



Imagerie microscopique en génétique et développement

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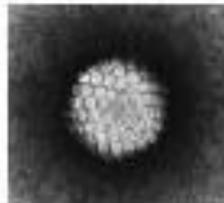
A variety of scales for a variety of systems to image...

Electron Microscopy

Photonic Microscopy



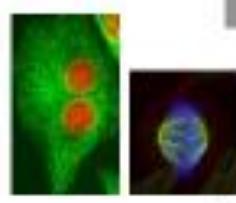
Virus



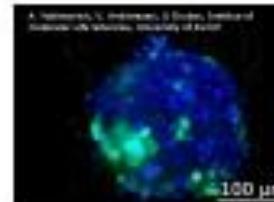
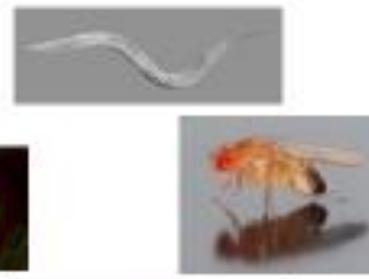
Prokaryotes



Eukaryotic cells



multicellular model





Why Imaging facilities ?

Imaging in Biology:

- State-of-the-art required for publishing in high impact factor journals.
- Costly equipment.
- Difficult to use.
- need efficiency

MRic provides:

- Easy access to the latest imaging technologies.
- Advice and support from engineers.
- Individual or group training to use the systems autonomously.

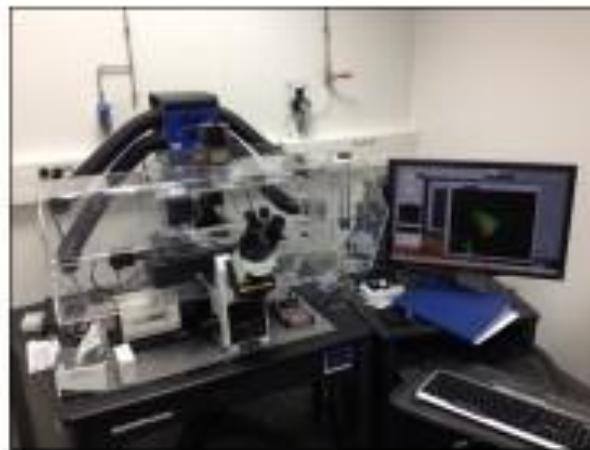


MRic Photonics equipment



- 6 confocal microscopes (SP8, SP5 w/ two-photon, Airyscan, ...).
- 2 Spinning-disk (CMOS, EmCCD Cameras...)
- Fast-FLIM prototype

- 4 widefield microscopes
(Videomicroscopes, deconvolution, EmCCD camera, ...)

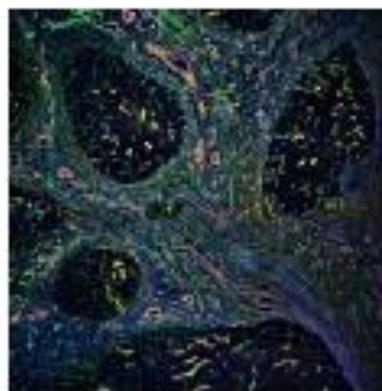
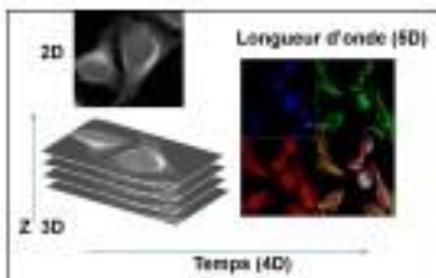


- Image analysis workstations.



