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alliance nationale
pour les sciences de la vie et de la santé

Institut Thématique Multi-Organismes
Technologies pour la santé

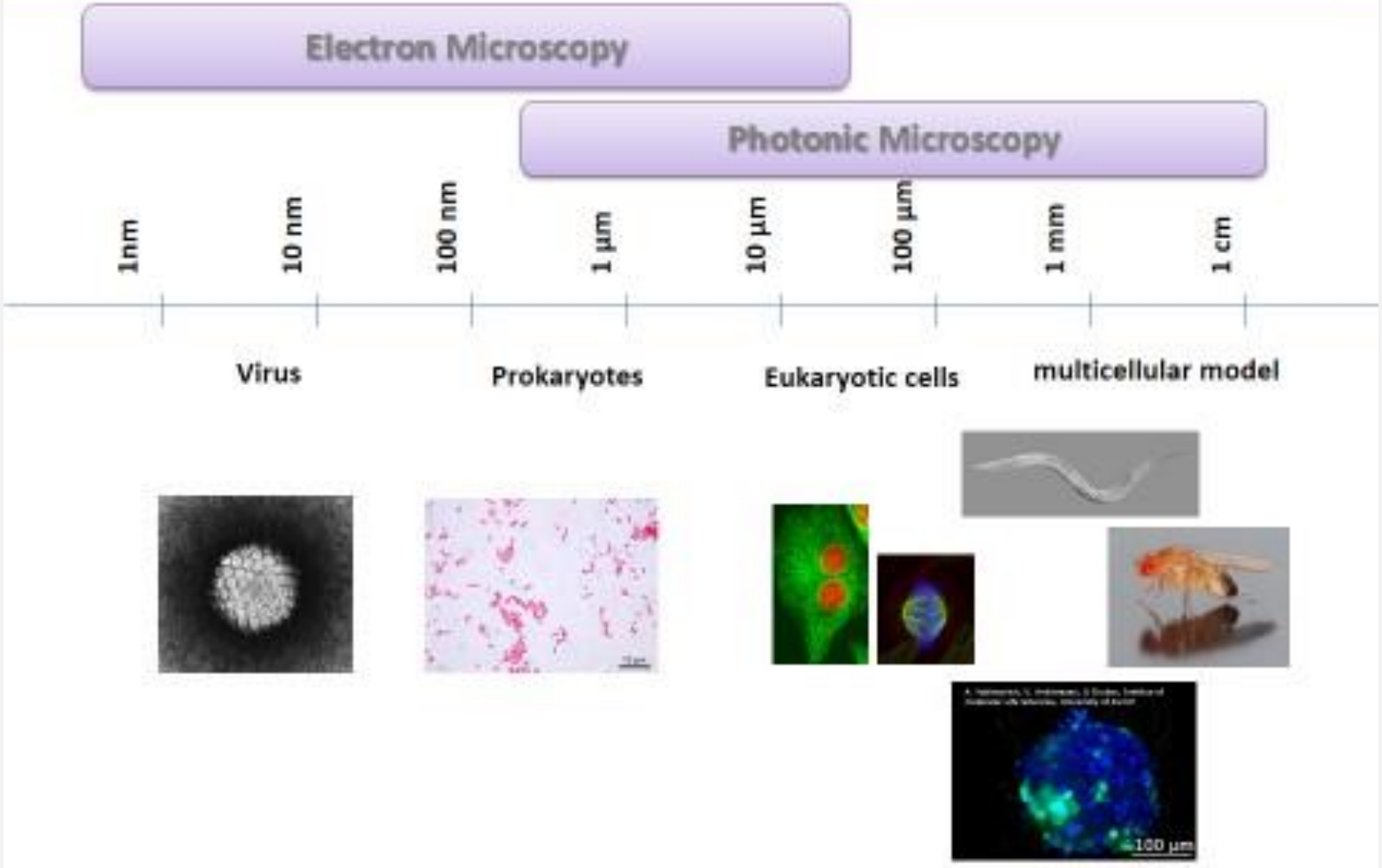
Imagerie microscopique en génétique et développement

Marc Tramier

Institut de Génétique et Développement de Rennes, CNRS – UR1

MRic

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



MRic
MICROSCOPY RENNES IMAGING CENTER

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:

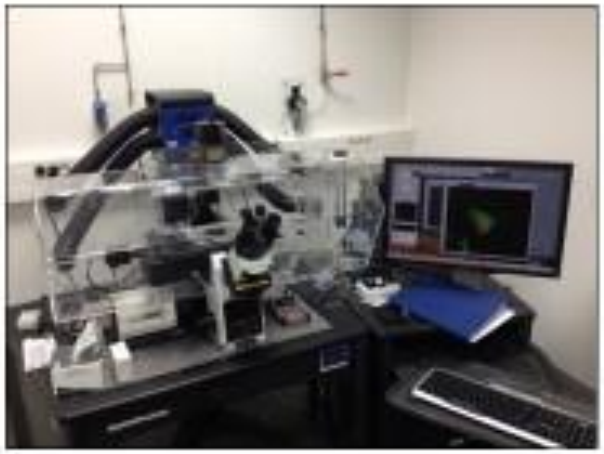
- Easy access to the latest imaging technologies.
- Advice and support from engineers.
- Individual or group training to use the systems autonomously.

http://microscopie.univ-rennes1.fr

Inserm cnrs UNIVERSITÉ DE RENNES 1

MRic Photonics equipment

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



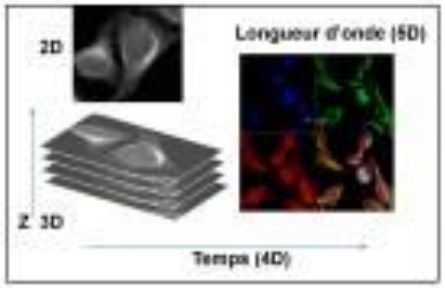
- 4 widefield microscopes (Videomicroscopes, deconvolution, EmCCD camera, …)

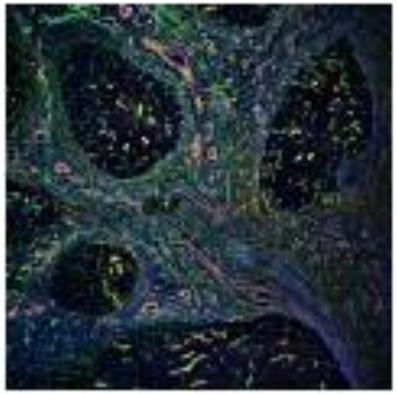
- Image analysis workstations.

