INSCOPER

User Guide Inscoper I.S. Interface version : 9.0

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1. IMAGING SOLUTIONS

From core solution to custom techniques

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

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

1.1. Inscoper I.S.

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

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

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

1.1.1. User journey

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



The user journey has 3 successive stages:

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



1.1.2. Getting started

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

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

TIN SCOPER	File	🔯 Settings	? Support		User Mode - 🔍 X
Configuration					Project Name Project 2022-09-05
Camera Settings C Exposure (ms) Advanced		Snap Live	Projes	Calibration Time Go to Light Path Axis Microscope Filters pE-800 fur Shutters Light Dath Objection et Name Project 2022-09-05 OK XY > Y-Axis 0 Step (um) 1 Focus 0 X = Step (um) 1	
			<u></u> ව ව	Channel Not saved Microscope IL Lamp O Subornel Cubornel	Add SpX-Q V
				Filters I Lambda A I Lambda A	xda C 🗸
		Ed	It Chart Crop Image		Go To Acquisition

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

1.1.3. Display settings

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



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

- | 1 Imaging solutions | 1.1 Inscoper I.S.
 - 1. Edit your well plate in the Well PLate Editor .

Display Settings							
Well Plate Editor	Wall Dista Edit	lor.					
Snap & Live	weir Plate Euri	lui					
Change Password	Name		\square	~	Save		
Project Name							
Tiff Name	X Size		~	X Spacing (µm)	9000		
Metadata File Name	Y Size	8	÷	Y Spacing (µm)	9000		
Logs			Ě		L		
Camera	Well Type		~)				
FRAP	Well Width (µm)	5					
Memory			=				
Image Display	Well Height (µm)	5					
Preferences							

- a. Name the support.
- **b.** You can edit the saved well plate by directly selecting it in the drop-down menu, make corrections and save these new settings.
- c. Enter the number of horizontal and vertical wells.
- d. Enter the horizontal and vertical spacing between the wells.
- e. Enter the well type (circle, square, rectangle, ...).
- f. Add the dimensions of the wells.
- g. Save this new well plate.

i

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

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

	Displa	y Settings		- 🗆 ×
Well Plate Editor Snap & Live	Snap & Live			
Change Password Project Name	FPS limit in live	30	Î.	
Tiff Name	✓ Save Snap	snaps		
Metadata File Name Logs Camera FRAP Memory Image Display Preferences	Channel Live Add			
				Save

In this tab, you can:

- a. Set a frame rate (FPS) limit for Live mode.
- **b.** Change the directory where snapshots are saved.
- c. Define a default channel for Live mode.
- **3. Change Password**: Set or change the password to switch from user mode to expert mode.

	Display Se	ettings	- 🗆 ×
Well Plate Editor Snap & Live Change Password Project Name Tiff Name Metadata File Name Logs Camera FRAP Memory	Change Password Current Password New Password Confirm New Password		Confirm
Image Display Preferences			

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

	Display Settings	
Well Plate Editor Snap & Live Change Password Project Name	Project Name	
Tiff Name Metadata File Name Logs Camera FRAP Memory Image Display Preferences	Text Date Project yyyy-MM-dd Preview : Project 2025-04-17	Save

5. Tiff Name: Change the format of the image saved.

For example, by default, one file is saved per position (including all other dimensions). However, you can choose to save the image as one file per position per channel instead.

_						
Well Plate Editor Snap & Live Change Password Project Name	Tiff Name All in One 5 Name Add					
1 Tiff Name Preferences	2 Text Dimension Index Dimension Name Text Date Channel Time Channel Position Z Position Axis Camera Extra					
	 3 Text Extra Dim. image_ Position Name Min Digit Nb. 0 4 Preview : image_MyPos 6 Save 					

- a. Select the Tiff Name tab.
- b. Here are all the elements that can be automatically added to the standardized name of saved files. Add them by dragging and dropping them to the name construction tools below. Generally the name composition is the text + dimension name + dimension

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

- c. Name construction tool that shows schematically the name of the future saved file.
- d. Name preview.
- e. Enter a name and click on the **Add** to save this new default name.
- f. When you like the name structure, click **Save**.
- 6. Metadata File Name: Change the format of metadata file saved.

	Display S	Settings	- 🗆 ×
Well Plate Editor Snap & Live Change Password Project Name	Metadata File Name	All in One	
Tiff Name	Text	Dimension Index	Dimension Name
Metadata File Name	Text Date	Channel	Channel Position
Logs			
Camera		Z	Axis Camera
FRAP		Extra	
Memory			
Image Display	Text		
Preferences	metadata		
	Preview : metadata.json		
			Save

7. Logs: Modify logs level.

- Display Settings							
Well Plate Editor Snap & Live	Logs						
Change Password Project Name	Firmware Log	LOG_INFO	~				
Tiff Name	API Log	LOG_INFO	~				
Metadata File Name	IIS Log	LOG_INFO	~				
Logs Camera FRAP							
Memory							
Image Display Preferences							

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

	Display	y Settings			- 🗆 ×
Well Plate Editor	Camera				
Snap & Live Change Password	Hamamatsu_Fusion	Add	PCO_Panda	Add	
Project Name					
Tiff Name					
Metadata File Name					
Logs					
Camera					
FRAP					
Memory	No content i	n table	No c	ontent in table	
Image Display					
Preferences					

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

	Display Se	ettings		
Well Plate Editor Snap & Live	FRAP			
Change Password	X Mirror			
Project Name	Y Mirror			
Tiff Name	XY Permuted			
Metadata File Name	Calibration Point Offset	0.1	<u>^</u>	
Logs				
Camera	Joystick Resolution 1	50		
FRAP	Joystick Resolution 2	500	<u>^</u>	
Memory	sofuler neodiation 2			
Image Display	Joystick Resolution 3	5000	\sim	
Preferences				Save