Bioimaging

- Multidimensional acquisitions

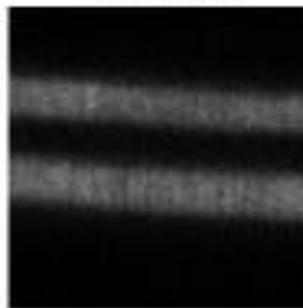


Immunomarquage
coupe de tissu
ganglionnaire M.
Gueret, MicMac

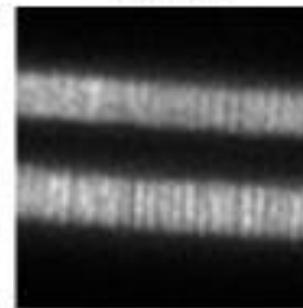
- Confocal super-resolution



Unprocessed



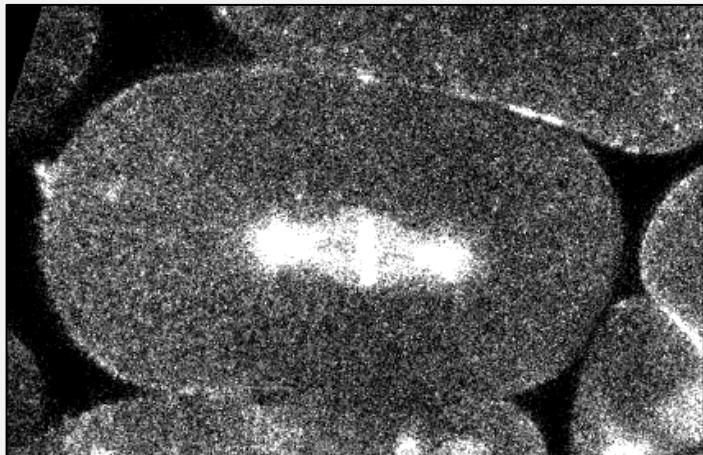
Processed



Micrilli of *C. elegans* intestine. Ezrin::GFP

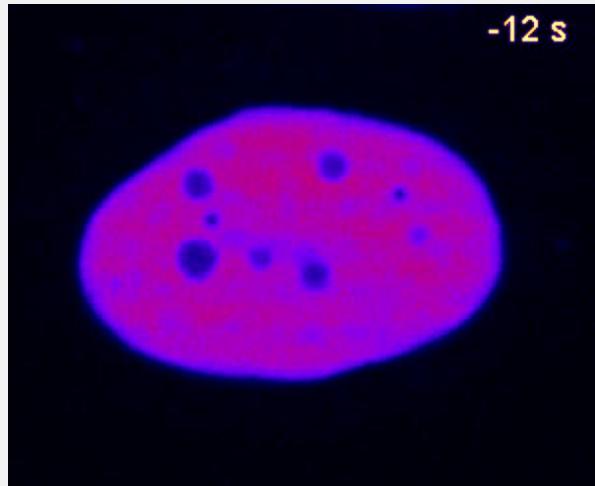


Applications using photo-manipulation



> Photo-ablation

centrosome ablation in the dividing one-cell *C.elegans* embryo.
(Anne Pacquelet, IGDR)



> Photo-damage

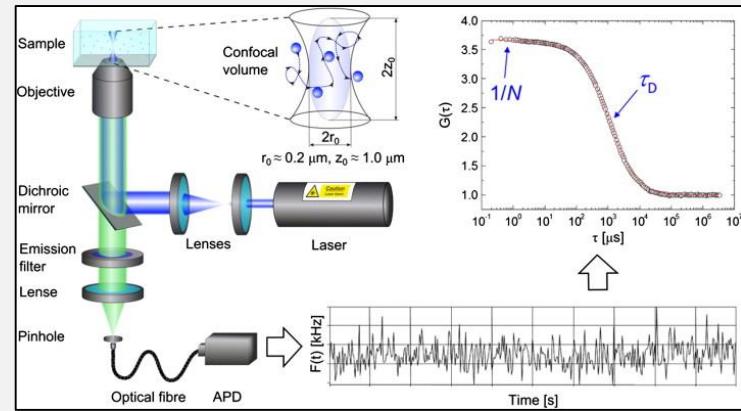
DNA damage in eukaryotic cell
(Sébastien Huet, IGDR)



Analysis of protein dynamics and interaction

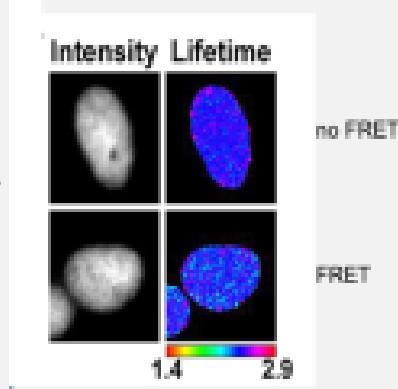
F-techniques

- Fluorescence Correlation Spectroscopy (FCS)
- Fluorescence Cross Correlation spectroscopy (FCCS)

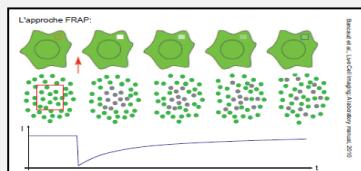


From Koynov & Butt, 2012

- FRET by FLIM.



- FRAP



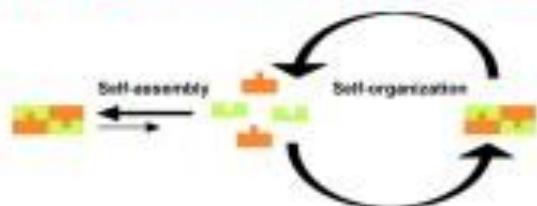
	FRAP	FRET / FLIM	FCS
Diffusion	✓		✓
Fraction Mobile	✓		
Concentration			✓
Interactions		✓	✓
KD (binding kinetics)	✓	✓	✓



Why and how to develop quantitative fluorescence microscopy methods in Cell Biology?

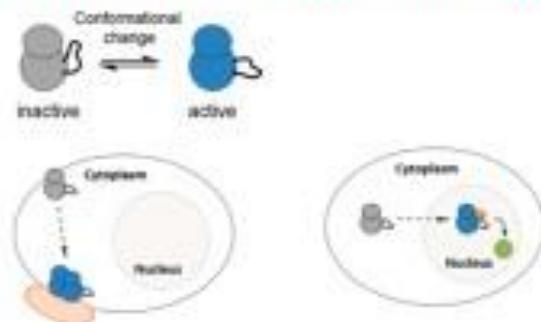
Cellular adaptation to external cues involves

