INSCOPER

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scanFRAP

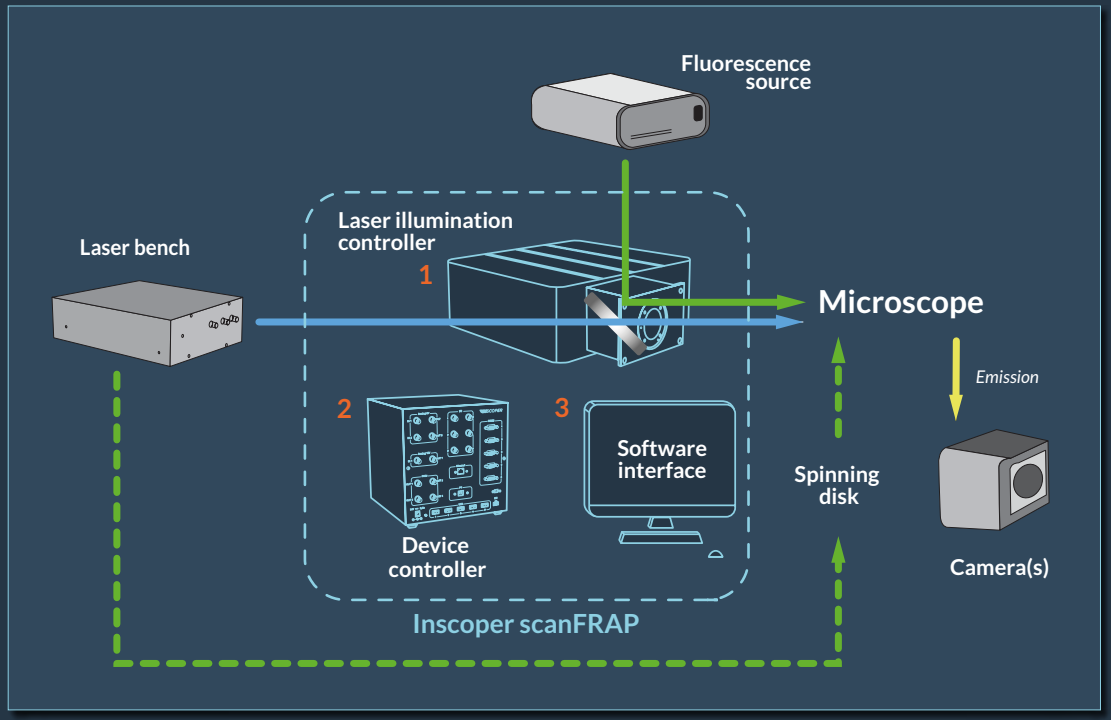
Solution for Photomanipulation & Optogenetics

ADDING AN ADVANCED IMAGING MODALITY TO THE MICROSCOPE ›

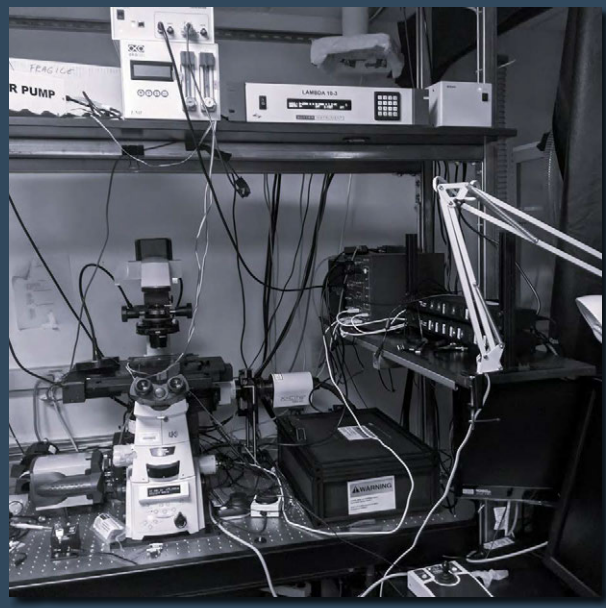
Inscoper scanFRAP is a turnkey solution for **FRAP** and **photomanipulation** applications, compatible with all brands of microscopes. It consists of (1) an optical module for controlling the laser illumination, (2) an electronic unit for triggering and synchronizing the microscope signals, and (3) complete image acquisition and processing software for operating all devices and functions of the microscope.

