INSCOPER
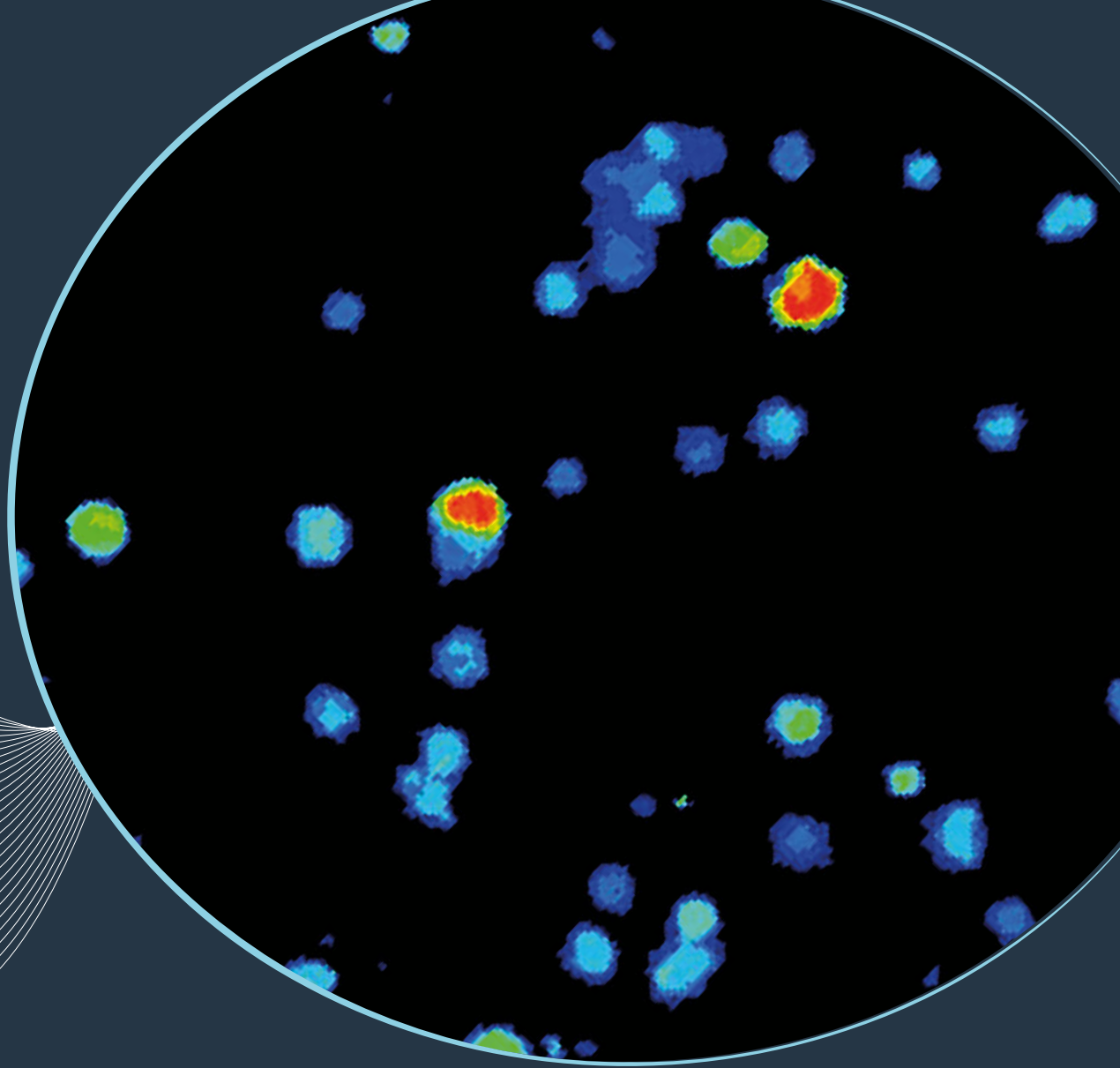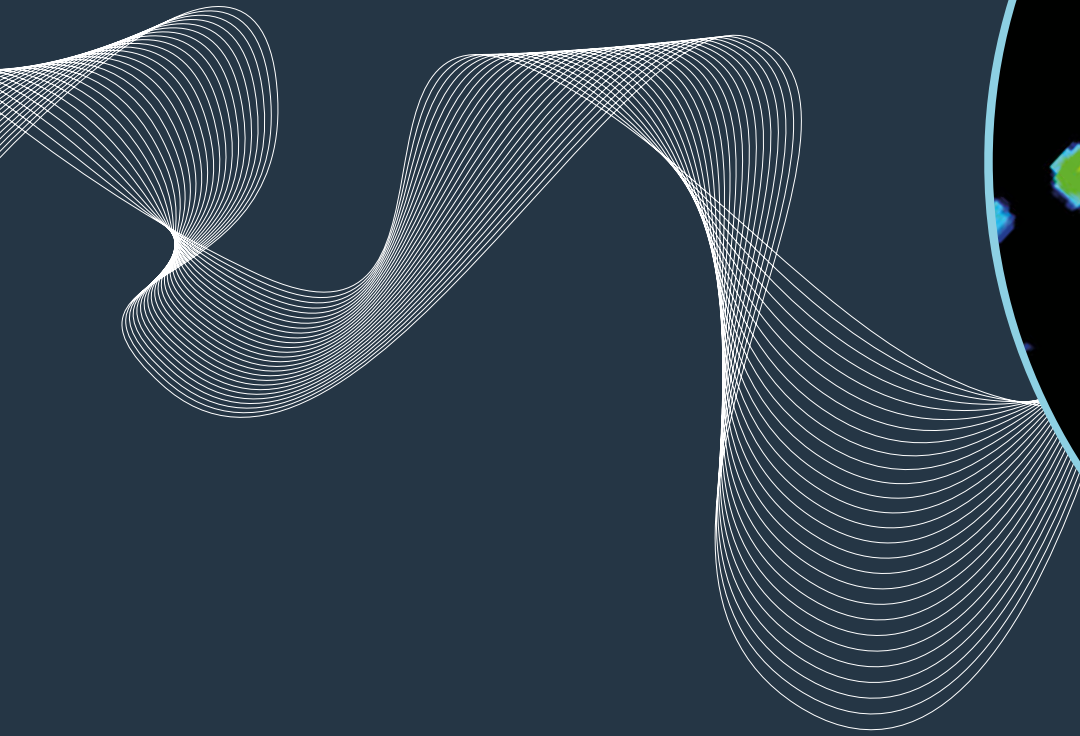