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

	Displa	y Settings		- 🗆 ×
Well Plate Editor Snap & Live Change Password Project Name Tiff Name Metadata File Name Logs	Memory Maximum memory	9.5547	GB	Save
FRAP				
Memory				
Image Display				
Preferences				

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

	— 🗆 × Display Settings
Well Plate Editor Snap & Live Change Password Project Name Tiff Name Metadata File Name Logs Camera FRAP	Image Display Smooth pixels Image Contrast Mode MAX Always Visible XY Coordinates Pixel Value Metadata
Memory Image Display Preferences	Metadata Name No content in table

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

	Display Settings $ \square$ $ imes$
Well Plate Editor Snap & Live	Preferences
Project Name Tiff Name	Time unit ms ~
Metadata File Name Logs	Distance unit um 🗸
Camera	Acquisition Start
FRAP	Confirm Acquisition before start
Memory Image Display	Delay before acquisitions 0 h 0 0 s 0
Preferences	Metadata
	Csv Delimiter ;
	Warnings
	✓ Display time interval warning
	Not enough space on disk as warning
	Default Data processor
	✓ Default Process Raw data ✓
	✓ Default Tiling Process Tiling
	Tiff File
	Tiff Compression Raw 🗸

1.1.4. Configuration

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

TINSCOPER Diffile Settings	? Support						(User Mode	- • ×
Configuration								Project 2022-09-	-05
Camera Settings C]^ [⊂] [Calibration	Tiling	С					
Exposure (ms) 100		Go to Light Path	Axis Micro	scope Filters	pE-800 fura	Shutters			D
Threshold	в	Light Path			~				
Contrast Max: 6985		Axis		X-Axis 0		$\langle \rangle$			
17.12 pages				Step (um) 1					
				Focus 0		÷			
<u>.</u>				Step (um) 1					
		Channel			Not say	ved 🗸	Add		
	<u>s</u>	Microscope	TL Lamp	0	÷	Cube	SpX-Q	~	
(inclust color preset)			Switch	FLUO					
(1023,682) = 400		Filters	Lambda A		~	✓ Lambda C		~	
X-Axis : 0.00um Y-Axis : 0.00um		pE-800 fura	✓ 340nm			✓ 380nm			
FPS : 9.95	Chart Crop Image							Go To Ac	quisition

The screen can be divided into 4 parts:

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

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

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

1.1.4.1. Process images

1.1.4.1.1. Camera settings

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

Configuration						
Camera Settings	G	8phases		~ 1	L	
Exposure (ms)			100		^	2
Binning			1x1		~	
Advanced 3				Snap	Live	

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

a Preset Create Preset Remove	Advanced Camera Settings	
Show Read-only properties		
Property	Value	
	1x1 V	0
Hamamatsu_Fusion-BIT PER CHANNEL	16	
Hamamatsu_Fusion-BUFFER FRAMEBYTES		
Hamamatsu_Fusion-BUFFER PIXEL TYPE		
Hamamatsu_Fusion-BUFFER ROWBYTES		
Hamamatsu_Fusion-BUFFER TOP OFFSET BYTES		
Hamamatsu_Fusion-BufferMode	LocalBuffer 🗸	
Hamamatsu_Fusion-Bus		

- **b.** Select the parameters which will be saved in the camera preset by checking boxes. You can filter the parameters by writing in the search bar.
- c. Then Confirm:

er crop		
b Property		
Hamamatsu_Fusion-SoftwareCrop_Enabled 1		
Hamamatsu_Fusion-SoftwareCrop_Enabled 2		
Hamamatsu_Fusion-SoftwareCrop_Height 1	0.5564	
Hamamatsu_Fusion-SoftwareCrop_Height 2	0	
Hamamatsu_Fusion-SoftwareCrop_Width 1	0.7135	
Hamamatsu_Fusion-SoftwareCrop_Width 2	0	
Hamamatsu_Fusion-SoftwareCrop_X 1	0.1081	
Hamamatsu_Fusion-SoftwareCrop_X 2	0	

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



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

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



1.1.4.1.2. Interaction with the live image



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

1.1.4.1.2.1. Tools to interact with the image

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

NB: Some LUT are presetted like:

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

1.1.4.1.2.2. ROI creation



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

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

Then, use the shape tools to draw desired shapes:

	Draw a straight line.
୰	Draw a freehand line.
	Draw the edge of a rectangle.
	Draw a filled rectangle.
Ο	Draw the edge of a circle.
	Draw a filled circle.
ß	Draw a free form edge.
8	Draw a filled free form.

1.1.4.1.2.3. ROI manipulation



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

1.1.4.2. User/Expert display modes

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



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

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

1.1.4.3. Control of motorized devices

1.1.4.3.1. Interaction with motorized devices

Light Path	Objective	~ A
Axis	X-Axis	0 • B
	< XY 🕨 Y-Axis	
	▼ Step (um	n) 1
	Focus	0 C
	▼ Step (um	n) 1

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

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

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

1.1.4.3.2. Create/Load a channel

				8	}			<u>7a</u>	_				7b
6	Channel	470nm	_Cyan		470nm_Cyar	ı `	~	Add		Remove	Loa	d	Save
	Spectra X	1 2	Cyan Cyan Cyan Cyan	On/Off nsity]	Teal On/Off	Blue Dn/Off Blue ntensity				
			 ✓ Intensity ✓ Green On/Off ☐ Green Intensity]	Red On/ Red On/ Red Intensity	, Off (),				
	Nikon Ti2	5	✓ TL Shutter			^	3	✓ IL Shutte✓ CubeFilt	er 🚺	AD Lumencor	~		
		4	✔ LightPath	Cam Le	eft	~							

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

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

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

1.1.4.4. Calibration protocols

1.1.4.4.1. Tiling calibration

I

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

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

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



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

Automatic

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

Manual

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



1

- NB: First, be sure that the camera is switched on Live.
- 1. Use the virtual joystick to move the X and Y and observe whether the joystick and the camera have the same orientation.
- 2. Adjust the step if necessary.
- 3. If the orientation is not the same, you can add a rotation.
- 4. You can add a mirror effect on the image if necessary.
- 5. Click on Confirm.

