi-Channels

Use the **Multi-Channels** dimension to configure one or more channels (e.g., fluorescence, brightfield) for the acquisition sequence. Channels must have been defined previously in the [Configuration Tab](#).



#### CONFIGURING CHANNELS

1. Click **Add Channel**.
2. Select a channel from the list.
3. Adjust the following parameters for each channel independently:
  - **Z-stack**: Enables the Z-stack dimension specifically for this channel. If you uncheck this box, the center plane will be acquired.
  - **Z-Offset**: Applies a spatial Z-offset. This is used when focal planes vary between emission wavelengths (chromatic aberration) or when targets are at different depths.
  - **Shutter Blink**: Optimizes hardware shutter actuation to minimize sample exposure time and prevent photobleaching.
  - **Camera**: Assigns the active camera (for multi-camera systems).
  - **Exposure (ms)**: Defines the sensor exposure time for the channel.
  - **Time**: Defines the acquisition frequency within a time-lapse (e.g., acquire this channel at all time points, only the first time point, only the last time point, or at intervals).
  - **Intensity**: Adjusts the excitation light source intensity (hardware-dependent, usually % power).
  - **Red cross**: Delete a channel
4. To start a real-time preview of a channel, click the button corresponding to the channel's name (located next to the **Live** label in the multi-channel header). To apply any subsequent configuration changes, click the button again.
5. Click **Confirm** to apply the configuration.

#### Note

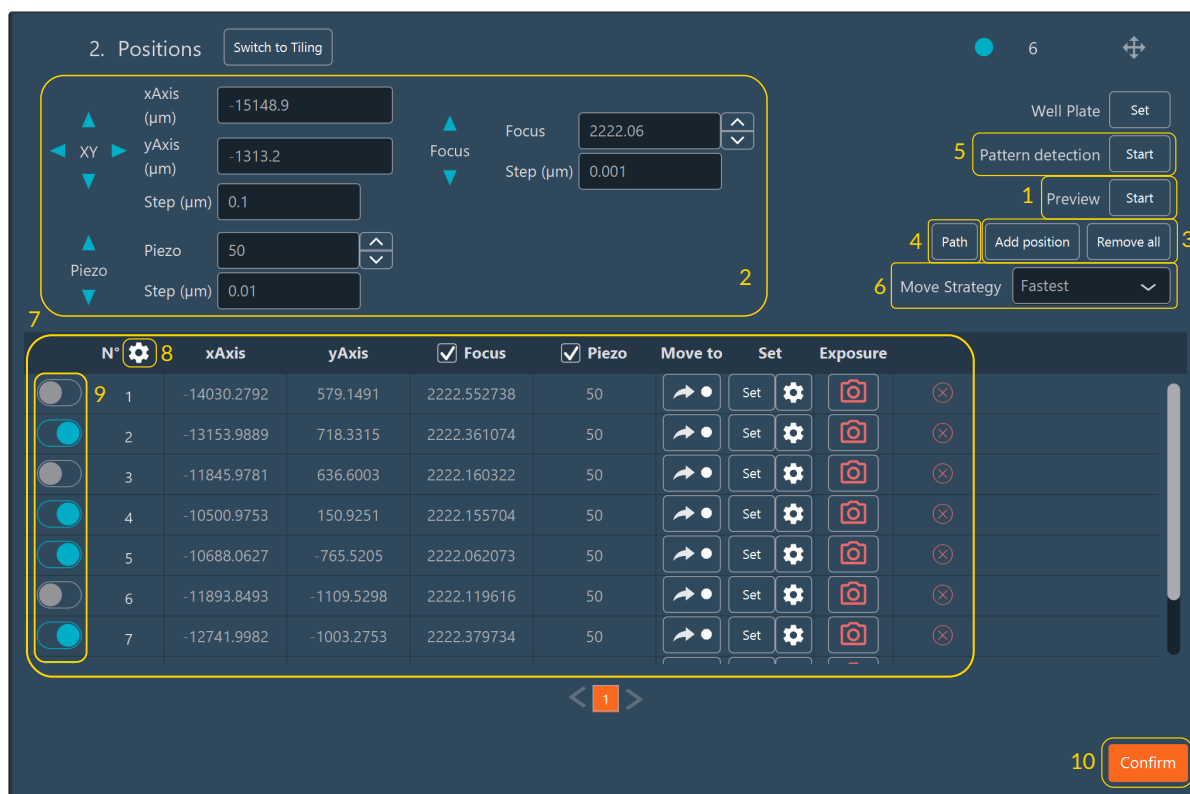
If you change the exposure time or intensity during a live preview, click **Live channel** again to refresh the hardware and update the image stream.

#### Tip

Reorder the execution sequence of the channels by dragging and dropping them in the list.

## 1.4.4 Positions

The **Positions** dimension captures multiple discrete XYZ locations across a sample.

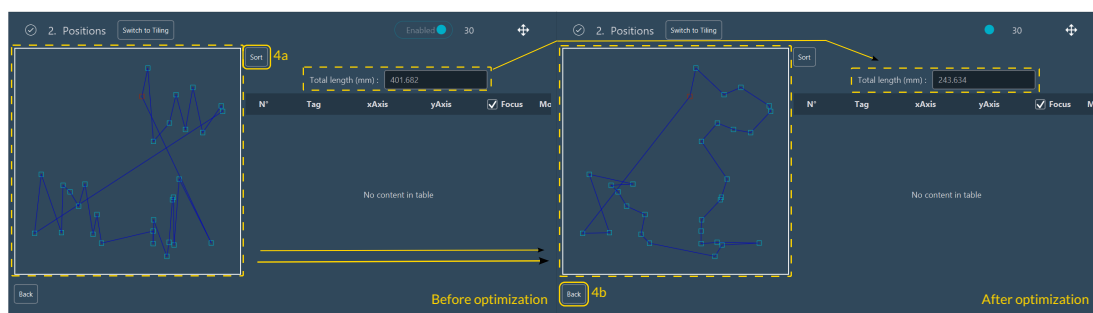


### Switch to Tiling

Toggle directly to the **Tiling** dimension using the **Switch to Tiling** button to capture a continuous mosaic rather than discrete points.

## Adding and Optimizing Positions

1. Generate a fast **Preview** of the sample to act as a navigation map.
2. Move the **stage** to the target area and adjust the **focus**.
3. Click **Add position** to record these XYZ coordinates in the position list.
4. To optimize the **stage movement** trajectory across multiple positions, click the **Path** button.



{ .center width=100% }

- a. By default, the stage visits positions in the order they were added. The interface visualizes this path and calculates the total travel distance. Click **Sort** to compute an optimized route (e.g., shortest path) that minimizes travel time.
  - b. Once optimization is complete, click the **Back** button to return to the principal position view.
5. Use the **Pattern Detection** feature to automatically find XYZ coordinates matching a visual pattern in the sample.



Pattern Detection

New Tiling

5b

5c

5d

5e

Pattern to use

Current live image

Roi

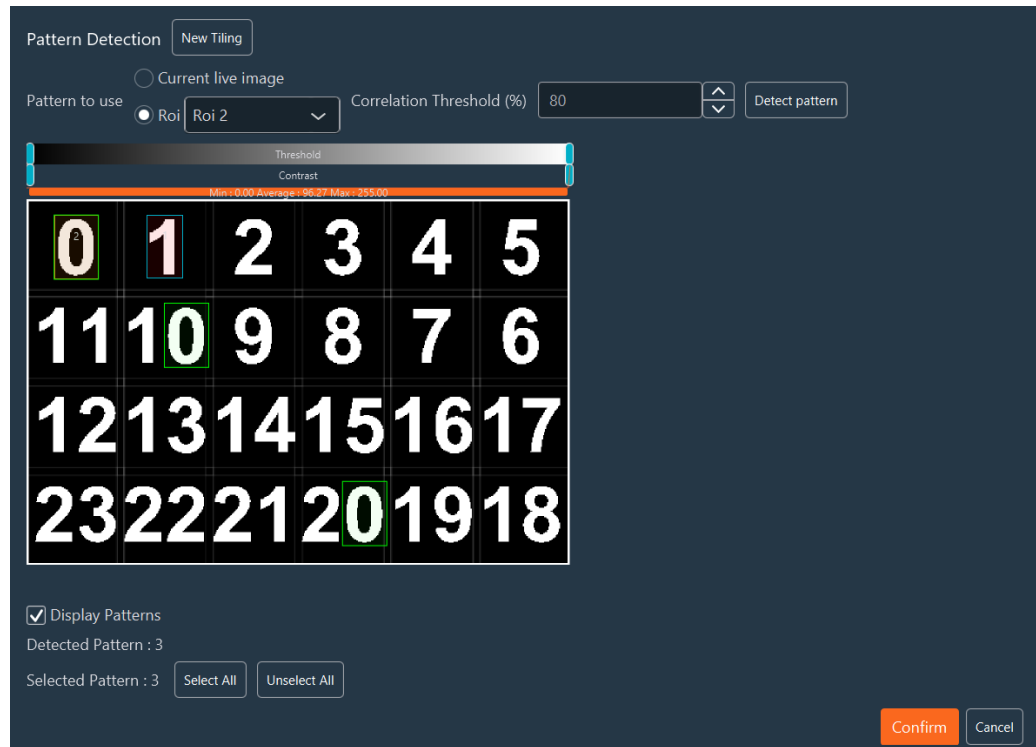
Roi 2

Correlation Threshold (%)

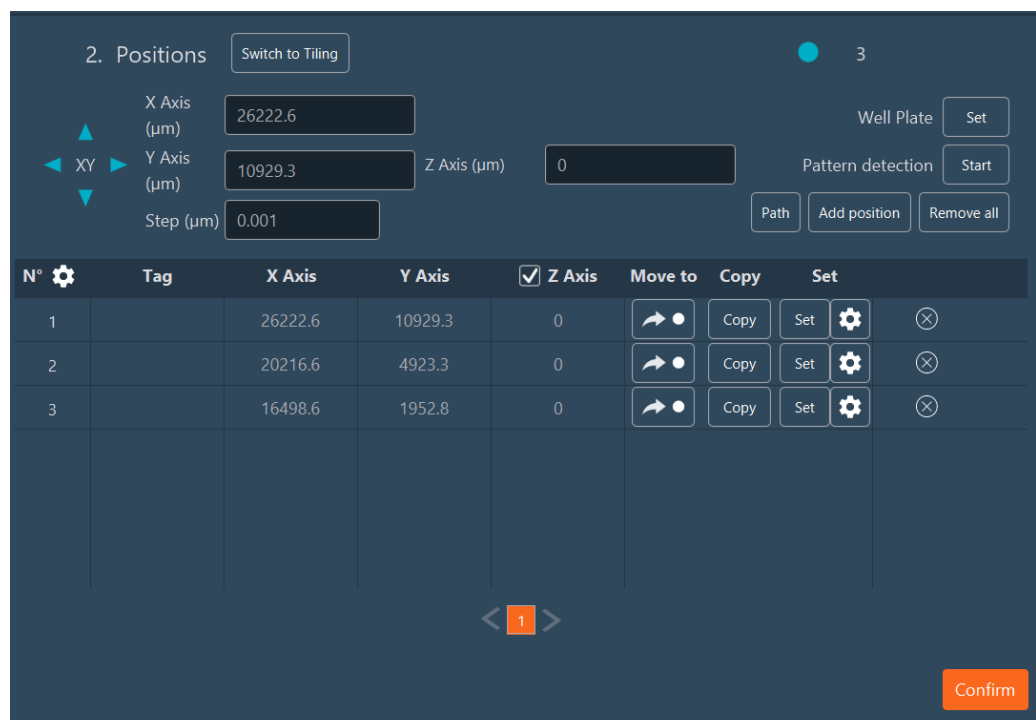
80

Detect pattern

- **a.** Click the **Start** button located in the Pattern Detection section.
- **b.** Create a reference scanning area by clicking **New tiling**. Configure it as described in the [Tiling dimension section](#).
- **c.** Define the source pattern to detect:
  - **Current Live Image:** Extracts the pattern from the current live FOV.
  - **ROI:** Draw an ROI around the specific biological pattern to detect.
- **d.** Set the **Correlation Threshold** (this defines the minimum similarity percentage to identify a match).
- **e.** Click **Detect pattern** to initiate the scanning algorithm.
- **f.** Recognized patterns are highlighted with bounding ROIs over the preview. Manage these selections using **Select All**, **Unselect All**, or by clicking individual ROIs to toggle them.



- **g.** Click the **Confirm** button. The geometric coordinates of each detected pattern are appended automatically to the position list.



6. Configure the **Move Strategy**: For multiposition acquisitions spanning large travel distances, select how the stage translates:
  - **Fastest**: Navigates directly from point A to point B at maximum speed.
  - **XY Split**: Translates between positions incrementally. Specify the movement interval in the **Step (µm)** field.

## 7. Position Editing & Overrides:

- **X-Axis/Y-Axis:** Manually modify coordinates.
- **Focus / Z-axis:** Check this box to force the system to use the Z-value saved for this position. If unchecked, the microscope maintains its current live Z-value upon arrival.
- **Move to:** Immediately drives the stage to this coordinate.
- **Set:** Overwrites the saved coordinates for this position with the current live XYZ stage position. Clicking the



modifies specific axes (X, Y, or Z) and applies global offsets.

×

xAxis

yAxis

Focus

Old

31000.3

23256.2

5126.28

New

☒ 35275.8

☐ -23663.6

☐ 5126.22

Offset

☐ 4275.5

☐ -407.4

☐ -0.06

Apply to

Selected Position

All Positions

- **Custom Exposure per Position:** Assign unique camera exposure times to specific spatial positions (useful if sample brightness varies drastically across a tissue section).

[illegible]

1. Click the **camera icon** on the row corresponding to the position where you want to adjust the exposure time.
2. Check the **Custom** box to unlock exposure time modification.
3. Modify the exposure value in milliseconds. This override applies only to the currently active channel.
4. Click **Apply** to confirm the changes.

When a row is modified, the **camera icon color** changes from **red to green**, serving as a visual indicator that a custom exposure time is active.

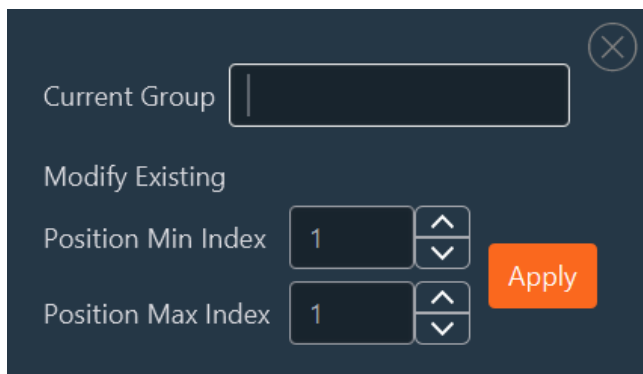
| N° | xAxis    | yAxis   | <input checked="" type="checkbox"/> Focus | Move to                                                                              | Set                                                                                       | Exposure                                                                                                                                                                    |
|----|----------|---------|-------------------------------------------|--------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1  | -12360.6 | -1497.9 | 2389.58                                   |  | Set  |   |
| 2  | -14100   | -959.4  | 2389.56                                   |  | Set  |   |



: Permanently deletes this position from the list.

8. **Grouping:** To manage large numbers of positions, cluster them into logical groups. Click the **gear icon** to access group settings:

- Assign a semantic name to the group.
- Indicate the contiguous range of position indices to group together (e.g., 1-15).
- Click **Apply**.
- Repeat these steps to create multiple, non-overlapping groups.



The screenshot shows a dark-themed dialog box for grouping settings. At the top right is a close button (X). Below it is a text input field labeled "Current Group". Underneath is the section "Modify Existing", which contains two rows: "Position Min Index" and "Position Max Index". Each row has a numeric input field (both containing "1") and a vertical spinner control with up and down arrows. To the right of these controls is an orange button labeled "Apply".

9. **Activate** or **Disable** the position. This allows you to store a specific position without actively acquiring it.

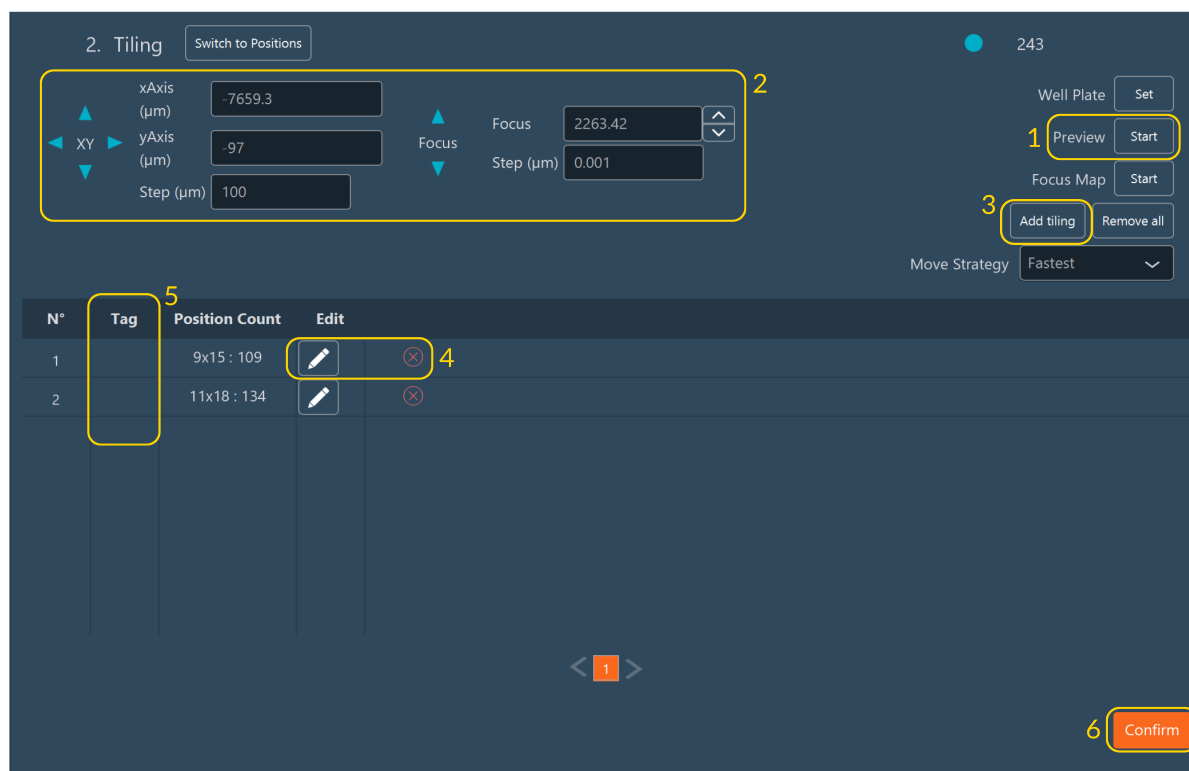
10. When all position geometries and parameters are finalized, click **Confirm**.

🕒 2026-07-17

## 1.4.5 Tiling

### Creating a Tiling

Use the **Tiling** dimension to capture large sample areas through automated mosaic acquisition.



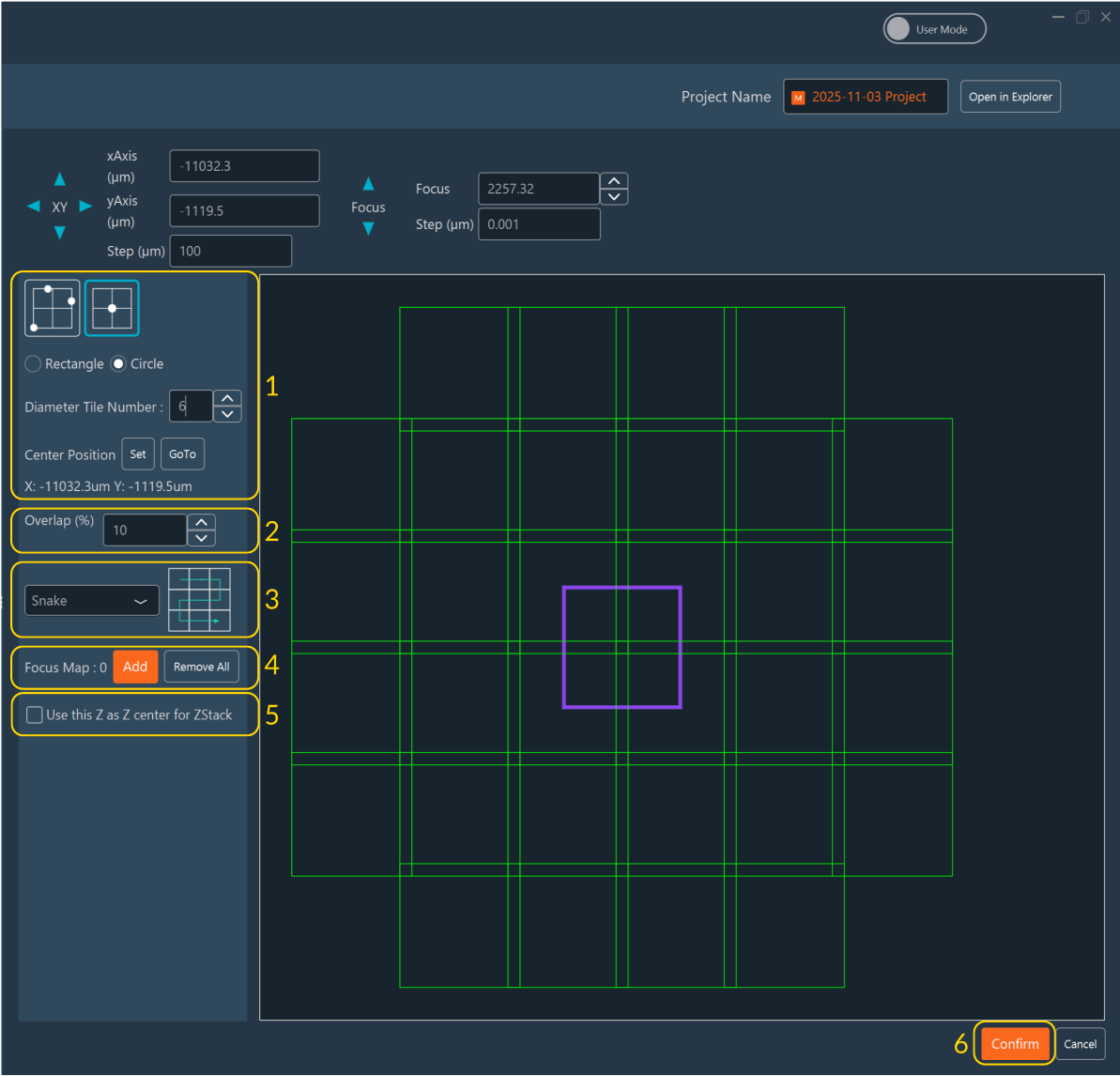
#### Switch to Positions

You can toggle directly to the [Positions dimension](#) using the **Switch to Positions** button if you require discrete capture points instead of a continuous mosaic.

1. Generate a low-resolution [Preview](#) of the sample.
2. Move the stage to the region of interest and focus the image.
3. Click **Add tiling** to append a new tiling configuration block to the dimension list.
4. To configure the parameters of a tiling block, click the **Pen** icon. To delete a block, click the **Red Cross**.
5. Assign a label to each tiling block by modifying its field in the **Tag** column.
6. Click **Confirm** to save the tiling configuration.

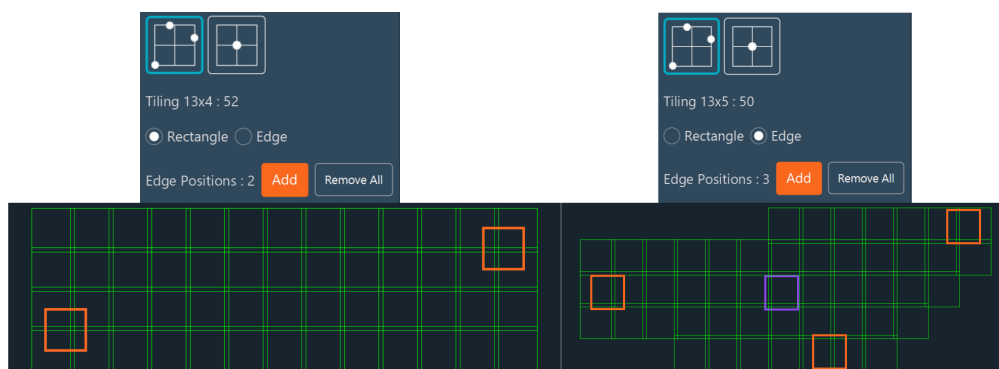
### Setting Up a Tiling Layout

Define the physical tiling boundary using two topological methods:

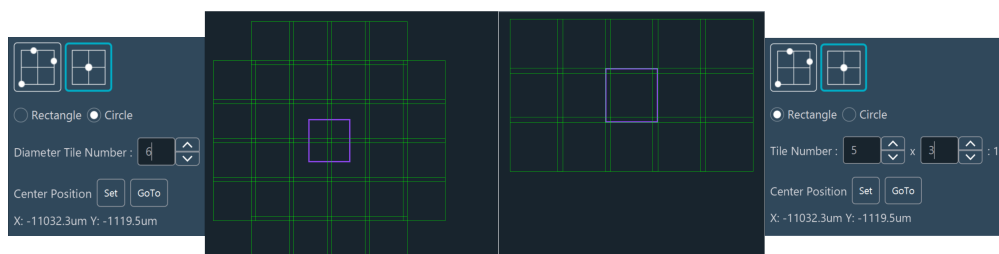


## 1. Define Boundaries:

1. **By Edge:** Click **Add** to drop coordinates outlining the region of interest. The software connects these points to form a polygon.
  - Clear the bounding box using **Remove All**, or delete a single vertex by right-clicking it.



2. **By Center:** Drive the stage to the center of the acquisition region and click **Set**. Then, select either a rectangular or circular tiling geometry.
  - For a rectangle, specify the bounding grid dimensions (e.g., 3x3 tiles). For a circle, specify the bounding diameter.



### Note

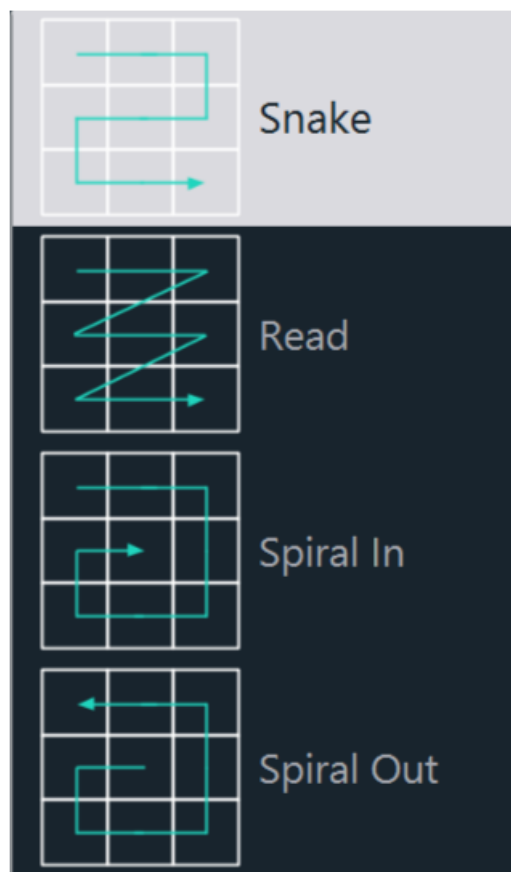
Use the right-hand panel controls to manually jog the hardware stage and manage your defined coordinate vertices.

