INSCOPER

fastFLIM

Solution for Fluorescence Lifetime Imaging Microscopy

www.inscoper.com

THE EASY-TO-USE FLIM SOLUTION FOR LIVE CELL IMAGING

Inscoper fastFLIM is a turnkey solution with **real-time calculation of the lifetime image**, compatible with all brands of microscopes. It can be used with any type of illumination in camera detection such as wide field, spinning disk, TIRF, SPIM.

The fastFLIM solution consists of:

(1) an optical module that connects to the camera port of a conventional microscope body, composed of a high-rate imager (to generate time gates) and a scientific camera,

(2) a pulsed white laser with a wavelength selection system,

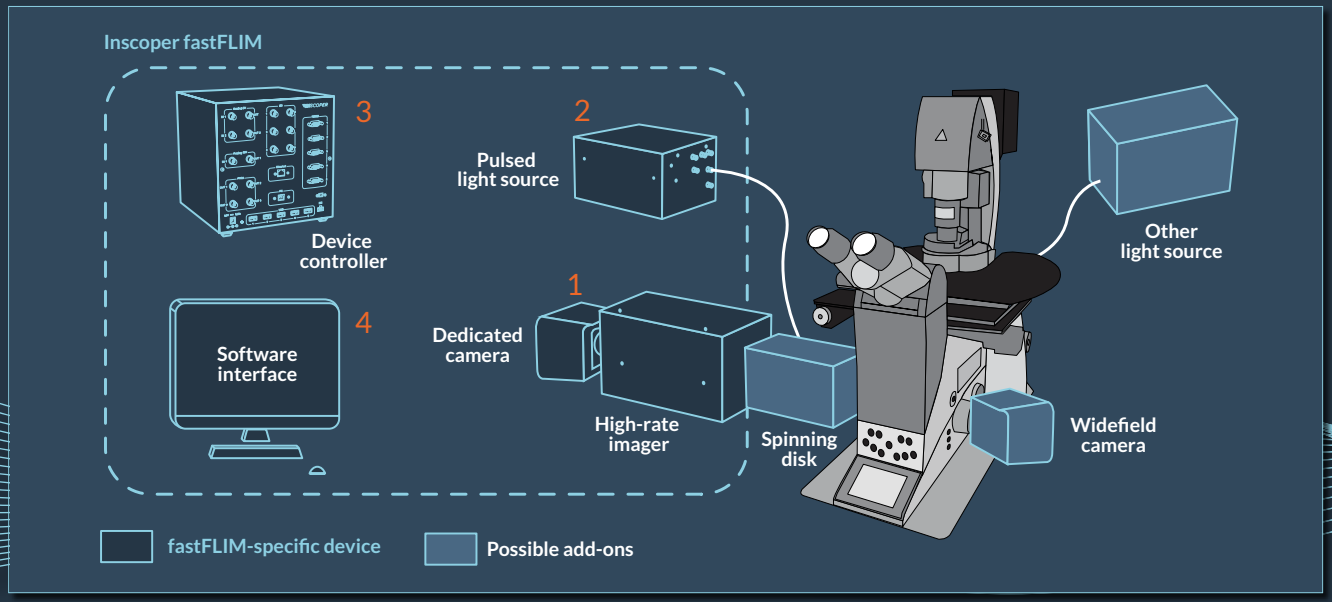
(3) a device controller for triggering and synchronizing the microscope signals,

(4) a complete image acquisition software.

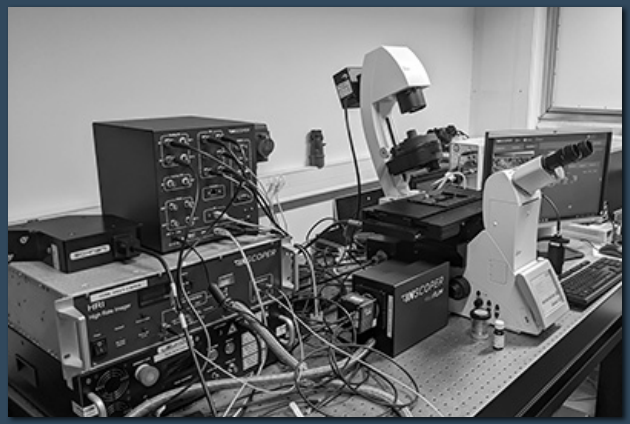
THE FIRST TIME-DOMAIN FLIM FOR CAMERA MICROSCOPES

The Inscoper fastFLIM solution is perfectly suited for live cell imaging. It can be used for a large panel of applications, including lifetime monitoring, FRET measurement (protein-protein interactions), biosensor analyzes, or control of the environment in the sample (pH, viscosity, etc.).