FOR LIVE CELL IMAGING

The Inscoper scanFRAP is developed for photomanipulation and optogenetics experiments in microscopy. Based on galvanometric mirror and optimized control technologies, this multi-purpose solution offers users capabilities for a wide range of *in vitro*, *ex vivo* and *in vivo* applications.
These applications can be combined within multidimensional acquisition sequences in the same software environment.



USER CASE @ BERKELEY UNIVERSITY



Originally designed as a TIRF system for PALM imaging, this microscope has been equipped with an additional photomanipulation module (FRAP). The two modalities are now combined in a user-friendly interface.

The system is used for rapid tracking of single molecules in living samples.

WHILE IMPROVING THE USER EXPERIENCE

TIME SYNCHRONIZATION

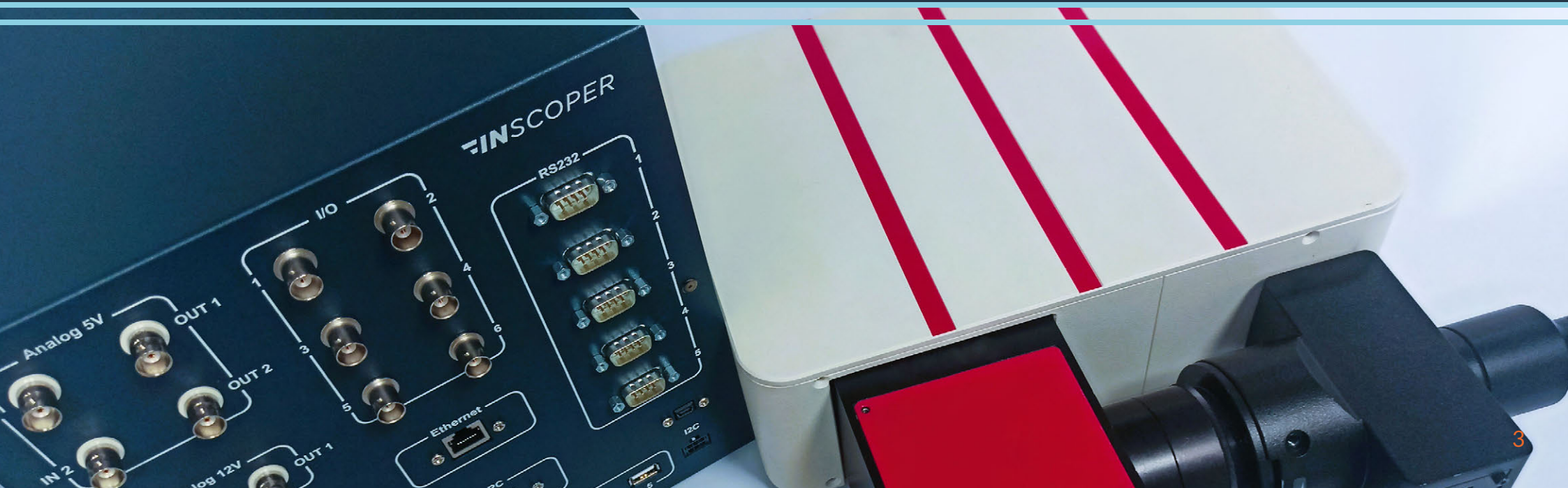
Accurate time measurement is often critical for FRAP experiments on living cells. The proprietary Inscoper control solution removes all software latency for maximum acquisition speed and perfect reproducibility between replicate sequences. It allows microsecond-accurate signal synchronization (laser illumination, camera exposure time, etc.). All the timings of the acquisition sequence are recorded in a *performance file* for further analysis.