MRic

Bioimaging

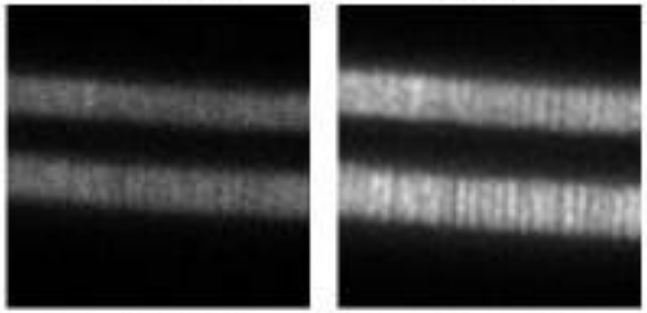
- Multidimensional acquisitions





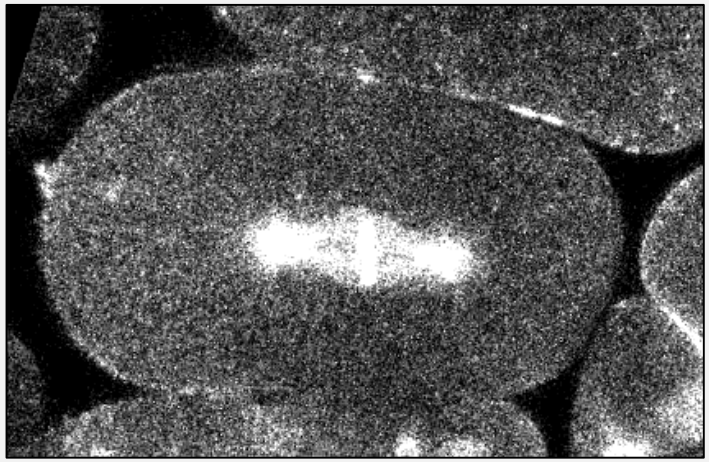
Immunomarquage coupe de tissu ganglionnaire M. Gueriec, MicMac

- Confocal super-resolution



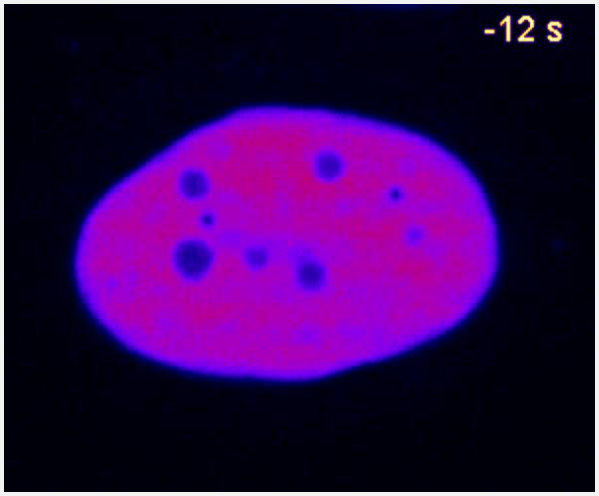
Microvilli of *C. elegans* intestine. Ezrin::GFP

Applications using photo-manipulation



> Photo-ablation

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



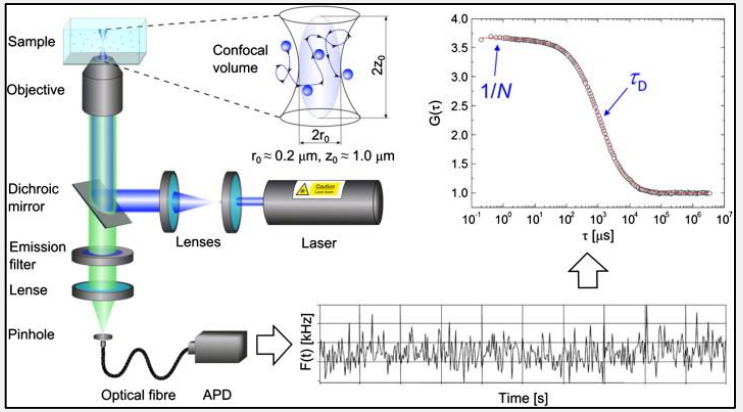
> Photo-damage

DNA damage in eukaryotic cell
(Sébastien Huet, IGDR)

Analysis of protein dynamics and interaction

F-techniques

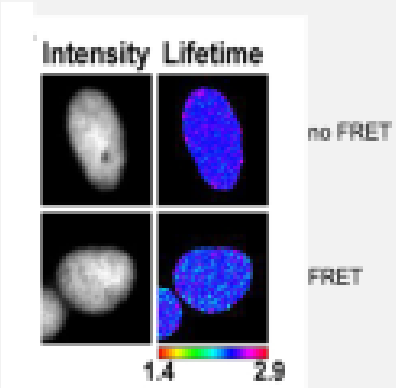
- Fluorescence Correlation Spectroscopy (FCS)



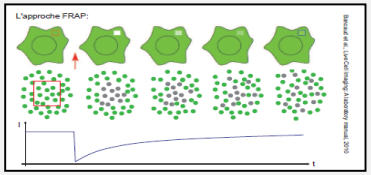
From Koynov & Butt, 2012

- Fluorescence Cross Correlation spectroscopy (FCCS)

- FRET by FLIM.