With its simplicity, fastFLIM democratizes the use of FLIM as a complementary measurement to quantitative fluorescence imaging. It is fully integrated into the Inscoper Imaging Solution to offer a robust and user-friendly product that can be combined with any other fluorescence imaging technique and third-party equipment.



USER CASE @ RENNES, FRANCE



The microscopy system combines the fastFLIM solution in addition to the Inscoper scanFRAP, which is a dedicated solution for laser photomanipulation. It equips a microscopy core facility, where users are regular biologists with various levels of practice in microscopy.

This system is mainly used for rapid imaging of biosensors expressed by transfected living cells. The combination of FLIM and FRAP modalities in the same acquisition allows interacting with living cells to analyze a large variety of biological processes such as protein expression, nuclear protein recruitment or mitosis.

PERFECTLY SUITABLE FOR MULTIDIMENSIONAL ACQUISITIONS

SEAMLESS AND COMPLETE INTEGRATION OF THE MICROSCOPE

The Inscoper fastFLIM solution is compatible with all camera-based microscopes from Leica, Nikon, Zeiss, and Olympus. It allows a seamless integration of various imaging microscopy techniques in the same software environment.

The third party devices that equip the microscope are fully controllable: cameras from leading manufacturers such as Andor, Hamamatsu, PCO and Photometrics, microfluidic devices (pumps, temperature...), light sources and filter wheels, XYZ stages, etc.

This offers great flexibility and simplicity in setting up multidimensional image acquisition sequences combining FLIM with various modalities (high-throughput in multi-well plates, photomanipulation, environmental perturbation, ...).

REAL-TIME IMAGING FOR LOW PHOTO-TOXICITY

The Inscoper technology for optimized microscope control guarantees the highest possible acquisition rate to observe the fastest biological phenomena. It also allows the illumination only during the exposure time of the camera, thus limiting the phototoxicity development in living samples.

FLIM images are **displayed live**. They can be processed using dedicated LUTs and with background subtraction while the sequence is still running.

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.