2. **Define Tile Overlap:** Set the overlap percentage between adjacent frames. A 10% overlap is standard for downstream stitching.
  - Enable **Auto Mode** to let the software calculate the overlap ratio based on your specified coordinate boundaries.
  - To manually dictate the translation distance between individual tiles, enable the **Custom Step** option via the [Display Settings](#) menu.

### Important

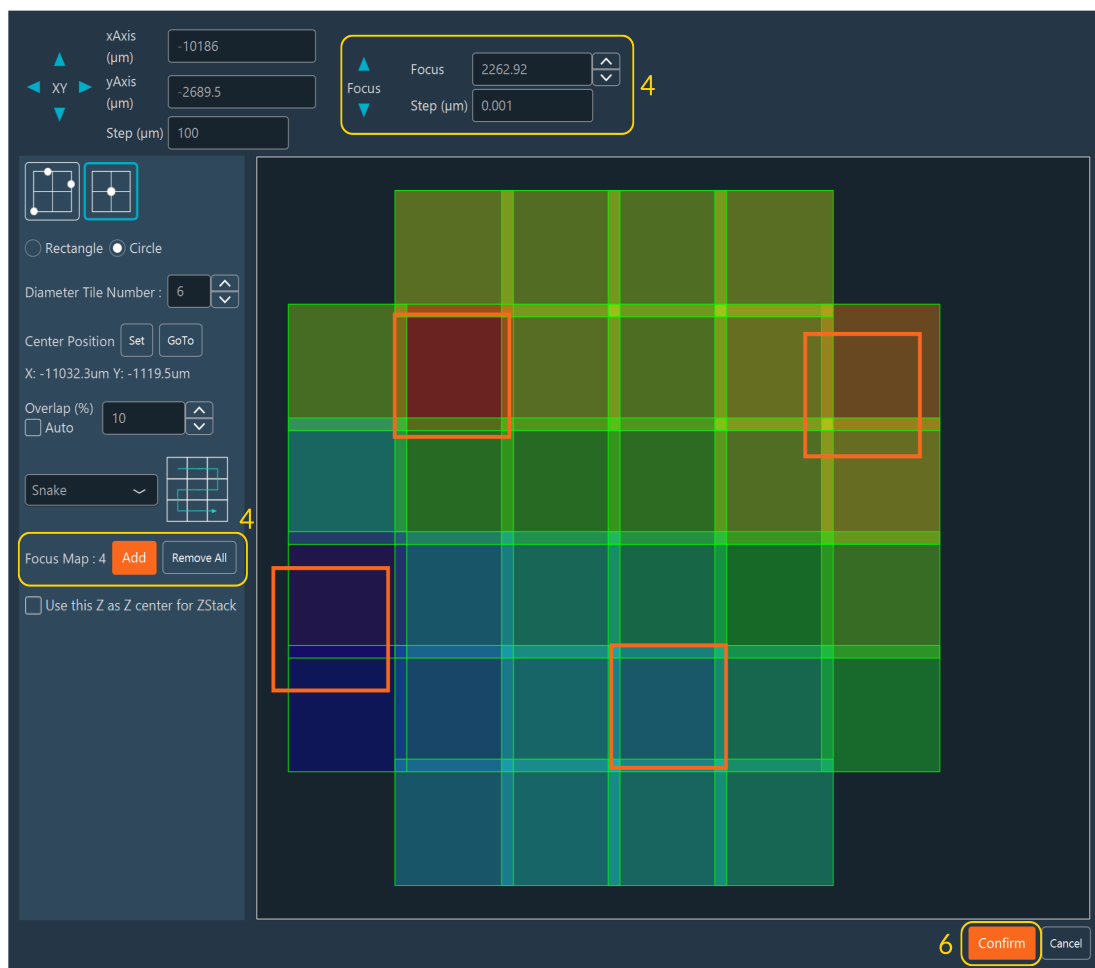
Accurate overlap is necessary for successful post-acquisition image stitching.

3. **Select Read Mode:** Choose the scanning path (e.g., Spiral, Snake) the stage executes during acquisition.



4. **Generate a Focus Map:** To compensate for uneven sample topology across a tiling area, create a Focus Map. Navigate the XY plane using the interface joystick or by clicking within the tiling map. Adjust the Z-focus at various locations, and click **Add** to store each point.
- Add points as necessary to model the sample's topography. Right-click any point (orange indicator) to remove it.
  - The software applies a color gradient overlay to the map, representing the interpolated Z-surface altitude across the acquisition region.





### 5. Configure Z-Stack Integration:

- Select the corresponding checkbox inside the tiling editor to center Z-Stacks around the interpolated Z-values stored in the Focus Map.
- If unchecked, the sequence uses either global Z-Stack parameters or the current live Z-position.

### 6. Click **Confirm** to save the spatial tiling configuration.



#### Note

- **Orange squares:** Indicate saved boundary vertices or focus map reference anchors.
- **Purple square:** Indicates the real-time physical position of the XY stage.



#### Tip

Use the **GoTo** button to rapidly drive the mechanical stage back to a previously saved topological reference position.

## Focus map automated setup for simple and multiple tilings

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

2. Tiling

Switch to Positions

243

XY

xAxis (µm)

-7659.3

yAxis (µm)

-97

Step (µm)

100

Focus

Focus

2263.42

Step (µm)

0.001

Well Plate

Set

Preview

Start

1 Focus Map

Start

Add tiling

Remove all

Move Strategy

Fastest

| N° | Tag | Position Count | Edit                              |
|----|-----|----------------|-----------------------------------|
| 1  |     | 9x15 : 109     | <div><div></div><div></div></div> |
| 2  |     | 11x18 : 134    | <div><div></div><div></div></div> |

<

1

>

Confirm

1. Click **Focus Map Start** within the **Tiling** dimension to begin configuration.
2. Set the following parameters for both simple and multiple tilings:
  - a. Select the step and size of the Z-stack.
  - b. Select an existing pattern.
  - c. Select the channel used to acquire the stack.
  - d. (Optional) Select **Filter unsure position** and configure the corresponding parameters:
    - i. **Filter Radius**: Smooth noise by replacing each pixel with the median of its neighbors (e.g., 0 = no smoothing, 1 = median of a 3x3 square surrounding the pixel, 2 = median of a 5x5 square surrounding the pixel).
    - ii. **Background ROI**: Select the Region of Interest (ROI) used to automatically calculate background values.
    - iii. **Background**: Define the mean background value.
    - iv. **Background Delta**: Define the acceptable noise amplitude.
    - v. **Valid Pixels**: Define the minimum percentage required to validate a position.
3. Select the tiling region for which to create the Focus Map.
4. Click **Start**. The system scans all points, generates the Focus Map, and displays the determined focal points. A progress bar indicates the current progression per tiling region. Once the Focus Map is complete, the software redirects you to the **Tiling** dimension interface.

5. Click **Confirm** to validate and proceed with the acquisition sequence setup.

## Live Visualization of a Tiling

To follow the mosaic as it is built during acquisition, configure a [Data Processor](#) before starting the sequence.

**RECOMMENDED: LIVE PREVIEW, STITCHING AFTER ACQUISITION**

1. Open the Data Processor and add a new node to the **RawData** root node.
2. Set the node **Type** to **SIMPLE\_TILING**.
3. Start the acquisition. Each frame is placed at its grid coordinates as it arrives, so the mosaic assembles live in the visualization panel. No stitching is applied.

The **SIMPLE\_TILING** output is not written to disk. It remains available for review in the **Visualization** tab after the acquisition, listed as **SIMPLE\_TILING**.

To obtain the final stitched image, run a separate post-acquisition processing with the **STITCH\_TILING** operation and export the result to disk. See [Tiling Stitching](#).

**Tip**

Stitching after the acquisition allows the stitching parameters to be tested and adjusted on the acquired data, and repeated if the result is unsatisfactory.

**ALTERNATIVE: STITCHING DURING ACQUISITION**

A serial pipeline can also stitch on the fly:

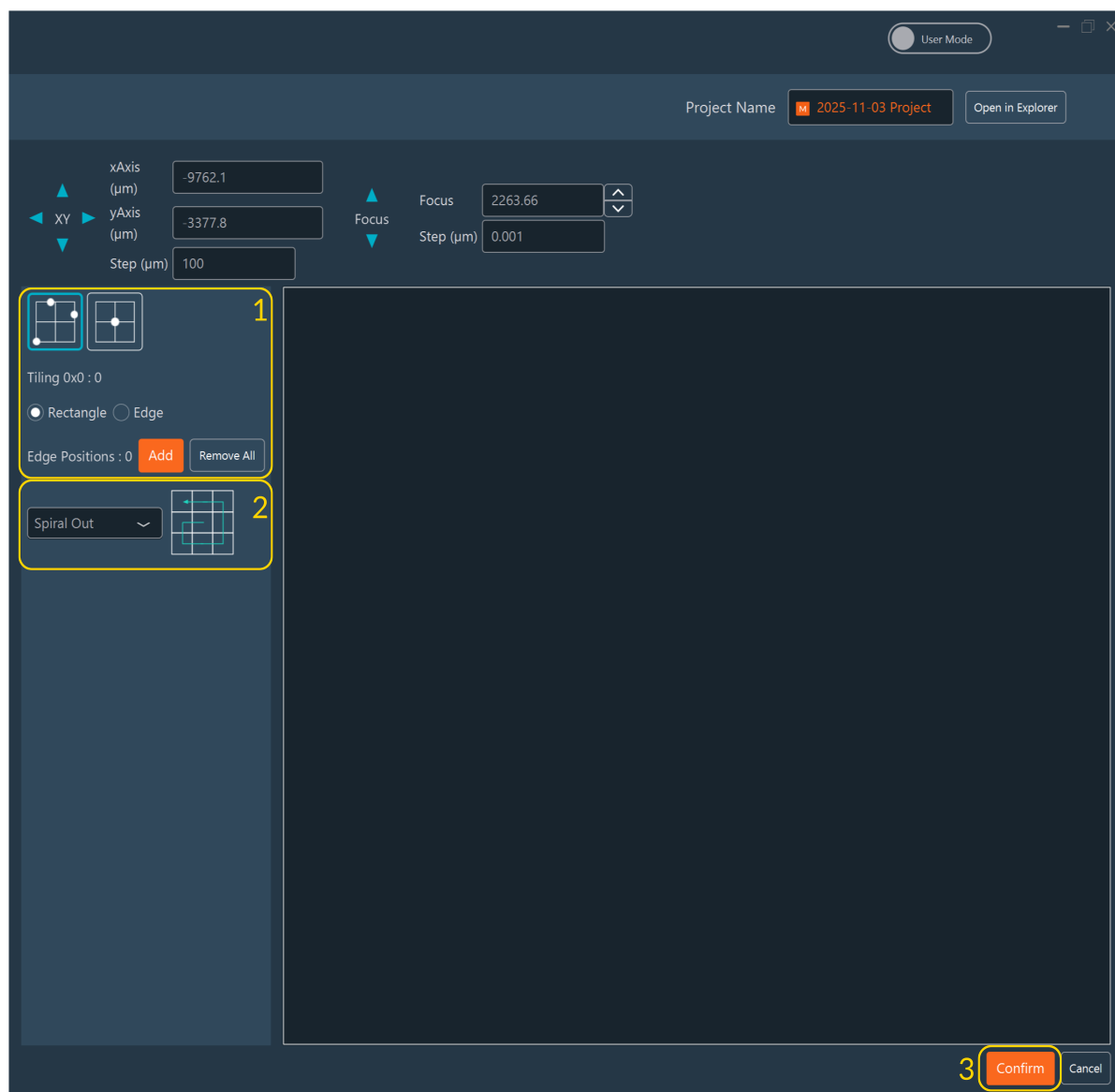
1. Add a **SIMPLE\_TILING** node to **RawData**, then chain a **STITCH\_TILING** node to it.
2. Before starting the acquisition, click **Edit parameters** next to the Data Processor and set the stitching options.
3. Start the acquisition. Stitching is performed at the end of the sequence.

**Warning**

With this pipeline, the tiling is not displayed live, and the stitching parameters cannot be adjusted once the acquisition has started. Unsuitable parameters produce a failed stitching that can only be corrected by reprocessing the data. Post-acquisition stitching is therefore recommended.

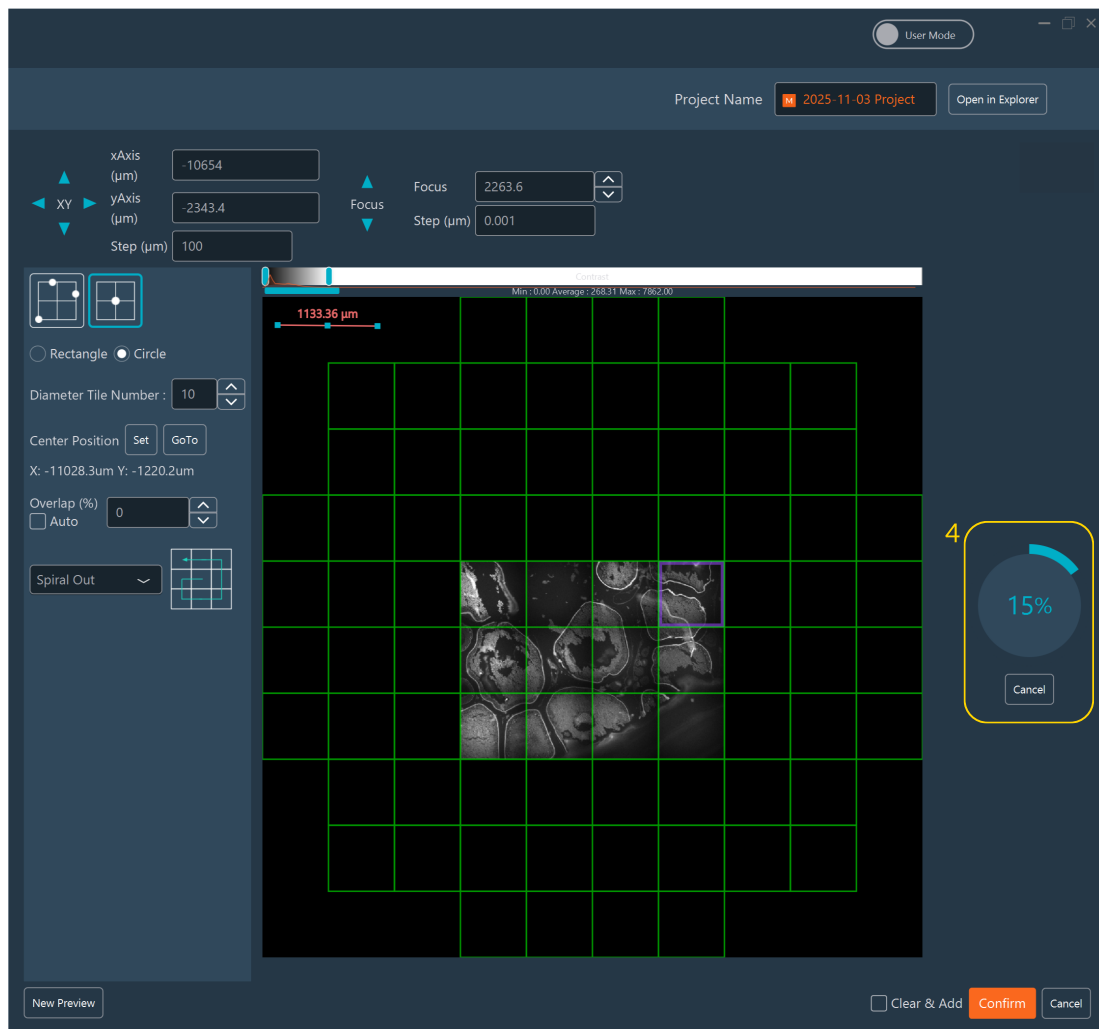
### 1.4.6 Preview Feature

The **Preview Feature** performs a fast, low-resolution scan of the sample to generate an interactive map. Use this map to identify regions of interest, set up multiposition coordinates, or define mosaic boundaries without exposing the entire sample to high-intensity light or capturing full-resolution data.

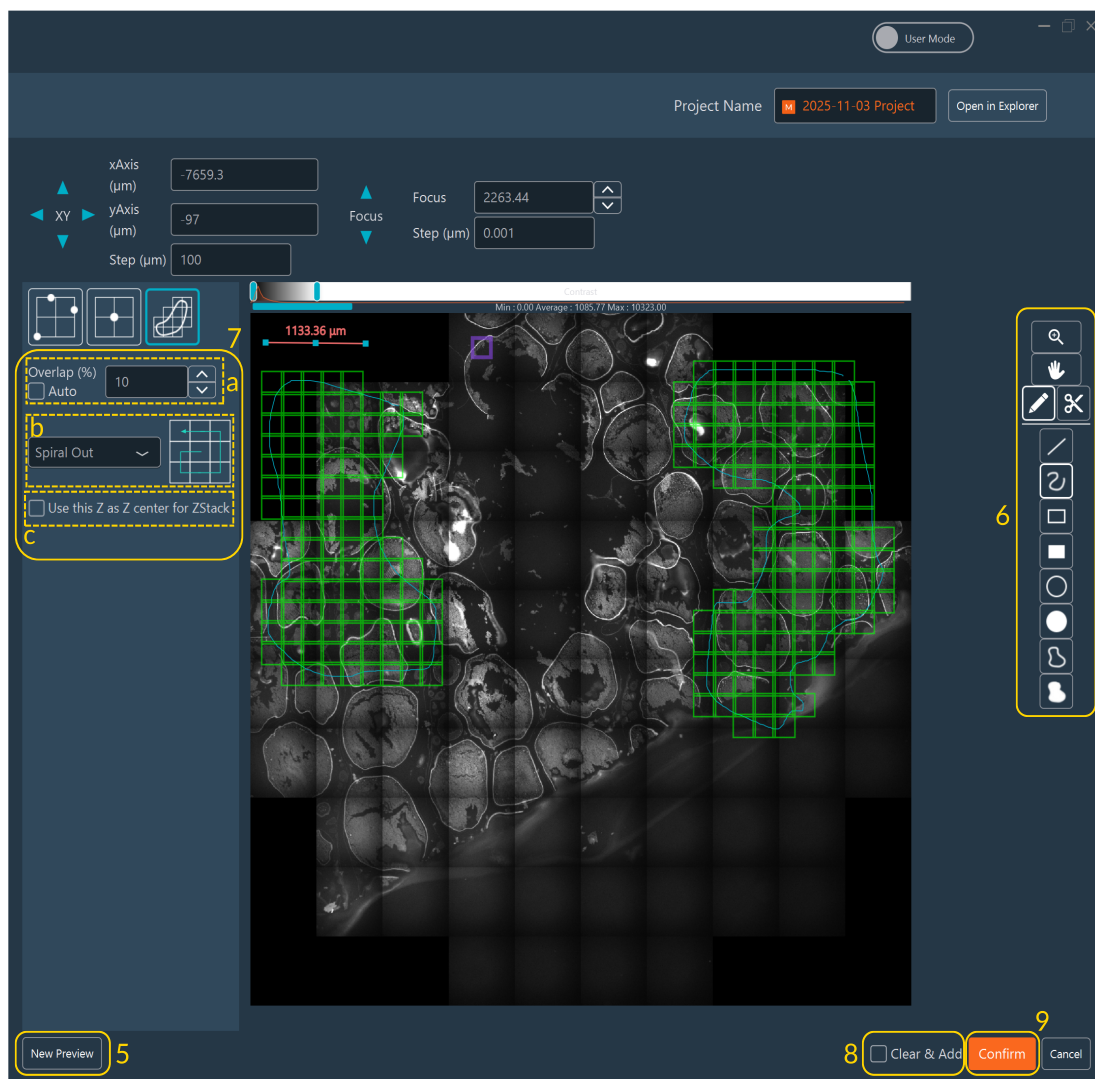


#### Setting up a Preview Scan

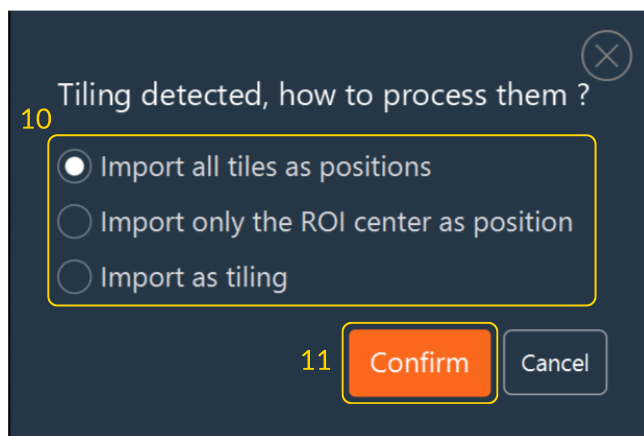
1. Configure the boundaries of the **Preview** area. Similar to the [Tiling dimension](#), you have two setup options:
  - Click **Add** at multiple boundary points while moving the stage to trace a custom polygon outlining your region of interest.
  - Alternatively, click **Set** to define the center coordinate, then specify the bounding grid dimensions (horizontal/vertical tile count).
2. Select a **Read Mode** for how the stage traverses the area (e.g., Spiral, Snake).
3. Click **Confirm** to lock in the configuration.



4. The preview scan begins, rendering the map in real-time. You can abort the acquisition at any time by clicking **Cancel**.



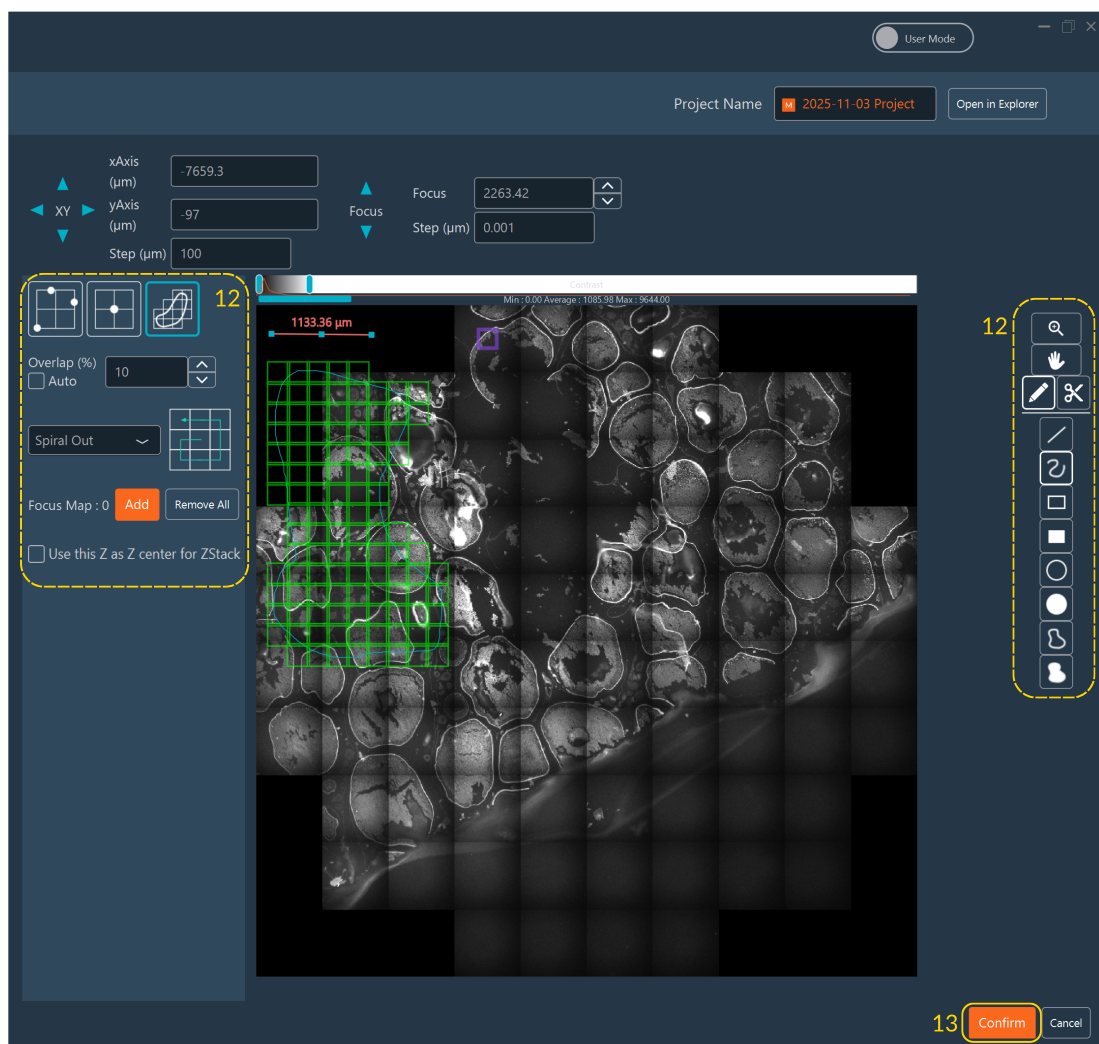
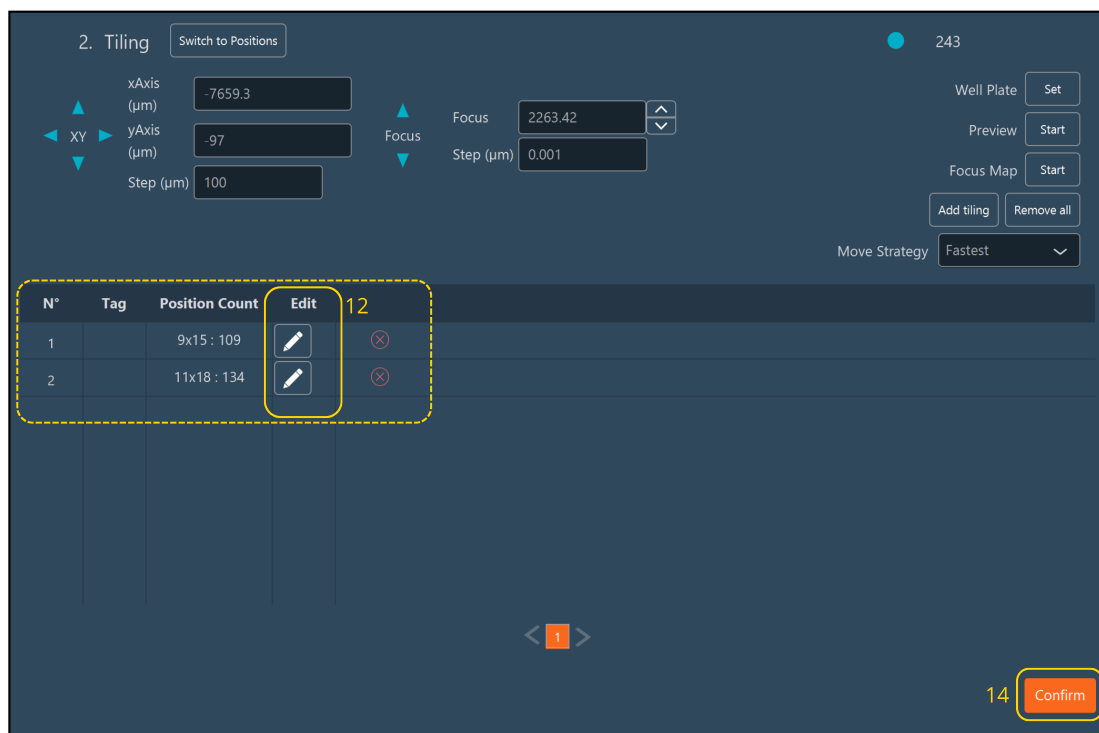
5. Click **New Preview** to discard the current map and start over in a different region.
6. Draw **Regions of Interest (ROIs)** directly onto the map to mark targets. Use the provided tools: **point** (ideal for discrete focal points used in the Positions dimension), **freehand**, **circle**, or **rectangle**.
7. Adjust the overlapping tiling settings for the selected ROIs:
  1. Enable **Auto Mode** to let the software calculate the image overlap threshold (available only for Tiling imports).
  2. Choose the specific scanning **Read Mode**.
  3. Check the **Z-reference** box to explicitly center subsequent Z-Stacks around the Z-value established during this preview/focus map phase. If unchecked, the system reverts to the global Z-Stack parameters.
8. Check **Clear & Add** if you want these new targets to overwrite previously defined tilings or positions. Leave it unchecked to append them.
9. Click **Confirm** to validate the ROI selections.
10. If you drew ROIs that encompass multiple camera tiles but intend to import them into the **Positions dimension**, the software will prompt you on how to handle the spatial discrepancy:
  - **Import all tiles as positions:** Generates a discrete capture point for every tile covered by the ROI area.
  - **Import only ROI center as position:** Registers a single coordinate at the geometric center of the ROI.
  - **Import as tiling:** Reroutes the coordinate set to the Tiling dimension for continuous mosaic acquisition.