HARDWARE COMPATIBILITY

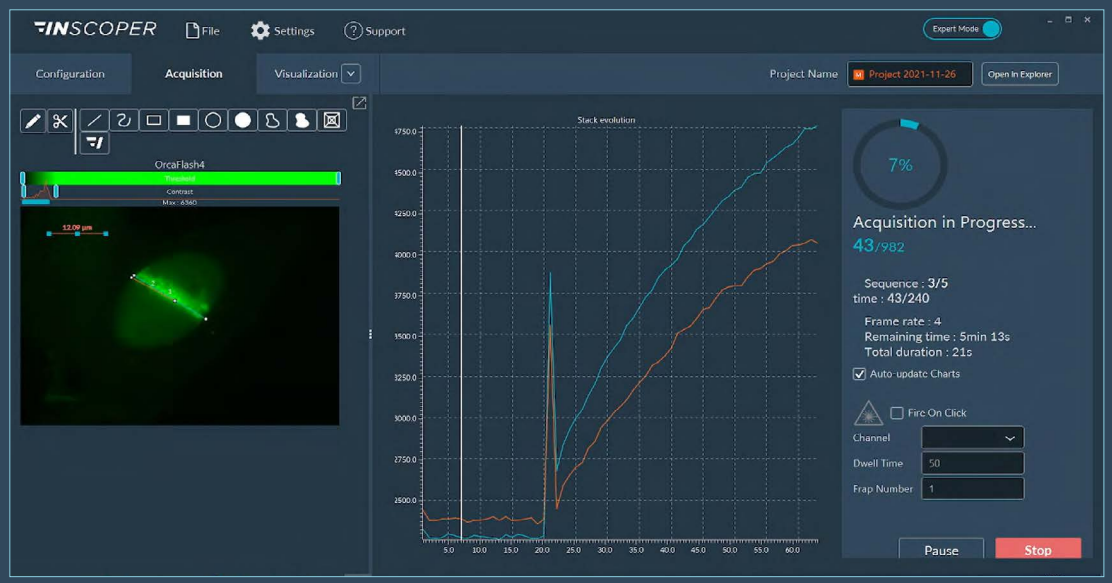
The scanFRAP solution is compatible with all camera-based microscopes from Leica, Nikon, and Zeiss, and with IX-81 and IX-70 series from Olympus. It comes with all the benefits of the Inscoper imaging solution for light microscopy, which includes the full control in the software of all third-party devices that equip the microscope stand: camera(s), spinning disk, X-Y stage and motorized Z-focus, light sources, etc., to offer great flexibility and simplicity in setting up advanced image acquisition sequences.

SUPPORT SERVICE

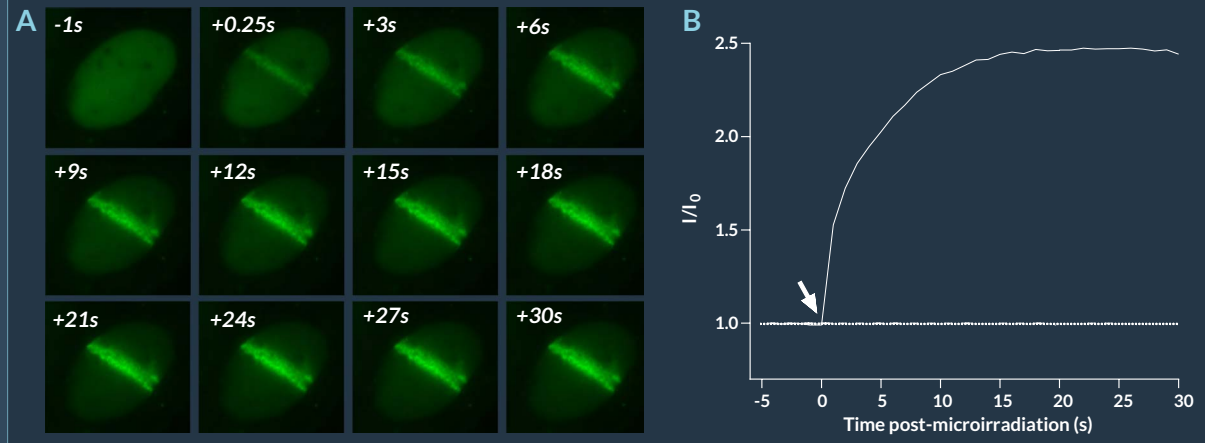
The INSCOPER staff likes to say that microscopes are like living organisms. Our engineers are here to make your microscope work properly, and to adapt it to the changes in equipment and user cases that might arise during its lifetime. We can frequently optimize the system rapidly using remote tools but we are available to work on your site when needed.