INNOVATIVE TECHNOLOGY FOR LIVE & AUTOMATED FLIM IMAGING ▸

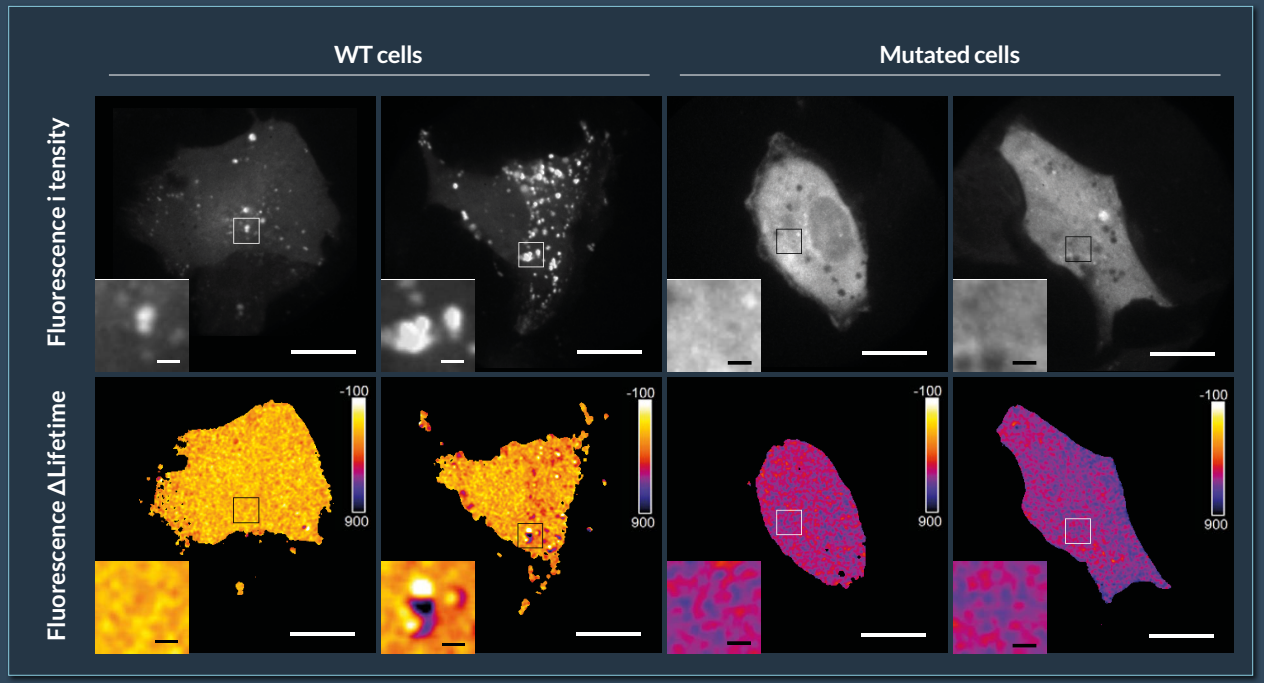


Figure 1: Biosensor imaging with FRET/FLIM using the Inscoper fastFLIM

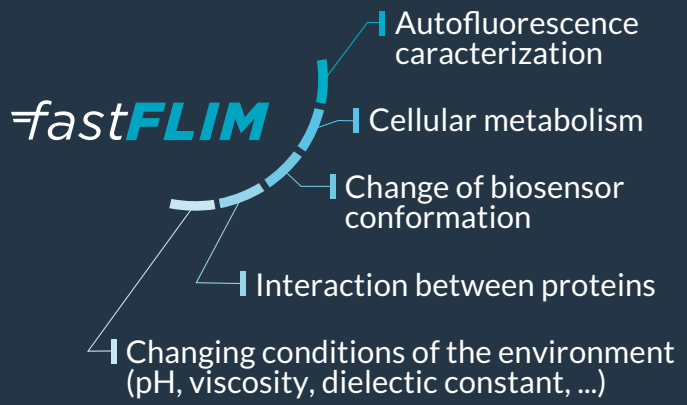
Representative fluorescence intensity and ΔLifetime images of WT and mutated U2OS cells, co-expressing biosensor and analyzed by FRET/FLIM. Squares on the images illustrates the location of the zoomed images. Pseudocolor scale: pixel-by-pixel ΔLifetime. Scale bars: 40 µm (large images) and 5µm (enlarged images).

Courtesy of Dr. Elif Begüm Gökerküçük, IGDR, Rennes, France

fastFLIM renews the way to do FLIM imaging on a camera microscope. The FLIM measurement is done in time domain, with a picosecond pulsed laser and a time gate generator. It has the advantages of doing FLIM with a camera detection, namely the low photo-toxicity and the high acquisition speed, but without the need to use a reference sample or a fitting calculation to perform the measurement.

Temporal gates of 2 ns in a time window of 10 ns are sequentially generated to obtain a stack of 5 time-gated images. These images are then used to **calculate the pixel-by-pixel mean fluorescence Δlifetime in real time**, according to the following equation: $\tau = \Sigma \Delta t_i \times Ii / \Sigma I_i$, where Δt_i corresponds to the delay time of the i^{th} gate while I indicates the pixel-by-pixel time-gated intensity image.

This method ensures rapid FLIM measurements: no fitting or binning steps are required, and lifetime is calculated in live, with minimal photon budget.