11. Click **Confirm** to apply your routing choice. After confirmation, all generated positions or tilings appear in their corresponding dimension tabs.
12. In the Tiling dimension tab, click the **Pen** icon next to an imported entry to edit it against the original preview map:
  - **Move, modify, or delete** an ROI visually.
  - Change the scanning **Read Mode**.
  - Add a local **Focus Map** within the ROI if the sample is not flat, or if the Z-focus plane needs adjusting across the ROI.

Select whether to use the Z-position saved during this preview as the absolute center of your Z-Stack. If left unchecked, the Z-Stack will default to the Z-value defined in the standalone Z-Stack Dimension parameters.





13. Click **Confirm** to save your modifications to the individual ROI.

14. Finally, click **Confirm** in the main panel to validate the entire **Tiling Dimension** sequence.



Tip

To save the visual output of your preview scan as a reference image, **right-click** anywhere on the preview rendering window.

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1.4.7 Multiwell Plate Mode

Overview

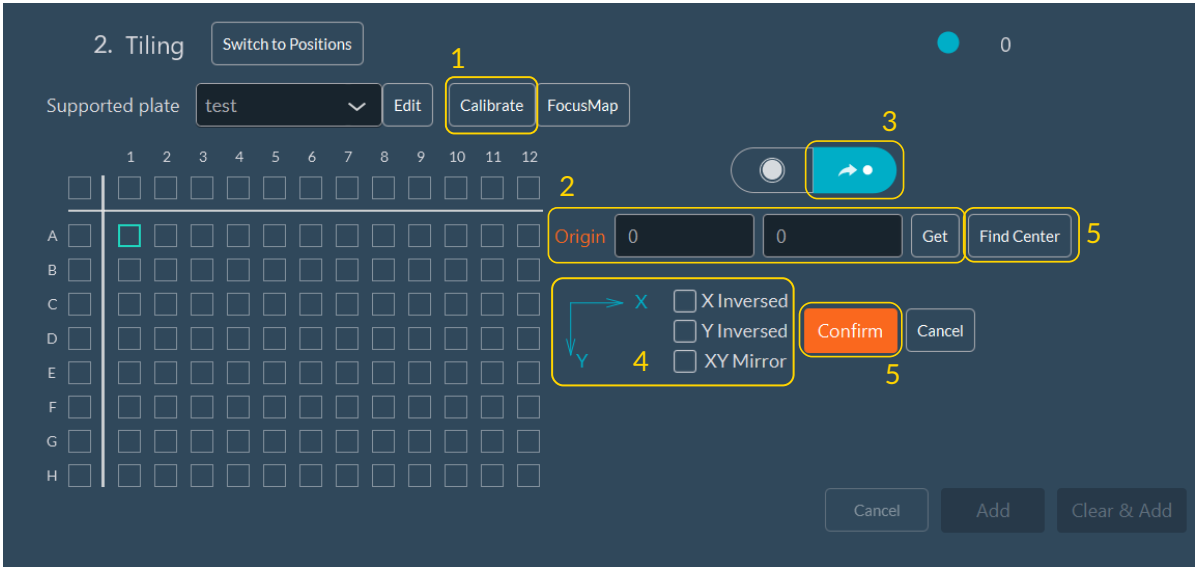
The **Multiwell Plate Mode** automates the imaging of standardized microplates. Access this feature from either the **Positions** or **Tiling** dimensions by clicking the **Set** button.



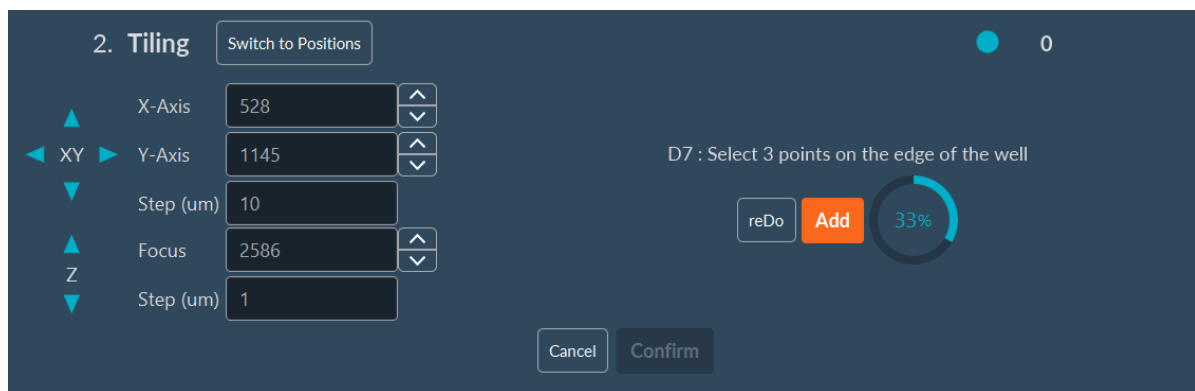
| Button | Description                                                                                                                                |
|--------|--------------------------------------------------------------------------------------------------------------------------------------------|
|        | <b>Select Wells:</b> Click to highlight the wells you intend to image.                                                                     |
|        | <b>Auto Move:</b> Once the plate is calibrated, activate this toggle to automatically physically move the XY stage when you select a well. |
|        | <b>Unselected:</b> The well is excluded from the acquisition.                                                                              |
|        | <b>Selected:</b> The well is included in the acquisition.                                                                                  |
|        | <b>Current Position:</b> Indicates the real-time position of the objective relative to the plate.                                          |

Calibration

Calibrate the system to map the virtual coordinates of the software to the physical dimensions of the well plate. Without calibration, XY stage moves do not land on the intended well.



- 1. Click **Calibrate** to align the stage movement with the multiwell plate.
- 2. Click **Get** to set the origin point of your plate.
- 3. **Move** the stage from the origin to a different well to test the alignment.
- 4. If the stage movement is not correct, **adjust** the orientation.
- 5. Click **Confirm** to save the stage alignment, or click **Find Center** to calibrate the center of the well.



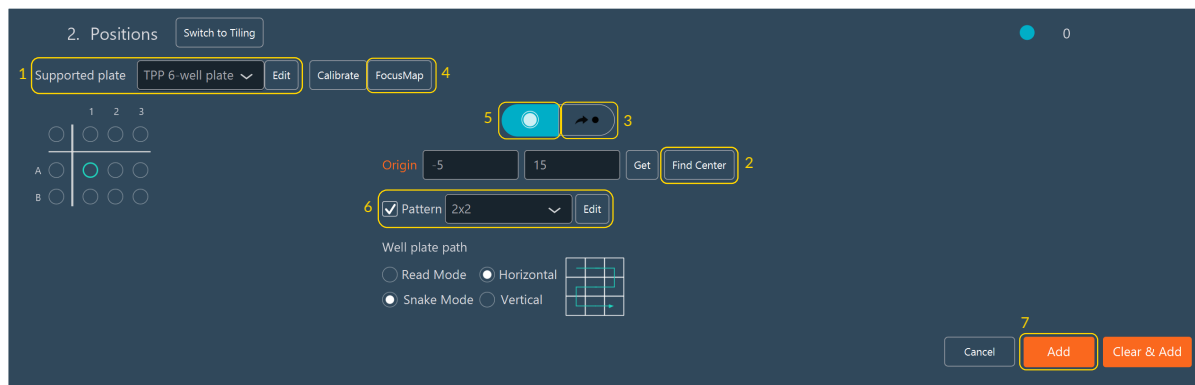
#### HOW TO PERFORM FIND CENTER CALIBRATION?

- Click **Find Center** to start the semi-automated calibration.
- Select a well on the virtual map to serve as the calibration reference.
- Move the stage to that well, if it is not already there.
- While viewing the live image, move the XY stage to align the virtual crosshair with the edge of the well, then click **Add**.
- Repeat this alignment process two more times on different sides (e.g., top, bottom, left) of the same well to establish the geometry.
- Click **Confirm** to complete the calibration.

Once calibration is complete, the software navigates to any well on the plate.

#### Multiwell Mode in the Positions Dimension

Use this mode to capture distinct, single-field-of-view images within designated wells.



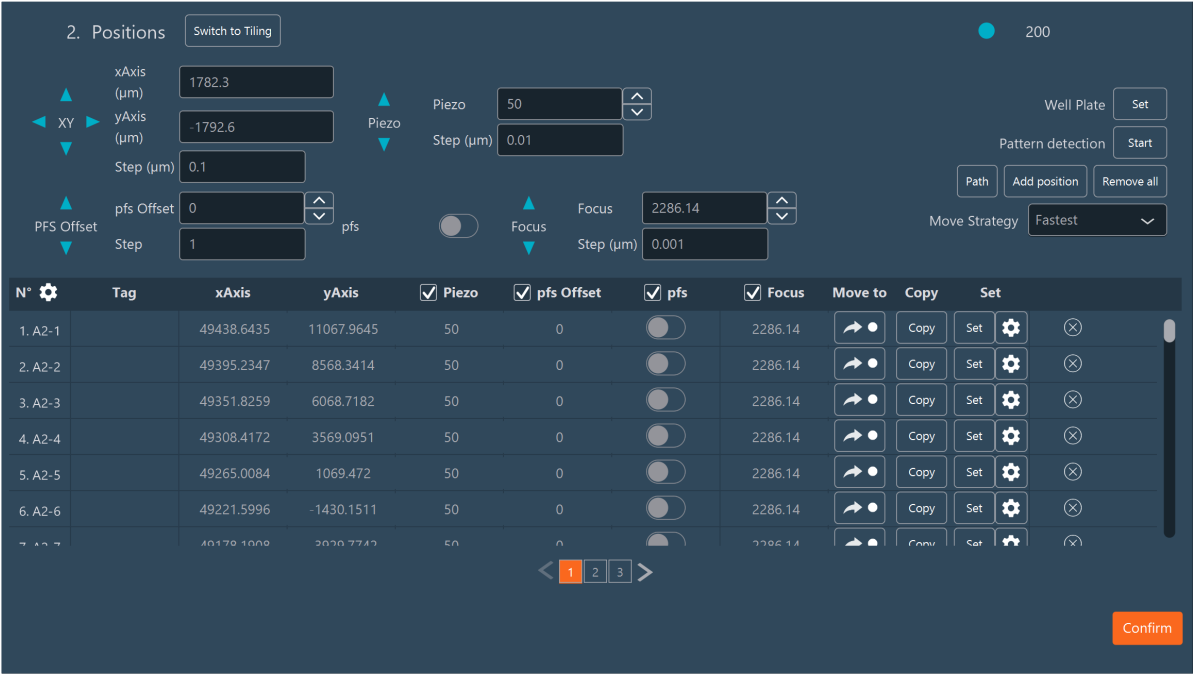
1. Select the plate format (e.g., 96-well, 384-well) from the **Supported plate** dropdown list.
2. Calibrate the well plate using the **Find Center** tool. If this has not already been done.
3. You can now automatically move the stage directly to specific wells.
4. Create a **Focus Map** for the desired wells, if necessary. A focus map maintains focus across uneven plate bottoms.
5. Select the wells to image on the virtual map.
6. To generate an automated imaging pattern within each well, check the **Pattern** box and choose the pattern desired in the drop-down menu. If another pattern is needed, click **Edit** to modify existing pattern or create a new one.
7. Click **Add** to confirm the settings and populate the position list.



#### Tip

**Fast Selection:** In the virtual well plate map, click a row letter or column number to select the entire row or column. You can also click the top-left corner to select the entire plate. To select multiple discontinuous wells, click and drag your mouse to create a selection box.

All generated coordinates are added to the Positions dimension list.



EDITING A PATTERN

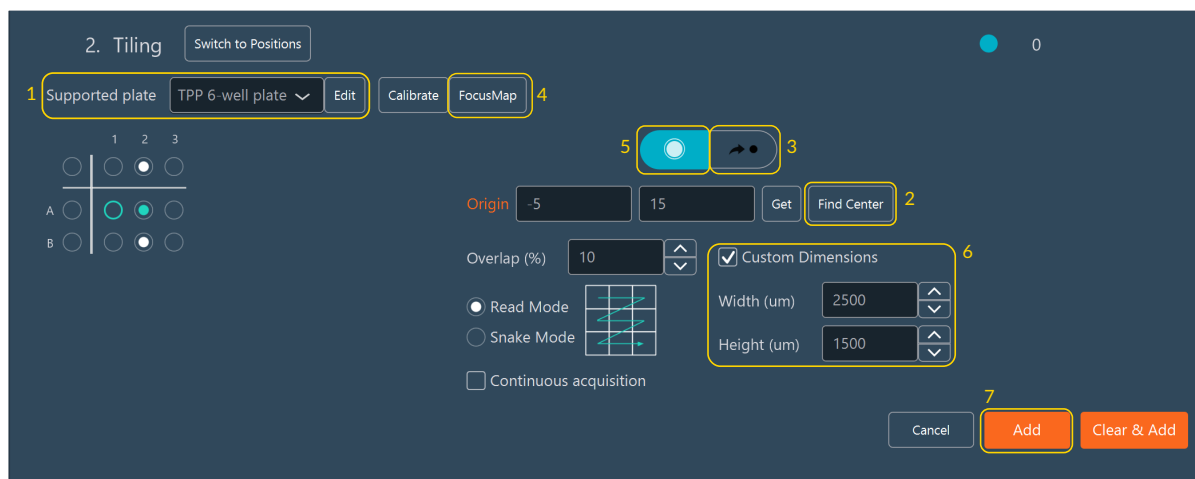
Click **Edit** next to a pattern to modify its distribution parameters:

1. Select the pattern strategy: **Grid** or **Random**.
  - For a **Grid** (a), specify the number of horizontal and vertical images, the spacing distance between adjacent positions, and the X/Y offset (to translate the entire grid relative to the well center).
  - For a **Random** pattern (b), specify the total number of positions and the minimum required exclusion distance between them to prevent overlap.
2. Click **Add** to update the pattern.
3. Click **Switch to WellPlate** to return to the interactive plate overview.



Multiwell Mode in the Tiling Dimension

Use this mode to acquire large, stitched mosaic images covering entire wells or significant portions of them.

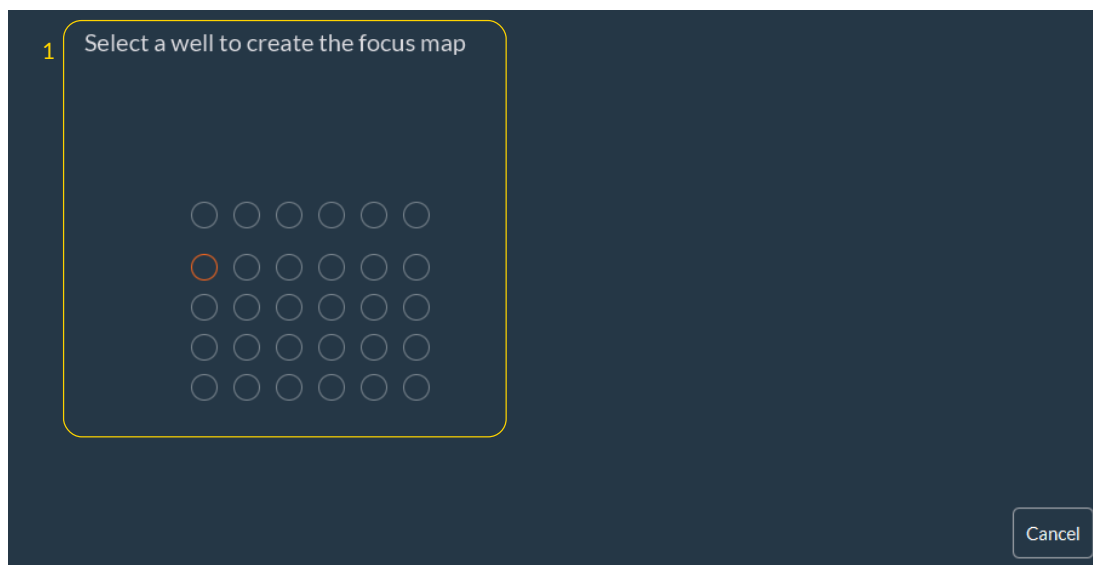


1. Select your plate format from the **Supported plate** dropdown list.
2. Calibrate the well plate using the **Find Center** tool. If this has not already been done.
3. You can now automatically move the stage exactly to specific wells.
4. Create a **Focus Map** for the desired wells, if necessary.
5. Select the wells you intend to image on the virtual map.
6. Check the **Custom Dimensions** box to configure the specific tiling size, tile overlap percentage, and scanning read mode to be applied uniformly within each selected well.
7. Click **Add** to confirm your settings and generate the tiling matrices.

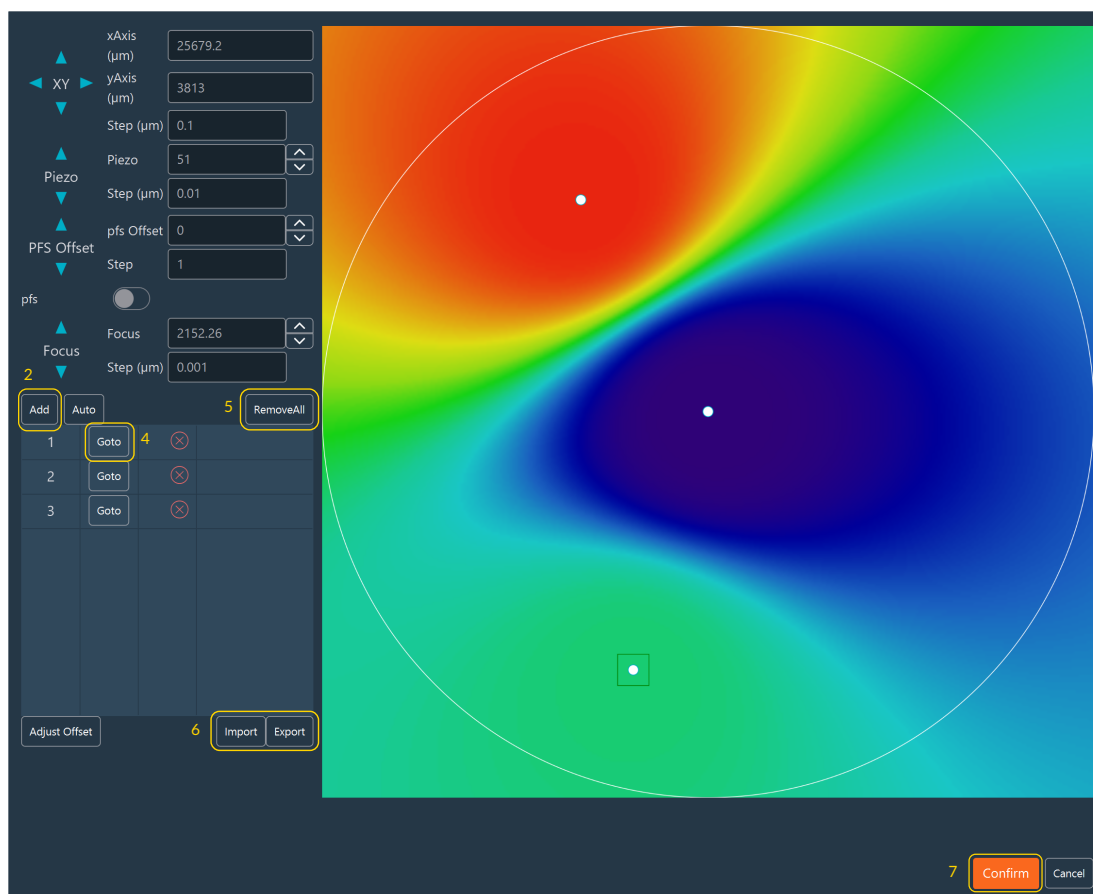
## Manual Focus Map Setup

A Focus Map creates a topological Z-surface to compensate for sample drift or uneven plate substrates.

1. Select the specific well for which you want to create a Focus Map.



2. Move the stage to a desired topological anchor point within the well, manually adjust the focus until sharp, and click **Add**.
3. Repeat this process at multiple disparate points until the Focus Map adequately covers the imaging area. The software interpolates the focus between these points.
4. Click **Go to** to move the stage back to a previously saved focus anchor point for verification.
5. Click **Remove all** to clear all points from the current map.
6. You can also import or export a saved Focus Map using the respective buttons (useful for standardized, recurring assays).
7. Click **Confirm** to lock in your Focus Map.

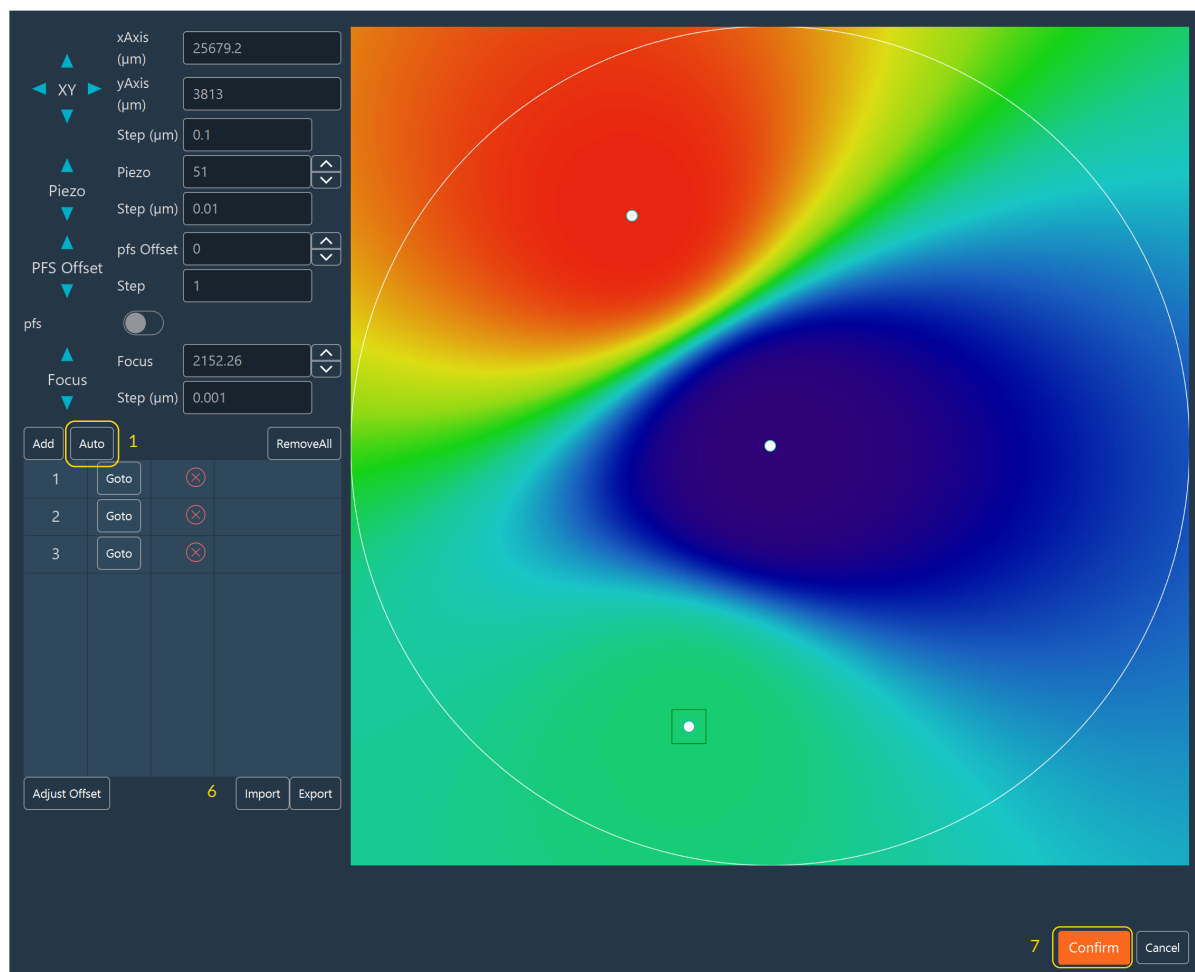


## Automated Focus Map Setup

The **Automated Focus Map** is a software feature of the Inscoper I.S. that automatically generates a hardware-driven relief map of the biological sample before executing tiling imaging.

auto on one well:





1. click Auto
2. Define the **step** interval and **size** for the Z-stack.
3. Select an existing sampling pattern from the dropdown list (and proceed to step 9), or click **Edit** to define a new spatial pattern.

**Auto FocusMap**

2 Step (um) 1

Stack Size 5

3 Pattern 2x2\_Gap800 Edit

Channel Blue\_395\_DA...

☐ Filter unsure position

Filter radius 1

BackgroundRoi

Background 0

Background Delta 0

Valid pixels (%) 5

Start Cancel

4. If creating a new pattern, select either a **Grid** or **Random** geometric distribution:
  - **Grid (a):** Specify the total number of images to acquire horizontally and vertically, along with the gap distance between adjacent image boundaries. The X and Y offset values translate the entire pattern grid across the well.
  - **Random (b):** Specify the total number of positions and the minimum required exclusion distance between coordinate points.
5. Click **Add** to save the distribution pattern.
6. Click **Switch to FocusMap** to return to the primary dimension configuration.

The image shows a software interface for editing wellplate patterns. It consists of two side-by-side panels, both titled 'Wellplate pattern edition'. The left panel is labeled with a yellow '5' and shows a 'Grid' pattern configuration. It has a dropdown for 'Existing Patterns' set to '2x2\_Gap800', an 'Add' button, and a 'Type' dropdown set to 'Grid'. Below these are input fields for 'Horizontal Number' (2), 'Vertical Number' (2), 'Gap (um)' (800), 'Offset X (um)' (0), and 'Offset Y (um)' (0), each with up/down arrow controls. The right panel is labeled with a yellow 'b' and shows a 'Random' pattern configuration. It has an empty 'Existing Patterns' dropdown, an 'Add' button, and a 'Type' dropdown set to 'Random'. Below these are input fields for 'Position number' (200) and 'Minimal Distance (um)' (100), each with up/down arrow controls. At the bottom left of the interface is a button labeled 'Switch to FocusMap' with a yellow '6' next to it. A yellow 'a' is also present near the bottom of the left panel.

7. Select the **channel** designated for acquiring the Z-stack during the Focus Map generation.
8. To reject optical artifacts and empty fields, select the **Filter unsure position** checkbox and configure the rejection parameters:
  - **Filter Radius:** Applies a spatial median filter to reduce high-frequency noise before evaluation (e.g., 0 = no smoothing; 1 = median based on a 3x3 pixel kernel; 2 = median based on a 5x5 pixel kernel).
  - **Background ROI:** Automatically calculates absolute Background and Background Delta values by evaluating a user-drawn Region of Interest (ROI). The Background equals the mean pixel intensity of the ROI, and the Background Delta equals two times the standard deviation.
  - **Background:** Specifies a static mean background intensity value.
  - **Background Delta:** Specifies the permitted noise amplitude threshold.
  - **Valid Pixels:** Sets the required percentage of pixels whose intensity must exceed the background threshold to validate the coordinate position.
9. Click **Start** to generate the focus map.