- self organization of intracellular macromolecular complexes



Transient interactions
between macromolecules

- activation of biochemical activities

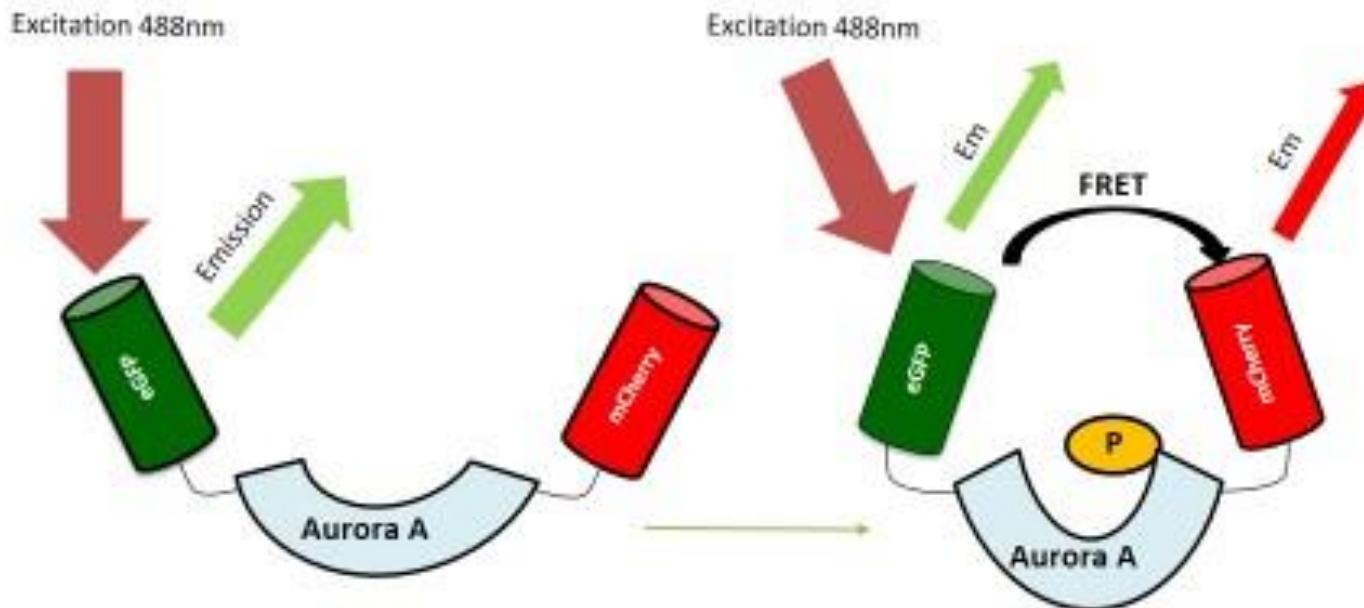


Transient activation
and/or conformational changes

To monitor, in real time, the spatio-temporal changes of macromolecular interactions, biochemical activities, conformations...



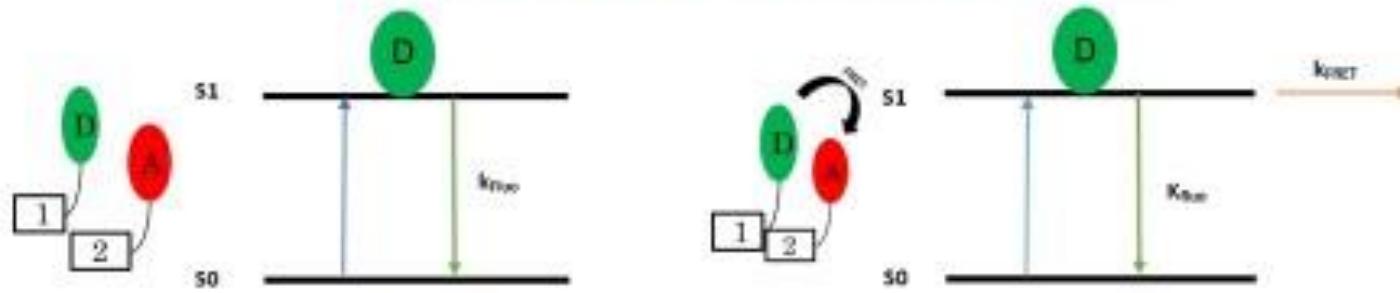
THE AURORA A-BIOSENSOR



Activation of Aurora A by T288 phosphorylation changes its **conformation allowing FRET** between GFP and mCherry



FRET BETWEEN 2 FLUOROPHORES



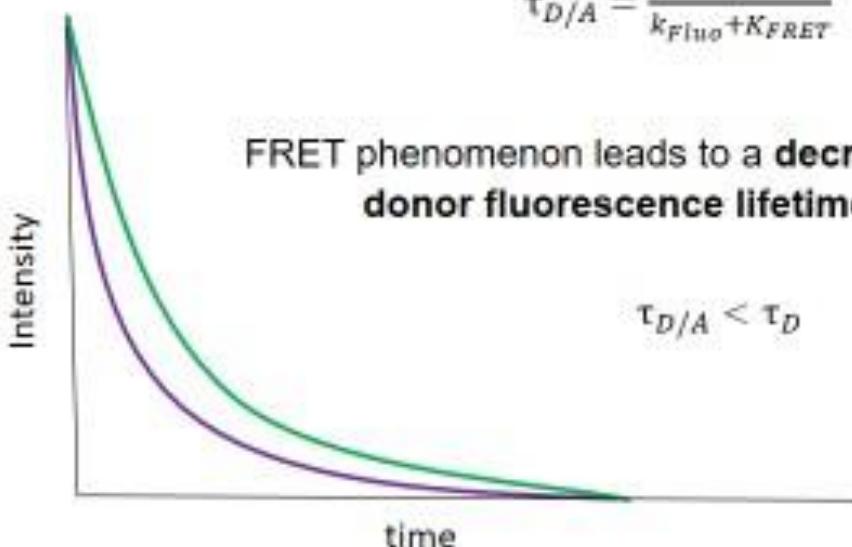
$$I(t) = I_0 e^{-\frac{t}{\tau_D}}$$

$$\tau_D = \frac{1}{k_{Fluo}}$$

$$I(t) = I_0 e^{-\frac{t}{\tau_{D/A}}}$$

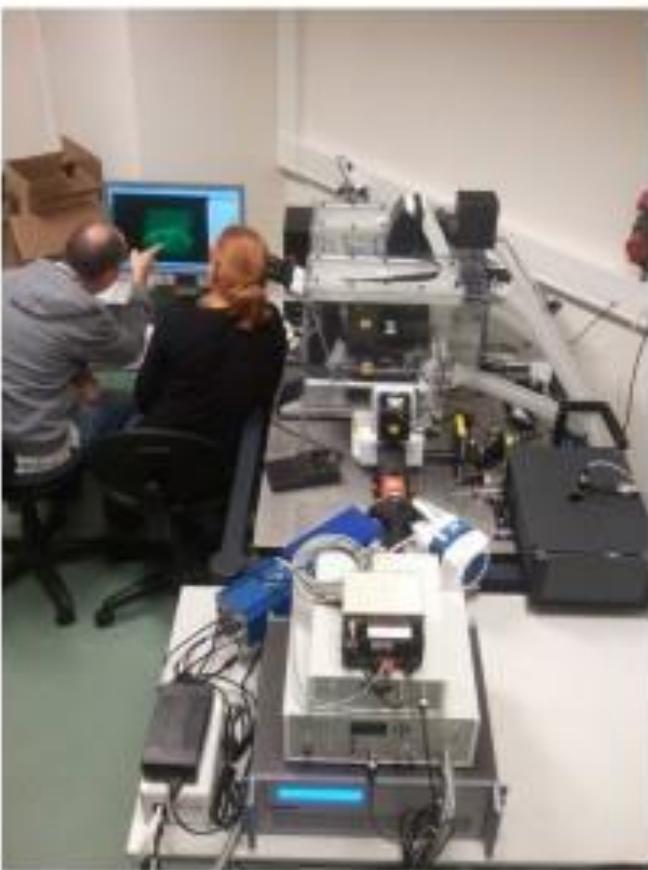
$$\tau_{D/A} = \frac{1}{k_{Fluo} + K_{FRET}}$$