Once the calibration finished, the Tiling button turns green.

1.1.5. Acquisition

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

TINSCOPER 🕒 File 🏟 Settings	⑦ Support	User Mode – C X
Configuration Acquisition		lame Project 2022-09-06
Camera Settings C	Sequence 1	Config Add Show All
Exposure (ms) 100	⊘ 1. Time	● 0 / 0
Contrast Max: 6985	 2. Tilling Switch to Positions 	● 0 🖉 🕁
195.79 pixels	S. Z-Stack	● o 🖋 🕂
Con Anton and	 ✓ ✓	● 0 💉 🕂
	2 Data Processing and Charts Call Parameters	4
	Save Acquisition In RAM On Disk Save as BigT Save as BigT	Total images 0 Total size 08 Minimal duration 00:00min:00:00ms Start Acquisition
FPS: 9.97	t Chart Crop Image	

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

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

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

1.1.5.1. Dimensions

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



1.1.5.1.1. Time

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

	1. Time		• 15
1	Number of Time Points	15	
2	Interval	0 h 0 min 5 s 0 ms	Burst Model 4
3	Total Time	0 h 1 min 15 s 0 ms	
			Confirm

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

1.1.5.1.2. Multichannels

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

Image: Ward of the system I	
1 Blue_395 ∨ 0 ↓ Hamamat ∨ 25 ⊗ 2 Cyan. 470 ∨ ✓ 0 ↓ Hamamat ∨ 25 ⊗	
2 Cyan_470 🗸 🔽 0 🔂 🗖 Hamamat 🗙 25 🕅	
5	
Солбд	

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

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

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

1.1.5.1.3. Positions

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

			2.	Pc	ositions								•				
	1	A	<pre> </pre> </th <th></th> <th>xAxis (µm) yAxis (µm) Step (µm) Focus Step (µm)</th> <th>10000 2000 1 3000 1</th> <th></th> <th></th> <th></th> <th></th> <th></th> <th>3 5 Move</th> <th>2 4 Pa Path .</th> <th>We ttern de Add posit / Fast</th> <th>ell Plate etection</th> <th>Set Start Lemove all</th> <th></th>		xAxis (µm) yAxis (µm) Step (µm) Focus Step (µm)	10000 2000 1 3000 1						3 5 Move	2 4 Pa Path .	We ttern de Add posit / Fast	ell Plate etection	Set Start Lemove all	
6	N	• 🏟	7		Тад	хАх	is	уАх	is	V Foc	us	Move to	Сору	Se	t		
						1000	×	2000	~	3000	^		Сору	Set		\otimes	F
						20000	< >	3000	^	3000	×		Сору	Set		\otimes	
l										< 1 >							
																8	onfirm

To use the **Positions** dimension:

- 1. Move the stage and the focus to the desired position.
- 2. Click on Add position to add the XYZ coordinates in the position list.
- 3. Click on the **Path** button if you need to optimize the **stage mouvement** when you have multiple positions.
 - **a.** The stage moves following the order in which positions were added. You can view the complete movement path and calculate the total distance traveled. To optimize the path and reduce travel distance, click the **Sort** button.
 - **b.** Once optimization is complete, click the **Back** button to return to the position dimension view.



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

- a. Click the Start button located in front of the Pattern Detection section.
- **b.** Create a new tile to find the pattern. Click on **New tiling**. Set it up as described in the Tiling dimension.
- c. Select the pattern you wish to detect:
 - Current Live Image: Use the pattern from the live image
 - **ROI**: Draw an ROI around the specific pattern you want to detect.
- **d.** Indicate the **Correlation Threshold** (that mean the percentage of similarity between pattern and other form).
- e. Click on **Detect pattern** to start the detection.

Pattern Detection New Tiling 4b	<u>4d</u>	4e
Current live image		Detect pattern

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



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

2	2. Positions	Switch to Tiling					93	
	X Axis (μm)	26222.6					w	ell Plate Set
	γ ト Y Axis (μm)	10929.3	Z Axis (µ	m) 0			Pattern d	etection Start
	Step (µm)	0.001				Pa	th Add posi	ition Remove all
N° 🏚	Tag	X Axis	Y Axis	🗸 Z Axis	Move to	Сору	Set	
1						Сору	Set 🔯	\otimes
2		20216.6				Сору	Set 🔯	\otimes
3		16498.6				Сору	Set 🏚	\otimes
				< 1 >				
								Confirm

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

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 - i. You can modify the axis (X,Y and focus), apply offset on the axis for all positions or for the

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

	xAxis	ا بر	/Axis	Focus	
Old					
New 🔘	35275.8	-23663.6		5126.22	,
Offset 🔵					
Offset 🔵		-407.4 -407.4	;\$C) -0.06	



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

		\otimes
Current Group		
Modify Existing		
Position Min Index		
	Apply	
Position Max Index		

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

1.1.5.1.4. Tiling

1.1.5.1.4.1. Tiling creation

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

:	2. Tiling	Switch to Position	15				• 243
A	xA3 (µr yA3 (µr Ste	kis -20465.7 n) -5787.2 n) 0.1		Focus	Focus 484,14 Step (µm) 0.001	pfs	Well Plate Set FocusMap Start 2 Add tilling Remove all Move Strategy Fastest
		3					
N°	Тад	Position Count	Edit	4			
1				\otimes			
2							
						<.	5
							Confirm