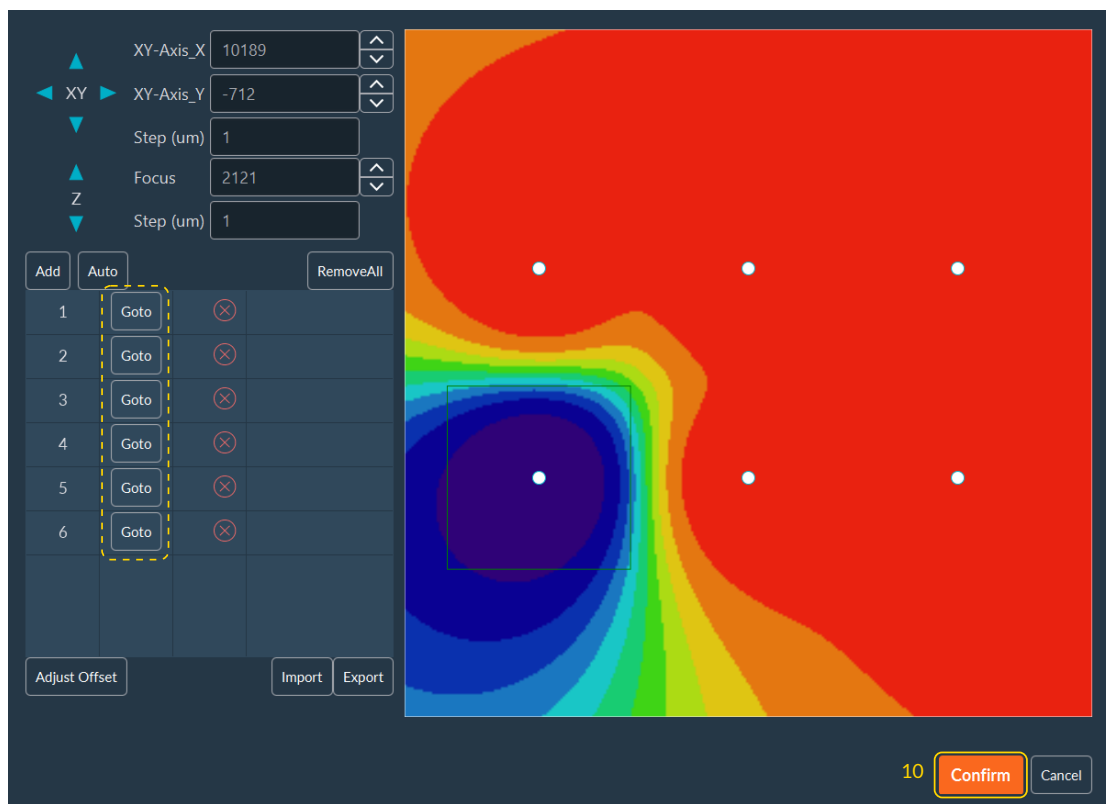
### Auto FocusMap

|                                                   |                  |        |
|---------------------------------------------------|------------------|--------|
| Step (um)                                         | 1                | ↑<br>↓ |
| Stack Size                                        | 5                | ↑<br>↓ |
| Pattern                                           | 2x2_Gap800       | ▼ Edit |
| Channel                                           | Blue_395_DA... ▼ | 7      |
| <input type="checkbox"/> Filter unsure position 8 |                  |        |
| Filter radius                                     | 1                | ↑<br>↓ |
| BackgroundRoi                                     | ▼                | Auto   |
| Background                                        | 0                | ↑<br>↓ |
| Background Delta                                  | 0                | ↑<br>↓ |
| Valid pixels (%)                                  | 5                | ↑<br>↓ |

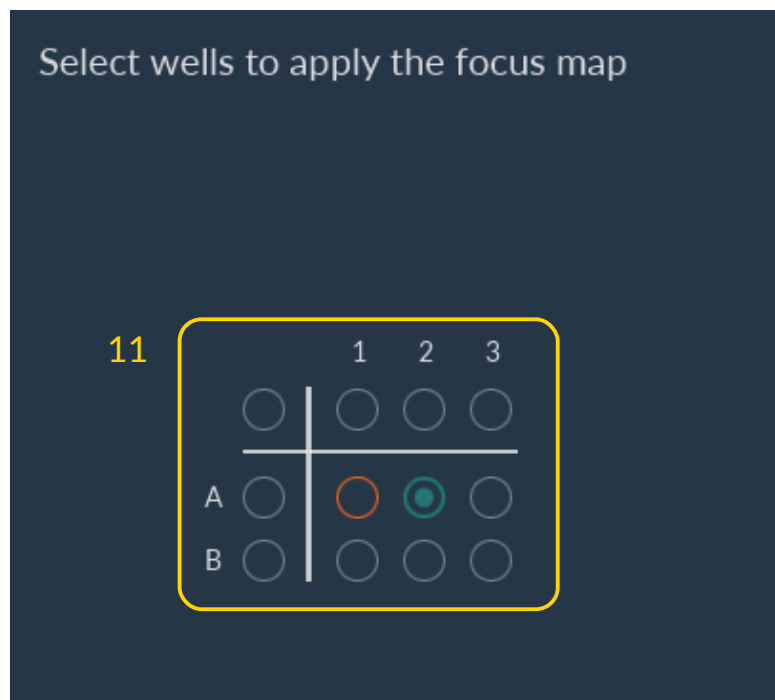
9 **Start** Cancel

The system navigates to all defined coordinates to create the Focus Map and displays a list of validated anchor points. Click **GoTo** adjacent to the coordinate or click directly on the thumbnail projection to verify physical focus at any anchor point.

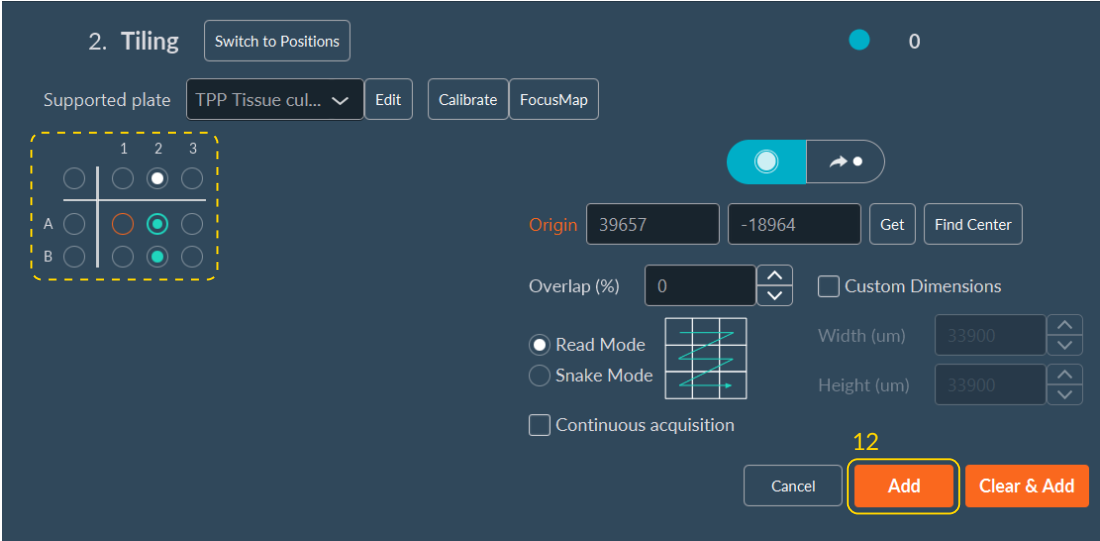
10. Click **Confirm** to finalize the generated Focus Map.



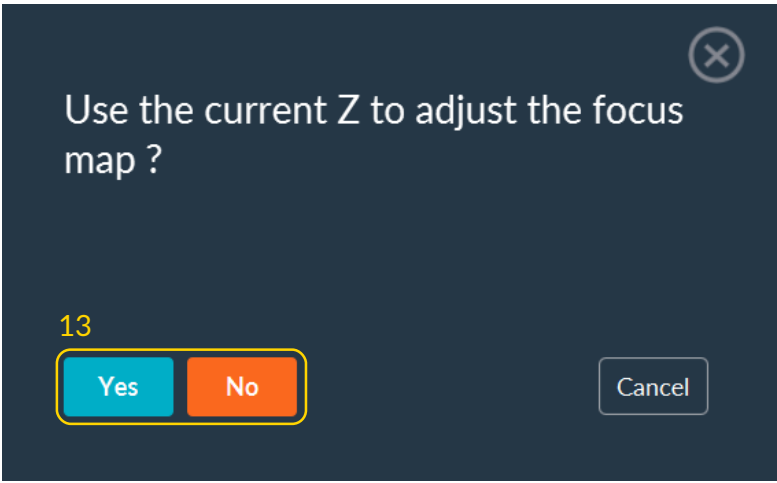
11. Select the wells to apply the Focus Map to, then click **Confirm**.



12. Select the wells to acquire, then click **Add**.



13. Select whether to use the current Z-position to adjust the Focus Map.



After this step, you can continue configuring your acquisition sequence.

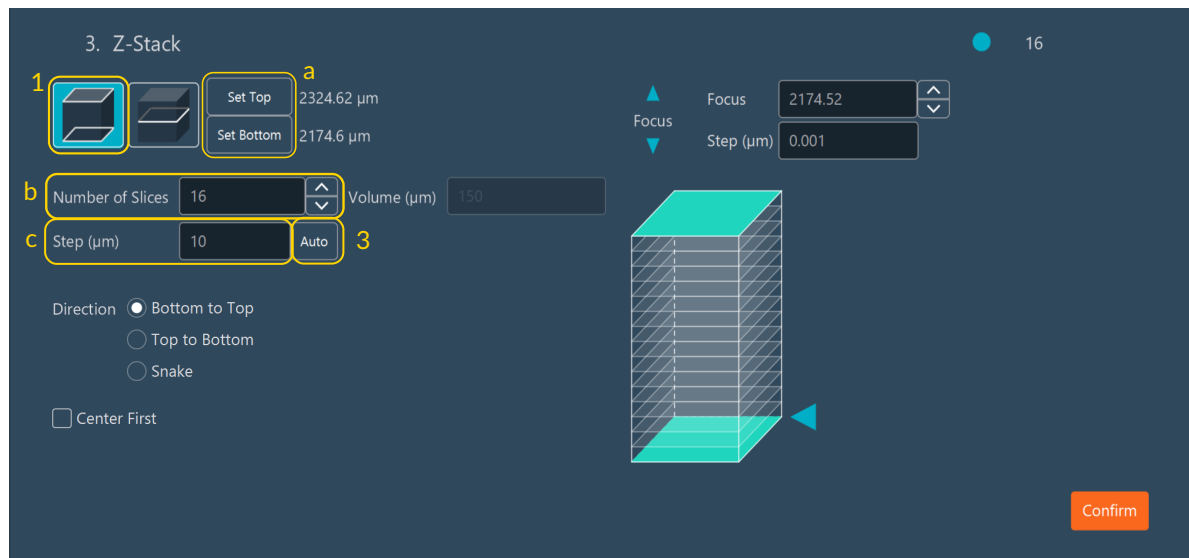
## 1.4.8 Z-stack

Use the **Z-Stack** dimension to capture volumetric data by acquiring a series of slices along the Z-axis.

### Configuring a Z-Stack

Configure the volumetric boundaries using one of two primary methods:

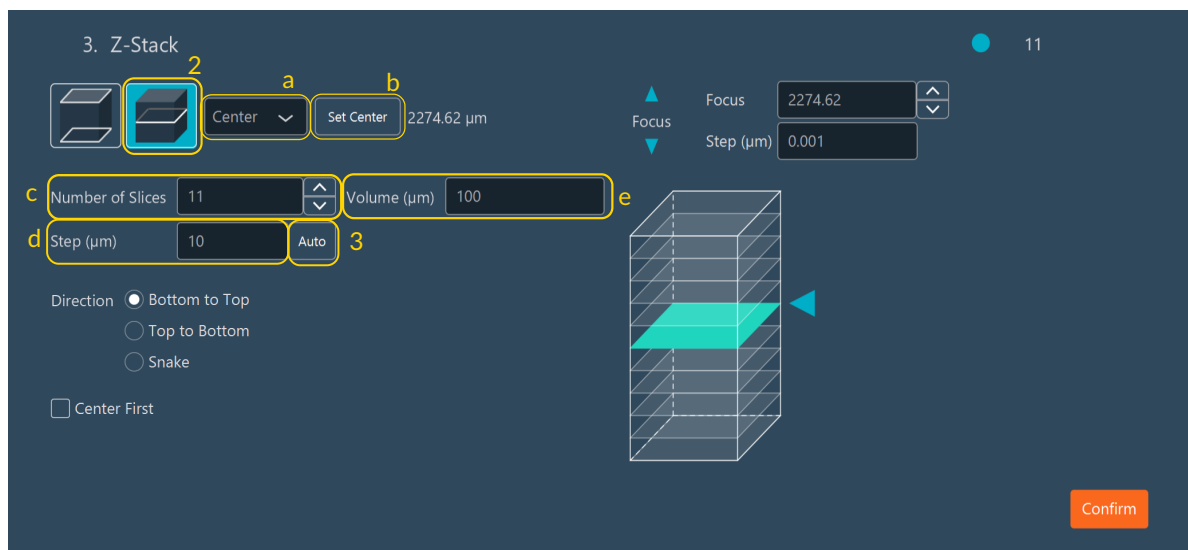
#### DEFINE TOP AND BOTTOM LIMITS



Move the focus to the desired upper and lower focal limits (1):

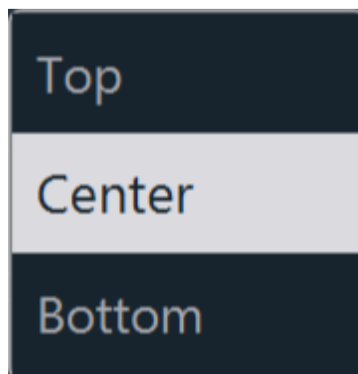
- a. click **Set Top** and **Set Bottom** to establish the boundaries.
- b. Specify the **number of slices** (focal planes) within this volume.
- c. Set the **step size** (in  $\mu\text{m}$ ) between adjacent focal planes. Enter your own value or click **Auto** (3) to use [Nyquist Calculation](#).

## DEFINE VIA A REFERENCE PLANE



Move the focus to the desired center focal plan (2):

- a. Select whether the current live focal point represents the **center**, **top**, or **bottom** of the intended Z-stack.



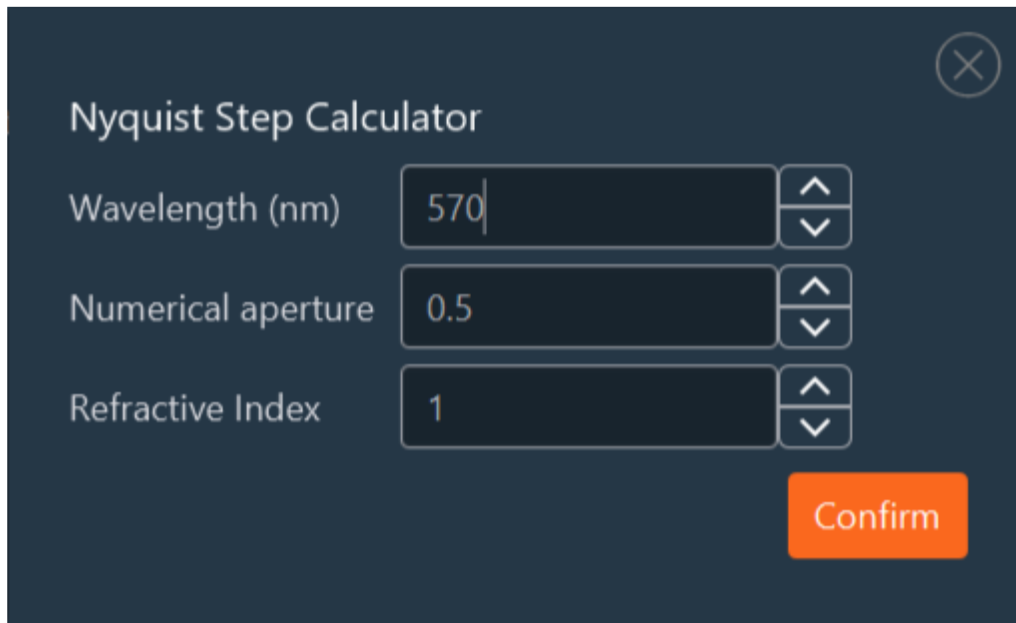
- b. Click the corresponding registration button (**Set Center**, **Set Top**, or **Set Bottom**) to log the current focal plane as the anchor.
- c. Specify the total **number of slices**.
- d. Set the **step size** (in  $\mu\text{m}$ ). Enter your own value or click **Auto** (3) to use [Nyquist Calculation](#).
- e. Alternatively, define the **volume depth** of the Z-stack (in  $\mu\text{m}$ ). The software computes the remaining variables automatically.



### NYQUIST CALCULATION

Use this method to automatically compute the optimal step size for maximum 3D optical resolution without oversampling the volumetric data. Click **Auto** to generate the ideal Nyquist Z-sampling rate.

- The software calculates the ideal physical step size based on the **longest emission wavelength** configured in the multi-channel parameters.
- These values remain **editable**. Manually override the calculated step size or tune the inputs, such as the emission wavelength, the objective's numerical aperture (NA), and the refractive index (RI) of the immersion medium.
- Click **Confirm** to save the computational settings.



**Nyquist Step Calculator**

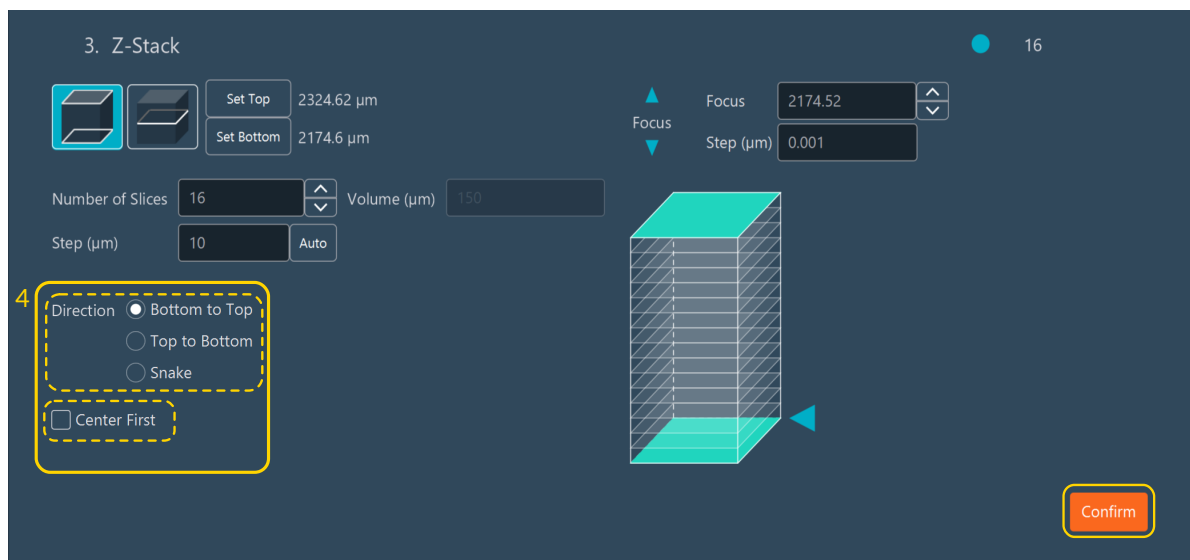
Wavelength (nm)

Numerical aperture

Refractive Index

**Confirm**

### Execution settings



**3. Z-Stack**

Set Top 2324.62  $\mu\text{m}$   
Set Bottom 2174.6  $\mu\text{m}$

Number of Slices  Volume ( $\mu\text{m}^3$ )

Step ( $\mu\text{m}$ )  **Auto**

Direction ☒ Bottom to Top  
☐ Top to Bottom  
☐ Snake

☐ Center First

Focus   
Step ( $\mu\text{m}$ )

**Confirm**

Once the geometric volume is defined, set the execution parameters (4):

- Select the **acquisition direction**: either **Top to Bottom**, **Bottom to Top**, or **Snake** (bidirectional alternating)
- Enable **Center First** to force the sequence to begin acquisition at the **central focal plane**.

#### Tip

This setting is required with hardware autofocus systems (e.g., PFS, Definite Focus), which must anchor before the volume is swept.

Click **Confirm** to save the Z-stack configuration block.

 **Tip**

Use the interactive **Z-stack diagram** to verify the setup — drag the **blue arrow** or click the diagram to drive the stage and preview the stack bounds.

 **Important**

When configuring a Z-stack from a center reference plane, the **Volume**, **Step**, and **Number of Slices** fields are linked. Adjusting one recalculates the others. If the **Positions dimension** is active and the Z-axis override is enabled for a position, the local focus value of that position overrides the center coordinate of the Z-stack.

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### 1.4.9 Software Autofocus

Use the **Software Autofocus** module to keep your sample in focus during the acquisition sequence. To calculate Z-axis corrections, the software captures autofocus evaluation frames on the fly. These frames are processed in memory and are not saved to the disk.

#### Configuring Software Autofocus

1. Specify the number of Z-planes to acquire and analyze in the **Focus Number** field.
2. Define the Z-axis step size in the **Step** field (in the units specified in the [software settings](#)) to determine the distance between successive planes.
3. Select the active **Channel** used to evaluate focus.
4. Select the active **Camera** and specify the sensor **Exposure** time (in ms) for the autofocus capture frames.
5. Select the autofocus algorithm from the **Autofocus Algo** dropdown menu:
  - **Maximum**: Optimized for bright fluorescent signals.
  - **Sharpness**: Optimized for dense morphological structures (such as brightfield or phase contrast), where the algorithm detects the highest high-frequency contrast.

#### Note

The sharpness algorithm calculation is performed exclusively on the central 20% area of the image.

6. Under **Do Autofocus**, specify the execution frequency of the autofocus routine. Enter the interval in the **Every Time** and **Every Position** fields to define how often the routine executes relative to time points and stage positions.
7. Select the **Advanced Parameters** checkbox to configure mechanical device integration:
  - Under **Do autofocus with**, select the mechanical device that drives the autofocus routine (such as a fast piezo stage or the microscope internal Z-drive).
  - Under **Apply offset to** (optional), select the mechanical device to which the system applies the calculated Z-offset.
8. Click **Test AutoFocus** to test the autofocus protocol on the sample immediately.
9. Click **Confirm** to save the configuration.

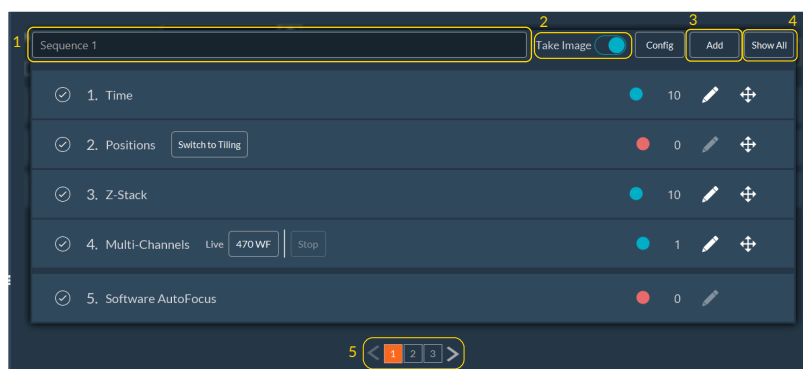
#### Note

Please note that if you are using a software mirror for your camera, the autofocus image will not have the same orientation as your live view.

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### 1.4.10 Multi-Sequence Acquisition

After configuring an isolated acquisition sequence, launch it immediately or append additional sequences to build a **Multi-Sequence Acquisition** for automated workflows.



Each sequence within the interface includes the following setup options:

- 1. Rename:** Assign a name to the sequence for identification during the experiment.
- 2. Toggle Image Acquisition:** Disable or enable image capture for a specific sequence by toggling the **Take Image** button (useful for setting up delays or hardware moves without saving data).
- 3. Add Sequence:** Append a new, empty sequence to the multi-sequence stack.
- 4. Show All:** View a summary of all prepared sequences in a bottom panel.
- 5. Navigation:** Jump between sequences. The currently selected sequence highlights in orange.

Clicking **Show All** (4) opens the **Summary Tab**, providing sequence management:

- 1. Loop Count:** Define how many times the entire multi-sequence stack should loop.
- 2. Intermittent Sequences (Optional):** Check this box to trigger the sequence intermittently based on a selected dimension (e.g., execute this sequence only on every 3rd time point).
- 3. Take Image Toggle:** Quickly enable or disable image capture for individual sequences from the summary view.
- 4. Active Status:** Activate or deactivate an entire sequence by clicking its colored indicator (red indicates inactive, blue indicates active).
- 5. Edit:** Click the **Pen** icon to open the configuration for that sequence.
- 6. Duplicate:** Clone an existing sequence by clicking **Copy**.
- 7. Delete:** Remove a sequence by clicking **Delete**.

Number of loop

1

1

☐ Intermittent Sequences

2

Add Sequence

Remove All

|            |                         |                                  |   |   |      |   |
|------------|-------------------------|----------------------------------|---|---|------|---|
| Sequence 1 | Take Image <div>3</div> | Time10 Z-Stack10 Multi-Channels1 | 4 | 5 | 6    | 7 |
| Sequence 2 | Take Image              | Positions2 Multi-Channels2       |   |   | Copy |   |
| Sequence 3 | Take Image              | Time5 Tiling25 Multi-Channels1   |   |   | Copy |   |

### 1.4.11 Data Processor

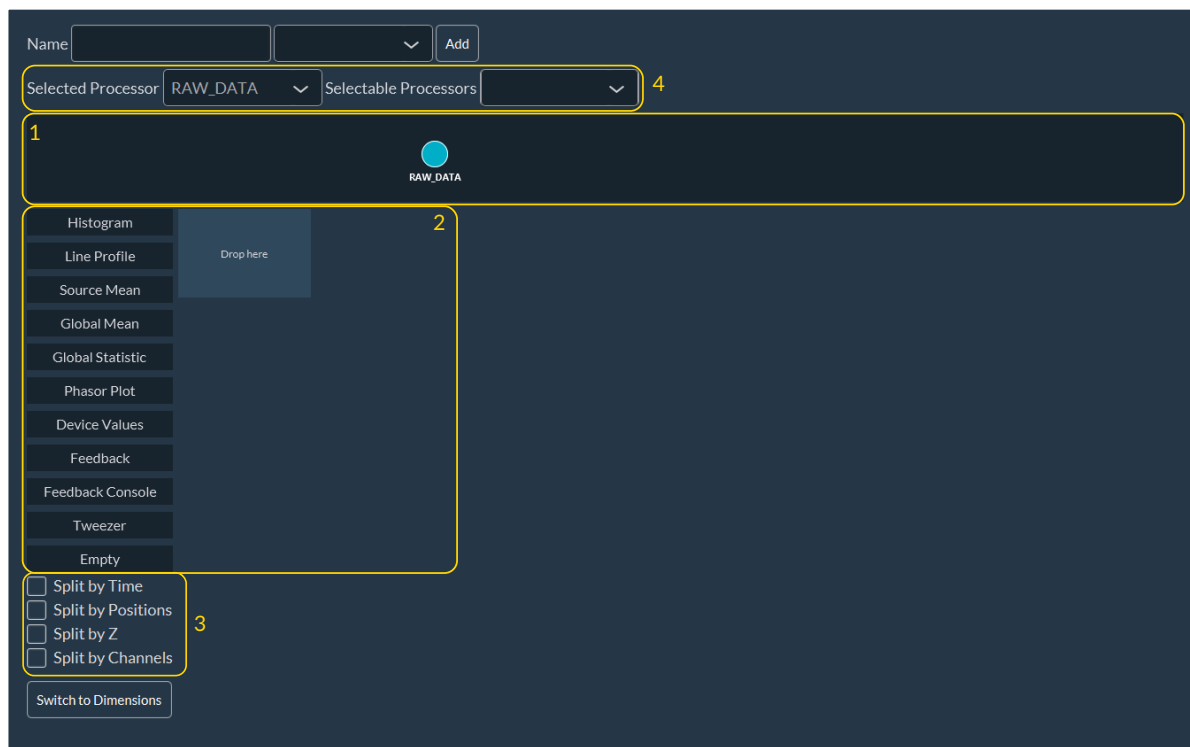
Use the **Data Processor** to customize data visualization and analysis pipelines during and after image acquisition. View raw image streams, monitor fluorescence intensity graphs, or apply post-processing.