BIOLOGY APPLICATIONS ▸



Interface view of the Inscoper software during FRAP acquisition. Acquisitions are real-time monitored with images or graphics. The "Fire-on-Click" can be activated at any moment to add a new ROI to photobleach while the acquisition is running.



ALC1 recruitment to DNA damages following laser micro-irradiation in living cells

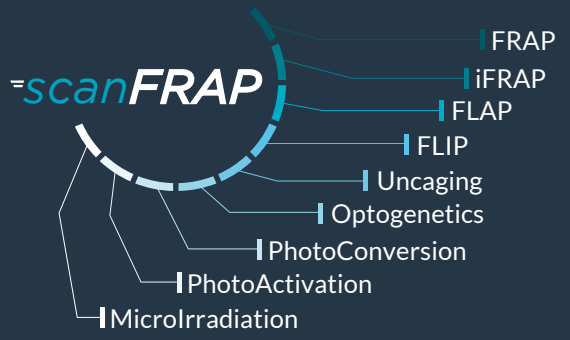
(A) U2OS cells were transiently transfected with an ALC1-GFP plasmid. ALC1 protein is mainly involved in DNA repair following damages. A progressive recruitment of the GFP-tagged ALC1 protein on DNA lesions was induced by laser micro-irradiation using Inscoper scanFRAP solution. (B) This phenomenon can easily be quantified with the software. The arrow indicates the start of protein recruitement following laser damage.