liveRATIO

Solution for real-time ratiometric imaging

THE NEW STATE-OF-THE-ART SOLUTION FOR FRET & RATIOMETRIC IMAGING ›

Inscoper liveRATIO is a comprehensive image acquisition and processing solution developed for ratiometric and FRET imaging. It consists of an electronic unit for triggering and synchronizing the microscope signals, and complete image acquisition and processing software for operating all devices and functions of the microscope.
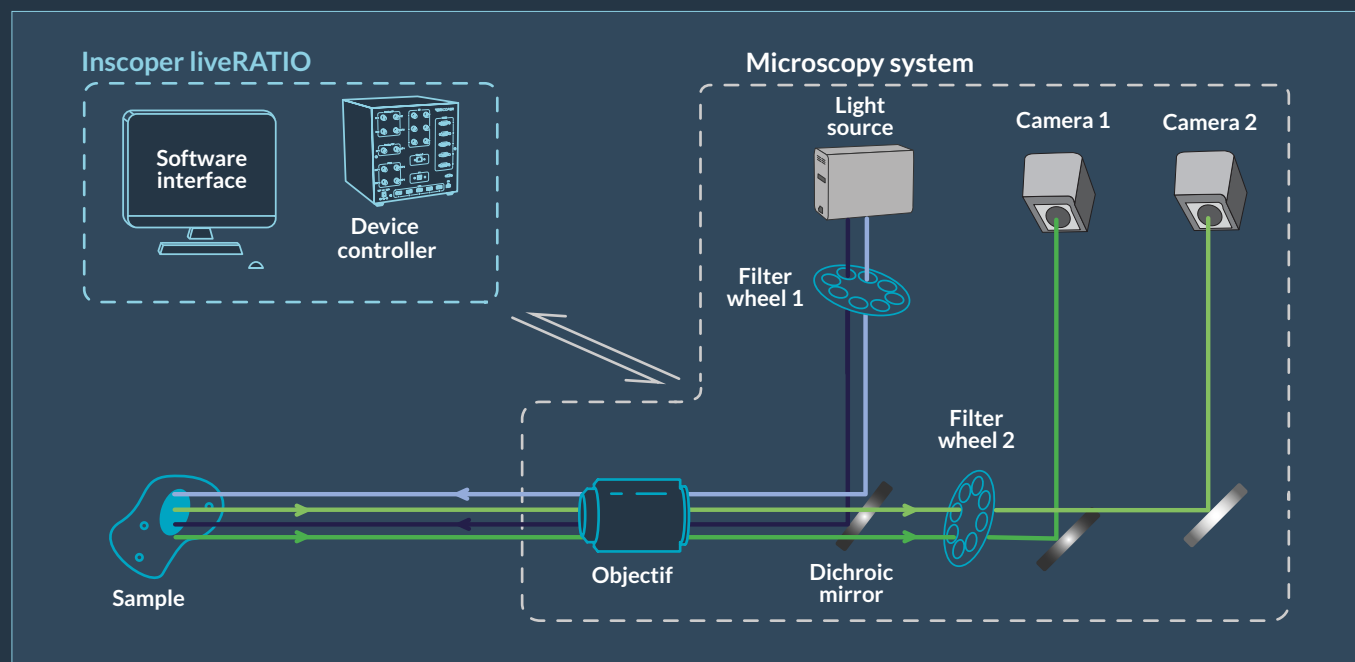
The solution is compatible with all microscope equipment that is commonly involved for ratiometric imaging: fluorescence illumination source, filter wheel, one or more camera detectors, etc.
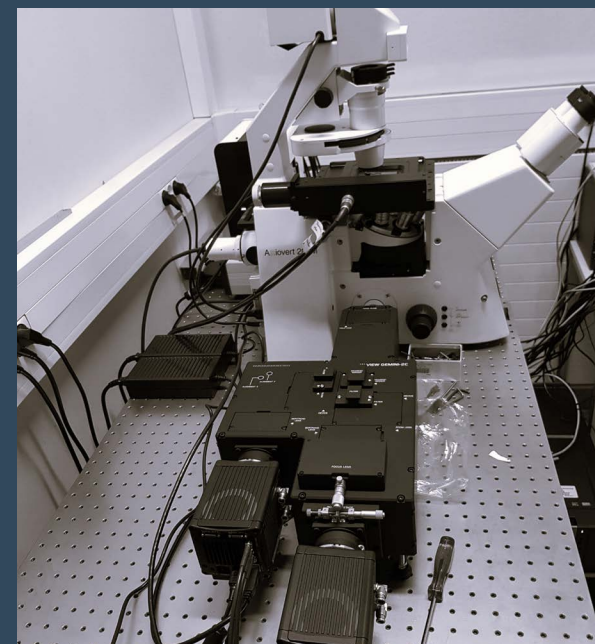
FOR RATIOMETRIC IMAGING

The liveRATIO solution is a variation of the Inscoper imaging solution for fluorescence microscopy that is specialized for ratiometric imaging and its multiple applications.

The software allows biologists to monitor the evolution of the fluorescence ratios in real time and to replay them afterwards, with an acquisition frame rate at the maximum of the microscope's capabilities thanks to the elimination of all software latency through our proprietary technology.

Ratiometric imaging can easily be combined within multidimensional acquisition sequences in the same software environment and even with microfluidics.

USER CASE @ GRENOBLE, FRANCE

The system is a shared microscope used for different purposes: quantitative FRET imaging at the cell scale, fluorescence imaging over large scales, or fast imaging of micro-organisms.

Different methods are used depending on the project. The Inscoper software enables the simple use of the microscope for its various purposes: alternating excitation schemes, fast imaging with hardware cropping, large field tiling.

Inscoper liveRATIO
Software interface
Device controller

Microscopy system
Light source
Camera 1
Camera 2
Filter wheel 1
Filter wheel 2
Objectif
Dichroic mirror
Sample

◂ COMBINING PERFECTLY WITH MULTIDIMENSIONAL MICROSCOPY ACQUISITIONS

LATENCY-FREE AUTOMATION

The liveRATIO solution is compatible with all camera-based microscopes from Leica, Nikon, Zeiss, and Olympus (IX-81 and IX-70 series). It allows a complete and latency-free control in the software of all the third party devices that equip the microscope stand: cameras from leading manufacturers such as Andor, Hamamatsu, PCO and Photometrics, microfluidic devices (pumps, temperature...), light sources and filter wheels, XYZ stages, etc., to offer great flexibility and simplicity in setting up multidimensional image acquisition sequences combining ratiometric and/or microfluidics modalities.