- FRAP

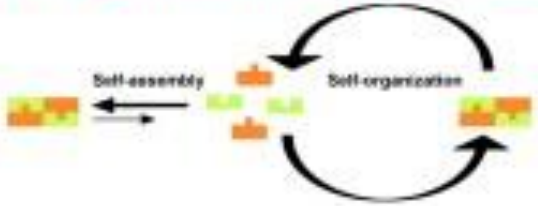


	FRAP	FRET / FLIM	FCS
Diffusion	√		√
Fraction Mobile	√		
Concentration			√
Interactions		√	√
KD (binding kinetics)	√	√	√

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

Cellular adaptation to external cues involves

- self organization of intracellular macromolecular complexes



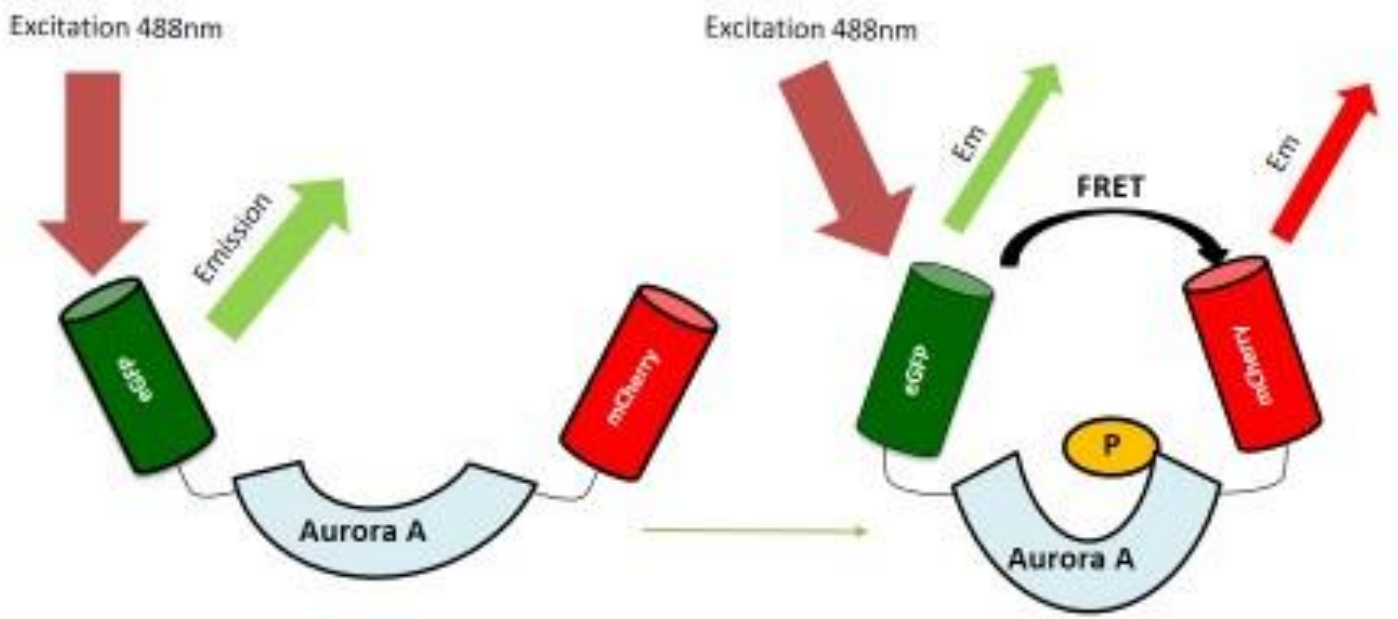
Transient interactions
between macromolecules

- activation of biochemical activities

Transient activation
and/or conformational changes

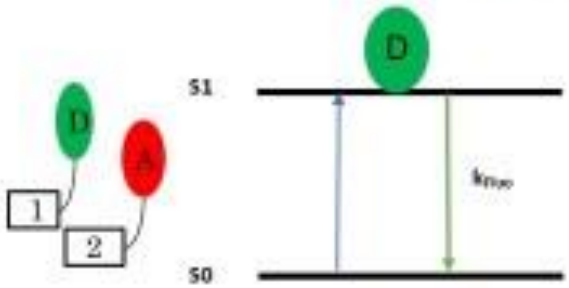
To monitor, in real time, the spatio-temporal changes of macromolecular interactions, biochemical activities, conformations...