EASILY ACCESSIBLE FOR REGULAR BIOLOGY USERS

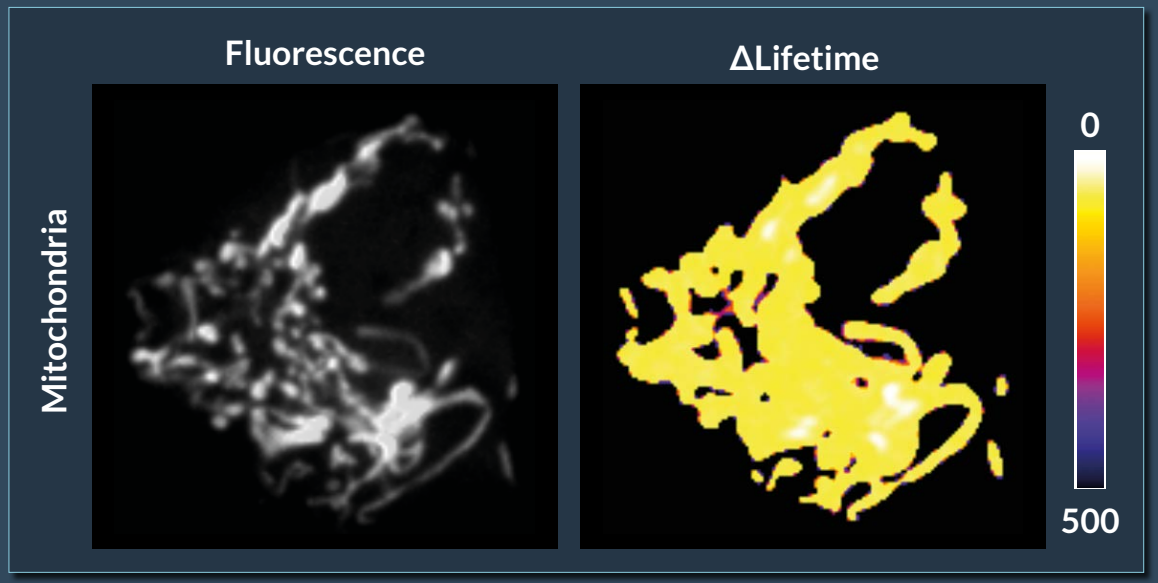


Figure 2: Mitochondria monitoring with FLIM/FRET using biosensors

Forster's Resonance Energy Transfer by Fluorescence Lifetime Imaging Microscopy analyses on MCF7 cells expressing a fluorescent biomarker. Fluorescent-labeled mitochondria are image according their fluorescence intensity (left) and ΔLifetime (right)

Courtesy of Dr. Giulia Bertolin, IGDR, Rennes, France

To make FLIM available to cell biologists, we needed a simple solution that could be automatically combined with other microscope imaging modalities.

The fastFLIM solution addresses the growing need for rapid lifetime imaging to solve many of the challenges brought by the scientific teams at our institute.

With this system our users are completely autonomous, which allows for a better workflow on our microscopy imaging facility.

Dr. Marc Tramier, Research team leader at the Institute of Genetics and Development of Rennes (France), Scientific director of the Microscopy Rennes Imaging Center, Scientific advisor of INSCOPER , Laureate of the CNRS Crystal Medal in 2019 .

- No preparation required. There is no need for a reference sample, users just have to position their sample and launch the acquisition of images.
- No complicated calculation. Users do not have to do the math, the result is direct as there is no fitting calculation to proceed.
- Fast. Five intensity images are enough to calculate one FLIM image, allowing an average frame rate of 2 to 10 images FLIM images per second (depending on the exposure time).
- Live. FLIM images are immediately displayed and processed in real time.
- Reproducible. The measurement is perfectly stable over time, whatever the duration and repetition of the acquisitions.
- Compatible. fastFLIM includes the Inscoper Imaging Solution that can make this convenient FLIM technique a stand-alone microscopy solution or an additional imaging modality.

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. The specific features for fastFLIM imaging combine seamlessly with the other microscopy techniques available on the system.

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, frequency), 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
- 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
- Customization of temporal gate width, number of temporal gates, and the gate spacing
- 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
- Real-time monitoring of fluorescence intensity with graphics during acquisition
- Real-time monitoring of fluorescence Δlifetime evolution with 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

VISUALIZATION
PROCESS & GET YOUR DATA

- Visualization of all raw images with dimensions filters
- Access to raw and fastFLIM-processed images for both gated intensity and fluorescence Δlifetime
- "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
- Export of all data related to ΔLifetime calculation
- All Inscoper metadata are Bio-Formats compatible
- "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 fastFLIM features

TECHNICAL SPECIFICATIONS

FASTFLIM IMAGING

EXCITATION	Excitation source	White light pulsed supercontinuum laser
	Repetition rate	60 MHz
	Optical fiber, illumination modality	Widefield: liquid fiber Spinning disk: monomode fiber
	Laser power at optical fiber output	25 mW
	Wavelenth selection	Microscope optics:filters, dichroics Tunable filter if needed
	Light spectrum	From 400 to 700 nm
TIME GATE GENERATION	Temporal gate	≤0.5ns to 4ns
	Trigger delay	Up to 25ps precision on 25ns range
DETECTION	Detection mode	Scientific camera
	Measurable life times	From 1 to 4 ns
	Temporal resolution	Up to 10 images per second
	Spatial resolution	500nm for 60x objective and 2x additional magnification
	Field of view	100um diameter for 60x objective and 2x additional magnification

CE FC

SOFTWARE INTEGRATION

Device control & Image acquisition	Inscoper Imaging Solution
Microscope compatibility	Leica DMI series Nikon TI series Olympus IX70 series and IX81 Zeiss Axiovert/AxioObserver series
Supported third-party devices	www.inscoper.com/supported-devices
System compatibility	Full integration of all hardware devices and other imaging techniques of the microscopy system in the same software environment
Recommended computer configuration	Windows 10 64 bits RAM 16 Go SSD 128 GB Large screen(s) 1920 x 1080 minimum

PHYSICAL INTEGRATION

Optical mounting	Camera port
Control equipment	RACK 19” cabinet, 15U

LET'S KEEP IN TOUCH

contact@inscoper.com
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.

INSCOPER