REAL TIME IMAGING

Perfect synchronization of the microscope trigger signals and the camera exposure time as allowed by INSCOPER technology is a key advantage for imaging fast biological events such as calcium flux or protein-protein interactions.

The solution also allows biology users to interact in real time with the sample for analysis purposes. The convenient management of the image background and the LUTs for colorization help the output of directly usable images.

SUPPORT SERVICE

The INSCOPER staff likes to say that microscopes are like living organisms. Our engineers are here to make your microscope work properly, to adapt it to the changes in equipment (including computer and Windows OS version) and user issues 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.

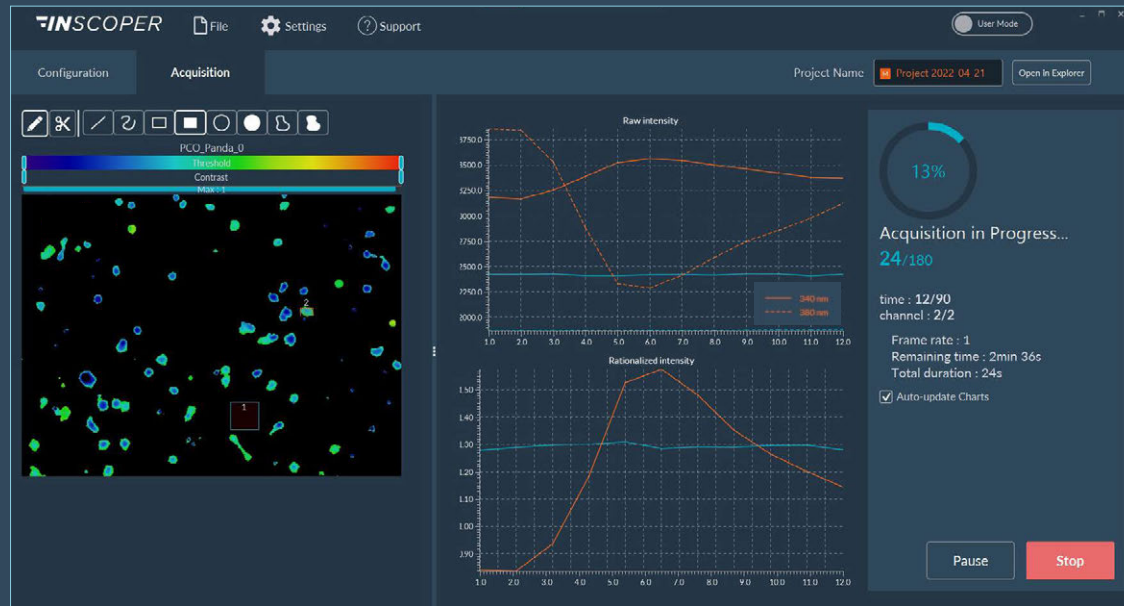*Figure 1: Inscoper liveRATIO user interface during the acquisition*