FRET phenomenon leads to a **decrease of donor fluorescence lifetime**





fastFLIM prototype



Spinning Disk

Fast way to get **confocal resolution** images

But need **powerful laser**

⇒ **Supercontinuum** laser (white laser)

Intensifier to acquire **time-gated images**

Non-fitting method to analyse data

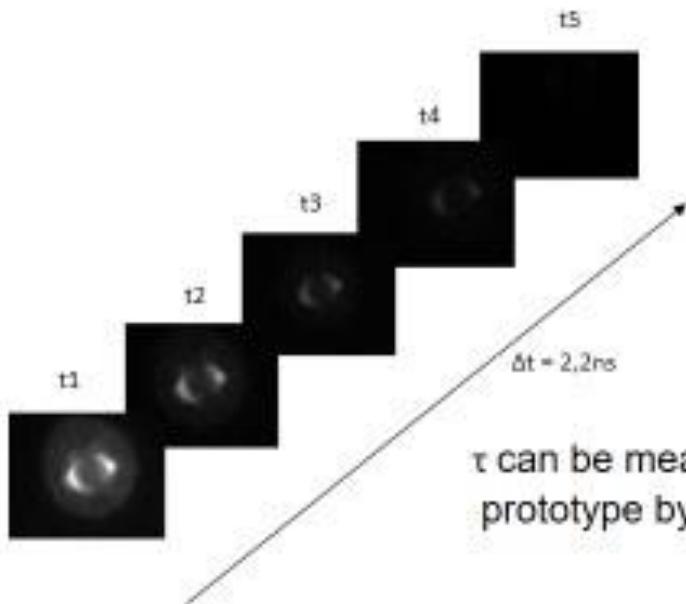
On-line treatment and calculation

Fast acquisition up to several images/ s

Leray et al., PlosOne, 2013

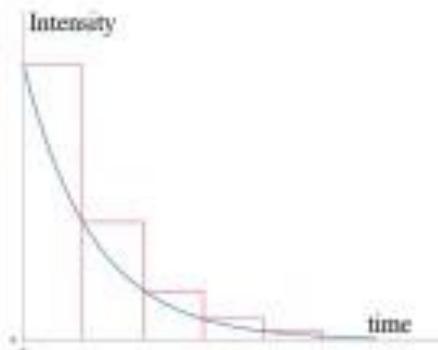


ACQUISITION AND DATA ANALYSIS OF FASTFLIM



$$\langle \tau \rangle = \frac{\sum \Delta t_i \times I_i}{\sum I_i}$$

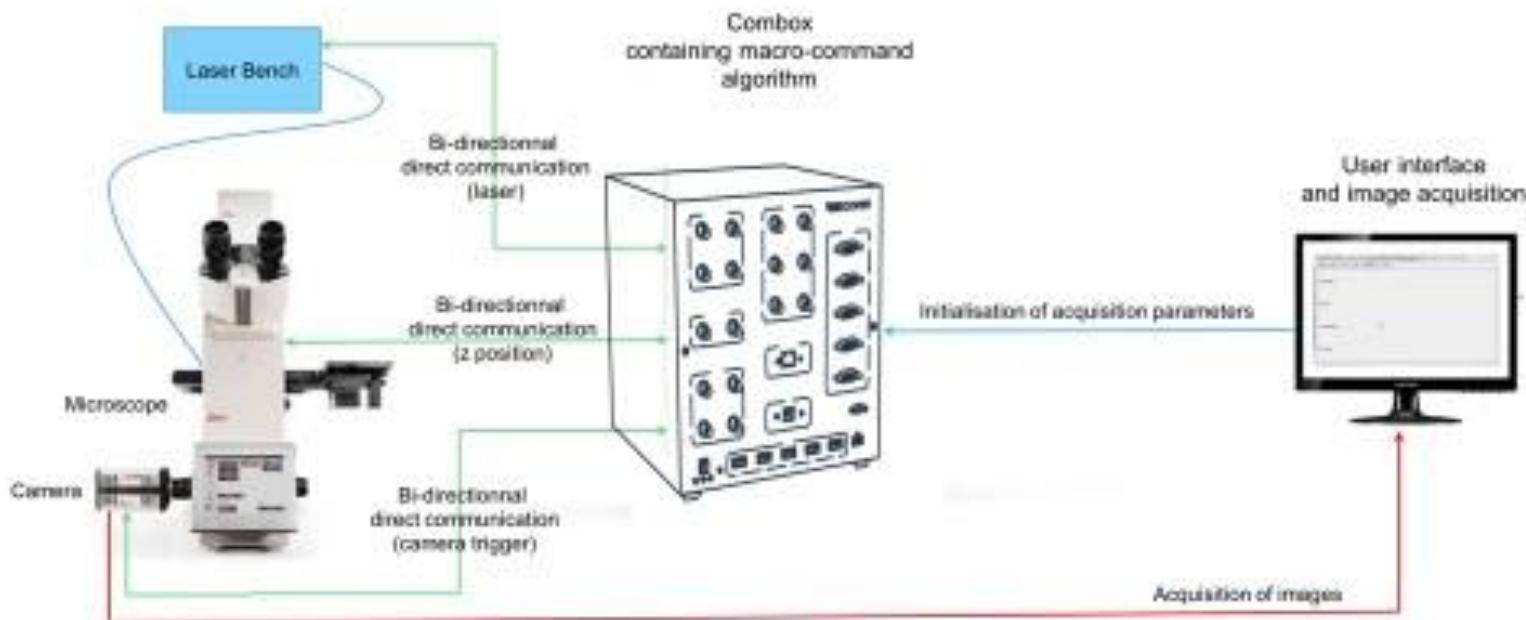
τ can be measured with our fast-FLIM prototype by using five gates of 2.2ns