PHOTOMANIPULATION FOR BIOLOGY

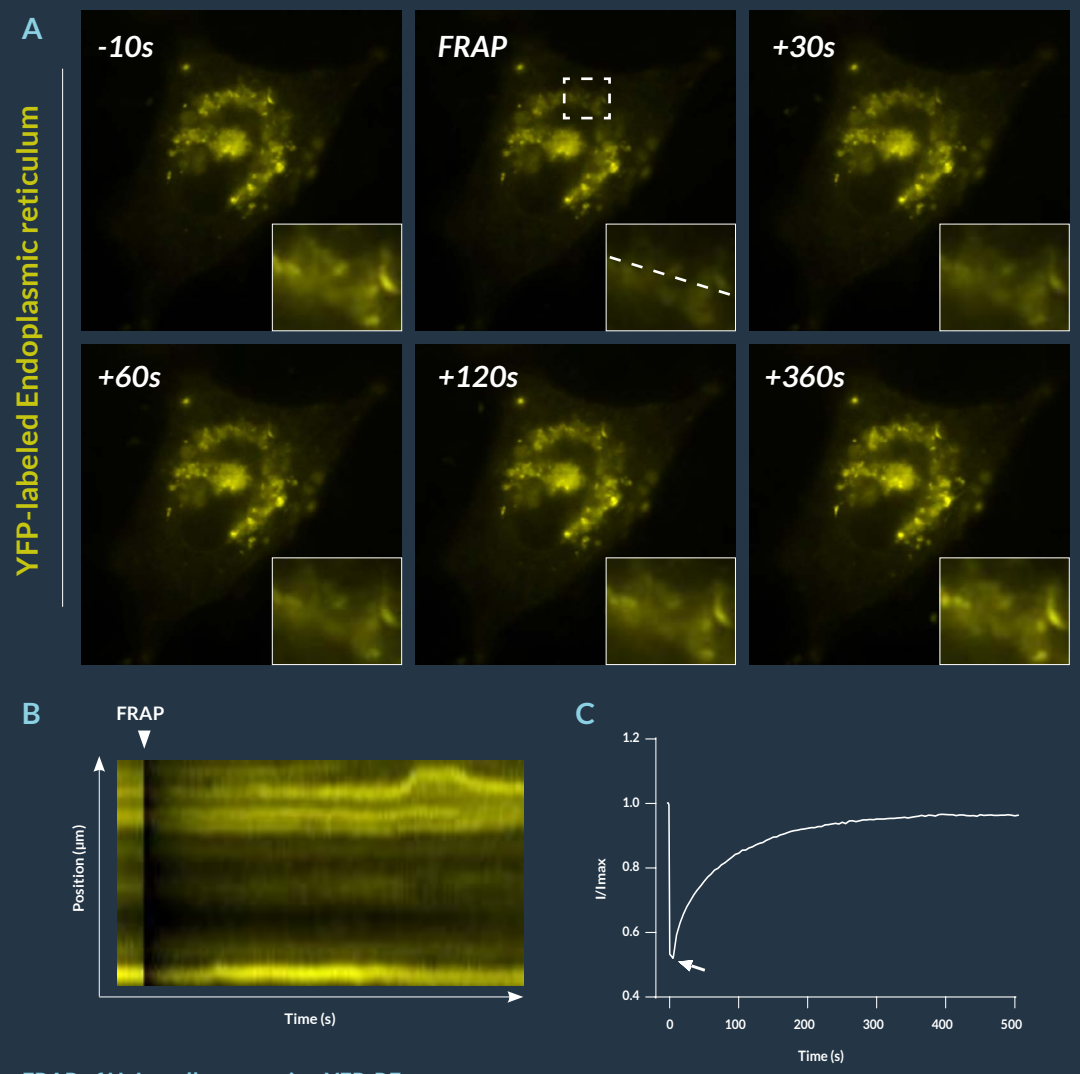
In microscopy, photomanipulation refers to a wide spectrum of techniques that allow biologists to easily interact with their living sample using a selected and specific illumination. These laser modulation techniques enable users to initiate/ modulate biological processes, to induce localized stress or to partially degrade a part of the specimen. One of the most common applications of photomanipulation in biology is Fluorescence Recovery After Photobleaching (FRAP). It enables the characterization of the diffusion process of fluorescent-labeled organelles or proteins. A selected part of a cell is selectively photobleached, then the recovery time of the fluorescence is measured.

A LARGE PANEL OF BIOLOGICAL APPLICATIONS

The Inscoper scanFRAP is a multi-application solution developed for high-performance photomanipulation and optogenetic experiments in microscopy. With hardware based on galvanometric mirror technology, the Inscoper scanFRAP offers users a large range of *in vitro*, *ex vivo* and *in vivo* applications such as iFRAP (inverse FRAP), FLIP (Fluorescence Loss In Photobleaching), FLAP (Fluorescence Localization After Photobleaching), photoactivation, photoconversion, uncaging, microirradiation and optogenetics.



BIOLOGY APPLICATIONS



FRAP of HeLa cells expression YFP-RE

(A) HeLa cells were transiently transfected with an ER-YFP plasmid to label the whole endoplasmic reticulum (ER). The photobleaching and a progressive fluorescence recovery was observed in a well defined ROI (dashed rectangle). This area is zoomed in the lower part of the figure. Dashed line is used for the following kymograph. (B) Evolution of the fluorescence intensity according to time in the dashed line (A). Photobleaching event is here tagged by the arrowhead. (C) This FRAP phenomenon can easily be quantified with the software and raw data can be exported in .csv file. The arrow symbolizes the photobleaching event.

CUSTOMIZABLE SOLUTION TO FIT YOUR EXPERIMENTAL DESIGN

With the same user-friendly interface as the Inscoper Imaging Solution, biologists can "draw" the region of interest (ROI) to bleach using conventional (line, circle, square) or custom-made (freehand drawing or new patterns) tools. The Inscoper scanFRAP also has a "Fire-on-Click" feature, that allows users to add/remove/modify ROI to photobleach in real time during acquisitions. Following an automated calibration, the Inscoper scanFRAP enables the use of customized photomanipulation protocol for each ROI (wavelength, pulse time, pulse iteration, laser diameter, laser power, dwell time).

