

## ChemPhysBio2025 - Interdisciplinary Summer School

### Imaging Biology: Interdisciplinary Tools and Techniques to Unravel Cellular Dynamics

#### Hands-On Sessions – Overview

There will be **two hands-on sessions**, held on **Tuesday and Thursday afternoons**.

Participants are invited to **select and rank their top three preferences** from the list below.

**Detailed descriptions, practical information, and contact persons** for each hands-on session are provided in the following pages.

- Proteins recruitment after DNA damages on living cells, imaging and analysis
- From 2D single-particle tracking using Total Internal Reflection Fluorescence Microscopy to 6D tracking with digital holography
- Synthesis and Characterization of an Organic Fluorophore
- Monitoring of intracellular pH with a ratiometric biosenseur in living cells
- Following and modulating lipid dynamics in cell membrane
- Fluorescence Lifetime Imaging Microscopy
- Structured Illumination Microscopy
- Nanofluidic for diagnosis
- How to characterize a fluorescent probe? Focus on fluorescent proteins
- Super Resolution Imaging
- mesoSPIM (mesoscale selective plane illumination microscopy)

## **ChemPhysBio2025 - Interdisciplinary Summer School**

### **Imaging Biology: Interdisciplinary Tools and Techniques to Unravel Cellular Dynamics**

**Hands-on Title: Proteins recruitment after DNA damages on living cells,  
imaging and analysis**

**A few key words on methods:**

- **Probes: PARP2-GFP**
- **Biological question: PARP2 recruitment after DNA damage**
- **Instrument: Laser Scanning Confocal Microscope and FRAP technique**
- **Data Analysis: ImageJ Macro**

**Objectif and short description :**

- To understand, depending on the biological question, which imaging modality should be used.
- To know how to highlight and/or follow cellular processes/markers thanks to the different methods and techniques of light imaging
- To know the limits of the imaging techniques used

After DNA damages, an acquisition timelapse allow to measure the recruitment of PARP2 protein in the damaged region.

**Location (address and meeting point):** Institut Curie - Centre de Recherche sur le Centre universitaire - **Bâtiment 110** – Rue Henri Becquerel – 91401 Orsay

**Map on website:** <https://institut-curie.org/platform/curiecoretech-multimodal-imaging-center-uar2016-us43#tab-where-do-you-find-us>

**Contact:** Marie-Noëlle Soler – Tél: +33 1 69 86 31 30 (Office)/ +33 1 69 86 30 01 (Entrance)

**Site internet:** <https://curie.fr/plateforme/curiecoretech-centre-dimagerie-multimodale-uar2016-us43>

## ChemPhysBio2025 - Interdisciplinary Summer School

### Imaging Biology: Interdisciplinary Tools and Techniques to Unravel Cellular Dynamics

**Hands-on Title: From 2D single-particle tracking using Total Internal Reflection Fluorescence Microscopy to 6D tracking with digital holography**

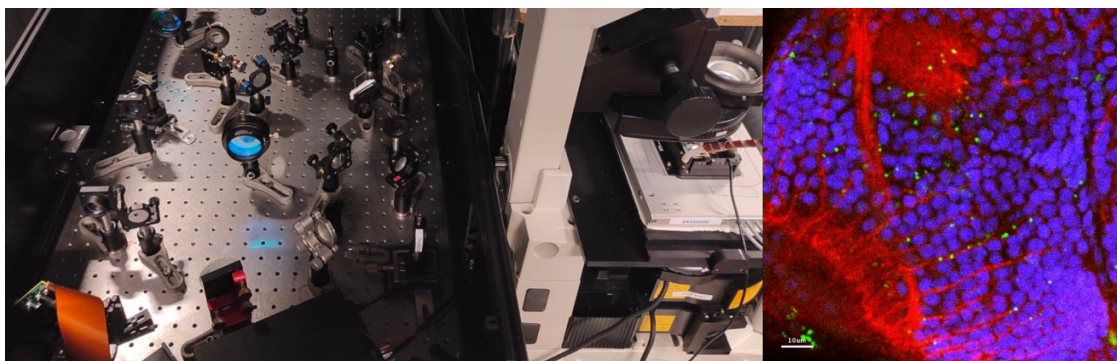
A few key words on methods:

- **Probes** : nanoparticles (fluorescent nano-diamonds or barium titanate NP)
- **Biological question** : intraneuronal transport
- **Instruments** : home-made TIRFM and two-photon microscopy device for single-particle holographic tracking
- **Data Analysis** : Modified Intraneuronal Nanoparticle Tracking (MINT)

**Objectif and short description :**

The goals of this labwork are

- Explain and show how TIRFM works
- Explain how holographic tracking works
- Acquire data from basic samples
- Introduce how we measure intraneuronal transport in cultured neurons
- Explain the analysis pipeline developed in the lab to extract pertinent parameters of this transport



Left: our set-up for holographic tracking. Right: non linear nano-particles (green) in axons (red) of zebra-fish larvae neurons (image from M. Frétaud, INRAE, Jouy en Josas)

**Location (address and meeting point):** ENS Paris-Saclay 4 avenue des Sciences 91190 Gif sur Yvette – wait in the main lobby.