U2OS GFP - Aurora A - mCherry



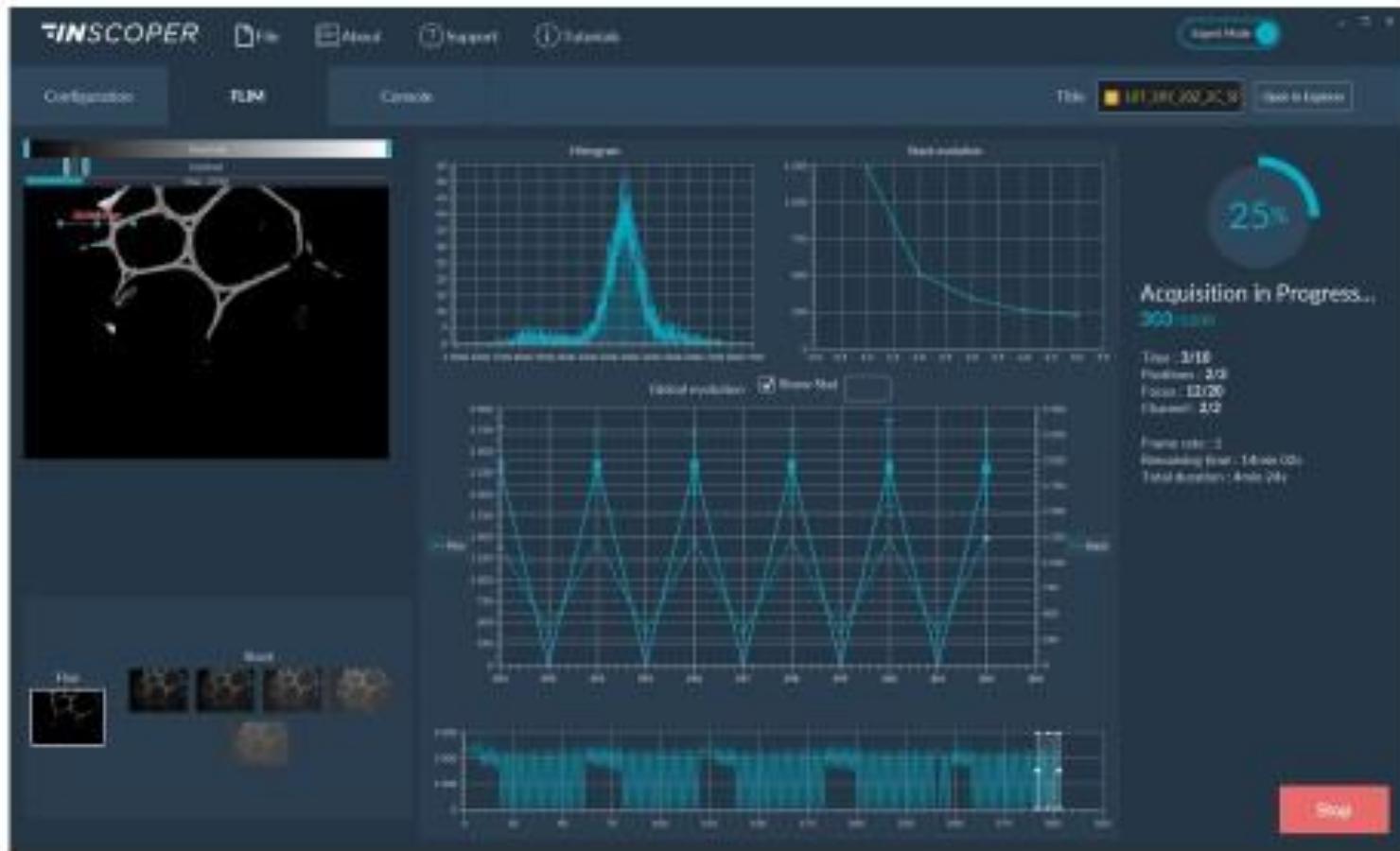
The spin off Inscoper to control microscopes and to increase acquisition speed



INSCOPER



fastFLIM interface





fastFLIM transfer

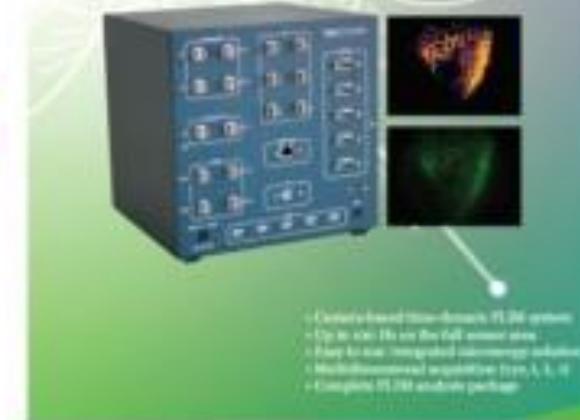


Microscopes fluorescents larges	Microscopes confocal	Vidéo-microscopie
Leica CM10XA2	Leica SP8	API CineVision
Zeiss AxioImager	Leica SP8 Multiphoton	Leica CM10B
Olympus IX2	Leica SPE	MicroImaging
Zeiss AxioPlan	Leica SPE Multiphoton	Oscilloscope de source
	Olympus	Cellules souches
	Alyxplan Zeiss	Analyse d'images
		Velocity
Microscopes de R&D	Spinning Disk	
PALM		ImageJ, Fiji
		Mesdrobox
	FastFLIM	