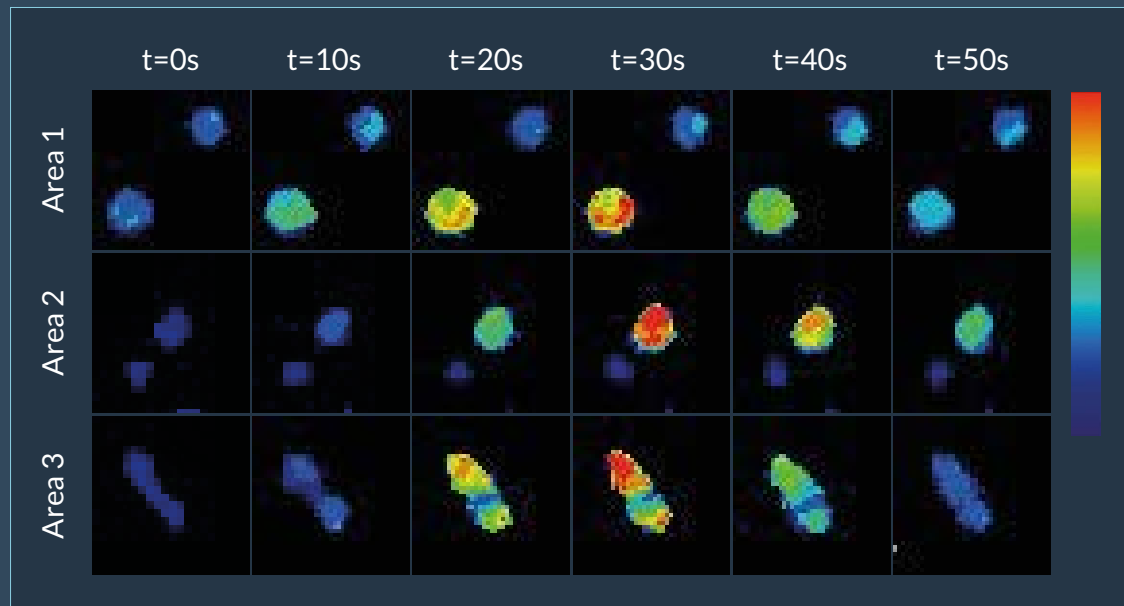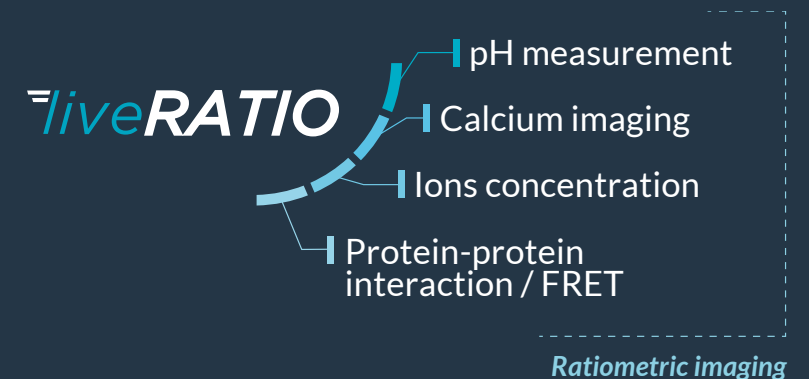## RATIOMETRIC IMAGING FOR A LARGE PANEL OF BIOLOGICAL APPLICATIONS

In microscopy, ratiometric imaging is based on the calculation of a ratio between (two or more) fluorescence intensities. This technique is used for sensitive measurements on living cells to prevent artifacts due to bleaching, modification of the laser intensity or variation of the fluorescent probe concentration. To obtain this ratio, we most often use either two excitation lasers (dual excitation indicator) or two detection ranges (dual emission indicators). More illumination modalities could be combined with this live imaging approach.

Inscoper liveRATIO is a multi-application solution developed for ratiometric imaging. It offers to users a large panel of in vitro applications such as FRET (Förster Resonance Energy Transfer) for protein-protein interaction, calcium imaging, ions concentration measurement (Mg2+, K+, Na+, Cl-,...) and intracellular pH characterization.

Figure 2: Ratiometric imaging of activated T cells labelled with Fura-2

pH measurement

Calcium imaging

Ions concentration

Protein-protein interaction / FRET

Ratiometric imaging

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Figure 3: T cells activation monitored by ratiometric imaging with the Inscoper liveRATIO

(A) Representative images of γδ T cells labeled with Fura-2 with DMSO or phospho-antigen (PA) stimulation. Surfaces plots are extracted from each dashed square. Scale bar = 100μm.

(B) Kymograph representing the evolution of the fluorescence intensity as a function of time. The black arrowhead represents the addition of DMSO or PA in the medium.

(C) Calcium fluctuation induced by DMSO or phospho-antigen on γδ T lymphocytes labeled with Fura-2. The black arrowhead represents the addition of DMSO or PA in the medium. Data are expressed according to the mean ± SEM.

CUSTOMIZABLE SOLUTION TO SUITE WITH YOUR EXPERIMENTAL DESIGN

The liveRATIO solution provides all the tools needed for ratiometric imaging. First, biologists can select ROI (regions of interest) to analyze their sample using the user-friendly software interface. Users also benefit from the performance of the Inscoper technology for optimized control, which guarantees the highest possible acquisition rate.