INSCOPER TECHNOLOGY FOR FAST IMAGING IN LIVE CELLS

Users also benefit from the performance of the Inscoper Imaging Solution for optimized control, which guarantees the highest possible acquisition rate. Experiments with INSCOPER technologies strictly synchronize the illumination time with the exposure time, thus limiting the phototoxicity in living samples. It also enables the user to image the evolution of the fastest biological phenomena following photomanipulation (protein recruitment, genesis and dynamics of intracellular organelles, cell-cell interaction, ...).

LIVE MONITORING OF FLUORESCENCE INTENSITY

Live analyses are performed to monitor the evolution of the fluorescence intensity in all ROIs simultaneously. Each acquisition leads to the generation of *.tif* images and a *.csv* file containing all raw data of the fluorescence intensity changes for each ROI. A *performance file* is also generated to archive the acquisition sequence and the chain of commands. Such a file can be used for metrology applications for example.

The Inscoper scanFRAP is an ideal solution to perform photomanipulation experiments on living samples, combining a fully customizable illumination beam and a high spatial/temporal resolution.

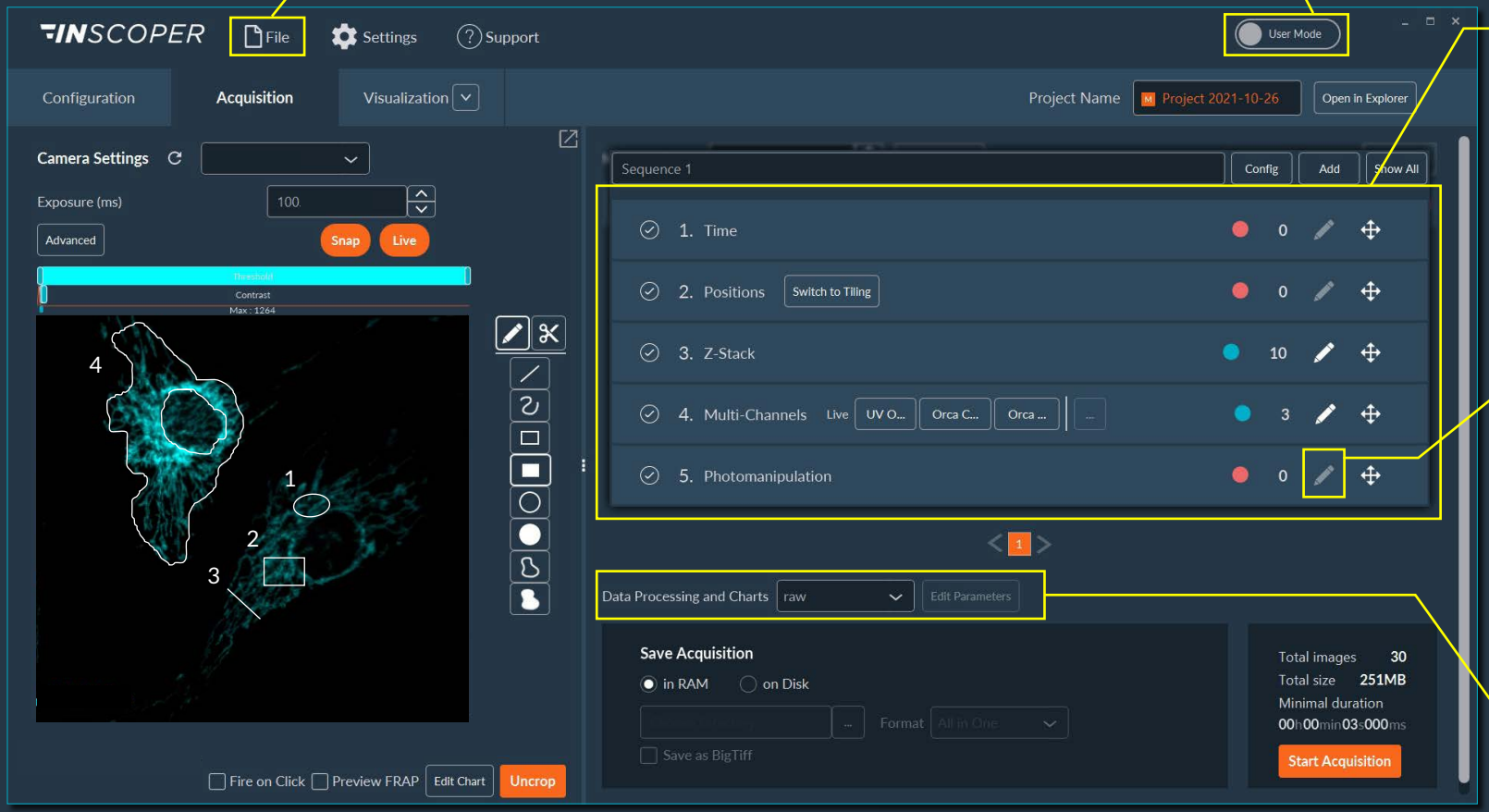
INTUITIVE SOFTWARE ENVIRONMENT FOR ALL MICROSCOPE USERS

The graphical user interface of Inscoper software has been designed to address all kinds of needs in microscopy acquisition, with the aim to always keep a simple and intuitive user experience. The interface has only one window, with 3 tabs corresponding to the 3 steps of the acquisition: Configuration, Acquisition, Visualization. There are no complicated drop-down menus, though all the features for advanced microscopy are there.

Storage & backup of user acquisition projects

Two user levels: “Expert” with access to all parameters, and “User” with limited access that can be customized

The photomanipulation protocols can be set from the acquisition tab as well as other dimensions



Illumination of as many ROIs as needed

Applying processing tools to visualize images or graphics in real time

SOFTWARE FEATURES

CONFIGURATION
AUTOMATE & CALIBRATE

- Access to all microscope settings and features, including third-party devices: filters, objectives, dichroic cubes, shutters, light sources (wavelength, intensity), cameras (exposure time, binning, crop, advanced settings)