**Contact :** [karen.perronet@ens-paris-saclay.fr](mailto:karen.perronet@ens-paris-saclay.fr) +33 1 81 87 55 87

## **ChemPhysBio2025 - Interdisciplinary Summer School**

### **Imaging Biology: Interdisciplinary Tools and Techniques to Unravel Cellular Dynamics**

#### **Hands-on Title: Synthesis and Characterization of an Organic Fluorophore**

##### **A few key words on methods:**

- Organic synthesis
- Purification
- Characterization
- Photophysical properties (absorbance, fluorescence)

##### **Objective and short description:**

In this practical course, we will look at the multiple steps in the process of synthesizing fluorescent molecules and analyzing their fluorescence properties.

The tutorial will comprise several stages:

- We will carry out the organic synthesis of a fluorophore using commercial molecules.
- The resulting compound will be further purified to ensure sufficient purity for photophysical characterization.
- The end of the course will be devoted to studying the photophysical properties (absorbance, fluorescence) of the isolated molecule.

##### **The goals:**

The objective of this practical work will be to raise awareness of the impact of chemical modifications on the fluorescence properties of molecules.

**Location (address and meeting point): ICSN (Bat 27). Access via the main gate of the campus, located 1 avenue de la Terrasse, Gif-sur-Yvette**

**Contact :** [arnaud.chevalier@cnrs.fr](mailto:arnaud.chevalier@cnrs.fr), Tel: 33 (0)1 69 82 30 58

**Site internet:** <https://icsn.cnrs.fr/>

## ChemPhysBio2025 - Interdisciplinary Summer School

### Imaging Biology: Interdisciplinary Tools and Techniques to Unravel Cellular Dynamics

#### Hands-on Title: **Monitoring of intracellular pH with a ratiometric biosenseur in living cells**

##### A few key words on methods :

- **Probes** : PHP sensor (derivative of GFP)
- **Biological question** : Measuring intracellular pH in *Sinorhizobium meliloti* bacteria to understand involvement of acidification in differentiated nitrogen-fixing bacteroids
- **Instrument** : Cytometer Cytoflex S
- **Data Analysis** : Cytexpert software and Rstudio

##### Objectif and short description (5-10 lines max or bullet points) :

The legume-rhizobium symbiosis is characterized by the formation of symbiotic root nodules, in which the bacteria differentiate into nitrogen-fixing bacteroids. In order to identify novel features of Terminal Bacteroid Differentiation (TBD), we tested on free-living bacteria and bacteroids undergoing TBD (*Sinorhizobium meliloti* in Medicago nodules) a novel genetically encoded ratiometric fluorescent biosensor (PHP, derived from GFP) to estimate intracellular pH. A complementary approach in flow cytometry and imaging makes it possible to calibrate the biosensor, and to combine the statistical power of cytometry and the resolving power at the tissue level of imaging.

##### Location (address and meeting point):

Bâtiment 21 - Avenue de la terrasse

91198 Gif sur Yvette cedex

Meeting point in the Hall of Building 21 (ground floor)

<https://g.co/kgs/XDzScjk>

**Contact** : mickael.bourge@i2bc.paris-saclay.fr

##### Site internet s'il existe :

<https://www.i2bc.paris-saclay.fr/bioimaging/cytometry/>

## **ChemPhysBio2025 - Interdisciplinary Summer School**

### **Imaging Biology: Interdisciplinary Tools and Techniques to Unravel Cellular Dynamics**

#### **Hands-on Title: Following and modulating lipid dynamics in cell membrane**

**A few key words on methods :** lipid biosensor-video microscopy-

- **Probes** : Lipid binding domain coupled to fluorescent proteins
- **Biological question** : How to follow and modulate phospholipid dynamics in cell membrane during cell stimulation ?
- **Instrument** : Spinning disk confocal microscopy (Imagerie-Gif platform)
- **Data Analysis** : Image analysis with metamorph software and ImageJ/Fiji

#### **Objectif and short description :**

**Aim:** Imaging phospholipid dynamic during stimulation of leukocytes

**Cells:** A leukocyte cell line (neutrophil-like) transfected by one or two lipid biosensors and expressing an inducible heterodimer system.