#### Interface Description



In **User Mode**, access pre-configured data processing pipelines. Select a pipeline from the drop-down menu and begin the acquisition.

In **Expert Mode**, click the **Switch to Data Processor** button to build custom data processing workflows.



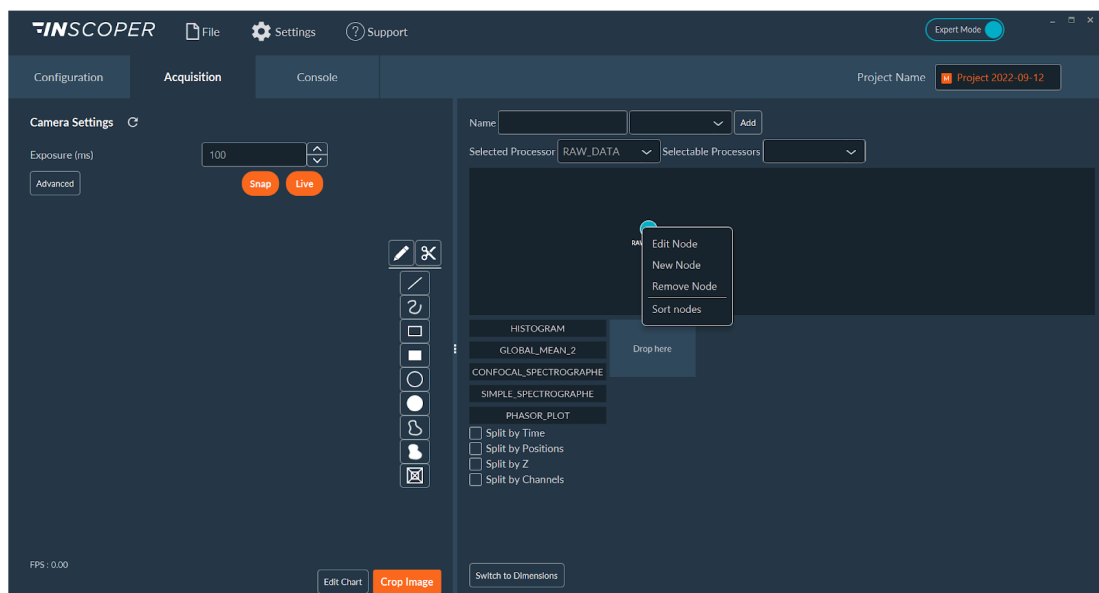
1. **Workspace:** The canvas for creating and routing data processing workflows.
2. **Visualization Tools:** Controls for customizing how data is displayed.
3. **Dimension Analysis Tools:** Tools for analyzing data separated by dimension (e.g., assessing Z-stacks or time-lapses).
4. **Image Visualization Customization:** Controls for viewing images post-processing:
  - **Selected Processor:** Choose which specific processing node's output to view live during acquisition.
  - **Selectable Processor:** Choose which node outputs will remain available for review in the visualization tab after the acquisition concludes. By default, the system selects all processing steps.

#### Note

The available processing options depend on the system's specific hardware and license configuration.

#### Creating a New Data Processing Workflow

1. Every workflow originates with the raw images, represented by a root node named **RawData**. To process these images, create a new node by right-clicking **RawData** and selecting **New Node**.



- The system generates a new, empty node. To assign a processing operation to this node, double-click it, or right-click and select **Edit Node**.



#### Tip

For improved readability, right-click the workspace and select **Sort Nodes** to automatically arrange the pipeline. You can also manually reposition nodes by dragging and dropping them.

- In the node properties window, use the **Type** drop-down menu to select the desired processing algorithm (e.g., Stitching, Maximum Projection, Background Subtraction).
  - Check the **Temp. Data** box to prevent saving the output of this specific step to disk. This reduces processing overhead and minimizes storage space requirements.



Data Processor

AddRemove

Name

NO\_PROCESSOR

Type

NO\_PROCESS...

▼

☐

Temp. Data

Confirm

Cancel

AVAILABLE PROCESSING OPERATIONS

| Processor                   | Description                                                                                                                       |
|-----------------------------|-----------------------------------------------------------------------------------------------------------------------------------|
| NO_PROCESSOR                | Passes the data through without modifying it.                                                                                     |
| SIMPLE_TILING               | Positions each image at its coordinates within a tiling grid.                                                                     |
| STITCH_TILING               | Stitches adjacent images of a tiling acquisition by blending overlap regions. (Note: Requires SIMPLE_TILING to be applied first). |
| STANDARD_DEVIATION_ON_FLY   | Calculates pixel intensity standard deviation across an image stack.                                                              |
| SHADING_CORRECTION          | Corrects uneven illumination and optical artifacts (e.g., sensor dust).                                                           |
| FILTER                      | Reduces noise by removing anomalous pixels (despeckling).                                                                         |
| TIME_MIN                    | Performs a minimum intensity projection across the Time dimension.                                                                |
| TIME_MEDIAN                 | Performs a median intensity projection across the Time dimension.                                                                 |
| TIME_MAX                    | Performs a maximum intensity projection across the Time dimension.                                                                |
| TIME_AVERAGE                | Calculates the average pixel intensity across the Time dimension.                                                                 |
| TIME_STANDARD_DEVIATION     | Calculates the intensity standard deviation pixel across the time dimension.                                                      |
| TIME_SUM                    | Calculates the sum pixel intensity across the time dimension. .                                                                   |
| FOCUS_MIN                   | Performs a minimum intensity projection across the Z-stack dimension.                                                             |
| FOCUS_MEDIAN                | Performs a median intensity projection across the Z-stack dimension.                                                              |
| FOCUS_MAX                   | Performs a maximum intensity projection across the Z-stack dimension.                                                             |
| FOCUS_AVERAGE               | Calculates the average pixel intensity across the Z-stack dimension.                                                              |
| FOCUS_STANDARD_DEVIATION    | Calculates the intensity standard deviation pixel across the Z-stack dimension.                                                   |
| FOCUS_SUM                   | Calculates the sum pixel intensity across the Z-stack dimension.                                                                  |
| POSITION_MIN                | Performs a minimum intensity projection across the position dimension.                                                            |
| POSITION_MEDIAN             | Performs a median intensity projection across the position dimension.                                                             |
| POSITION_MAX                | Performs a maximum intensity projection across the position dimension.                                                            |
| POSITION_STANDARD_DEVIATION | Calculates the intensity standard deviation pixel across the position dimension.                                                  |
| POSITION_SUM                | Calculates the sum pixel intensity across the position dimension.                                                                 |
| CHANNEL_MULTICOLOR          | Merges images from multiple channels into a multi-color composite.                                                                |
| SUBTRACT_BACKGROUND         | Subtracts the background signal to improve image contrast.                                                                        |
| CHANNEL_RATIO               | Calculates the ratio of intensities between channels to measure relative changes.                                                 |

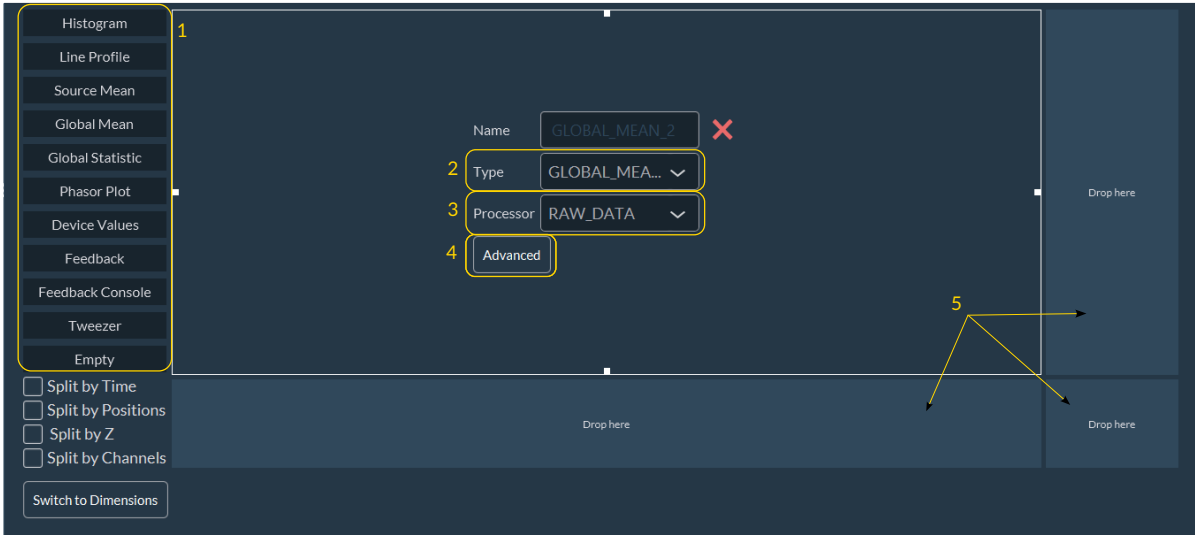
| Processor           | Description                                                  |
|---------------------|--------------------------------------------------------------|
| MULTI_CHANNEL_MERGE | Merges channels natively (primarily used in SPIM workflows). |

 **Tip**

You can execute all processing steps either during or after acquisition. When run during the acquisition, the processed data updates continuously and saves directly to the final output file.

Customizing Data Visualization

Customize the display of acquired data, such as tracking the evolution of fluorescence intensity over time.



SETTING UP THE VISUALIZATION WINDOW

- Select the Data Type:** Choose what metric to display during the acquisition:
  - Histogram: Monitors the evolution of the pixel intensity distribution.
  - Global mean: Monitors the average image intensity across an acquisition sequence.
  - Global statistic: Similar to Global mean, but includes additional statistical metrics (e.g., variance).
  - Device values: Periodically retrieves and plots telemetry from connected hardware devices.
- Assign to a Panel:** Drag and drop the selected data type into a view panel. Change this data type later using the panel's drop-down menu.
- Specify Source:** Define the data source (node) for the visualization.
- Customize Graph:** Adjust visual graph options, such as axis titles and curve labels.
- Add Graphs:** To display multiple graphs simultaneously, drag and drop additional data types into empty view panels.

GRAPH CUSTOMIZATION OPTIONS

- Set a custom title for the graph.
- Label the X and Y axes.
- Toggle the visibility of curve legends.
- Enable the **Always** option to ensure the graph remains visible throughout the experiment.

1 Name

Type GLOBAL\_MEA... ▾

2 X Axis

Y Axis

3 ☐ Show Legend

Processor RAW\_DATA ▾

4 H Priority ALWAYS ▾

V Priority ALWAYS ▾

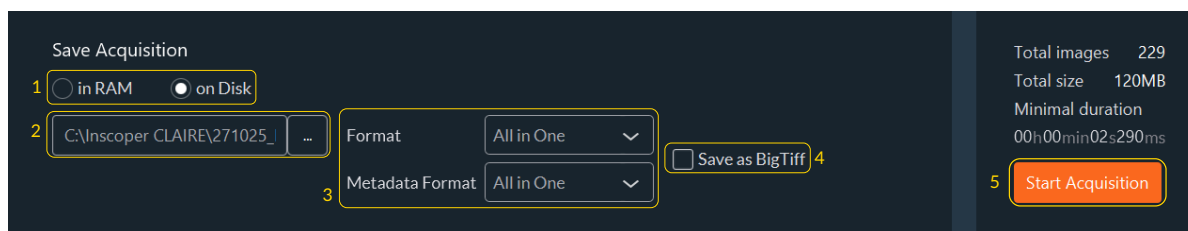
 Note

Visualization customization is optional. It aids in data interpretation during acquisition and does not affect the saved acquisition data.

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### 1.4.12 Saving Images and Metadata

The saving interface configures the destination path and data architecture for the automated sequence outputs.



Configure how the acquired images and metadata are handled:

1. Select the data storage destination:
  - **RAM:** (Not recommended for prolonged acquisitions). Data stored in RAM is volatile and is lost if the application closes or the system crashes. Use only for temporary test captures.
  - **Hard disk, SSD, or Server:** (Recommended). Writes data to a physical drive or network storage.
2. Specify the destination folder path.
3. Choose the image saving format:
  - **All in one file:** Combines the dimensional dataset (Z-stacks, Time points, Channels) into a single, multi-page image file.
  - **One TIFF per image:** Saves every captured frame as a discrete TIFF file (e.g., `Image_T01_Z03_C01.tiff`). Use this if post-processing tools require sequential files instead of multi-page containers.
4. (Optional) Check **.bigTiff** to support files exceeding the standard 4GB legacy TIFF limit (recommended for large volumetric datasets).

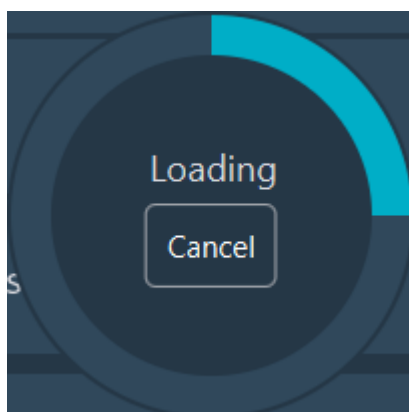


#### Tip

To configure standardized file naming conventions before initiating an automated acquisition, refer to the [Display Settings](#) guide.

1. Start acquisition

The sequence will be generated and uploaded to the Inscoper device controller. You can abort the process at any time by clicking **Cancel** on the loading progress indicator.

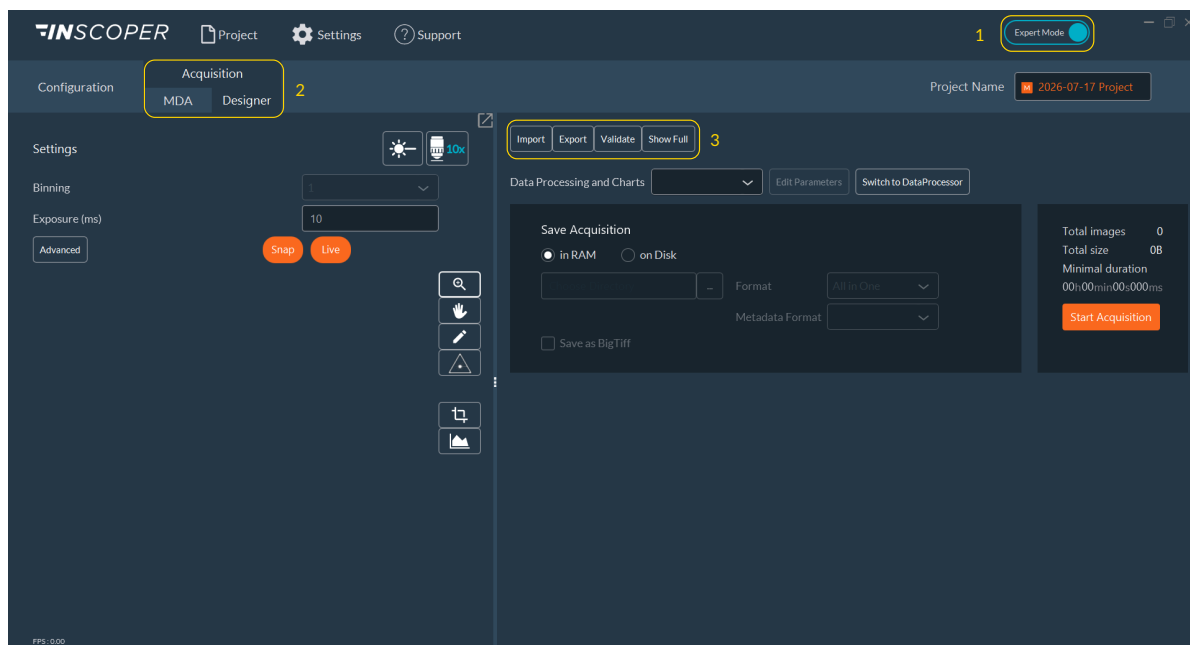


### 1.4.13 Acquisition Designer

The **Designer** module is a graphical workspace for building custom acquisition protocols. Combine logical and hardware blocks into an interactive flowchart to define a tailored, multi-dimensional workflow.

#### How to Access the Designer

Enable **Expert Mode** (1), open the main **Acquisition** tab, then select the **Designer** sub-tab (2).



#### Note

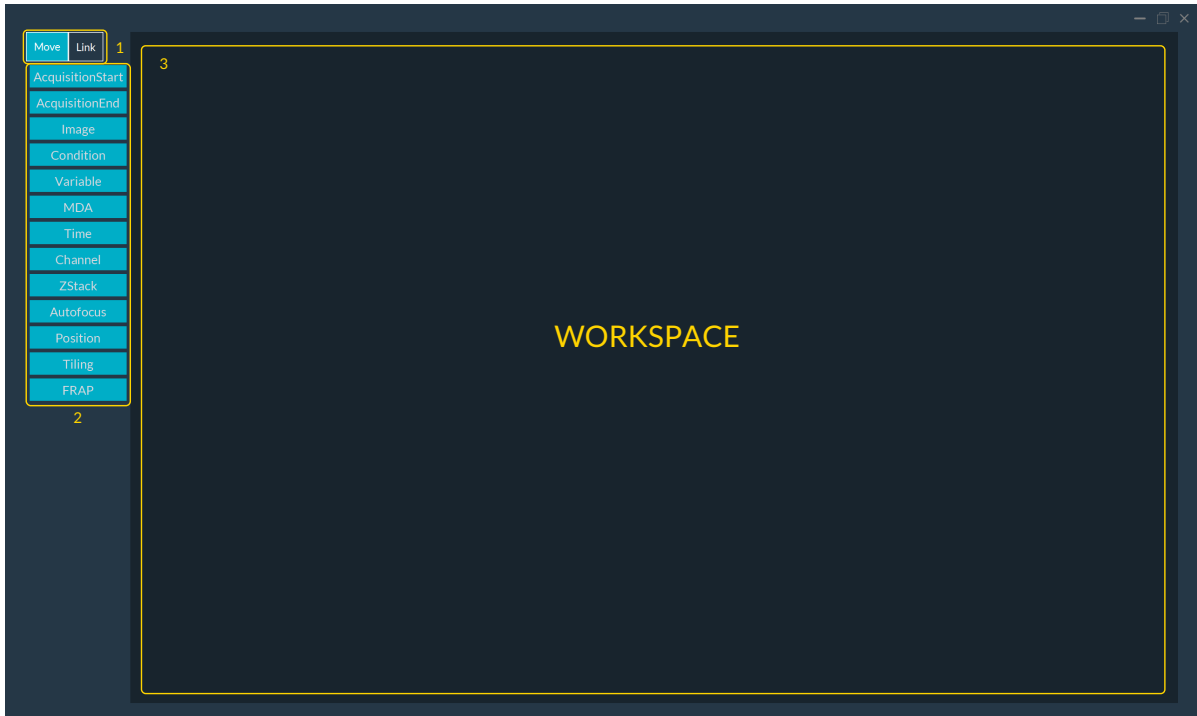
**Validate** and **Show Full** are only available in Expert Mode (1). In User Mode, the action bar (3) is reduced to **Import** and **Export**.

The action bar (3) at the top right of the tab provides four commands:

- **Import:** load an existing protocol from a JSON file. The file browser opens in the `complexProtocols` directory. A confirmation pop-up appears once the protocol is loaded.
- **Export:** save the current protocol as a JSON file, by default in the same `complexProtocols` directory.
- **Validate:** register the protocol modifications and recompute the total number of images.
- **Show Full:** open the complete graphical workspace used to build the flowchart.

#### How to Create a Protocol

Click **Show Full** to open the workspace. The workspace opens as a separate maximized window, on the secondary screen when one is available. Assemble the protocol from blocks placed and linked on the canvas.



The window is organized in three areas: the tool selector (1), which switches between **Move** and **Link**; the block palette (2), which lists every block type available; and the canvas (3), which holds the flowchart.

HOW TO INTERACT WITH BLOCKS

- **Add a block:** drag a block from the palette (2) onto the canvas (3) and release the mouse button at the target location.
- **Move a block:** activate the **Move** tool (1), then drag the block across the canvas.
- **Connect two blocks:** select the **Link** tool (1), click the source block, then click the target block. The Designer draws a directional flow arrow between them.
- **Configure a block:** switch back to **Move** mode (1), then double-click the block to edit its parameters.

LIST OF AVAILABLE BLOCKS

- **AcquisitionStart:** mandatory marker defining the protocol start point.
- **AcquisitionEnd:** mandatory marker defining the protocol end point.
- **Image:** captures raw data. Set the block **Name** (1), the **Camera** (2), the **Exposure** time in milliseconds (3), and the **Sequence Index** (4).

The screenshot shows a configuration dialog for the 'Image' block. It has a dark blue background and a close button (X) in the top right corner. The dialog contains four input fields: 'Name' with the value 'Image', 'Camera' with a dropdown menu showing 'OrcaFusion\_0', 'Exposure' with a numeric input '20', and 'Sequence Index' with an empty text field. Below these fields is a 'Specific Indexes' section with an 'Add' button. At the bottom, there is a table with two columns: 'Type' and 'Value'. The table is currently empty, with the text 'No content in table' centered below it. At the bottom right, there are 'Confirm' and 'Cancel' buttons. The input fields are numbered 1 through 4.

- **Condition:** evaluates a logical expression and splits the flow into two branches.



Four parameters are available: **Name** (1), **If True Text** (2), **If False Text** (3), and **Condition** (4). The two text fields (2) and (3) set the labels drawn on the outgoing branches; they default to **Yes** and **No** and may be renamed.

- **No** (loop back): if the expression is false, the flow returns to a designated block and repeats the step.
- **Yes** (loop exit): if the expression is true, the flow leaves the loop and advances to the next step.

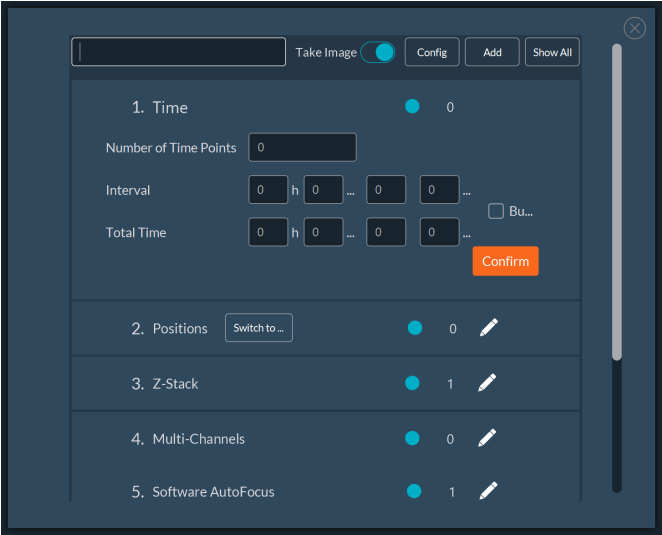
A numeric expression is accepted in the **Condition** field (4) in place of a boolean one: any non-zero result is treated as true. An expression left empty or set to **TRUE** always follows the true branch. See [Condition and Variable Syntax](#).

- **Variable**: declares one or more named values, each defined by a **Variable Name** (3) and an **Expression** (4). Use **Add** (1) to append a declaration and **Remove All** (2) to clear the list. Every expression is re-evaluated each time the flow passes through the block, so a variable may reference dimension indices and previously declared variables, not only constants.
- **Function**: stores or modifies a value used by a secondary dimension of a complex acquisition.
- **Prerequisite**: place the block before the first block that references the variable.

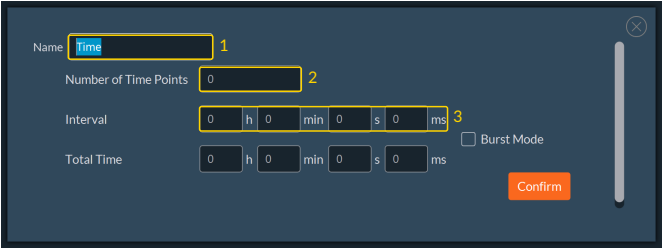
| Variable Name | Expression |
|---------------|------------|
|               |            |

- **MDA**: encapsulates a complete multidimensional sequence in a single block. Its dimensions are configured in the numbered list of the block window: **Time**, **Positions**, **Z-Stack**, **Multi-Channels**, and **Software AutoFocus**. Use it when a standard routine is sufficient inside a larger custom protocol, instead of building the equivalent chain of blocks manually.

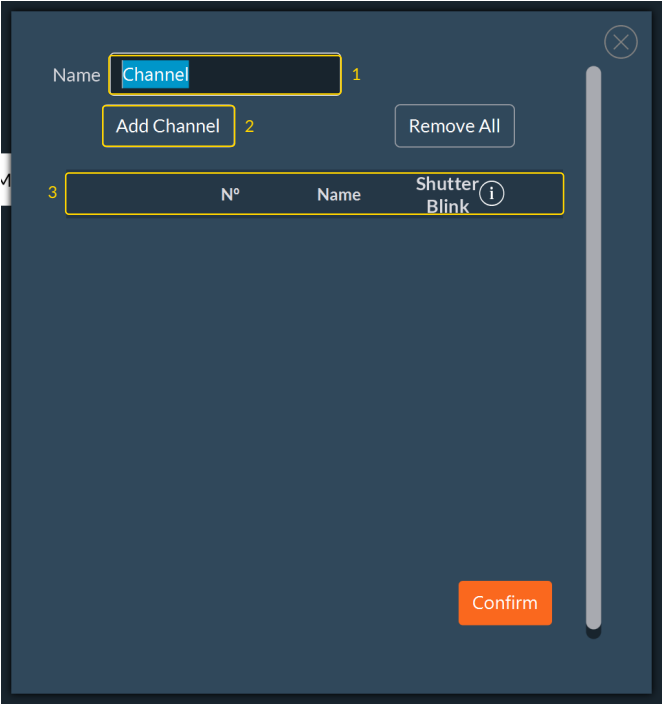




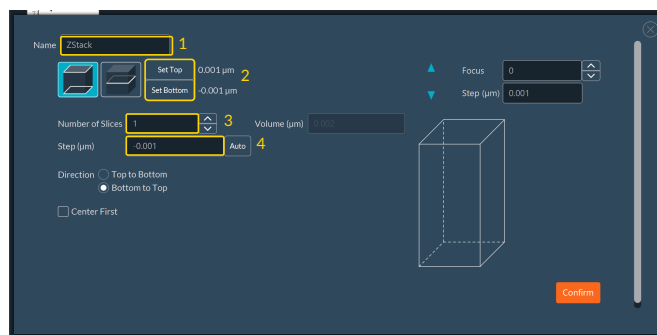
- **Time:** defines a time-lapse dimension from the block **Name** (1), the **Number of Time Points** (2), and the **Interval** (3). **Total Time** is derived from the two preceding fields.