- Simultaneous image acquisition on multiple cameras
- Customized design of channels that could be stored and reused
- Large panel of tools to create classic (line, square, circle) or personalized (hand-drawn) ROIs
- "ROI manager" to edit, duplicate or delete ROIs
- Software crop using a fully customizable tool
- Automated laser calibration for photomanipulation
- Advanced visualization of live images with possibility to zoom the sample in/out
- Automated stage calibration for tiling
- Manual and automated Look Up Table (LUT) to optimize live imaging
- Indicator of camera overload
- Virtual joystick to move according XYZ axes

ACQUISITION
MONITOR & REGISTER

- System of multi-sequences each consisting of several combinable and interchangeable dimensions: time, x-y position, tiling, z-stack, autofocus, channels (wavelengths)
- Photomanipulation: Selection of ROIs to photomanipulate according to a customizable protocol
  - Addition of a large panel of ROIs with geometrical forms or hand-drawn shapes
  - Opportunity to edit ROIs by removing areas inside ("donut-like" form to bleach the whole cell cytoplasm without altering nuclei for instance)
  - Setting of the laser for each ROI (pulse time, pulse iteration, ROI order)
  - Photomanipulation using different wavelength according to biological application needed

ACQUISITION - IN PROGRESS

- Real-time visualization of images and/or graphics during acquisition
- "Fire-on-click" mode to create/edit ROIs to photobleach while acquisition is running
- Creation/edition/deletion of ROIs during acquisition with automatic display update

VISUALIZATION
PROCESS & GET YOUR DATA

- Visualization of all raw images with dimensions filters
- "Play" option to replay the whole acquisition sequence according filters applied
- Processing of images using our algorithms (shading correction, tiling, merge channel, maximum projection, ...)
- Adding of new algorithms in our processing database (for expert users)
- Saving files according your preferences or using our saving module (in accordance with quality standards)
- Export of images to .TIF or .bigTIFF formats, graphics to .CSV files and videos to .AVI
- All Inscoper metadata are Bio-Formats compatible