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

1.1.5.1.4.2. Tiling edition

	2. Tiling swi	tch to Positions					0
	xAxis (µm) XY > yAxis (µm) Step (-4915.7 11884 (µm) 200	Focus Focus Step	s 484.94 (µm) 0.001	pfs (D	
1a	Туре	RECTANGLE 🗸	Diagonale 🗸				
		xAxis	yAxis	✓ Focus	✔ pfs		
	Point 1	-7678.7	11395.9	483.6	483.46	Get Goto	
	Point 2	-4915.6	11883.9	483.6	484.94	Get Goto	
1 b	Туре	RECTANGLE 🗸	Center 🗸]			5 Focus Map Edit
		xAxis	yAxis	✓ Focus	🖌 pfs		
	Center	-6297.15	11639.9	483.6	484.2	Get Goto	
	Width	2763.1					
	Height	488					
2	 Overlap (%) Auto 	10 ^					Show 🖌 Images
3	🔵 Custom Step (µm)	674.84	674.84				
4	Read Mode Snake Mode						
							6 Confirm Cancel

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

Type Point 1 Point 2 O Overlap (%)	RECTANGLE xAxis 0 1000 10	yAxis 0 v 1000 v	Focus 0 0 Cet 0 Cet 0 Cet	Soto
Туре	RECTANGLE 🗸	Diagonale 🗸	✓ Focus	Focus Map Edit
Type Point 1	RECTANGLE ~	Diagonale v yAxis 0	Focus	Focus Map Edit

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

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

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

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

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

1.1.5.1.4.3. Focus Map manual setup

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

	1 Focus Map Edit							
_								
Show ✔ Images								



To use the Focus Map:

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

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

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

1.1.5.1.4.4. Focus Map automated setup for simple and sultiple siling

The Automated Focus Map is a software optional feature of the Inscoper I.S. that automatically generates a relief map of the biological sample when performing tiling imaging. There are 2 types of the Automated Focus Map :

• Simple tiling;

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


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 - 2. The following parameters must be set for both simple and multiple tiling:
 - a. Choose the **step** and **size** of the stack.
 - b. Select an existing pattern or click Edit to create a new one.
 - c. If Edit is selected:
 - **Grid:** set number of images (horizontal and vertical), gap, offset X and Y. The offset X and Y allows you to move your pattern in the well.
 - Random: set number of positions and minimum distance between them.
 - d. Click Add to save the pattern.
 - e. Return to the Dimension via Switch to Tiling.
 - f. Select channel to acquire the stack.
 - g. Optional: Check Filter unsure position and set parameters:
 - Filter Radius: filter to smooth the noise by replacing each pixel by the median of the neighbor (0= no smoothing, 1= median on 3x3 square around the pixel, 2= median on 5x5 square around the pixel, ...).
 - BackgroundRoi: ROI for auto-calculating background values.
 - Background: Mean background value.
 - Background Delta: Noise amplitude.
 - Valid Pixels: Percentage needed to validate position.
 - 3. If using multiple tiling: select the tiling area for which you want to create a Focus Map
 - 4. Click on Start.
 - 5. Click **Confirm** to validate and proceed. You can continue to configure your acquisition sequence.

1.1.5.1.5. Multiwell plate mode

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

1.1.5.1.5.1. Multiwell plate feature

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



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

1.1.5.1.5.2. Multiwell plate calibration

2.	Tiling	Switch to Positions	• 0
	X-Axis		
≺ XY ►	Y-Axis Step (um)	10	D7 : select 3 points on the edge of the well
▲ Z	Focus	2586	
•	Step (um)	1	Cancel Confirm

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

- 1. Click on Find Center to start the semi-automated protocol.
- 2. Select the well to be used for well plate calibration.
- 3. Move manually the stage to this position if it was not previously done.
- **4.** Fit the edge of the well with the virtual cross by moving the xy stage on the live image and click on **Add**
- 5. Repeat twice on different sides of the well.
- 6. Click on **Confirm** to finish.

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

1.1.5.1.5.3. Multiwell plate mode in Positions dimension

TINSCOPER Project Settings (2) Support		User Mode – 🗇 🔿
Configuration MDA Complex		Project Name Project 2025-04-29
Comera Settings Tutal	1. Time 2. Positions Soluti to trop 3. Supported plats TVP 6 well plate V Extra FaceMan ⁴ 1 2 3 A O O O A A O O O A A O O O A A O O O O O A A O O O O O A A O O O O O O A A O O O O O O O O O O O O O O O O O O O	• • •
		Carrod Add Clear St Add
		• • 🖊
		• 1 🖍
	< <u> </u>	
IF5:20.2	Save Acquisition in RAM () on Disk Context Format Format	Total images 0 Total size 08 Minimal duration 00h00min00h050ms

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

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

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

You will find all your positions inside the dimension.

TINSCOPER Project Settings () Support		Uuer Mode - 🗇
Configuration MDA Complex		Project Name Project 2025-04-29
Camera Settings RUL RUL Camera Settings RUL		• 0 🖍
	A O O O O O O O O O O O O O O O O O O O	7 Gween Medde Cherr & Add
	G 3, 2-346K	• • •
	⊘ 5. Software AutoFreidus	• 1 🖌
	Call Processing and Cherls V Kell Presentation	
05.9.0 Est Cog Inigo	Sere Acquisition • In RAM O on Dik Tutper Acquise Status	Total images 0 Total size 08 Minimal duration Ob:00min0b:r50ms

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

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



1.1.5.1.5.4. Multiwell plate mode in Tiling dimension

TINSCOPER Project 🏟 Settings ③Support	🕒 ber Mode — 🗇 1
Configuration MDA Complex	Project Name Fright 2025-04-29
Camera Settings FULL	Sequence 1 Take Image 💽 Cooling 🔒 Add 🛛 Show Xil
Exposure (ms) 1x1 →	1, Time 🔹 0 🖌
Adverse Group Con	2. Trifing Suite's to Posson 0 ¹ Supported plate: TPP6 well plate > [44] Cateras [recenter] ⁴
	t t 2 3
	3, Z-Stack 🛛 1 🖊
	4, Mulü-Channels 🛛 🔍 🖊
	5, Software AutoFocus 🔍 0 1 🖌
	< <u> </u>
	Data Processing and Charts Control for Promotions
Edt. Crep Image	

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

1.1.5.1.5.5. Focus Map manual setup

1. Select the well to create the Focus Map.

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С			

- 2. Choose the position in the well where you want to make the focus and click on Add.
- 3. Repeat this process until you are ok with the focus of your well.