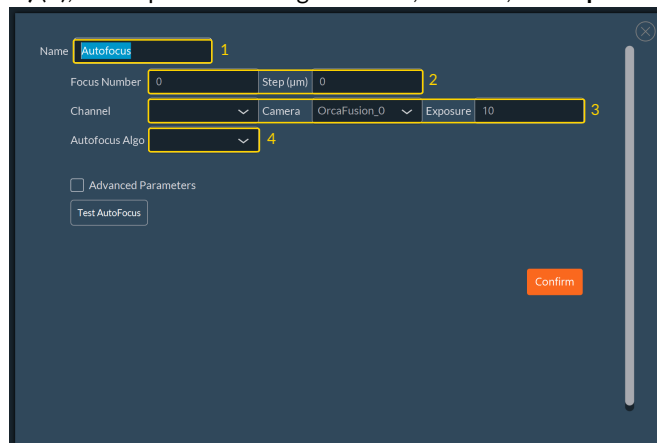
- **Channel:** defines one or several illumination wavelengths. Set the block **Name** (1), then use **Add Channel** (2) to append a wavelength to the channel table (3).



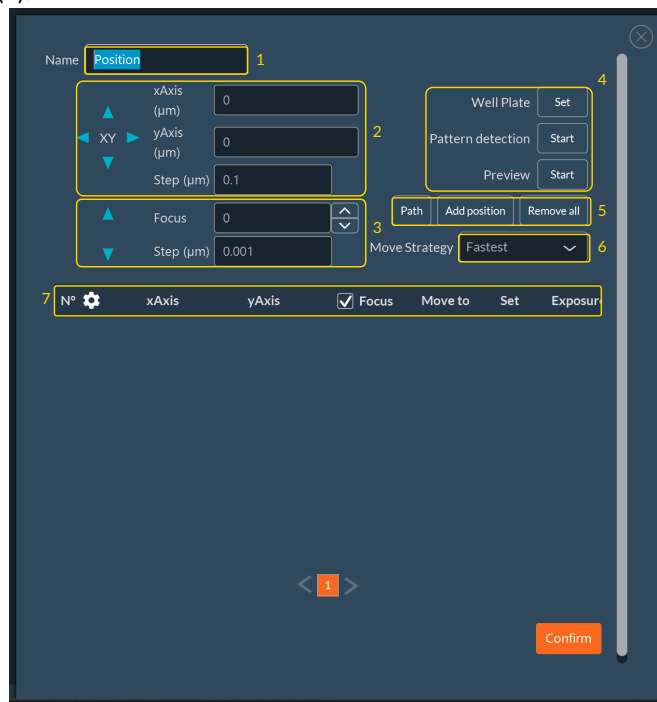
- **ZStack:** defines acquisition along the Z axis from the block **Name** (1), the top and bottom points set with **Set Top** and **Set Bottom** (2), the **Number of Slices** (3), and the **Step (µm)** (4).



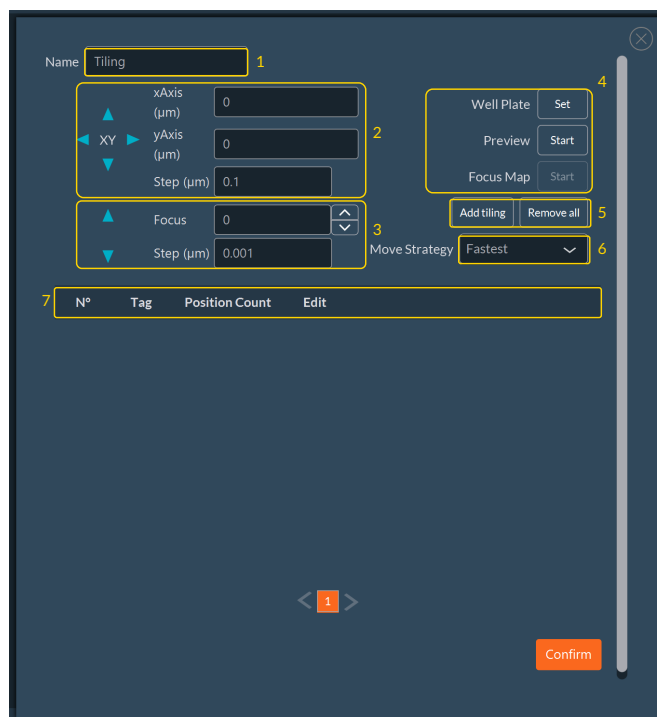
- **Autofocus:** finds the optimal focus plane. Set the block **Name** (1), the number of sampling images and the travel between them with **Focus Number** and **Step (µm)** (2), the acquisition settings **Channel**, **Camera**, and **Exposure** (3), and the **Autofocus Algo** (4).



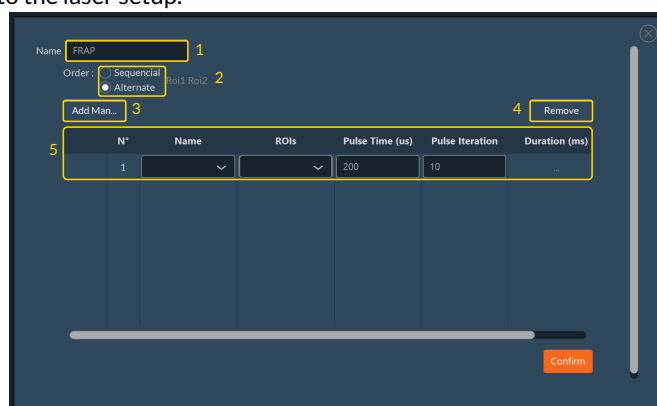
- **Position:** defines the XY stage coordinates visited by the protocol. Set the block **Name** (1), the **XY** coordinates and their step (2), and the **Focus** value and its step (3). The right column groups the **Well Plate** definition, the **Pattern detection**, and the **Preview** (4), the position commands **Path**, **Add position**, and **Remove all** (5), and the **Move Strategy** (6). Every position registered is listed in the table (7).



- **Tiling:** generates an image mosaic from adjacent fields of view. Set the block **Name** (1), the **XY** coordinates and their step (2), and the **Focus** value and its step (3). The right column groups the **Well Plate** definition, the **Preview**, and the **Focus Map** (4), the mosaic commands **Add tiling** and **Remove all** (5), and the **Move Strategy** (6). Every mosaic registered is listed in the table (7).



- **FRAP**: optional module block for photobleaching and photoactivation experiments. Set the block **Name** (1) and the **Order** in which the regions are illuminated, **Sequential** or **Alternate** (2). Use the manual entry button (3) to append a pulse and **Remove** (4) to delete the selected one. Each pulse is listed in the table (5) with its **Name**, **ROIs**, **Pulse Time (us)**, and **Pulse Iteration**. Configure the block according to the laser setup.



### Important

- **Mandatory unique naming**: every block in the workspace must have a unique name. Each dimension block publishes its counters under its own name, so two blocks sharing a name overwrite each other and **Condition** blocks evaluate the wrong values.
- **Structural errors are flagged, logical errors are not**: the workspace marks a block whose links are incomplete, for example a **Condition** block with an unconnected branch. It does not check the content of an expression. A condition that references a misspelled or undeclared name is only detected when the protocol runs.
- **Silent failure behavior**: the Designer does not display pop-up warnings for logical errors. If clicking **Start Acquisition** produces no action, inspect the links, the block names, and the loop criteria.

### CONDITION AND VARIABLE SYNTAX

**Condition** and **Variable** blocks share the same expression language. Expressions are evaluated against the acquisition context, which holds the counters published by every dimension block and the variables already declared. A name that is not present in the context at the moment of evaluation aborts the acquisition with an error, so a **Variable** block must always be placed upstream of the first block that references it.

## Available Names

Each dimension block publishes two names built from the block name:

| Name                           | Meaning                                                                                                                                                              |
|--------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>[Block Name]Index</code> | Number of steps already completed in that dimension. It is <code>0</code> before the first step and reaches <code>[Block Name]Max</code> once the last step is done. |
| <code>[Block Name]Max</code>   | Total number of steps in that dimension: the number of slices, time points, positions, or channels declared in the block.                                            |

A **Tiling** block publishes three additional names:

| Name                                     | Meaning                                      |
|------------------------------------------|----------------------------------------------|
| <code>[Block Name]TilingIndex</code>     | Index of the current mosaic.                 |
| <code>[Block Name]TilingNumber</code>    | Total number of mosaics.                     |
| <code>[Block Name]TilingLineIndex</code> | Index of the current line within the mosaic. |

A **Variable** block publishes each declared variable under its own name. The value of a variable is itself an expression, re-evaluated every time the flow passes through the block.



## Important

- Names are case-sensitive and must match the block name exactly. A name must start with a letter or an underscore and may contain only letters, digits, and underscores. Dotted names such as `ZStack1.Index` are not valid.
- An **MDA** block does not publish counters. Its internal Time, Channel, and ZStack dimensions cannot be referenced from a **Condition** expression. Use separate dimension blocks when the protocol has to branch on their indexes.

## Operators and Functions

| Category          | Symbols                                                                                                             |
|-------------------|---------------------------------------------------------------------------------------------------------------------|
| Arithmetic        | <code>+</code> , <code>-</code> , <code>*</code> , <code>/</code> , <code>%</code> (modulo), <code>^</code> (power) |
| Comparison        | <code>==</code> , <code>!=</code> , <code>&gt;</code> , <code>&gt;=</code> , <code>&lt;</code> , <code>&lt;=</code> |
| Boolean           | <code>&amp;&amp;</code> (and), <code>  </code> (or), <code>!</code> (not)                                           |
| Boolean constants | <code>TRUE</code> , <code>FALSE</code> (uppercase)                                                                  |
| Grouping          | <code>( )</code>                                                                                                    |

The following functions are available. Angles are expressed in radians.

| Function                                                           | Description                                                                 |
|--------------------------------------------------------------------|-----------------------------------------------------------------------------|
| <code>sqrt(x)</code> , <code>exp(x)</code> , <code>ln(x)</code>    | Square root, exponential, natural logarithm                                 |
| <code>abs(x)</code> , <code>floor(x)</code> , <code>ceil(x)</code> | Absolute value, rounding down, rounding up                                  |
| <code>cos(x)</code> , <code>sin(x)</code> , <code>tan(x)</code>    | Trigonometric functions                                                     |
| <code>acos(x)</code> , <code>asin(x)</code> , <code>atan(x)</code> | Inverse trigonometric functions                                             |
| <code>atan(y, x)</code>                                            | Two-argument arc tangent                                                    |
| <code>deg(x)</code> , <code>rad(x)</code>                          | Conversion between radians and degrees                                      |
| <code>if(condition, a, b)</code>                                   | Returns <code>a</code> when the condition is true, otherwise <code>b</code> |

Operator Precedence

Operators are grouped in the following order, from the highest precedence to the lowest. Operators of equal precedence are evaluated from left to right.

| Rank | Operators                                                                                                           |
|------|---------------------------------------------------------------------------------------------------------------------|
| 1    | <code>!</code> , unary <code>-</code>                                                                               |
| 2    | <code>^</code>                                                                                                      |
| 3    | <code>*</code> , <code>/</code>                                                                                     |
| 4    | <code>+</code> , <code>-</code>                                                                                     |
| 5    | <code>%</code>                                                                                                      |
| 6    | <code>==</code> , <code>!=</code> , <code>&gt;</code> , <code>&gt;=</code> , <code>&lt;</code> , <code>&lt;=</code> |
| 7    | <code>&amp;&amp;</code> , <code>&amp; \ </code>                                                                     |

 **Warning**

Modulo has a lower precedence than addition and subtraction, which is the opposite of the convention used in most programming languages. `TimeIndex - 1 % 2` is evaluated as `(TimeIndex - 1) % 2` , not as `TimeIndex - (1 % 2)` . Use explicit parentheses whenever `%` appears next to `+` or `-` .

Typical loop and interval expressions:

```
[Block Name]Index == [Block Name]Max
[Block Name]Index % [Variable Name] == 0
```

In the **Variable** block below, each variable name (1) is paired with the expression that defines it (2). The names `laser1` , `laser2` , and `laser3` are then available to every downstream **Condition** block.

Add

Remove All

|   | Variable Name | Expression |             |             |
|---|---------------|------------|-------------|-------------|
|   | laser1        | 2          | <div></div> |             |
| 1 | laser2        | 3          | 2           | <div></div> |
|   | laser3        | 4          |             | <div></div> |

Confirm

Cancel

 Note

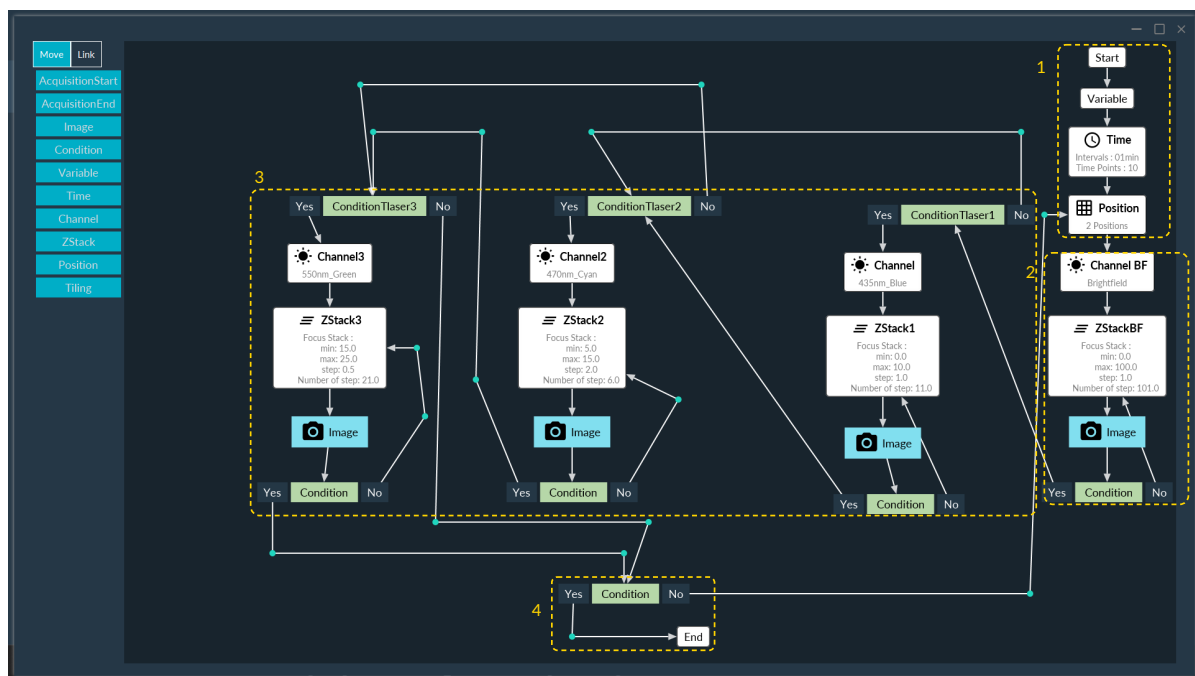
Condition examples:

- `ZStack1Index == ZStack1Max` : every Z slice declared in the **ZStack1** block has been acquired. Use it to close a Z-stack loop.
- `PositionIndex == PositionMax` : every position declared in the **Position** block has been visited. Use it to close a position loop.
- `TimeIndex % laser2 == 0` : the current time point is a multiple of the interval stored in the variable `laser2` . Use it to acquire a channel on a subset of the time points.
- A multi-channel loop (e.g., 405, 488, and 638) closes on the same rule: `[Block Name]Index == [Block Name]Max` .

COMPLEX ACQUISITION EXAMPLES

Example 1: One Interval Time per Channel

Time-lapse, multi-position, and Z-stack acquisition with four channels — BF, DAPI, GFP, and Texas Red — using a different interval time for each channel.



**Description:** the numbers below match the regions outlined on the flowchart.

## 1. Initial setup (1)

- Start the acquisition.
- Set the variables: declare the interval times used by the time-lapse.
- Set up the time-lapse: configure the timing parameters.
- Choose the positions: define the XY coordinates.

## 2. Default sequence: brightfield (BF) (2)

- Select the first channel (BF).
- Set up the Z-stack.
- Acquire the image.
- Condition: is the Z-stack finished?
  - **No**: continue the Z-stack acquisition.
  - **Yes**: proceed to the channel changes.

## 3. Channel conditional sequence (3)

- Laser 1 condition (DAPI): is the current time point a valid interval for DAPI acquisition?
  - **Yes**: select the DAPI channel, run the Z-stack, and acquire the image.
  - **Sub-condition**: is the Z-stack finished? If no, continue the acquisition. If yes, proceed to the next channel.
  - **No**: skip to the second channel.
- Laser 2 condition (GFP): is the current time point a valid interval for GFP acquisition?
  - **Yes**: select the GFP channel, run the Z-stack, and acquire the image.
  - **Sub-condition**: is the Z-stack finished? If no, continue the acquisition. If yes, proceed to the next channel.
  - **No**: skip to the third channel.
- Laser 3 condition (Texas Red): is the current time point a valid interval for Texas Red acquisition?
  - **Yes**: select the Texas Red channel, run the Z-stack, and acquire the image.
  - **Sub-condition**: is the Z-stack finished? If no, continue the acquisition. If yes, proceed to the position check.
  - **No**: skip to the position check.

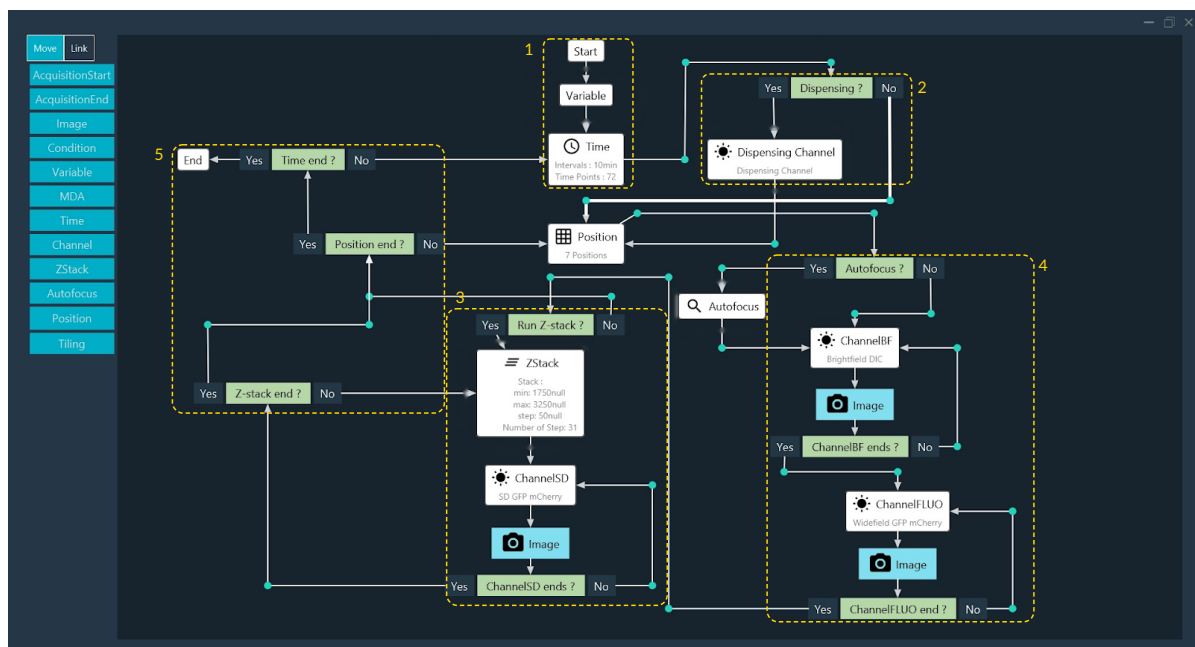
## 4. Position loop and end (4)

- Condition: are all positions acquired?
  - **No**: move to the next position, then restart the sequence from step 2.
  - **Yes**: end the acquisition.

## Example 2

The following protocol combines the same building blocks in a longer branched architecture. It alternates a spinning-disk Z-stack with a widefield channel pair, at seven positions and over 72 time points.





### Description:

1. Initial setup (1): start the acquisition, declare the variables, and configure the time-lapse.
2. Dispensing branch (2): a **Condition** block tests whether a reagent has to be dispensed at the current time point. If dispensing is required, the dispensing channel is selected before the flow reaches the **Position** block.
3. Spinning-disk sequence (3): a **Condition** block tests whether the Z-stack has to run. If it does, the **ZStack** block, the **ChannelSD** block, and the **Image** block are executed until the Z-stack loop closes.
4. Widefield sequence (4): an **Autofocus** condition runs the autofocus when required, then the **ChannelBF** and **ChannelFLUO** blocks are acquired in turn, each closing on its own channel condition.
5. Loop exits (5): the Z-stack, position, and time conditions are evaluated in that order. The protocol ends once the last time point has been acquired.

## Finalizing Acquisition Settings

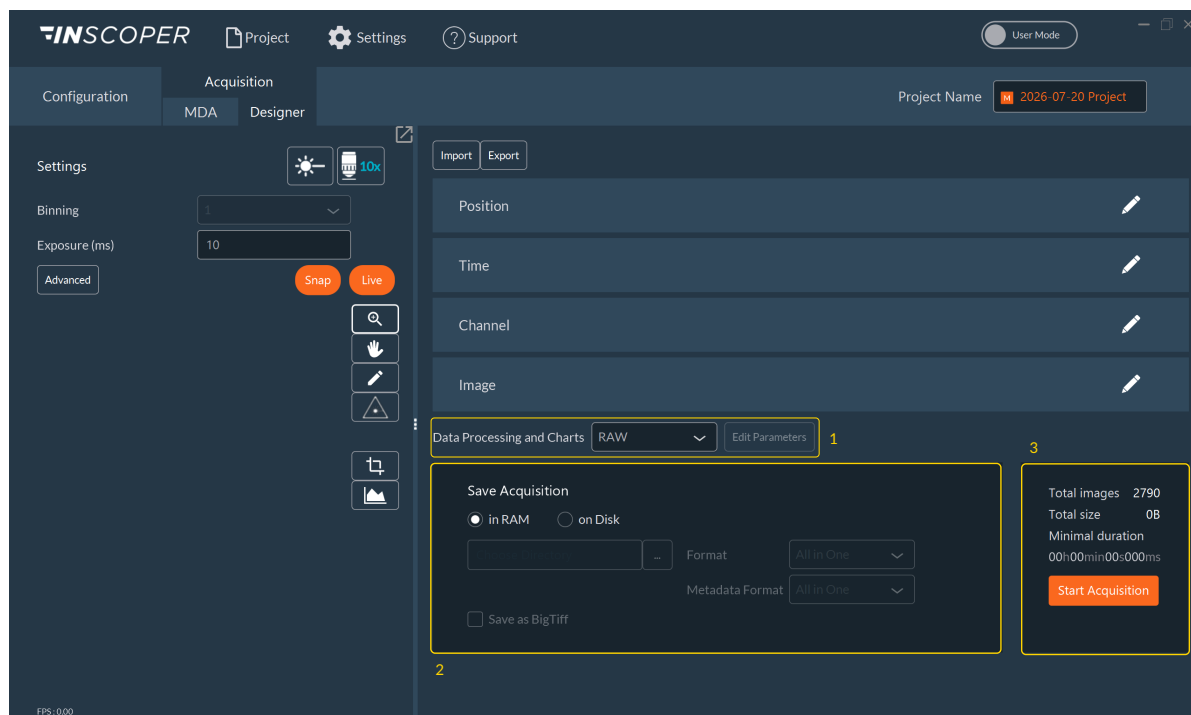
### REVIEW AND VALIDATE THE ACQUISITION PROTOCOL

Once the protocol is built, close the **Show Full** window to return to the main **Designer** tab for an overview and final adjustments.

Click **Validate** to register and verify the design.

#### Note

If the total image counter remains at **0** after clicking **Validate**, the flow architecture contains an error. Re-evaluate the block logic and the links.



Configure the following before launching the acquisition:

- The **Data Processing and Charts** selection (1), which controls real-time image visualization during and after the acquisition. Click **Edit Parameters** to configure the selected DataProcessor.
- The **Save Acquisition** settings (2): the saving location, **in RAM** or **on Disk**, and, for a saving on disk, the target directory, the **Format**, and the **Metadata Format**.

#### Note

The summary panel (3) displays the total number of images, the estimated file size, and the minimum duration of the experiment in real time.

Click **Start Acquisition** (3) to run the experiment.

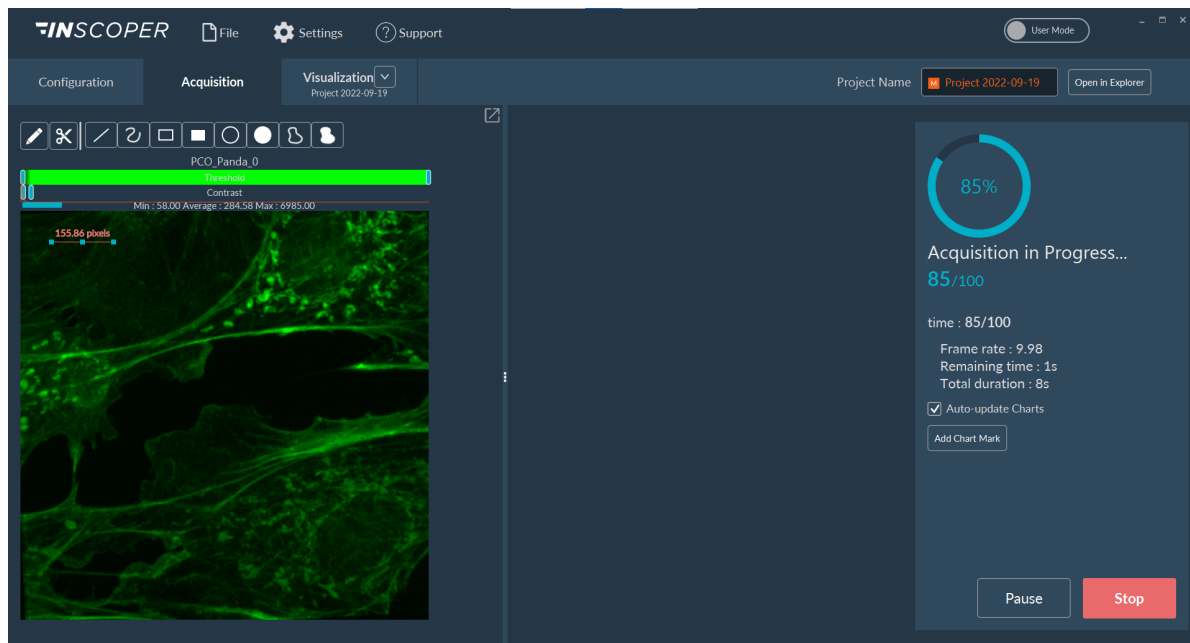
🕒 2026-08-31

## 1.5 Visualization & Analysis

Inscoper I.S. provides tools for visualizing data during and after acquisition.

### 1.5.1 Visualization During Acquisition

The **Visualization** tab allows for real-time monitoring of the acquisition sequence.