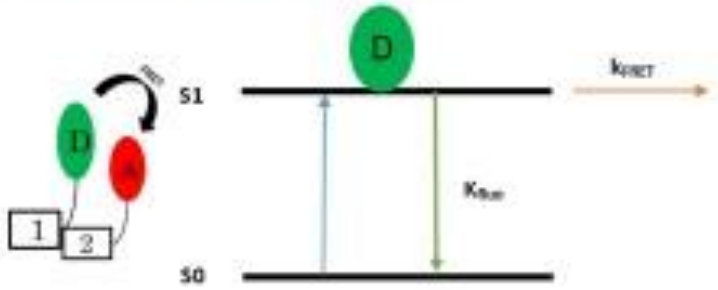
THE AURORA A-BIOSENSOR



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

FRET BETWEEN 2 FLUOROPHORES



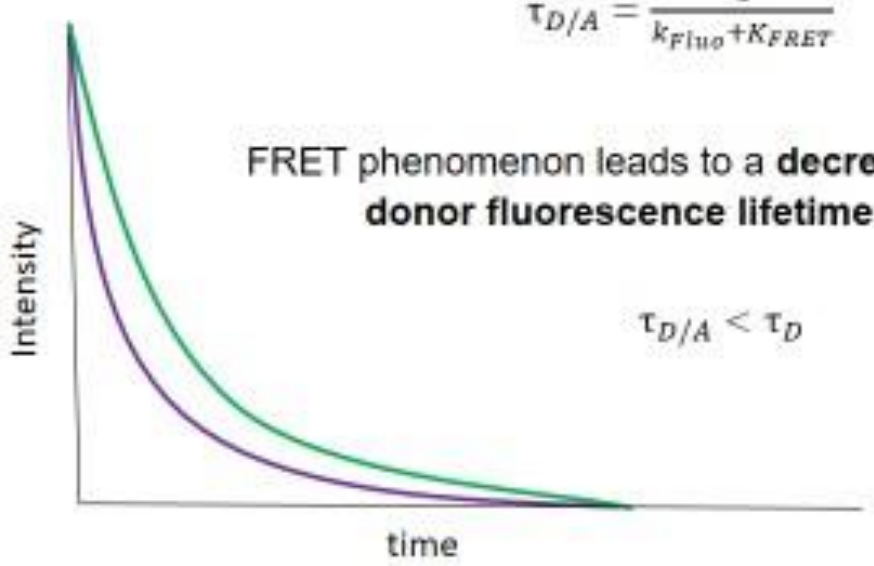


$$I(t) = I_0 e^{-\frac{t}{\tau_D}}$$

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

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

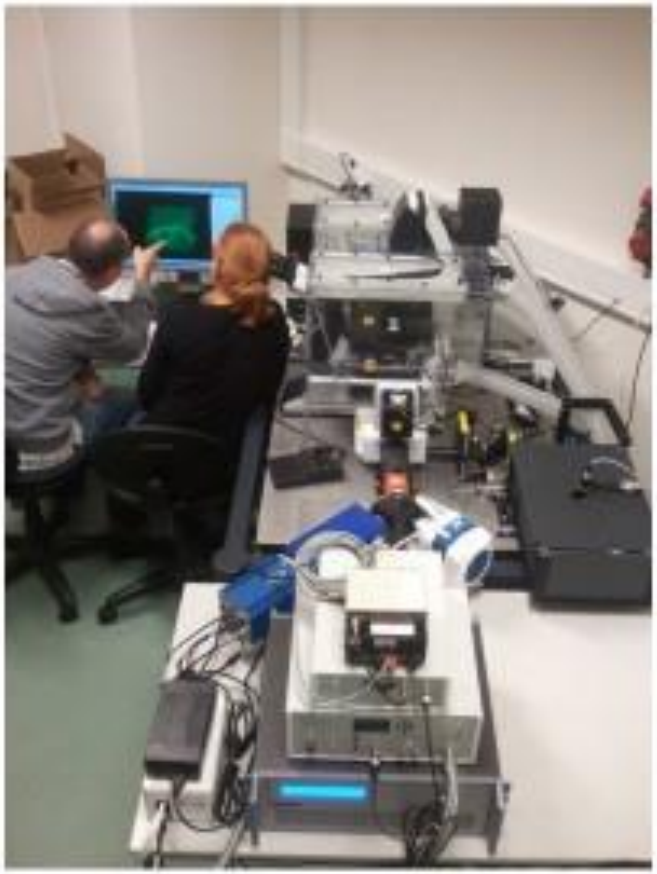
$$\tau_{D/A} = \frac{1}{k_{Fluo} + K_{FRET}}$$



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

$$\tau_{D/A} < \tau_D$$

fastFLIM prototype



Spinning Disk
Fast way to get **confocal resolution** images
But need **powerful laser**
⇒ **Supercontinuum** laser (white laser)

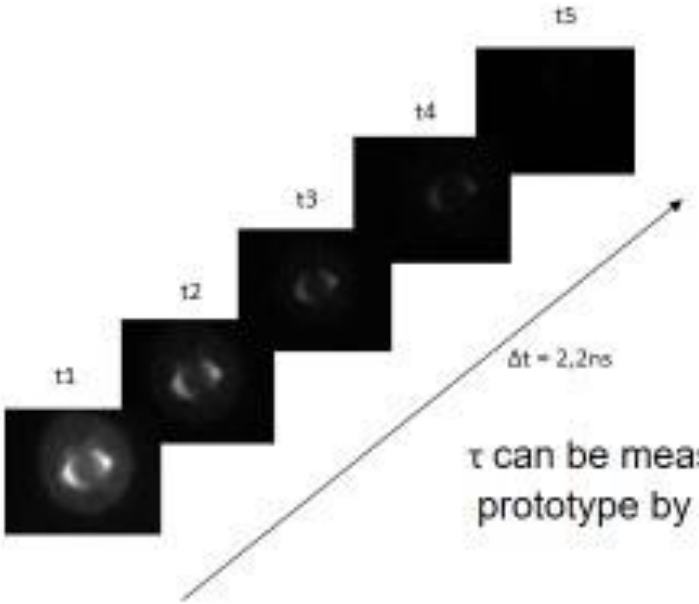
Intensifier to acquire **time-gated images**

Non-fitting method to analyse **data**
On-line treatment and **calculation**

Fast acquisition up to several images/ s

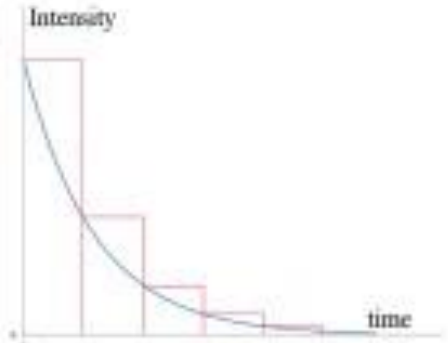
Leray et al., PlosOne, 2013

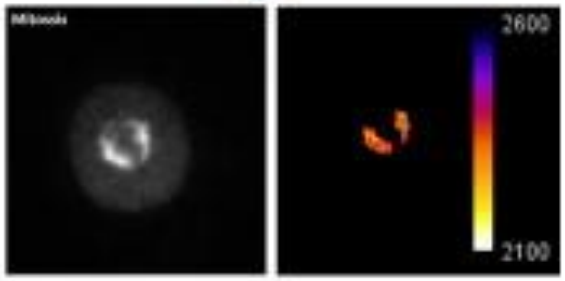
ACQUISITION AND DATA ANALYSIS OF FASTFLIM