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



1.1.5.1.5.6. Focus Map automated setup

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

2. Tiling Switch to Positions	• 0
XY-Axis_X -2292 XY ► XY ► XY ► XY-Axis_Y -18940 ✓ Step (um) 1 Focus 4557 Z Step (um) I 1	Well Plate Set I FocusMap Start Add tiling Remove all Move Strategy Fastest

- | 1 Imaging solutions | 1.1 Inscoper I.S.
 - **1.** Click on **Focus Map** to configure it.

2. Tiling Switch to Positions	1 0
Supported plate TPP Tissue cul 🗸 Edit	Calibrate FocusMap
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	
	Origin 39657 -18964 Get Find Center
в () () () ()	Overlap (%) 0 Custom Dimensions
	Read Mode Width (um) 33900
	Snake Mode Height (um) 33900
	Continuous acquisition
	Cancel Add Clear & Add

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



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



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

	Auto FocusMap		
4	Step (um)	1	$\mathbf{\hat{\mathbf{v}}}$
	Stack Size	5	
5	Pattern	2x2_Gap800 🗸	Edit
	Channel	Blue_395_DA 🗸	
	Filter unsure po	sition	
	Filter radius		
	BackgroundRoi	~	Auto
	Background		
	Background Delta		
	Valid pixels (%)		
		Start	Cancel

- 6. Select the pattern you want: Grid or Random:
 - a. For Grid, indicate the number of image you want to acquire horizontally and vertically, and the Gap (distance between 2 images).
 The offset X and Y allows you to move all your patterns in the well.
 - **b.** If you choose a **Random**, indicate the position number and the minimal distance between positions.
- 7. Save your pattern by clicking on Add.
- 8. Come back to the dimension by clicking on Switch to Tiling.

				/	7	-	
	Existing Patterns	x2_Gap800	~ /	Add		Existing Patterns	✓ Add
6	Туре	Grid	~			Туре	Random 🗸
а	Horizontal Number	2		Ŷ	b	Position number	200
	Vertical Number	2		♠		Minimal Distance (um)	100
	Gap (um)	800		♠	, I		
	Offset X (um)	0		Â ▼			
	Offset Y (um)	0		Â ▼			
L							
8	Switch to Tiling						

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

11. Click on **Start**.

	Auto FocusMap		
	Step (um)	1	↓ ↓
	Stack Size	5	↓ ↓
	Pattern	2x2_Gap800 🗸	Edit
9	Channel	Blue_395_DA 🗸	
10	Filter unsure po	sition	
	Filter radius		
	BackgroundRoi	~	Auto
	Background		
	Background Delta		
	Valid pixels (%)		
		11 Start	Cancel

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

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



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



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

2. Tiling Switch to Positions	• 0
Supported plate TPP Tissue cul 🗸 Edit	Calibrate FocusMap
	● ★●
	Origin 39657 -18964 Get Find Center
	Overlap (%) 0 Custom Dimensions
	Read Mode Width (um) 33900
	Snake Mode Height (um) 33900
	Continuous acquisition 14
	Cancel Add Clear & Add

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



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

1.1.5.1.6. Z-stack

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

	3. Z-Stack		• 11
1	▲ Focus 2246.62 Focus (μm) 2246.62 ▼ Step (μm) 0.001		
2	Min/Max Plane	Center Plane 3	}
	Min (µm) 2236.68 Get	Center (µm) 2241.68 Get	Stack Step (µm) 1 Nyquist
	Мах (µm) 2246.68 Get	Volume (µm) 10	Stack Size 11
Λ	Bottom to Top	Center First	
1	C Top to Bottom		_
			5 Confirm

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

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

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

Nyquist Step Calcı	llator	\otimes
Wavelength (nm)	550	^
Numerical aperture	1.4	^
Refractive Index	1.51	^
		Confirm

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

1.1.5.1.7. Software autofocus

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

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

	5. Softw	are AutoFocus					Enabled	1
1	Focus Number	0	Step (µm)	0]2			
3	Channel	~	Camera	Hamamatsu_F 🗸	Exposure	100	4	
5	Autofocus Algo	Sharpness 🗸 🗸						
	Add condition							
				On every im	ages			
	Advanced Pa	rameters						
	Test AutoFocus							
								Confirm

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

5. Softv	vare AutoFocus	• 1	
Focus Number	0	Step (µm) 0	
Channel	~	Camera Hamamatsu_F 🗸 Exposure 100	
Autofocus Algo	Sharpness 🗸]	
6 Add condition			
Every 1	position	∽]change	8
Advanced P Test AutoFocus	arameters		
			Confirm

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

Focus Number Channel	~	Camera Hamamat	su_F 🗸 Exposure	e 100	
Autofocus Algo	Sharpness 🗸]			
Add condition					
Every 1	∫ ♥ position	∼]change			(
7 Advanced Pa	Do autofoc rameters Apply offse	et to	~ ~		
8 Test AutoFocus					
					9 Conf

1.1.5.1.8. Multi-sequence acquisition

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

an



Each sequence has several options for setting up:

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

- | 1 Imaging solutions | 1.1 Inscoper I.S.
 - 4. You can have a synthetic view of the different sequences already prepared.
 - 5. You can seamlessly navigate between sequences. The selected sequence is highlighted in orange.

In the summary tab below you have the following options:

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

1	Number of loop		Add Semience			Remove All
2	Intermittent Sequences	Time 🗸 🗸		4	5	6 7
	Sequence 1	Take Image 🦲	Time10			Copy 🗙
	Sequence 2	Take Image 🦲	Z-Stack1			Сору
	Sequence 3	Take Image 🦲	Time0 Positions0 Z-Stack1 Multi-Channels0			Сору
		3				

1.1.5.1.9. Data Processor

1.1.5.1.9.1. Interface description

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



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



- 1. Space for creating data processing workflow.
- 2. Data visualization customizing tools.