FAST FLIM

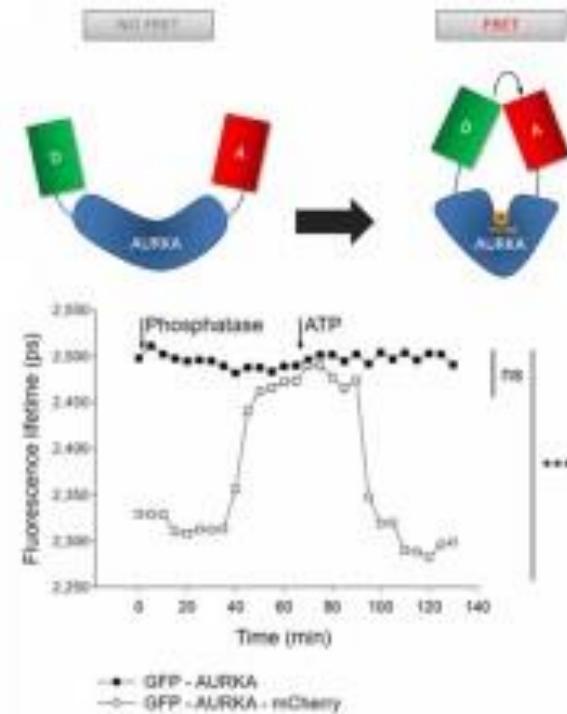
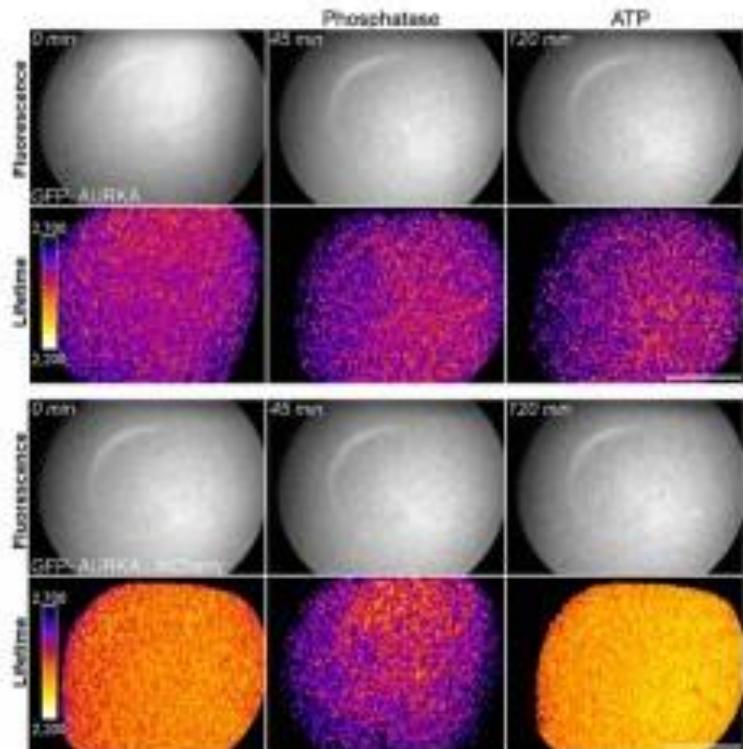
THE FASTEST TIME-DOMAIN FLIM PLATFORM



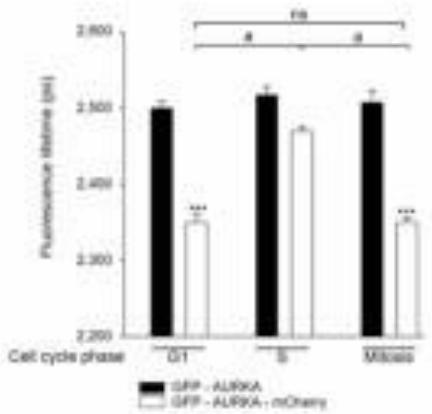
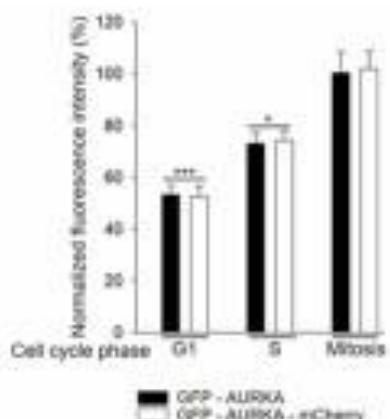
- Camera-based fluorescence: 15.04 nm
- Up to 100 Hz on the full sensor area
- Ready-to-use integrated fluorescence module
- Multidimensional acquisition (xy, z, t)
- Complete FLIM analysis package