$$<\tau>=\frac{\sum \Delta t_i \times I_i}{\sum I_i}$$

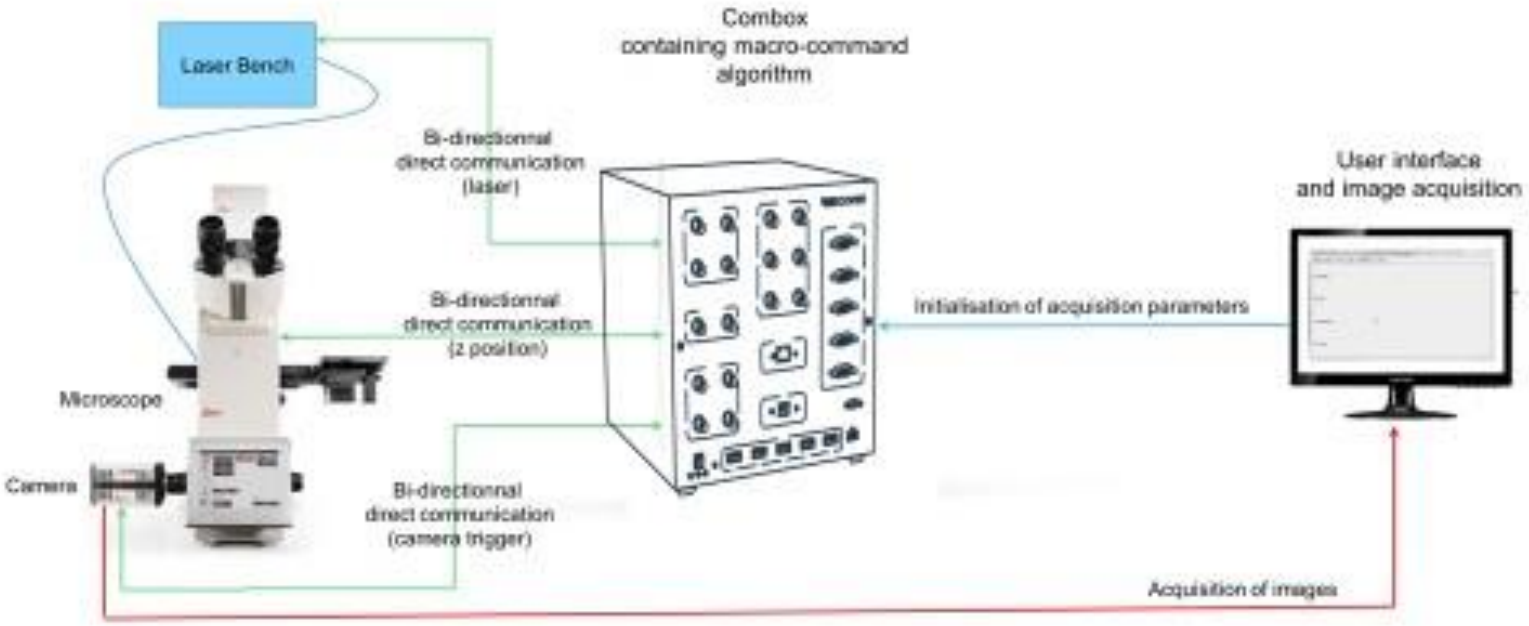
τ can be measured with **our fast-FLIM** prototype by using five gates of 2,2ns





U2OS GFP - Aurora A - mCherry

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



INSCOPER

fastFLIM interface

fastFLIM transfer

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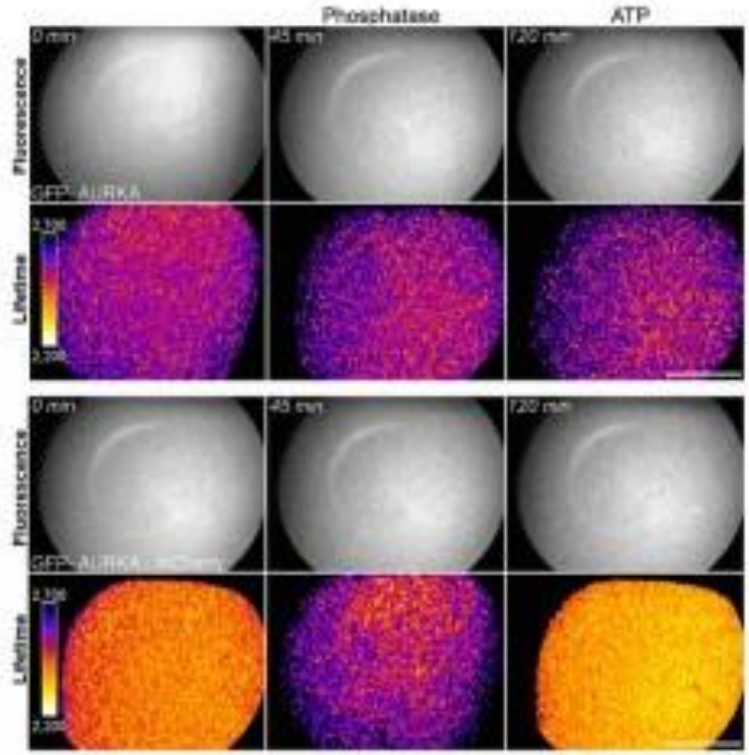
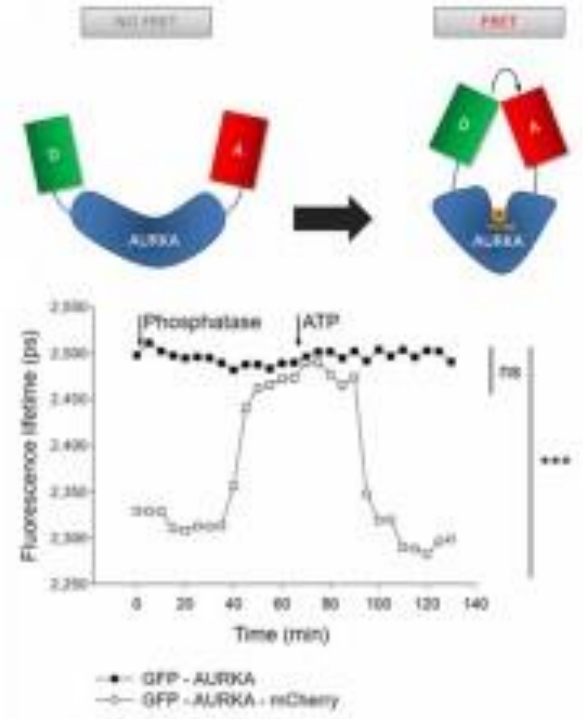
Microscopes champs large	Microscopes confocaux	Vidéomicroscope
Leica DMRXA2	Leica SP8	API DeltaVision
Zeiss Axiolmager	Leica SP5 Multiphoton	Leica DMRB
Olympus IX2	Leica SP5	
Zeiss Apotome	Leica SP5 Nomarski	Microprojecteurs
	Olympus	Oocytes de souris
	Airyscan Zeiss	Cellules adhérentes
		Analyse d'images
Microscopes de R&D	Spinning Disk	Velocity
PALM		Imagej, Fiji
	FastFLIM	Metafluor