1

- 3. Tools for analyzing data after splitting by dimension.
- 4. Tools to customize the image visualization after pre-treatments:

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

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

1.1.5.1.9.2. Create a new data processing workflow

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



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

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



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

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

Data Processor Add Remove]					
Name NO_PROCESSOR]					
Type NO_PROCESS 🗸						
🗌 Temp. Data						
Confirm)					

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

NO_PROCESSOR	No effect.
SIMPLE_TILING	Position each image at its theoretical position on a global tiling.
STITCH_TILING	Stitch adjacent images of a tiling, taking into account the overlap (requires SIMPLE_TILING before).
STANDARD_DE- VIATION_ON_FLY	Calculates the standard deviation of the intensity pixel by pixel of an image stack.
SHADING_CORRECTION	Removes unwanted signals from the image (misalignment, dust in the optical system,) Given an image of the background, the fol- lowing calculation is made for each pixel: (current intensity of the pixel / initial intensity of the pixel on the background) x average in- tensity of the background.
FILTER	Noise reduction by removing anomalous pixels from an image (de- speckle). For each pixel, the intensity values of the surrounding pix- els (according to a radius value) are recovered. It is then possible to apply a median or average filter to each pixel from these values. The pixels with an intensity value lower than this calculated value are then ignored (value equal to 0) thanks to a median or average filter (the median filter is recommended).
TIME_MAX	Keep the maximum value for each pixel of a stack from the "Time" dimension.
FOCUS_MAX	Keep the maximum value for each pixel of a stack from the "Z-stack" dimension.
TIME_AVERAGE	Keep the average value for each pixel of a stack from the "Time" di- mension.
FOCUS_AVERAGE	Keep the average value for each pixel of a stack from the "Z-stack" dimension.
CHANNEL_MULTICOLOR	Merge images from different channels. The maximum intensity val- ues for each pixel are retrieved and a blend of LUTs is performed.
SUBTRACT_BACKGROUND	Removes background from an image to improve contrast.
CHANNEL_RATIO	Rationalize the intensity of a single pixel using several different channels. The "SUBSTRACT_BACKGROUND" is directly present in "CHANNEL_RATIO" to optimize the output.
MULTI_CHANNEL_MERGE	Merges channels. Mainly used for SPIM with two excitation beams. 59

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

1.1.5.1.9.3. Data visualization personnalization

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

1	HISTOGRAM	•	
	GLOBAL_MEAN_2	→	
	GLOBAL_STATISTIC_2		
	CONFOCAL_SPECTROGRAPHE		
	SIMPLE_SPECTROGRAPHE	Type GLOBAL_MEA 🗸	Deer barr
	PHASOR_PLOT	3 Processor RAW_DATA 🗸	Drop here
	DEVICE_VALUES	4 Advanced 5	
	Split by Time		
	Split by Positions	$ $ $ $ $ $ $ $	
	Split by Z		
	Split by Channels	•	
		Drop here	Drop here
	Switch to Dimensions		

To customize the data visualization window:

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

To customize the graphics:

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

1	Name	
	Туре	GLOBAL_MEA 🗸
2	X Axis	
	Y Axis	
3	Show Le	egend
	Processor	RAW_DATA 🗸
4	H Priority	ALWAYS 🗸
	V Priority	ALWAYS 🗸

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

1.1.5.1.10. Saving images and metadata

1.1.5.1.10.1. Interface presentation

1 in RAM Image: On Disk 2 Choose Directory 4 Image: Directory	Save Acquisition	
Choose Directory Format All in One	1 in RAM O on Disk	3
	2 Choose Directory	Format All in One 🗸
Save as BigTiff	4 Save as BigTiff	

To save the acquired images and metadata:

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

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

1.1.5.2. Data export

1.1.5.2.1. Video exportation

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

	Export vi	deo				\otimes
1	Path					
2	Frame rate	10				0%
3	Dimension	472	X 350	O Preser	ve ratio	
	GenericSta	ckImageAll	InOne Sta	ck first image		
4	Export					

To export a video:

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

1.1.5.2.2. Stack exportation

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

	Export im	nages			\otimes
1	Choose File				
2	Sequences	Sequence 1	~	0%	
3	Format	All in One	~		
	Save as Big	gTiff			
	Use Filters				
	Crop		~		
					4 Export

To export a stack:

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

1.1.5.2.3. Metadata access

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

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

1.1.5.3. Semi-automated feedback microscopy feature

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

	Me	etadata		4 Tag	
	5	Roi	6	Tag	7
		1		Neurons	
		2		Glial cells	
		3		Blood vessels	
ð	Add	as tiling			
9	Overlap Read Snak	d Mode			
Í	Add To S	equence	(New	/ Sequence) 🗸 🛛 A	dd

To use this feature:

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

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

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

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

1.1.6. Visualization

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1.1.6.1. Visualization during acquisition



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

1

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

1.1.6.2. Visualization after acquisition

1.1.6.2.1. Interface presentation



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

1.1.6.2.2. 3D viewer

TINSCOPER Project 🖨 Settings ⑦ Support	Uter Mode -	o ×
Configuration Acquisition Visualization V MDA Complex Project 2025 01: 20.3	Project Name 🔟 Project 2025-01-20 Open in Diploier	
a nov. c	✓ Filters Channel ● ● 1 · 2 Sin ● ■ Inte OCust 2.Stack ● ● 1 · 11 Sin ● ■ Inte OCust Selected Images RAW_DATA ● New Sequence 1 Total: 22 1/2 D-14 Export: Voice Stack I = 1 · 12 · 14 · 12 · 14 · 12 · 14 · 12 · 14 · 14	ł
 ✓ Fusion Huo Cyan ✓ ✓	< 12>	Â