NEW ACQUISITION FROM ACQUIRED IMAGES

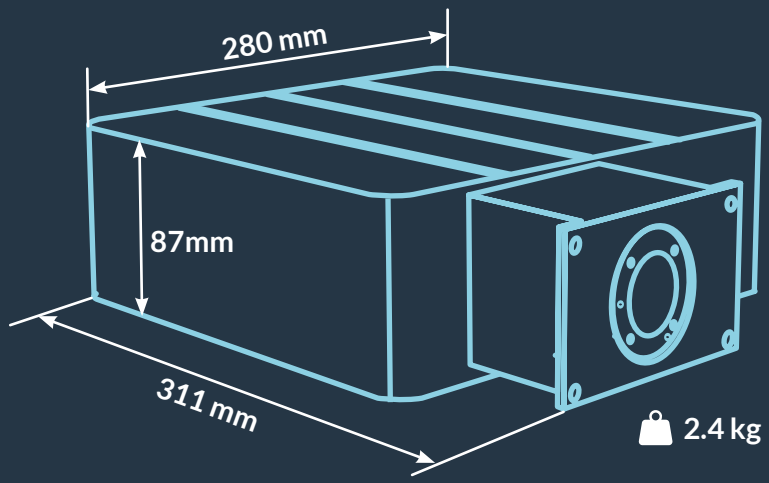
- "See in Live" mode to automatically center an element of interest on acquired image for later acquisition
- Large stitched image can be used to directly parameter new tilings from the visualization view

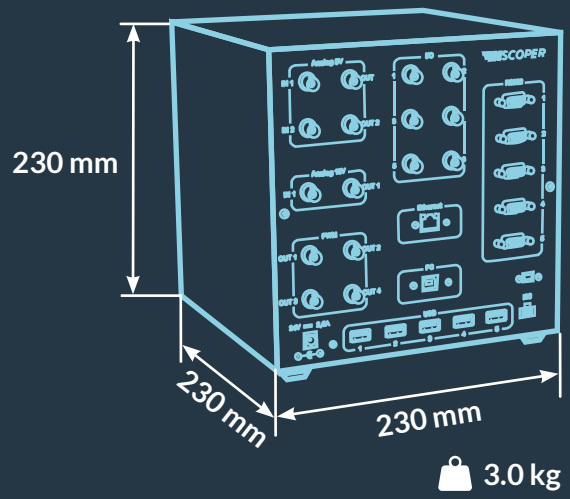
Specific scanFRAP features

TECHNICAL SPECIFICATIONS

LASER ILLUMINATION

Spectral bandwidth	355-750 nm
Optical connectivity	Single-mode fiber on the illumination port
Laser intensity modulation	0-100 % up to 6 lines, 12-bit resolution
Response time	≤ 1 ms
X-Y positions	Up to 100,000 points, resolution 0.5 µm per point
X-Y frequency scanning	45,000 points per second
Variable focus by electrical lens	-6 to +10 diopters, software control
Adjustable numerical aperture in back focus plane	ø1 to ø8 mm for depth of field adjustment, manual control (can be motorized)
Microscope compatibility	Leica DMI series Nikon TI series Olympus IX70 series and IX81 (excluding IX83) Zeiss Axiovert/AxioObserver series





MICROSCOPE CONTROL

Control inputs/outputs	4x analog 0-5V 12 bits 2x analog 0-12V 12 bits 4x PWM 6x TTL, expandable 5x USB A-type 1x USB B-type (PC) 1x Ethernet 5x RS232 D-type 1x I2C (auxiliary)
Recommended computer configuration	Windows 10 64 bits RAM 16 Go SSD 128 GB Two screens 1920 x 1080

CE FC

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GET A QUOTE,
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Provide us with the list of your system's devices and a short description of the application / manips that you carry out.

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