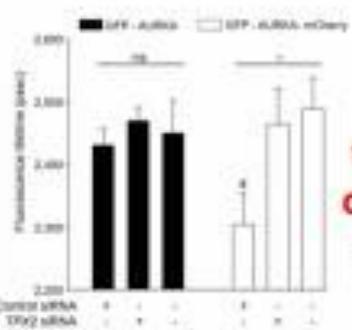
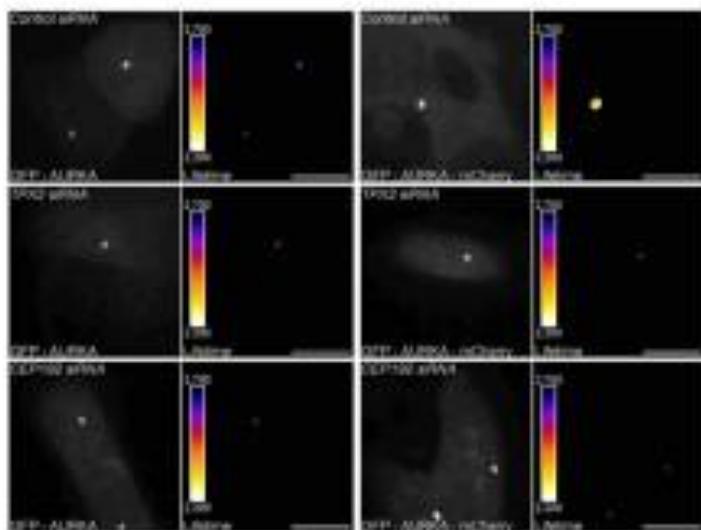
A FRET biosensor reveals the spatiotemporal activation and the functions of AURKA in living cells



The AurkA biosensor shows
phosphorylation-linked FRET



The AurKA biosensor
decorrelates
quantity and activation



The activation of
the AURKA biosensor
during the G1 depends
on TPX2 and CEP192

Bertolin et al., Nat Commun, 2016



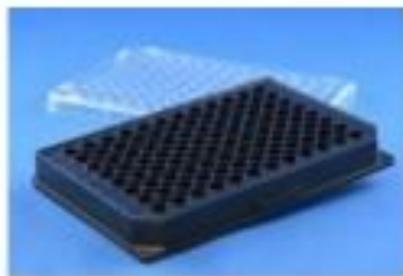
NEW METHODOLOGY

2 tools

A sensor of AurkA activity in cells A fast-flim microscope to perform rapidly FLIM



Performing an automated inhibitory drug assay





Performing a drug screening test

The screenshot shows the TINSCOPE software interface. On the left, there is a live fluorescence microscopy image of a sample with several bright spots. Below the image is an orange button labeled "Acquisition". Above the image, camera settings are displayed: Camera model: QImaging, Exposure time: 100ms, and two buttons for "Save" and "Exit". In the center, the experimental setup is defined in five steps:

- 1. Flu:** Includes a "Flu" button and a "0 / 0" status indicator.
- 2. Positions:** Includes a "Supernatant" dropdown set to "Multi", a "Map" button, and a "0 / 0" status indicator.
- 3. 2 Wash:** Includes a "Wash & Aspirate" button and a "1 / 1" status indicator.
- 4. Multi Channels:** Includes a "0 / 0" status indicator.
- 5. Flu:** Includes a "0 / 0" status indicator.

Below the steps are buttons for "Cancel", "Run", and "Run & Exit". At the bottom right, there are buttons for "Close", "Run", and "Run & Exit".

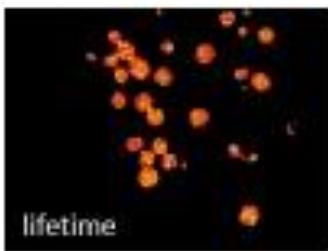
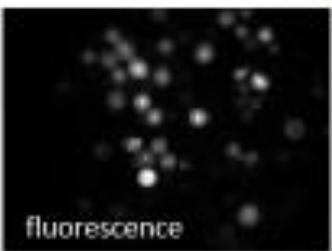


Analysis of data

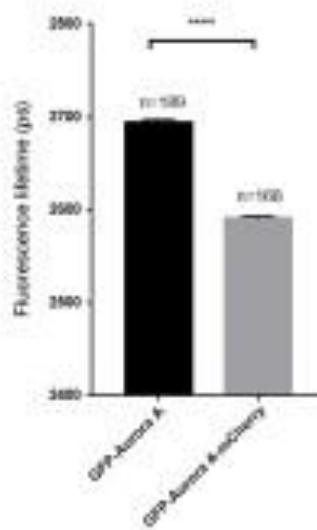
Need to analyze hundred of images (up to 6000)