Experiments with Inscoper products strictly synchronize the illumination time with the exposure time, thus limiting the phototoxicity development in living samples. It even enables imaging the evolution of the fastest biological phenomena. Image visualization can also be improved using a dedicated LUT for ratiometric imaging and the background subtraction feature while the sequence is still running.

LIVE MONITORING OF FLUORESCENCE INTENSITY MODIFICATION

Real time analyses of all ROI allow researchers to visualize the evolution of their experiment. 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 liveRATIO is a modern and versatile solution to perform ratiometric imaging experiments on living samples with high spatiotemporal resolution.
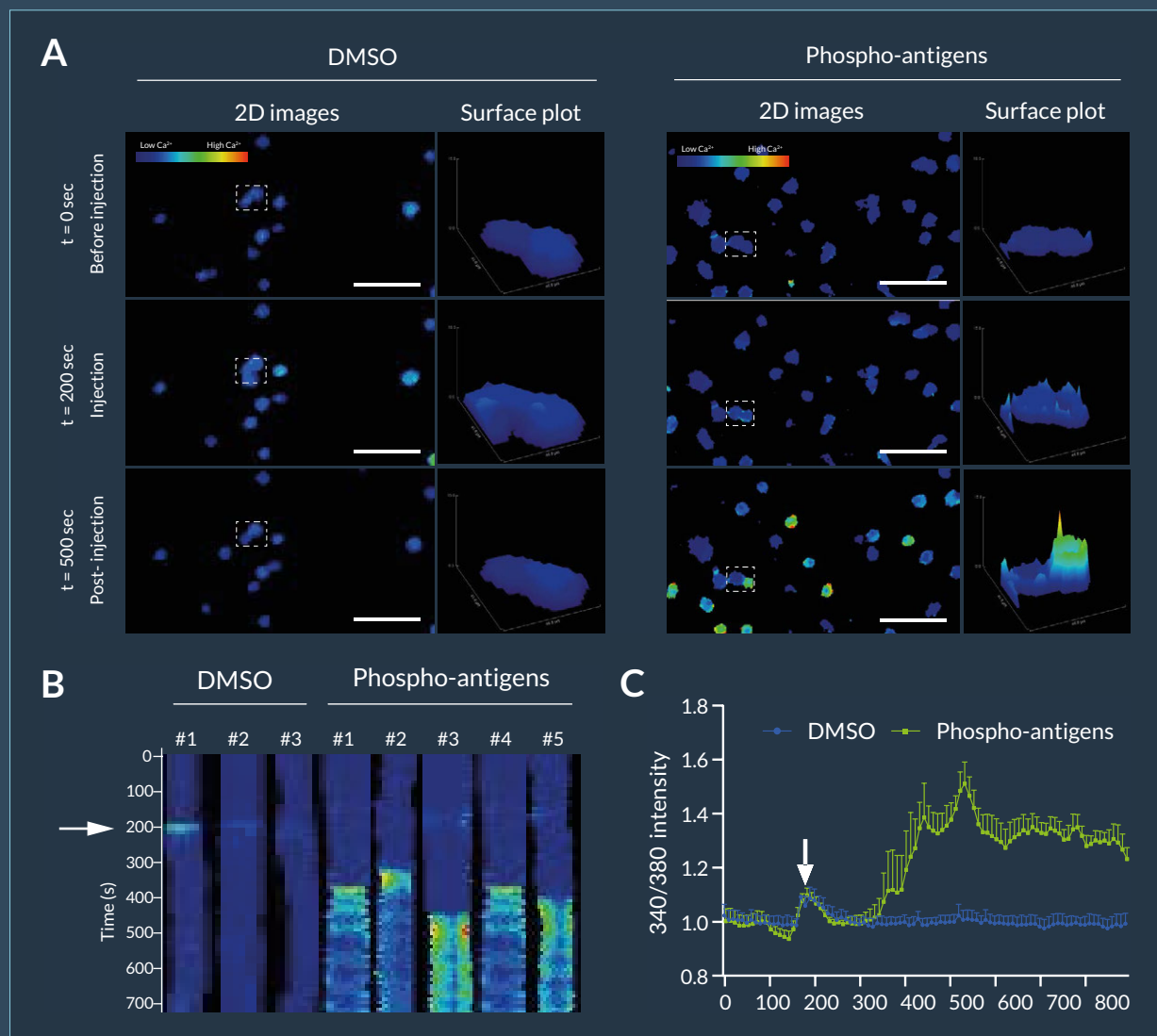
INTUITIVE SOFTWARE ENVIRONMENT FOR ALL MICROSCOPE USERS

The graphical user interface of Inscoper software has been designed to address all kinds of needs in microscopy acquisition, while always maintaining 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, and Visualization. There are no complicated drop-down menus, though all the features for advanced microscopy are there.