PHOTON LINES
Research

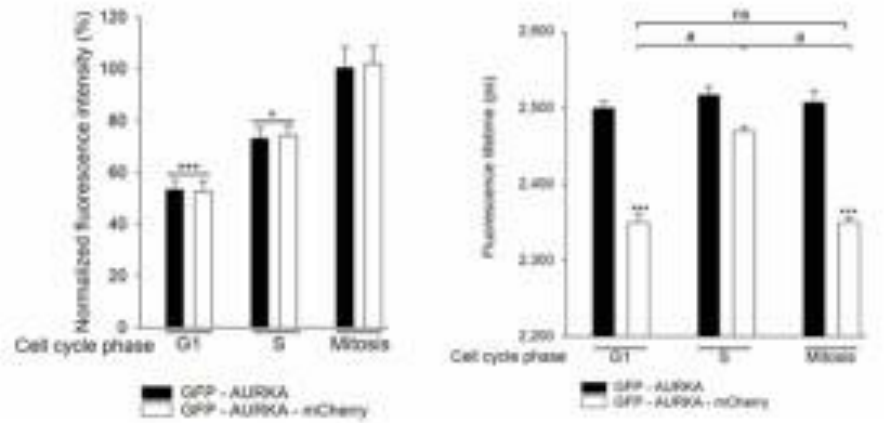
FAST FLIM
THE FASTEST TIME-DOMAIN FLIM PLATFORM