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

ClearVolume advanc	ed parame	eters			
Custom Voxel Size	X (nm) 6	500			
	Y (nm)	500			
	Z (nm) 1	000	^		
Channel	Gan	nma	Quality	Dithering	Render Algo
405		•			MaxProjection 🗸
488		•		•	MaxProjection 🗸
561		•			MaxProjection 🗸
					MaxProjection
					IsoSurface

1.1.6.2.3. Interacting with filters



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

1.1.6.2.4. Image Processing

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

Image to Process	RAW_DATA	~]	\otimes
Process type	CHANNEL_M	~	Ĵ	
			C	Confirm

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

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

Here is an example with tiling:

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Image to Process	simple_tiling 🗸	
Process type	Stitching 🗸	
Correlation threshold (%)	70	
ZStack Reference		
Channel Reference	(No Reference) 🗸	
Invalid Tile	INTERPOLATE 🗸	
	HIDE	Confirm
	DEFAULT_OFFSET	Commit
	INTERPOLATE	

- | 1 Imaging solutions | 1.1 Inscoper I.S.
 - **1.** Select the image to process.
 - 2. Select the process type.
 - 3. Indicate the correlation threshold (%).
 - 4. Check if you want to apply ZStack reference and select the good plane to calculate the stitching.
 - 5. Select the Channel Reference if needed.
 - 6. Select the action to apply to the invalid tile:
 - HIDE: ignore this tile
 - **DEFAULT_OFFSET:** use the offset of this tile in the non stitched tiling
 - **INTERPOLATE:** create a linear model of all valid relative offsets, and use it to generate a valid offset for the tile
 - 7. Click on Confirm.

1.1.6.2.5. Interacting with graphics

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

It is possible to:

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



- | 1 Imaging solutions | 1.1 Inscoper I.S.
 - **1**. Enable XY zoom mode.
 - 2. Enable X zoom mode.
 - 3. Enable Y zoom mode.
 - 4. Zoom to origin and enables auto-ranging
 - 5. Modify the range of XY axes values for the graph visualization.
 - 6. Export all data to a .csv file.
 - 7. Show/hide curves legends
 - 8. Tool to add some markers to the graph (see below).

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

Chart Markers Add				Remove All
Name	Start	End	Color	
Start	00h00min00s000m	00h00min01s000m		
Agonist	00h04min50s000m	00h04min51s000m		
Inhibitor	00h06min30s000m	00h06min31s000m		
End	00h09min00s000m	00h09min01s000m		

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

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



1.2.1. Configuration & Calibration



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

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

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

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

1. Manual calibration

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

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



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

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

2. Automatic calibration

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- NB: It is important to ensure that the focus is good throughout the calibration process. The laser is most accurate when in focus. Do not hesitate to adjust the focus before the calibration to get the best result.
- a. Click on Live if it is not already done (then you see Stop).
- **b.** Use the virtual joystick to move the laser pointer to any of the virtual crosses on the screen.
- c. When the laser pointer is on the cross, click **Select**. Repeat steps a and b three times.
- **d.** A fully automated protocol will make a pattern on the image. You have to validate the regularity of this pattern and click on **Confirm**.

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



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

1.2.2. Acquisition

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

To use the **Photomanipulation** dimension:

1. Select the scanning order.

The ROI can be scanned one at a time (Sequential) or alternately (Alternate).

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



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

1.2.3. Acquisition in progress

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

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



1.2.4. Visualisation

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



| 1 - Imaging solutions | 1.3 - TIRF

1.3. TIRF

Total Internal Reflection Fluorescence

1.3.1. Calibration

TIN SCOPE	ER Projec	t 🏟 Settings	? Support		Lipert Mode 🔵 — 🗇 🗙
Configuration			2		Project Name Project 2024-05-28
Mr. 493	Contrast D Average: 104.39 Max (375.00			Calibration Go to Nikon Ti2 Nikon Ti2	FRAP Time scanFRAP ON/OFF SeanFRAP ON/OFF V Intensity V Intensity V TL Itshutter V V TL Itshutter V V TL V TL V U V U
				scanFRAP	Image: Weight of the second
	Add		5 Confirm Cancel	ON/OFF	♥ Tirf On/Off ● ♥ Tirf Angle -90 ♥ Tirf Radius ● ▲8
	Clear				Go To Acquisition

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

1.3.2. Configuration



- **1**. Activate or deactivate Tirf using the switcher.
- 2. Select Tirf Azimuthal (circle illumination) if you need.
- 3.

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

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

1.4. fastFLIM

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

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

-fastFLIM

1.4.1. Configuration



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

- 1. Gate Width: Width of the temporal gate.
- 2. Gate Voltage: Multichannel Plate (MCP) Voltage to amplify the input signal.
- **3. Delay:** Time in picoseconds which corresponds to the delay generated regarding the synchronization signal of the pulsed laser.
- 4. Frequency: Frequency of the delay generator.
- **5. Ecl Threshold:** Threshold to set the noise level on the input synchronization signal for the intensifier.
- **6. Polarity:** Parameter to be set to ensure that the intensifier detects the delay generator synchronization signal
- **7. Impedance:**Parameter to be set to ensure that the intensifier detects the delay generator synchronization signal
- 8. Trigger Threshold: Threshold to set the noise level on the input synchronization signal for the delay generator.
- **9. Trigger Feedback:** Parameter for checking the synchronisation between the delay generator and the pulsed laser. The value must be close to 0 in this interval [-100 +100]. (Specific to the old generation of delay generators. Not present on the new generation.
- **10. Polarity** -/+: Parameter to be set to ensure that the generator delay detects the laser synchronization signal.
- **11. Impedance Hi/50:** Parameter to be set to ensure that the generator delay detects the laser synchronization signal.