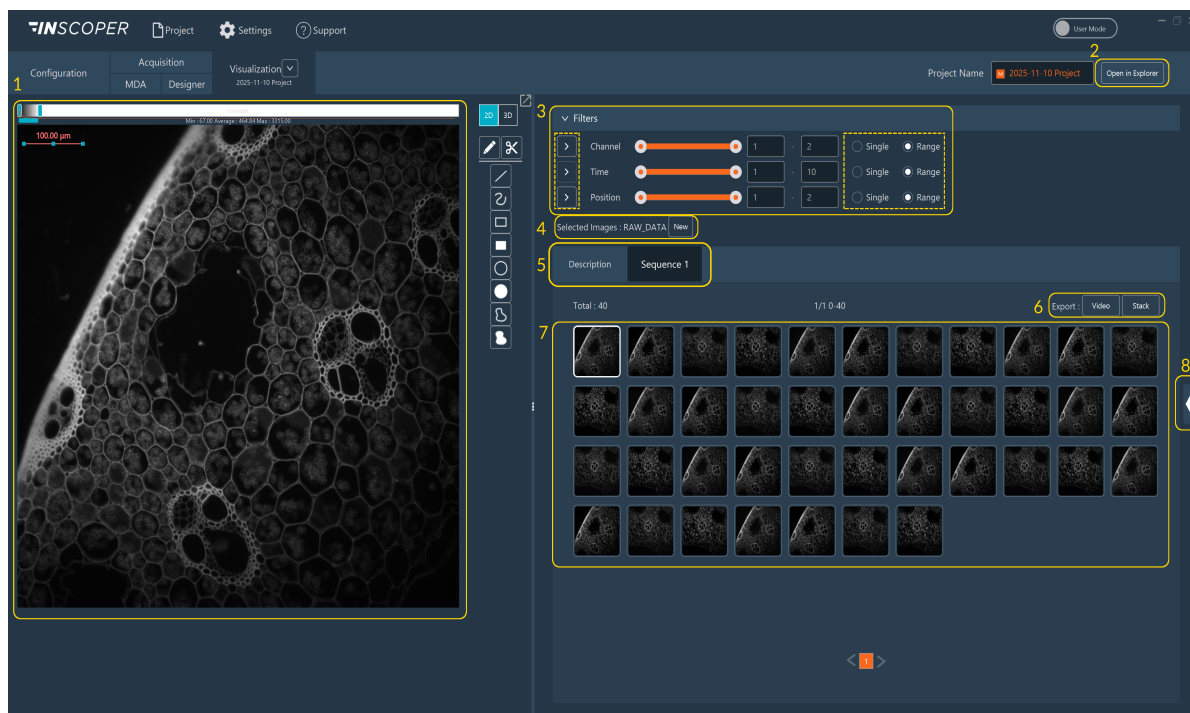
The interface is divided into three functional areas:

1. **Left Panel:** Displays the live, incoming images from the current acquisition sequence.
2. **Center Panel:** Renders corresponding graphical data (e.g., intensity evolution or statistical plots), depending on the configured Data Processor pipeline.
3. **Right Panel:** Provides acquisition progress metrics and system controls to pause or abort the active sequence.

#### Large Area Tiling

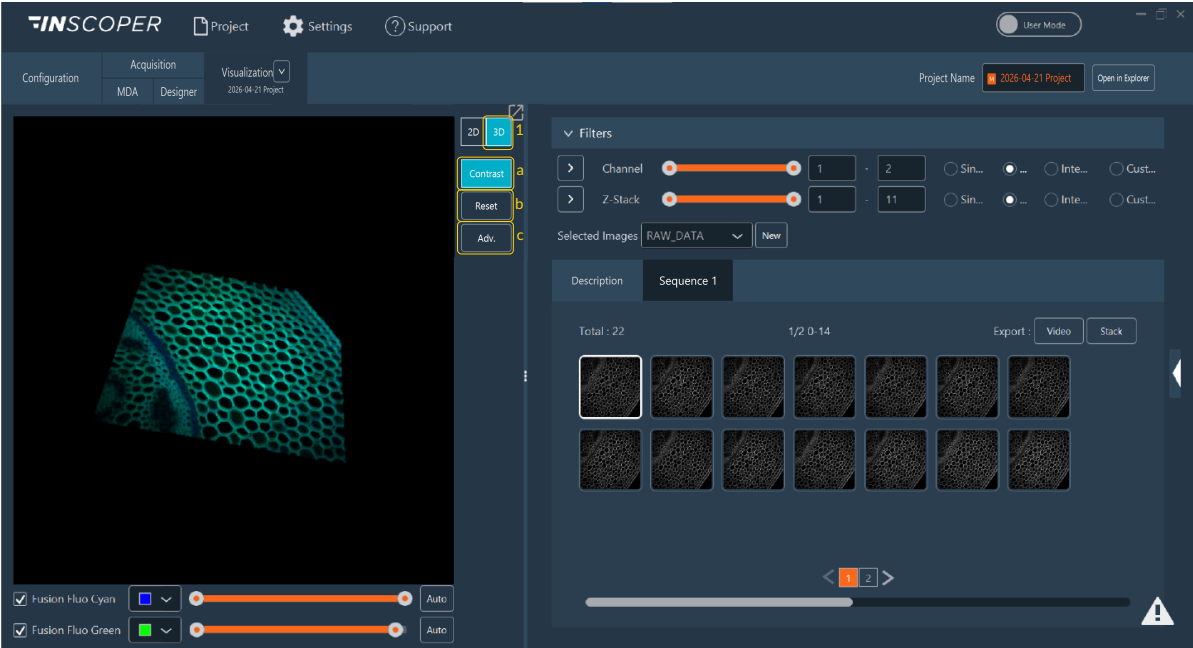
During extensive tiling acquisitions, the left panel dynamically updates to display the full macroscopic mosaic as individual tiles are acquired and aggregated into the global view.

## 1.5.2 Visualization After Acquisition

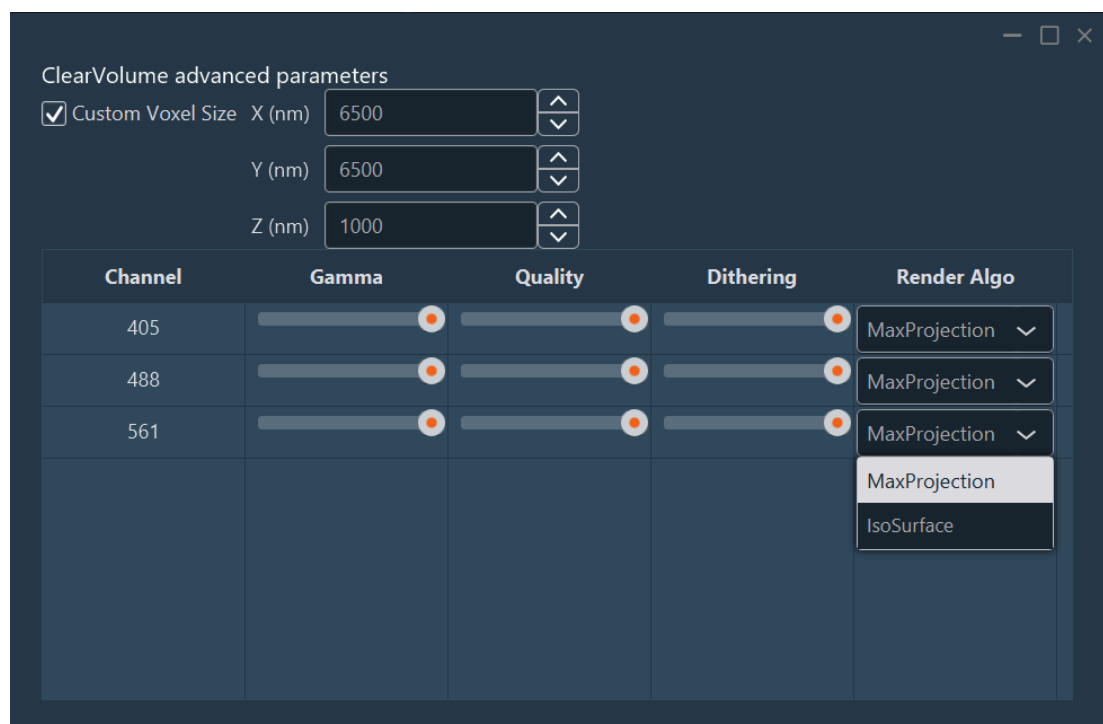


1. **Image Canvas:** View images in the central viewer.
2. **Open Directory:** Navigate directly to the local dataset storage path (applicable only if data is saved to disk).
3. **Filters:** Use dimension-based filters to search your dataset. Select individual images or full groups. Click the **Play** button adjacent to a dimension name to animate the sequence.
4. **Data Processing:** Select output images for post-acquisition processing.
5. **Mode:** Switch viewing modes to visualize quantitative data charts and Description of your acquisition.
6. **Export Options:** Save the currently viewed sequence as an MP4 **Video** or a TIFF **Stack**.
7. **Thumbnail Gallery:** Browse miniature representations of all acquired images.
8. **Metadata Access:** Reveal the hardware and experimental parameters tagged to the images.

3D Viewer



1. Click the **3D** button to launch the volumetric viewer.
2. 3D visualization canvas.
3. Adjust rendering options and parameters:
  1. **Contrast**: Display the LUT and adjust the contrast bar below the 3D view.
  2. **Reset**: Restore the default 3D camera view.
  3. **Advanced**: Access detailed rendering parameters per channel:
    - **Voxel Size** (nm).
    - **Gamma**: Apply a non-linear gamma curve to adjust contrast.
    - **Quality**: Adjust rendering quality for navigation.
    - **Dithering**: Reduce banding/aliasing artifacts.
    - **Render Algorithms**:
      - **MaxProjection**: Displays only the voxels with the maximum intensity encountered along the viewing ray.
      - **IsoSurface**: Renders a 3D surface connecting points of equal intensity value.



#### Limitations

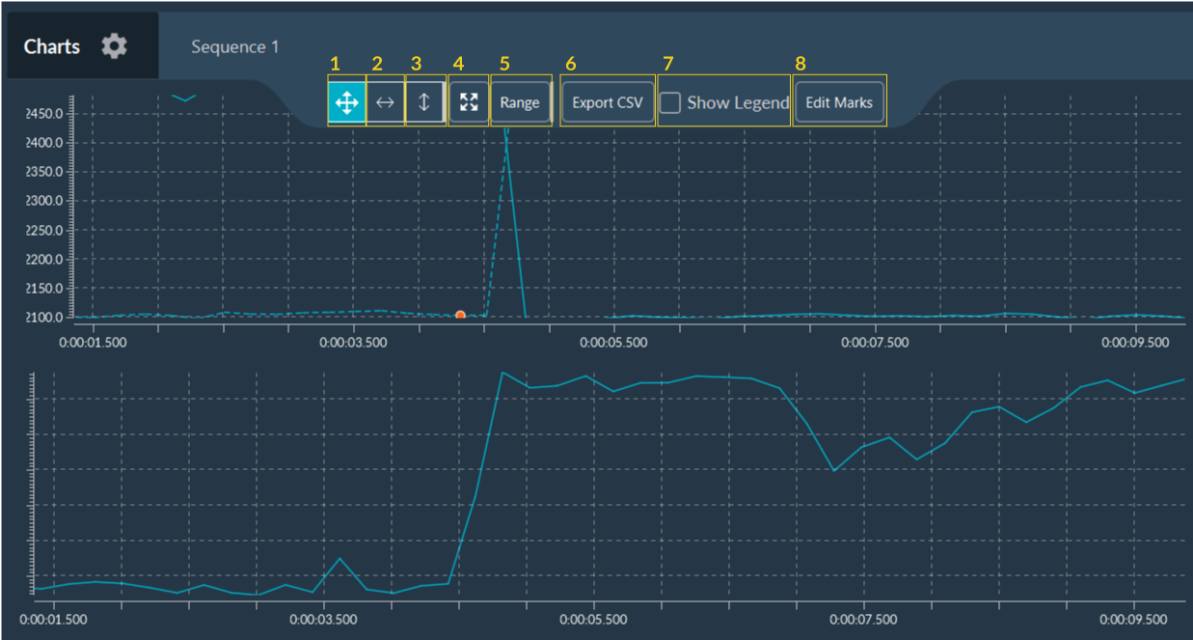
The 3D viewer accepts raw data only, and renders one position at a time:

- Use the dimension filters to select the position to render.
- Processed outputs are not supported. Selecting a channel overlay ( `CHANNEL_MULTICOLOR` ) or a tiling ( `SIMPLE_TILING` , `STITCH_TILING` ) opens the viewer on an empty black volume, without an error message.

### 1.5.3 Interacting with Graphics

Interact with charts by adjusting their appearance, adding time markers, or exporting raw data. Hover over a graph to access its toolbars.

- **Pan**: Click and drag the mouse wheel (middle click) to pan across the graph.
- **Zoom**: Scroll the mouse wheel to zoom in and out. Click and drag to select a region to magnify.
- **Jump to Image**: Left-click a data point on the graph to view the corresponding image.
- **Reset View**: Right-click within the graph to restore the default axis scaling.



Graph Tools

- 1. Enable the XY zoom mode
- 2. Enable the X zoom mode
- 3. Enable the Y zoom mode
- 4. Reset zoom or enable auto-ranging.
- 5. Manually override the axis ranges.
- 6. Export the raw graph data to a `.csv` file.
- 7. Toggle curve legends.
- 8. **Add Markers:** Annotate the timeline with event markers. Markers can be exported to a `.csv` file.

Chart Markers

Add

Remove All

| Name      | Start           | End             | Color                  |                        |  |
|-----------|-----------------|-----------------|------------------------|------------------------|--|
| Start     | 00h00min00s000m | 00h00min01s000m | <div><div></div></div> | <div><div></div></div> |  |
| Agonist   | 00h04min50s000m | 00h04min51s000m | <div><div></div></div> | <div><div></div></div> |  |
| Inhibitor | 00h06min30s000m | 00h06min31s000m | <div><div></div></div> | <div><div></div></div> |  |
| End       | 00h09min00s000m | 00h09min01s000m | <div><div></div></div> | <div><div></div></div> |  |
|           |                 |                 |                        |                        |  |

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.

### 1.5.4 Image Processing

Apply post-acquisition data processing from the Visualization tab.

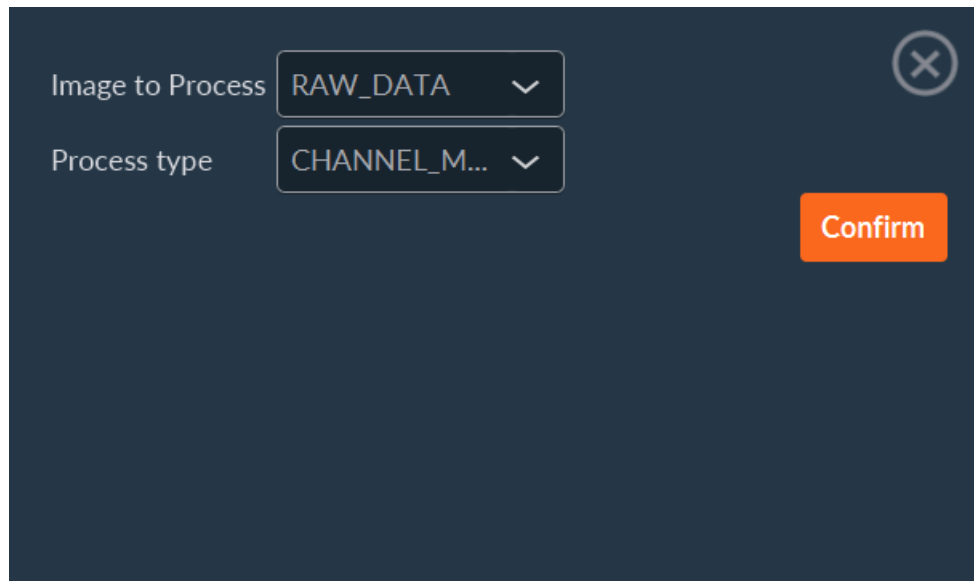
A dark-themed dialog box for image processing. It contains two labels, 'Image to Process' and 'Process type', each followed by a dropdown menu. The first dropdown shows 'RAW\_DATA' and the second shows 'CHANNEL\_M...'. Both dropdowns have a downward arrow icon. In the top right corner is a circular close button with an 'X'. In the bottom right corner is an orange 'Confirm' button.

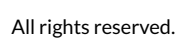
Image to Process RAW\_DATA ▼

Process type CHANNEL\_M... ▼

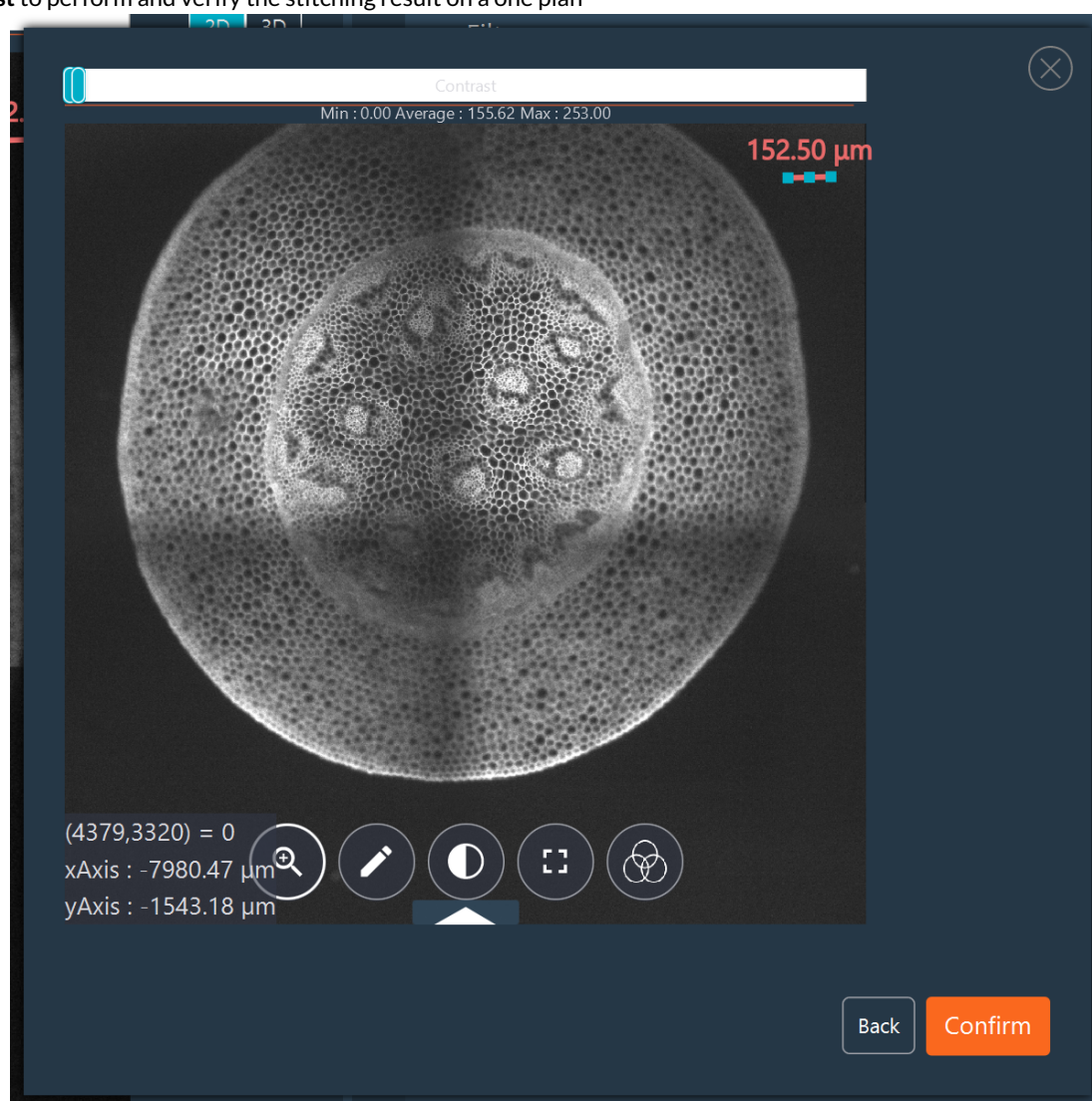
Confirm

1. Select the target sequence from the image drop-down list.
2. Choose the desired data processor.
3. Click **Confirm** to execute.





1. Select the **image to process**.
2. Select the **Stitching** **process type** (refer to the [stitching algorithm](#) for details on the registration and blending calculations).
3. Set the **correlation threshold (%)** for image stitching (the minimum correlation score,  $T_{\text{corr}}$ , required to validate the alignment between adjacent tiles; boundaries scoring below this threshold are flagged as invalid, triggering the configured fallback behavior).
4. Optional: Select a **Z-stack reference**, i.e. the focus plane used to calculate the stitching coordinates.
5. Optional: Select a **channel reference**, i.e. the channel used to calculate the stitching coordinates.
6. Define the fallback behavior for **invalid tiles** (uncorrelated):
  - **HIDE** : Discard the tile from the final image.
  - **DEFAULT\_OFFSET** : Place the tile based solely on its recorded mechanical stage coordinates.
  - **INTERPOLATE** : Interpolate the tile's position based on the calculated offsets of surrounding valid tiles.
7. Click **Test** to perform and verify the stitching result on a one plan



8. Click **Confirm**.

### 1.5.5 Data Export

Export sequences as MP4 videos or multi-dimensional Tiff stacks via the Export menu (Toolbar Item 6).

#### Video Export

Use dimension filters to isolate the image sequence, then click **Video** from the Export dropdown.

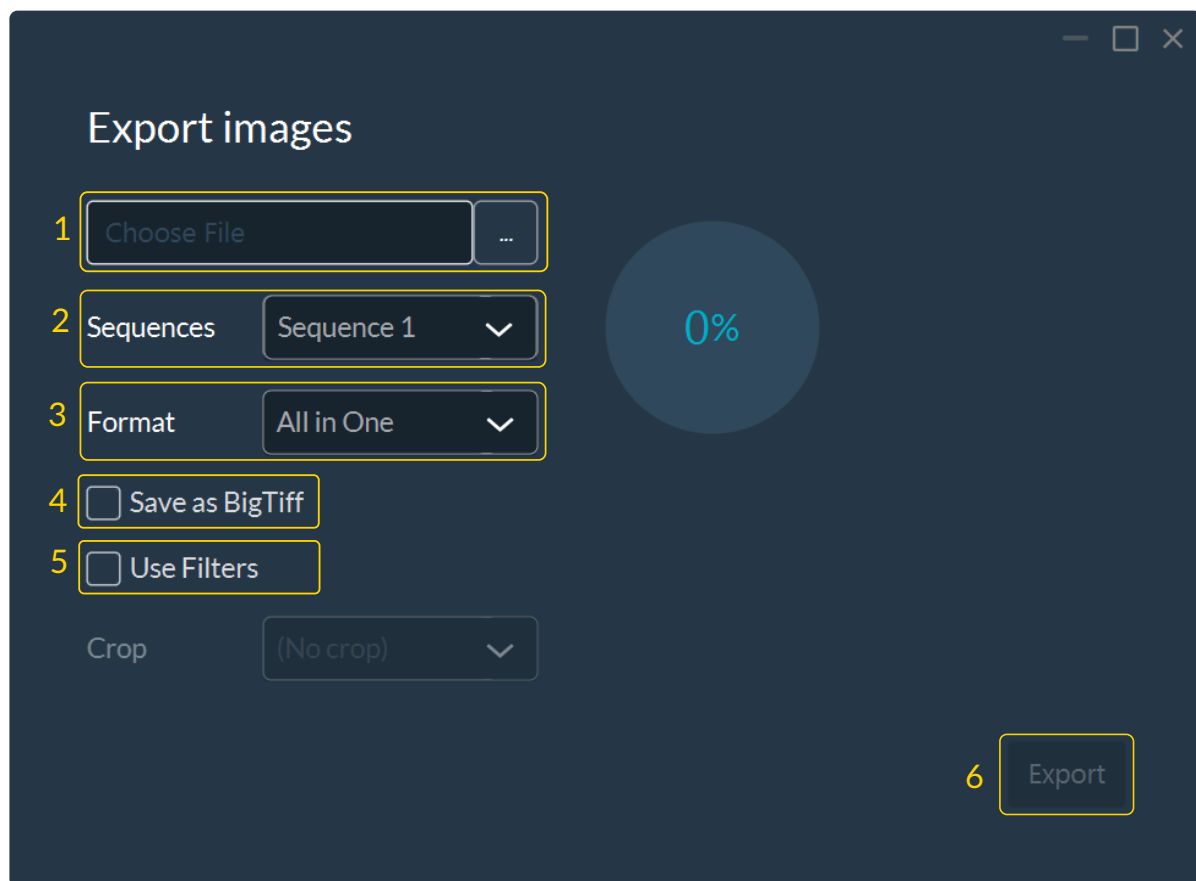


To export a video:

1. Select the destination file path.
2. Verify the sequence to export.
3. Select the video format/codec.
4. Click **Export**.

### Stack Export

Use dimension filters to isolate images, then click **Stack** from the Export dropdown to generate a multidimensional TIFF.



To export a stack:

1. Select the destination file path.
2. Verify the sequence to export.
3. Select the data format.
4. (Optional) Check **Save as BigTiff** if the expected file size exceeds 4GB.
5. (Optional) Check **Use Filters** to apply your visualization filters to the export.
6. Click **Export**.

### 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

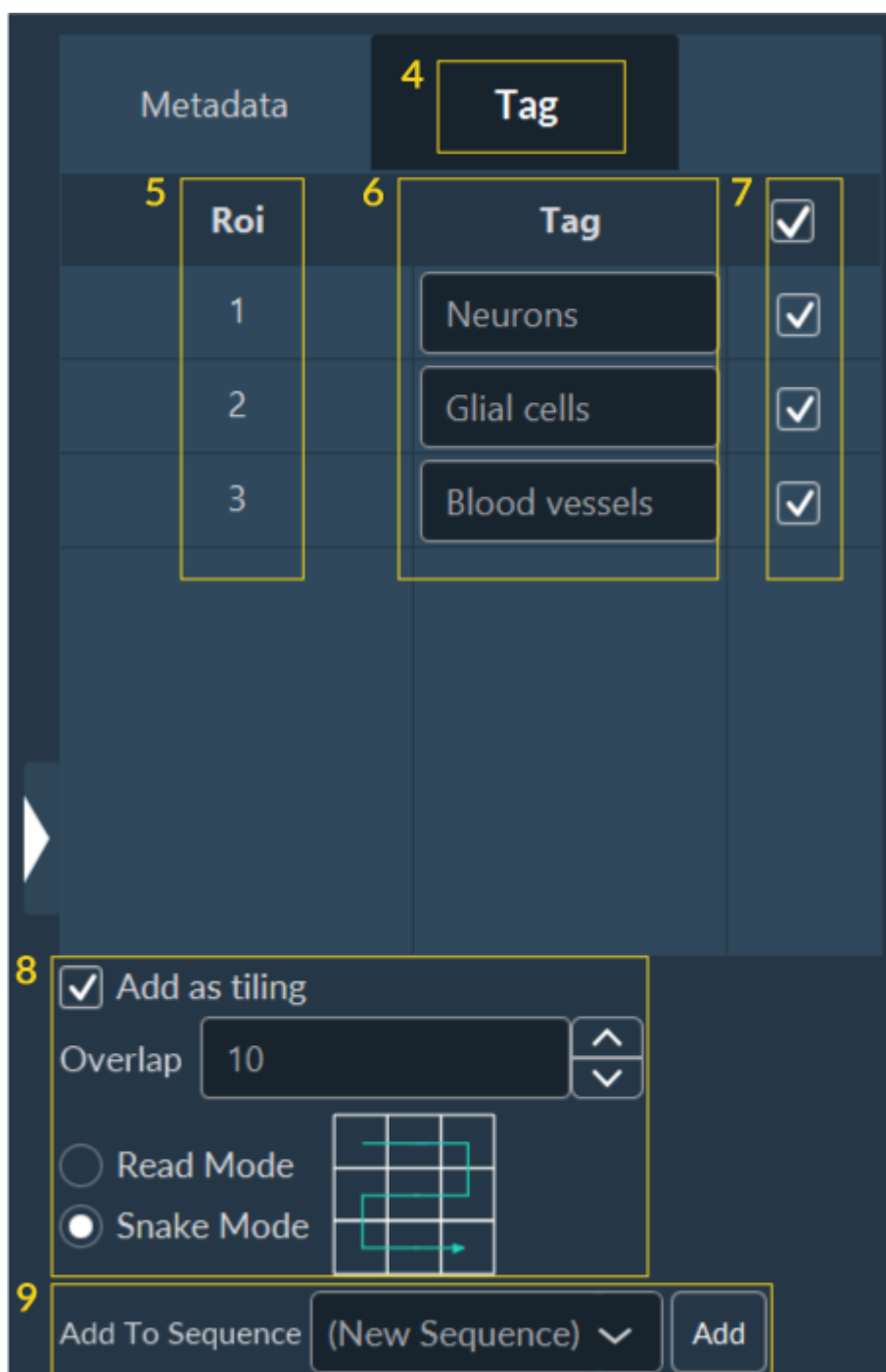
Filter

| Property                     | Value                         |
|------------------------------|-------------------------------|
| Leica_DMi8_Microscope-X-Axis | 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

1.5.6 Semi-automated feedback microscopy feature

The software supports feedback microscopy workflows. For example, you can acquire a low-magnification overview scan, tag regions of interest, and export those coordinates into a new, high-magnification acquisition sequence.