U2OS GFP-AuraKA-mcherry



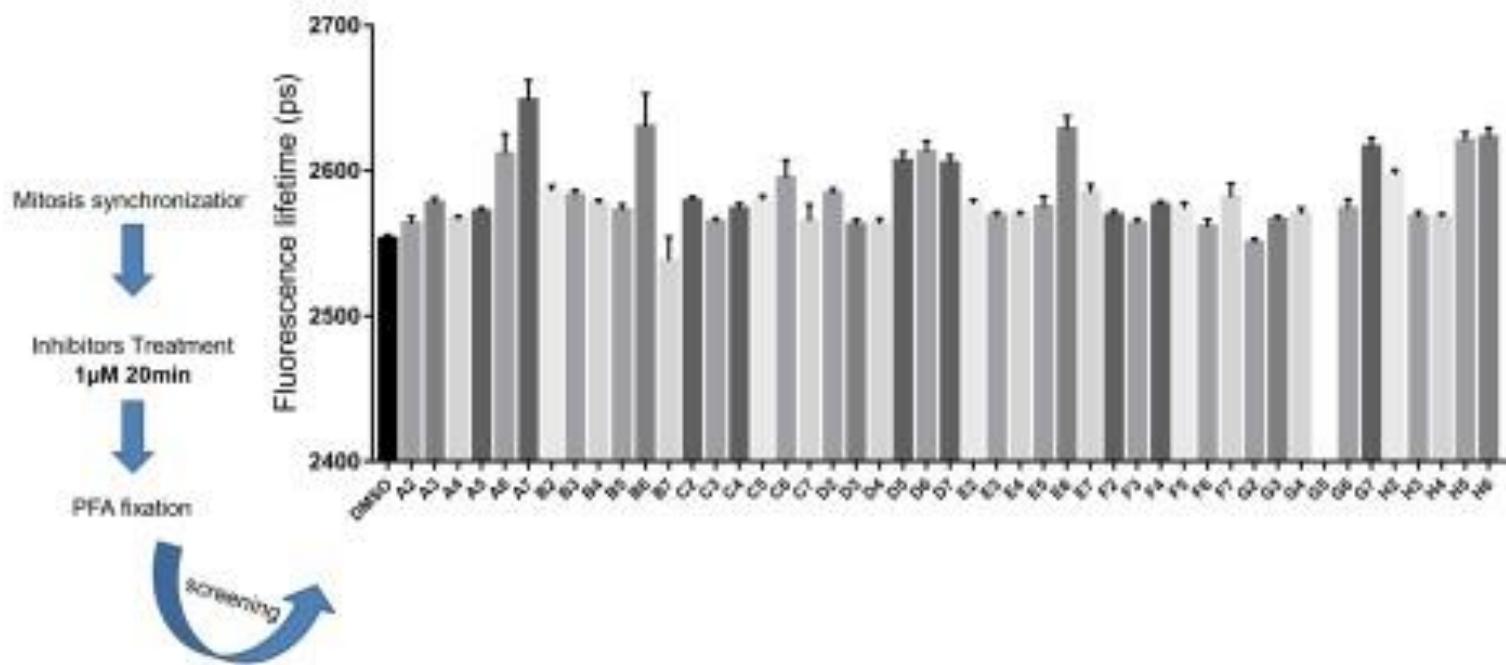
Cell segmentation



Assign the lifetime value to each cell counted



First screening



Collaboration S Ruchaud (Roscoff)
unpublished data



Perspective and vision

Technological developments

Gilles Le Marchand

Mael Balluet

In collaboration with

Jacques Pécreaux and Inscoper

The Roboscope

Methodological developments

Florian Sizaire, Begum Gokerkucuk,

Giulia Bertolin

3-color Multiplex FRET

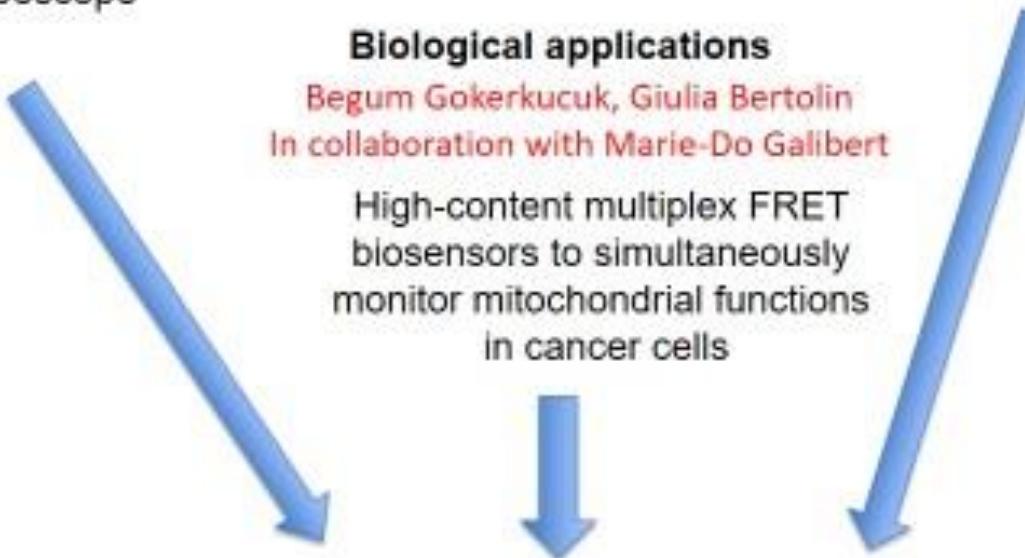
Automated FRET/FLIM for HCS

Biological applications

Begum Gokerkucuk, Giulia Bertolin

In collaboration with Marie-Do Galibert

High-content multiplex FRET
biosensors to simultaneously
monitor mitochondrial functions
in cancer cells



Automated platform to monitor mitochondrial signature
in the context of cancer progression

Vision of a tool for personalized medicine and drug design



MFQ team at IGDR

Thanks!



Gilles Levertchand Florian Sizaire Giulia Bertolin

Alumni:
Julien Roul
Claire Demeautis

In collaboration with
Claude Prigent (IGDR)
Sandrine Ruchaud (Roscoff)
Otmane Bouchareb (Inscoper)
Olivier Chanteux (Inscoper)



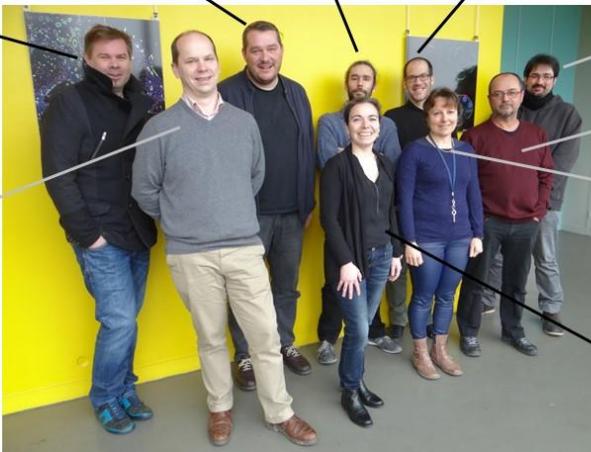
Directeur scientifique –
Ingénieur R&D: Marc
Tramier

Responsable scientifique:
Sébastien Huet

Ingénieur
plateforme:
Xavier Pinson

Responsable
scientifique:
Frédéric Mourcin

Responsable
scientifique:
Grégoire
Michaux



Ingénieur
plateforme:
Aurélien Dupont

Responsable
scientifique:
Denis Chrétien

Ingénierie
plateforme:
Agnès Burel

Ingénierie
plateforme:
Stéphanie
Dutertre

Mric core facilities at UMS Biosit