12. Output Status: Shutter.

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

1.4.1.1. Autodelay calibration

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



To calibrate the autodelay:

1. Click on Autodelay.

Calibration will start automatically.

2. Once a value has been found, click on **Confirm**.

The value is automatically transferred to the interface - Delay.

1.4.2. Acquisition

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

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

| 1 - Imaging solutions | 1.4 - fastFLIM

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

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

TINSCOPER CFile Settings (?) Support	(Uter Made - • ×
Configuration Acquisition Visualization MDA Complex Project 2023-02		Project Name Project 2023-02-27
Camera Settings C FLIM_SMALL_AREA V		Sequence 1 Take Image 🔵 Config Add Show All
Exposure (ms) 100 Binning 4x4		⊘ 1. Time Disabled● 0 🖌
Advanced Snap Live		⊘ 2. Tiling Switch to Pesitions 🕒 4 🖌
Contrast Μητ: 1/39/50 Ανκητές: 98/78 61 Μακ: 1/955.00 145.83 μm	✓ X	⊘ 3. Z-Stack ● 0 🖌
		🥥 4. Multi-Channels Live mcDenryCamera_FLIM
$\mathcal{H}(\mathcal{I})$		🧭 5. AutoFocus 📃 0 🧪
		6. FLIM • 5
$\prec \mathcal{H} \prec \mathcal{I}$	2	Switch to Advanced Mode
RJ V		Deita T Stack 5
		Contim
Edit Chart	Crop Image	/ .

• Advanced mode: if you have more than one FLIM channel during acquisition. In this mode you can set:

A. a specific Gate Delay and Delta T Size for each channel.

B. Select the channel on which you wish to perform the FLIM measurement by checking the **Do Flim** box.

	6. FLI	М			•	Var.
	Switch to Simple Mode		A			
B	Do Flim	Channel	Gate Delay	Delta T Size		
		GFP_FLIM	2000	5]	
	\checkmark	mCherry_FLIM	3000	5]	
						Confirm

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

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

	Data Processor Pa	arameters	\otimes
	FLIM	FIRST STACK	
	Threshold Min	3000 Dimension flim	~
	Threshold Max	65535 ^ Index 1	÷
1 2 Data Processing and Charts fastFLIM 3 Save Acquisition		Total images 24	Confirm
in RAM on Disk F∖Imagerie\fastflim Format All in One ✓ Sa	ive as BigTiff	Total size 2300kB Minimal duration 00h00min02:400ms 4 Start Acquisition	

1.4.3. Visualization

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

- A. FLIM image
- B. Intensity images used for the FLIM calculation
- C. Intensity decay
- D. Lifetime distribution



| 1 - Imaging solutions | 1.5 - liveRATIO

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

1.5. liveRATIO

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



1.5.1. Configuration

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

1.5.2. Acquisition

- 1. Wavelengths set up
 - a. In the Acquisition tab, go to Multichannels dimension.

4	. M	ulti-Channel	S Live 340	0nm 380nm	Stop		•	2	
Add	Chanr	nel						Remove	e All
	N٥	Name	Z Stack	Z Offset	Shu Bl	utter ink	Camera	Exposure (ms)	
-		340nm •	✓	0			PCO_Pan 🗸	100	
Intensity		10	^						
-		380nm •	~	0			PCO_Pan 🗸	100	
Intensity		10	^						
									Confirm

b. Select two or more wavelengths.

2. Data processing

- a. Select the Ratiometric imaging_Despeckle in the Data processing and Chart drop-down list.
- b. Click on the Edit Parameters to set and to optimize the ratiometric module.



- c. Select the ratio to measure during the experiment.
- d. Check if you want to use the module to remove the background noise.
- e. If checked, select a ROI without any signal.
- **f.** If checked, select the number of times the standard deviation will be considered for the calculation.
- g. Select the filter type to apply (median or average).
- h. Select the radius to consider for this filter.
- i. Click on Confirm.

Data Process	\otimes			
Ratiometric		FILTER		
Ratio	380nm / 🗸	Filter Type	Median	~
✓ Threshold	ROI 1 🗸	Radius	1	
Standard Dev.	2]	L	
			Con	firm

| 1 - Imaging solutions | 1.6 - MAICO I.S.

1.5.3. Visualization

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



1.6. MAICO I.S.

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

1.6.1. MAICO I.S.: before to start

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

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

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

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

1.6.2. Configuration

In the **configuration tab**, you have access to the laser and detector parameters. You can change the laser power and the gain of each detector. If you change the existing channel you can save it for your experiment by clicking on the **Add**. If you want to create a new channel or save your channel on the disk, you need to pass from **User Mode** to **Expert Mode** and click on **Save** (See here).

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



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

Configuration		
Camera Settings	Maico405 🗸 C	
Frame Averaging Averaging Frames 5 Advanced 4	 Maico405 Maico488 Maico561 Maico638 	

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

Advanced Camera Settings					
Preset Create Preset Remove					
Filter	C				
Show Read-only properties					
Property	Value				
Maico-Bus					
Maico-Camera Model	C15890-50				
Maico-COLORTYPE	B/W 🗸				
Maico-CONFOCAL SCANLINES	960 🗸				
Maico-CONFOCAL SCANMODE	SIMULTANEO 🗸				
Maico-CONFOCAL ZOOM					
Maico-DISTORTION CORRECTION	SEQUENTIAL				
Maico-Exposure	0.000008				

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

1.6.3. Acquisition

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

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

On the Multi-channels dimension:

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

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



1.6.4. Visualization

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

TINSCOPER I File Settings (?) Support	Expert Mode U ×
Configuration Acquisition Visualization V Project 2023-07-17	Project Name Project 2023-07-17 Open In Explorer
Common Common Marca 131 Marca 14400 5338 pm	✓ Filters > Channel ● 1 · 3 Si ● Inte Cus Image Processing Advanced
	 Charls 😍 Sequence 1 Total : 3 1/1 0-3 Export : Video Stack
	< 1>

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