To use this feature:

1. Acquire an overview image (e.g., a **Tiling** scan).
2. Use the ROI tools to draw regions around structures of interest.
3. In the **Visualization** tab, click the white triangle on the right edge to expand the side panel.
4. Navigate to the **Tag** sub-tab.
5. This lists all drawn ROIs. You can manage them directly from this list.
6. (Optional) Assign descriptive names/tags to each ROI.
7. Select the ROIs you wish to target.
8. If the new targets require their own sub-tilings, configure those spatial parameters here.
9. Choose to inject these new coordinates into a fresh sequence or append them to an existing one.

### Smart Microscopy

Use this workflow for:

1. A low-magnification pre-scan followed by targeted high-resolution imaging.
2. A brightfield pre-scan, applying fluorescence imaging to identified structures to reduce global phototoxicity.

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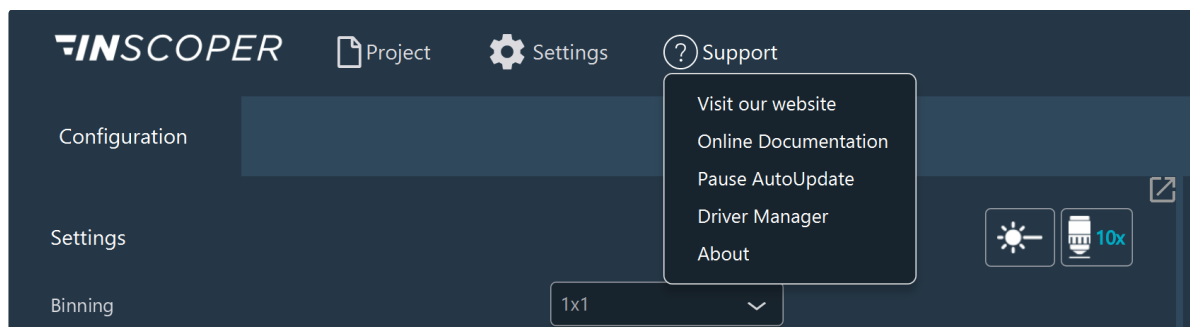
## 1.6 Support

If you have any questions or encounter any issues with your software, please contact us through our ticketing tool, Mainti4 (your login credentials will be provided after installation). To help us diagnose the issue quickly, we will need your software version and logs folder.

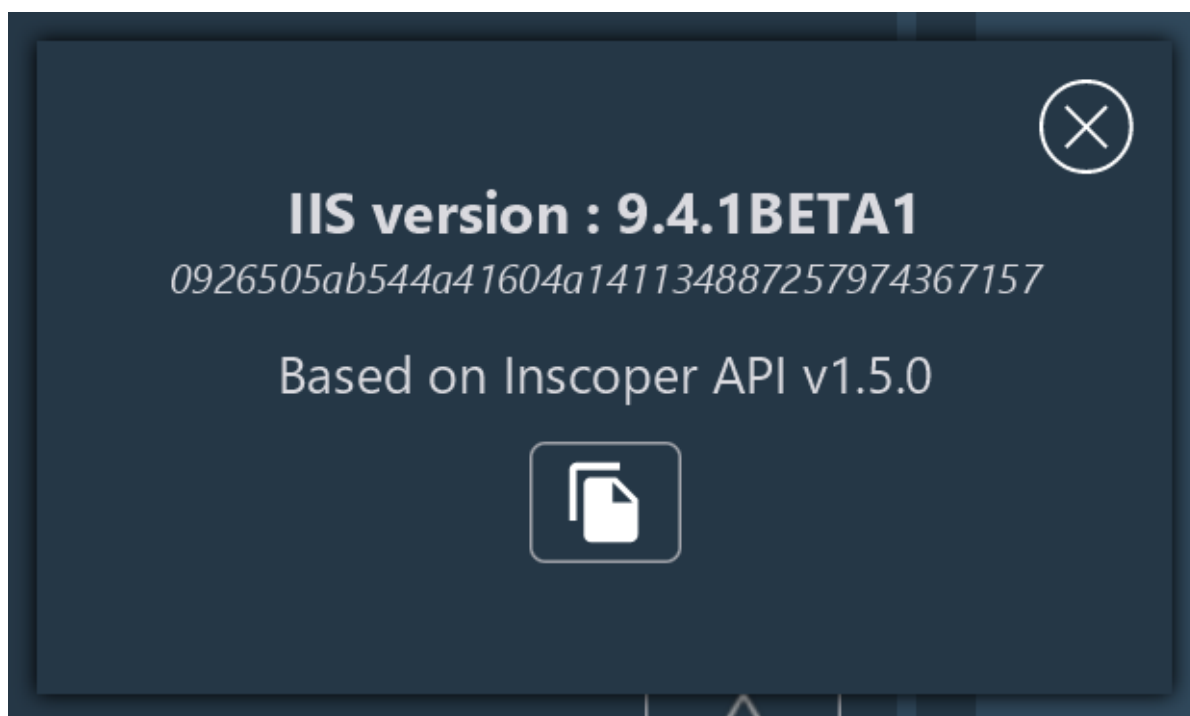
This page explains exactly where to find this information.

### 1.6.1 Inscoper Software Version

To find your Inscoper version, open the **Support** drop-down menu and click **About**.



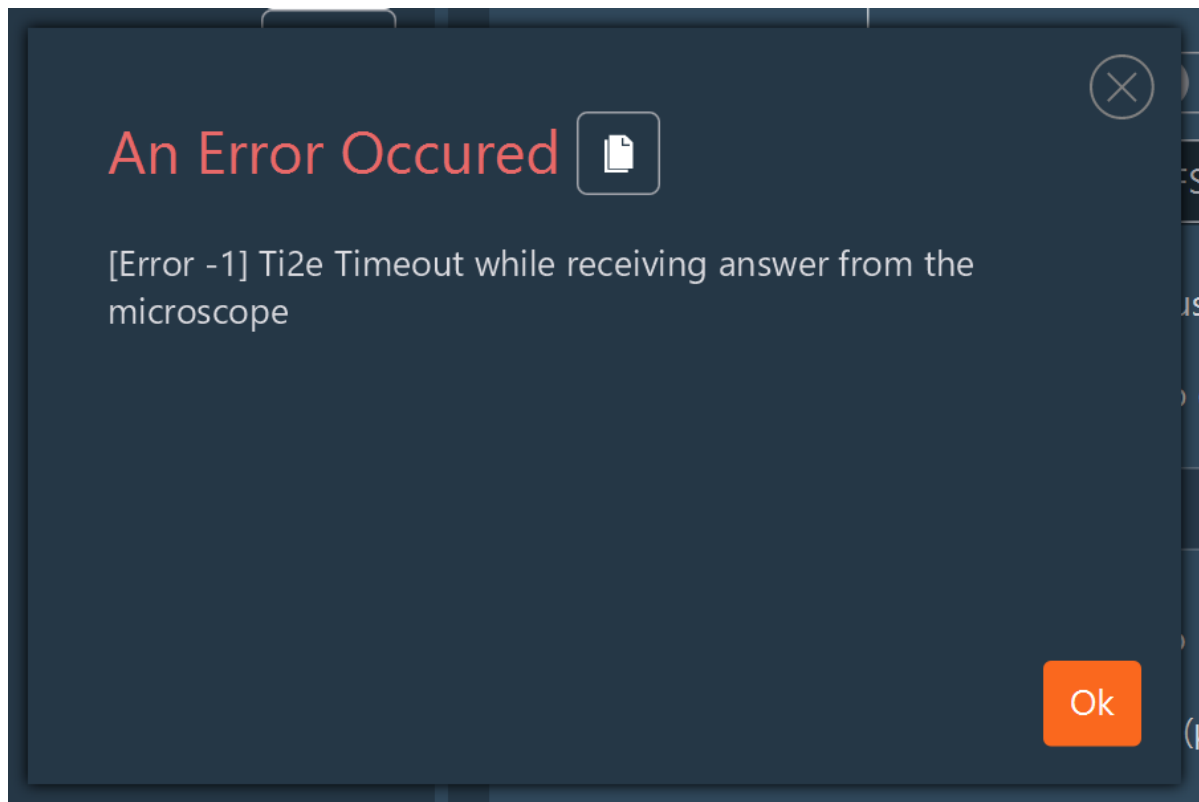
A pop-up will appear displaying all technical details, including the version and API numbers. If you encounter any issues with your software, you can copy and paste this information directly into your support ticket.



### 1.6.2 Error Windows

If you encounter an error while using the software, please copy and paste the error message directly into your support ticket.

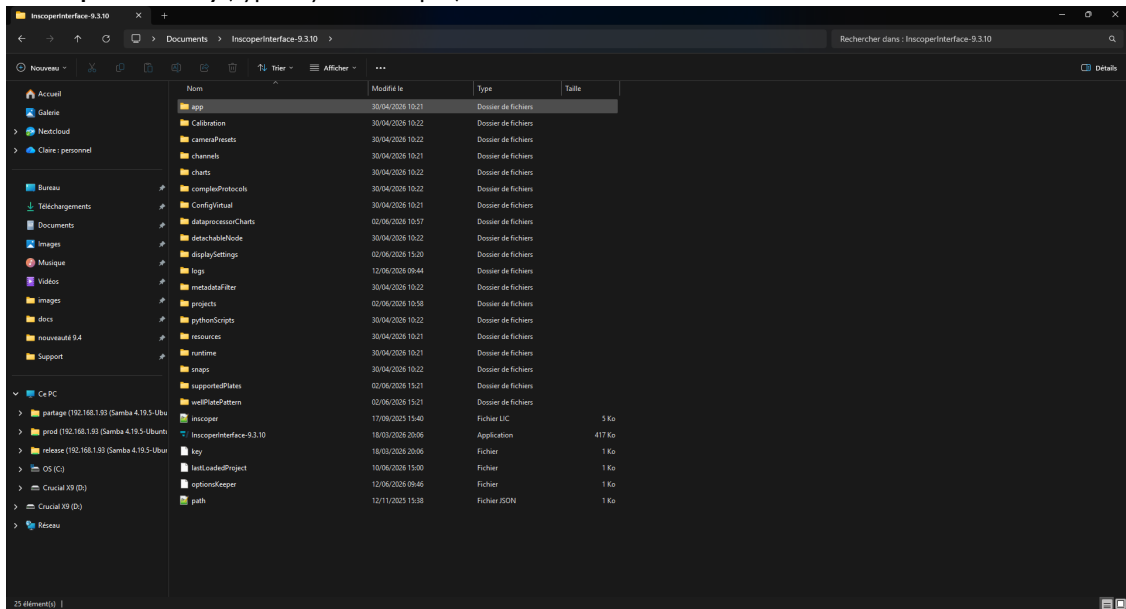




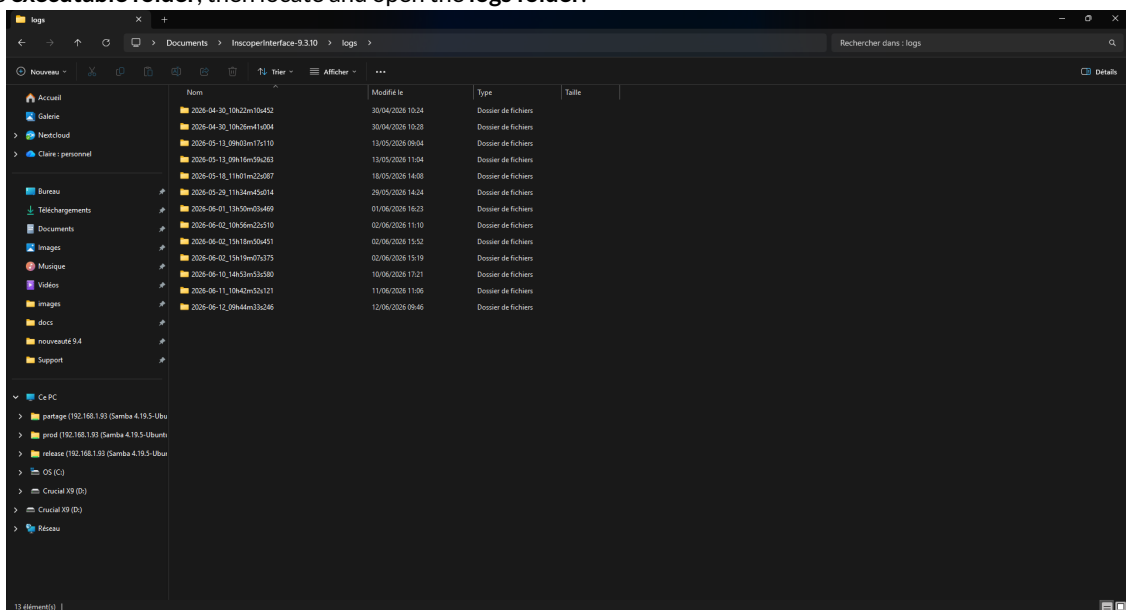
### 1.6.3 Find log folder

Follow these steps to locate your latest log folder:

- Open your **Inscoper** directory (typically: C:\Inscoper).



- Open the **executable** folder, then locate and open the **logs** folder.



- Select the most **recently created** folder inside.
- Attach this **folder** to your support ticket.

#### Note

A new log folder is generated every time the software interface is opened, so the newest folder will contain the details of your current session.

## 1.7 Add-ons

### 1.7.1 Custom Python Scripting

#### Introduction to Python Scripting

The **Python Scripting** module bridges the gap between automated image acquisition and custom computer vision algorithms. By executing Python scripts after each image capture event, it enables smart microscopy: dynamic, adaptive workflows that use computational analysis to control microscope hardware. This module is built on two core components: a graphical interface and a development toolkit.

#### THE GRAPHICAL INTERFACE MODULE

##### Licensing Requirement

The **Custom Scripting** software license option is required to use this module.

Located natively within the Inscoper I.S. GUI, the module serves as the bridge between your custom scripts and the acquisition engine. It is found within the **multi-dimensional acquisition (MDA)** tab, immediately below the standard sequence settings.

- **Automated Settings UI:** Upon importing a Python script, the module parses the code to identify all functions (potential entry points). Parameters defined in your Python functions are mapped to graphical controls (e.g., `bool` arguments generate toggle switches, `str` become text fields, enumerations become dropdown lists).
  - The **Image Provider** function dictates which acquired images are passed to the Python processing function at any given time point.
  - The **Processing Function** executes the analysis (e.g., computer vision, intensity measurements) and returns the updated values for microscope hardware parameters.
- **Sequence Execution:** The system engine triggers these functions as callbacks following each image capture. The Image Provider is invoked first to assess if analysis is needed; if confirmed, the Processing Function runs, adjusting designated hardware parameters, or pushing figures to the interface.

#### THE DEVELOPMENT TOOLKIT ( `inscoper-scripts` )

Provided as a local package, this toolkit serves as the foundation for custom script development. It contains a collection of Python scripts and utility sub-modules:

- **Application Scripts:** A suite of ready-to-use smart microscopy master applications, including modules for:
  - Mechanical tracking
  - Single-cell population screening/tracking
  - Autofocus
  - FRAP workflows
- **Structural Templates:** Simplified application examples that serve as starting points for generating custom scripts.
- **Utility Sub-modules:** A suite of helper libraries containing predefined image providers, ROI objects, and mathematical functions for tracking, measurement, segmentation, and filtering. These utilities simplify interacting with the core software environment, allowing you to display figures in the GUI, manipulate metadata dictionaries, and generate analytical plots.

## Calling Scripts in Inscoper I.S.

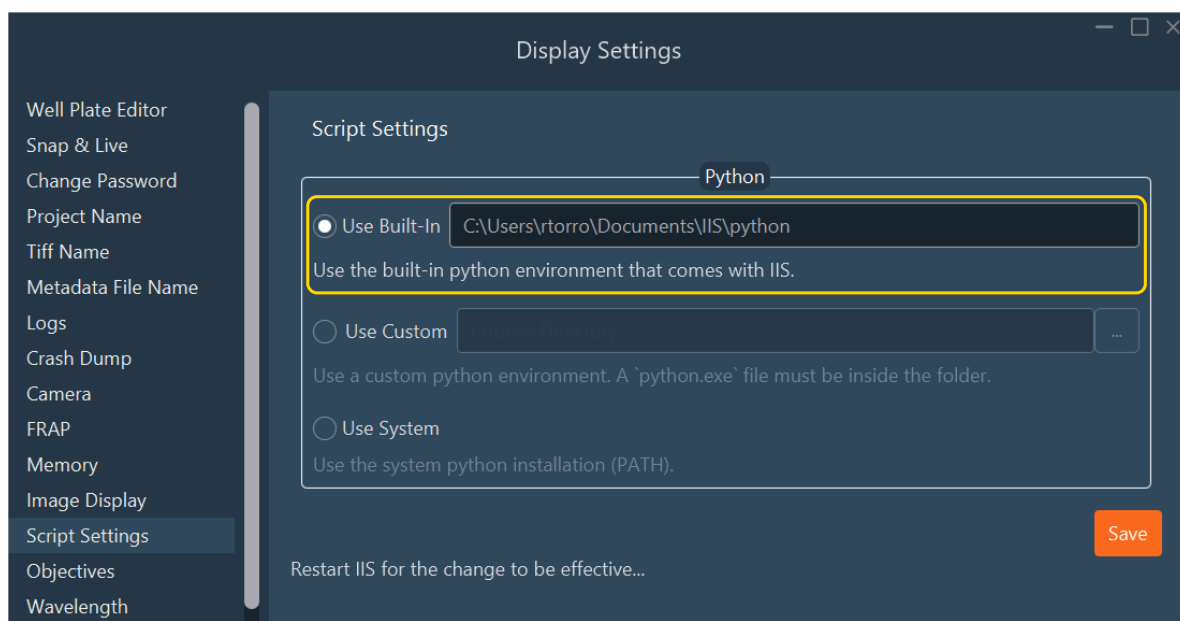
The Python Scripting module enables the execution of custom Python algorithms within an MDA sequence.

To ensure compatibility, scripts must adhere to the templates provided in the `inscoper-scripts` local package. You may use the included sample scripts for testing.

### PYTHON ENVIRONMENT SETUP

Before running a script, select the Python environment the software uses.

1. Navigate to **Settings > Display Settings**.
2. Select **Script Settings** from the left-hand navigation pane.
3. Choose your preferred execution environment:
  - **Use Built-In (recommended):** Uses the Python environment bundled with the software installation. It includes the standard (non-GPU) data science and image analysis packages.
  - **Use Custom:** Point the software to a specific, user-managed local Python executable ( `python.exe` ). Note: This advanced route explicitly requires the `jep` library (see warning below).
  - **Use System:** Uses the Python installation found in the PATH of the operating system.
4. **Restart Inscoper I.S.** to fully instantiate the environment changes.



#### Jep Package Required for Custom Environments

If you elect to use a custom environment (such as a specific Conda environment), the `jep` (Java Embedded Python) package is strictly required to bridge the software architectures.

Install it by activating your target environment and running:

```
pip install jep
```

 Expanding the Built-In Environment

If your custom script requires specialized statistical or biological libraries not included in the standard distribution:

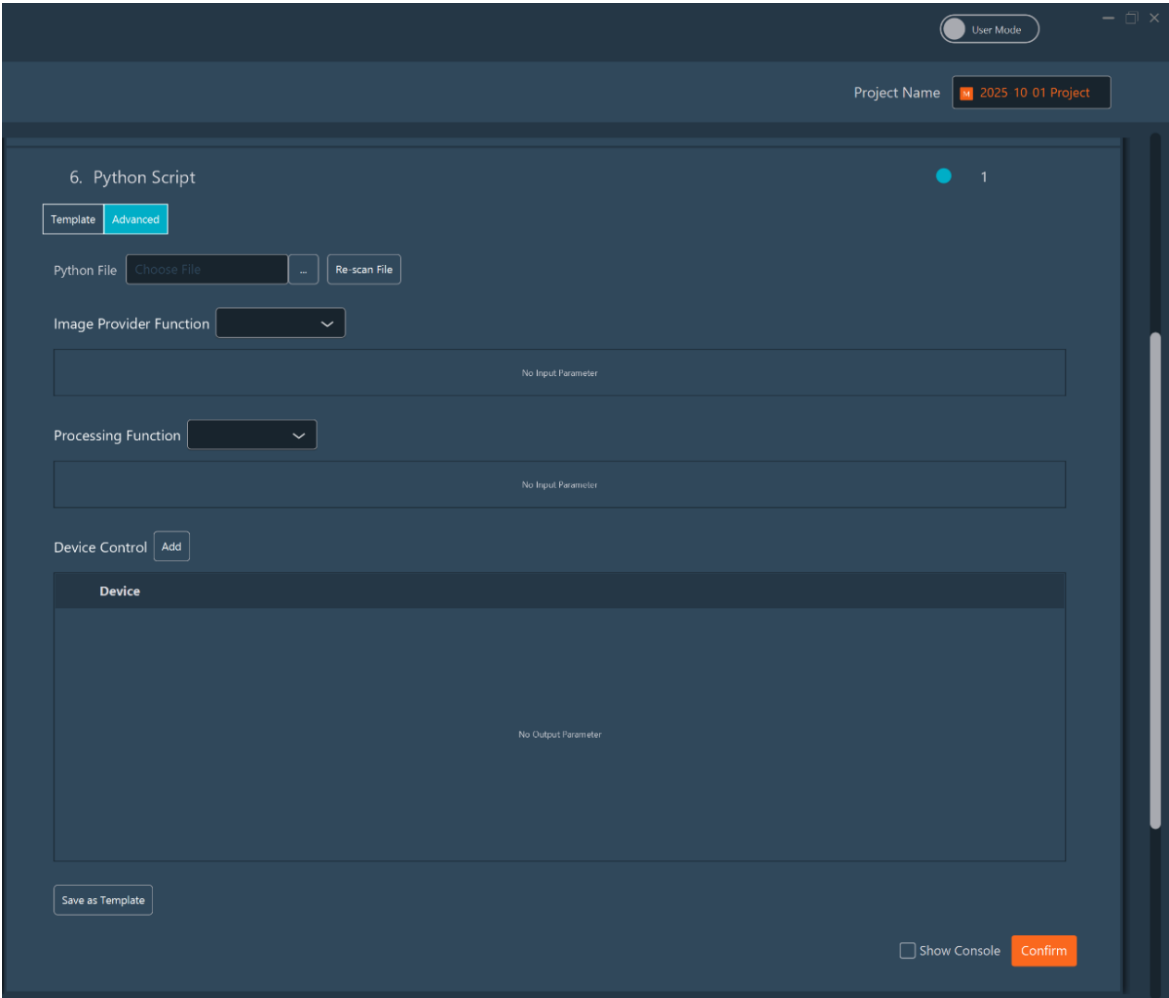
1. Open a command line terminal within the **I.I.S. installation directory**.
2. Navigate to the nested `python` subdirectory.
3. Execute the standard pip installation command:

```
./python.exe -m pip install <package_name>
```

**IMPORTING AND MAPPING A SCRIPT APPLICATION**

Once your Python interpreter is set, you can load your script into the MDA sequence definition.

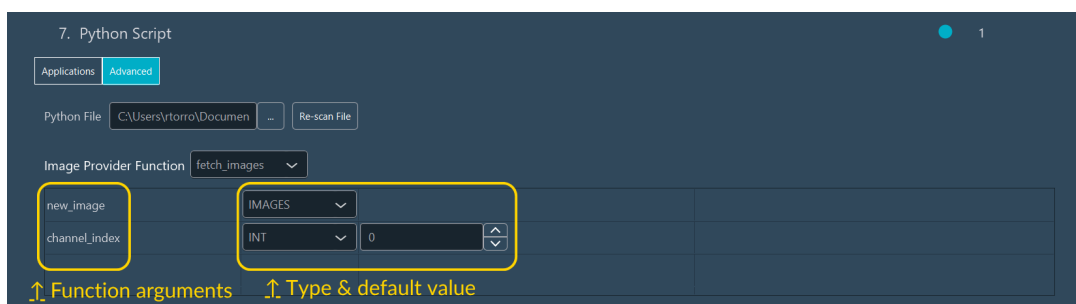
1. Navigate to the central **Acquisition** tab and select the **MDA** (Multi-Dimensional Acquisition) sub-tab.
2. Expand the **Python Script** module panel and toggle the **Advanced** switch to enable this mode.
3. Click the "..." button adjacent to **Re-scan File**, browse to the `inscopex-scripts` local package, and select your specific `.py` script.



1. **Map Python functions to I.I.S. parameters.** Define which function handles image fetching (image provider) and which handles heavy-computations (processing), then map the functions' arguments to the proper type and value.

1. **Step A (image provider):** Select the script function responsible for fetching images (e.g., `fetch_images` ).

- **Map Arguments:** Map the script variable `new_image` to the `IMAGES` data type. This allows the software to pass the most recently acquired image to your script.
- Map `channel_index` to `INT` and assign a default value (e.g., `0` ). These channel indices correspond to their order within the Multi-channels dimension.



1. **Step B (processing function):** Select the function responsible for data analysis (e.g., `correlation_tracking` ).

- **Map Arguments:** Map the `images` variable to the `IMAGES` type. This variable receives the images curated by the Image Provider function.
- Map hardware identifiers (e.g., `stage_x_id`, `stage_y_id`, `stage_z_id`) to the `SUBDEVICEID` type and set default values matching your hardware.
- Map logical switches (e.g., `correct_z`, `focus_on_motion`, `show_plots`) to `BOOL` and define initial states.
- Map `acquisition_context` to `IIS_CONTEXT`. This provides access to internal software APIs, such as rendering `matplotlib` figures in the UI (see [Interacting with Inscoper I.S.](#)).

2. **Step C (device control):** Declare any hardware sub-devices that will be updated by your processing function's outputs (it should match symmetrically with the return values of the processing function, e.g. if the processing function returns a tuple of new 'x', 'y', and 'z' values, then you should declare the X, Y, and Z sub-devices here). If your function only logs data without modifying hardware, leave this blank.

## 2. Save as a Reusable Template

1. Once mapping is complete, click **Save as Application Template**.
2. In the dialog box, select which parameters should remain editable by the user.
3. Unselected parameters will be hardcoded to their default values and hidden from the UI.