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

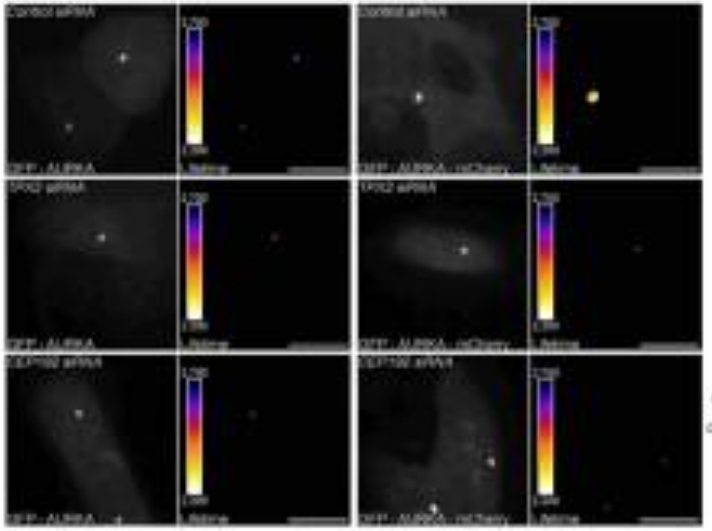


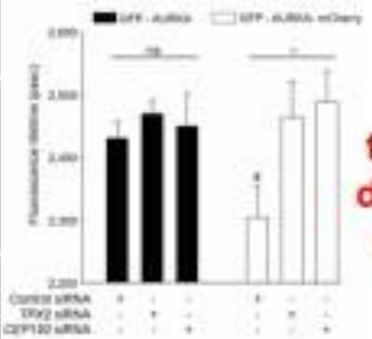


The AurkA biosensor shows phosphorylation-linked FRET



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

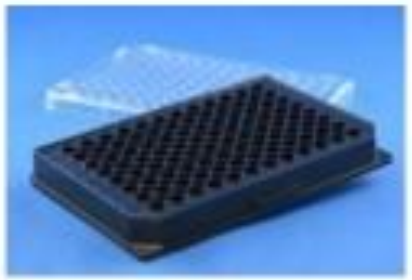
NEW METHODOLOGY

2 tools

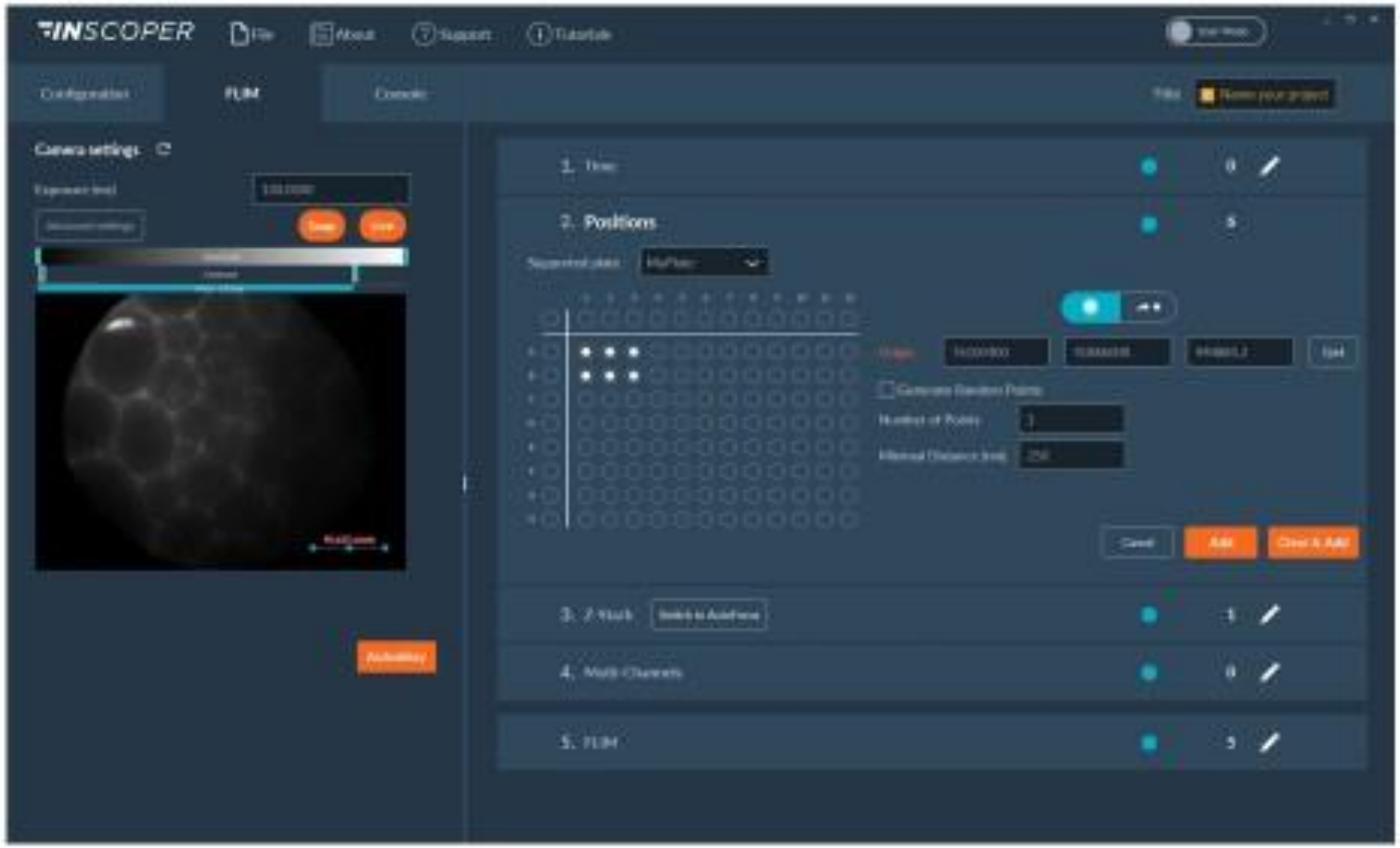
A sensor of AurkA activity in cells

A fast-flim microscope to perform rapidly FLIM

Performing an automated inhibitory drug assay

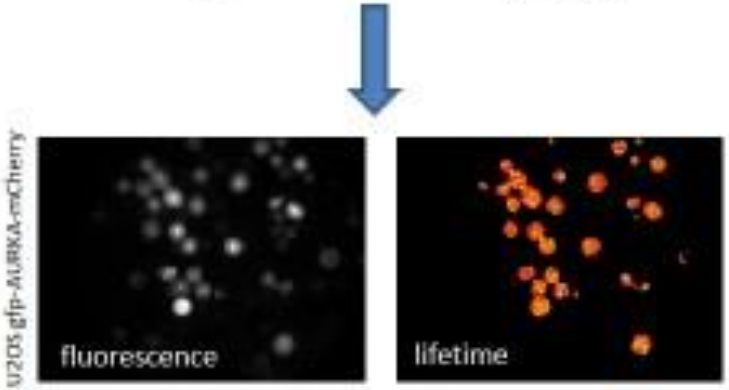


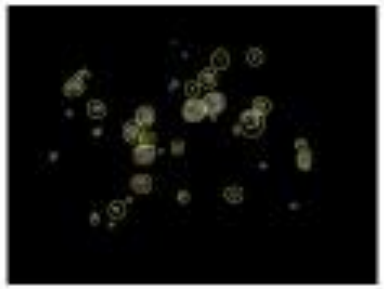
Performing a drug screening test



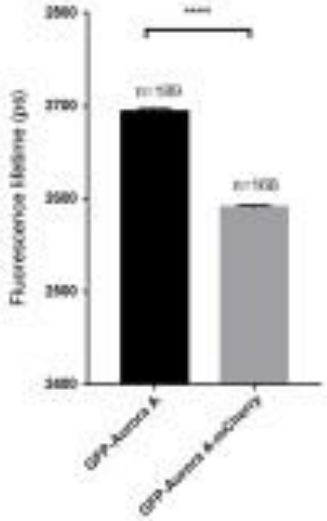
Analysis of data

Need to analyze hundred of images (up to 6000)