Replay of the sequence

Real-time image processing

Preview & export of images and videos

Visualization of raw and rationalized fluorescent signal in selected ROI

Possibility to substract the background

Customized LUT for ratiometric imaging

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)
- Virtual joystick to move according XYZ-axis
- Simultaneous image acquisition on multiple cameras
- Software with two accreditations level: experts (no limitation) and users (restricted access to configuration settings)
- Customized design of channels that could be stored and reused
- Large panels of tools to create ROI from classic (point, line, square, circle) to personalized (hand-drawn) forms
- "ROI manager" option to edit or delete ROI
- Software crop using a fully customizable tool
- Automated stage calibration for tiling
- Advanced visualization of live images with possibility to zoom in/out the sample
- Manual and automated contrast adjustment to optimize live imaging
- Specially designed Lookup Table (LUT) for ratiometric imaging
- Indicator of camera overload

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)
- Personalization of the dimension order
- Sequences creation that could be duplicated, with order changed or repeated using loop
- Customization with our "Data Processing" tool
  - Selection of the ratio needed with channels from the "multi-channels" dimension
  - Simultaneous measurement of different ratios during a single acquisition
  - Real time background subtraction

ACQUISITION - IN PROGRESS

- Real-time visualization of images and/or graphics during acquisition
- Live view of the tiling stitching during acquisition
- Creation/Edition/deletion of ROIs synchronized with an graphics auto-update when the acquisition is in progress
- Real-time visualization in graphs of the evolution of fluorescence signals for each channel, and rationalized values

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 algorithms (ratiometric calculation, 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

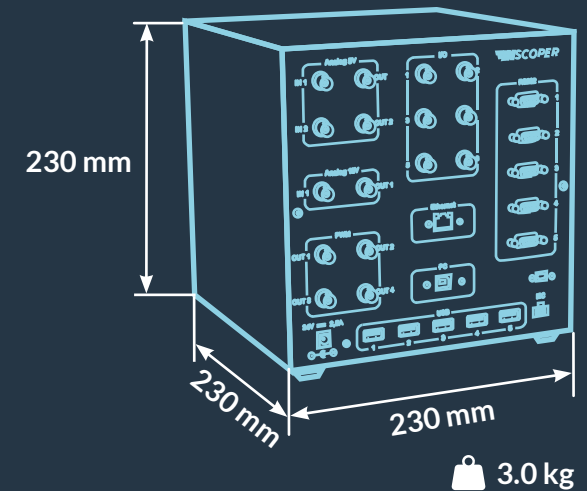NEW ACQUISITION FROM ACQUIRED IMAGES

- "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 liveRATIO features

DEVICE CONTROLLER SPECIFICATIONS

INPUTS / OUTPUTS

Analog 5 V	2x analog inputs & 2x outputs (0-5V) with resolution of 12 bits
Analog 12 V	1x analog input & 1x output (0-12V) with resolution of 12 bits
PWM	PWM 4x outputs which modulate devices that need duty cycle modulation with 5 V signals
Power	Supplied by 24 V-2.5 A
I/O	6x TTL ports that can be used as digital inputs or outputs (0-5 V)
Ethernet	Control devices via TCP/IP
PC	1x USB device, B-type
USB	5x USB Host ports, A-type
RS232	5x 9-pin female, D-type

230 mm

230 mm

230 mm

3.0 kg

RECOMMENDED COMPUTER CONFIGURATION

Operating system	▸ Windows 10 ▸ 64 bit
RAM	16 Go
Hard disk drive	128 GB SSD drive for fast image saving
Processor	Core i5 3.2 GHz
Screen	2 screens or 1 ultrawide screen

CE FC

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www.inscoper.com

GET A QUOTE,
REQUEST A DEMO,
ASK A QUESTION.

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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