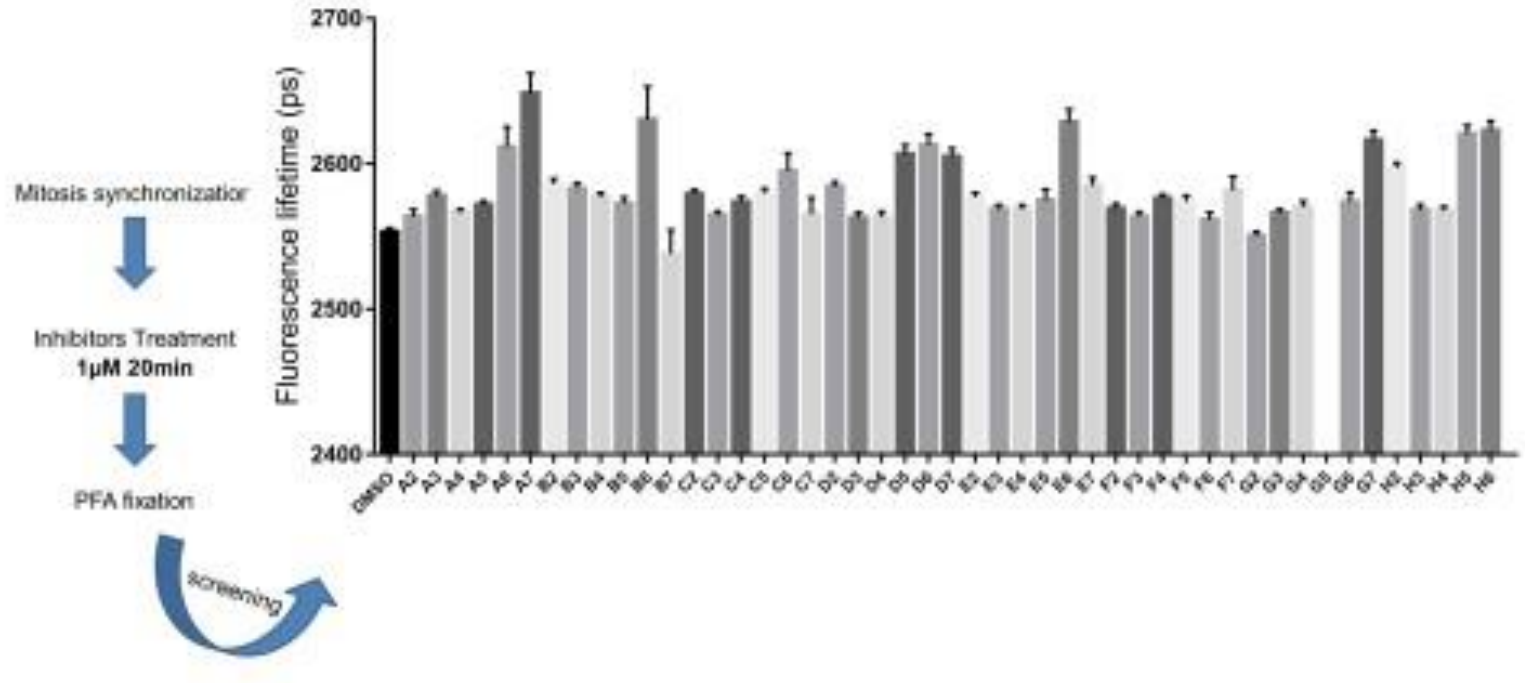


Cell segmentation



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 Pécréaux 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 plateform to monitor mitochondrial signature in the context of cancer progression
Vision of a tool for personalized medicine and drug design

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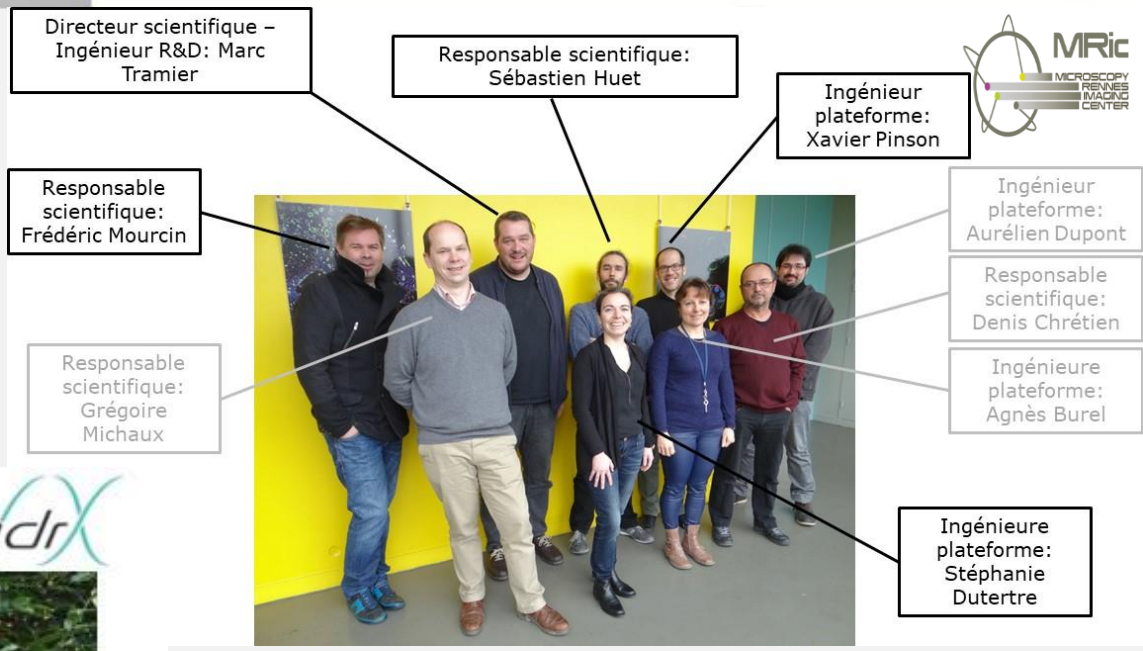
Institut Thématique Multi-Organismes
Technologies pour la santé

MFQ team at IGDR



Mric core facilities at UMS Biosit