**Method :** 4D video microscopy will be performed in order to follow the localization of the lipid (phosphoinositide) biosensors at the cell membranes during the stimulation by phagocytosis. An inducible heterodimer system will be used to modify lipid membrane composition.

#### **Location (meeting point):**

Imagerie-Gif  
I2BC, UMS9198 CNRS bâtiment 21  
1 avenue de la Terrasse  
91198 Gif-sur-Yvette

## ChemPhysBio2025 - Interdisciplinary Summer School

### Imaging Biology: Interdisciplinary Tools and Techniques to Unravel Cellular Dynamics

**Hands-on Title: Monitoring the tension at the plasma membrane by  
Fluorescence Lifetime imaging Microscopy (FLIM) on HeLa cells.**

**A few key words on methods:**

- **Probes** : FlipTR
- **Biological question**: Investigating the tension at the plasma membrane can reveal the biological state of a cell, and also highlight responses such as stress or interaction processes. The use of an exogenous mecanosensor allows us to probe the tension state of a membrane at the cell or tissue level.
- **Instrument** : SP8X confocal microscope equipped with FALCON module
- **Data Analysis** : exponential fitting and Phasor plot analysis

**Objectif and short description (5-10 lines max or bullet points):**

During this workshop we will first learn about the fluorescence lifetime of molecules, its properties and how to measure it live on a microscope. We will use one control molecule to calibrate the instrument and then apply the membrane mecanosensor FlipTR on living HeLa cells. This biosensor has the advantage of changing its fluorescence lifetime as a function of the tension applied to the membrane. We will test different tension states and analyse the data in different ways to discuss about the advantages of the different approaches.

**Location (address and meeting point):**

Bâtiment 21 - Avenue de la terrasse

91198 Gif sur Yvette cedex

Meeting point in the Hall of Building 21 (ground floor)

<https://g.co/kgs/XDzScjk>

**Contact** : sandrine.lecart@i2bc.paris-saclay.fr

**Site internet s'il existe :**

<https://www.i2bc.paris-saclay.fr/bioimaging/light-microscopy-facility/>

## ChemPhysBio2025 - Interdisciplinary Summer School

### Imaging Biology: Interdisciplinary Tools and Techniques to Unravel Cellular Dynamics

#### Hands-on Title: **Investigation of endoplasmic reticulum-mitochondria contact sites using Structured Illumination Microscopy (SIM).**

##### A few key words on methods:

- **Probes:** HeLa cells labeled with Seipin-Alexa-488 and Mitotracker red
- **Biological question:** Is it possible to increase the comprehension of the lipid droplets biogenesis at membrane contact sites between mitochondria and ER using super-resolution microscopy?
- **Instrument:** Elyra7, Lattice SIM (Zeiss)
- **Data Analysis:** Zeiss Zen blue software for SIM processing

##### Objectif and short description :

From recent research, we currently know that mitochondria and ER are dynamic, interconnected and able to communicate. The ER is the major site for lipid biosynthesis and  $\text{Ca}^{2+}$  storage. The question is: are these organelles able to exchange ions, metabolites and lipids in the specific membrane contact sites named MAM for Mitochondria Associated ER Membranes. Is this peculiar environment the cradle for the lipid droplets biogenesis. To answer these questions, high resolution imaging is needed.

##### Location (address and meeting point):

Bâtiment 21 - Avenue de la terrasse

91198 Gif sur Yvette cedex

Meeting point in the Hall of Building 21 (ground floor)

<https://g.co/kgs/XDzScjk>

**Contact:** valerie.nicolas@i2bc.paris-saclay.fr

##### Site internet s'il existe :

<https://www.i2bc.paris-saclay.fr/bioimaging/light-microscopy-facility/>



## ChemPhysBio2025 - Interdisciplinary Summer School

### Imaging Biology: Interdisciplinary Tools and Techniques to Unravel Cellular Dynamics

#### Hands-on Title: Nanofluidic for diagnosis

**Objective and short description :** In the field of biomedical analysis, current macroscopic methods based on chromatography techniques coupled with mass spectrometry remain long, tedious and costly, which can prove detrimental for certain pathologies such as inborn errors of metabolism, where early and rapid diagnosis is often paramount. On-a-chip analytical methods are very promising, as analysis can be carried out in less than thirty minutes using a microliter of cerebrospinal fluid (CSF). By combining a new electrophoresis protocol, we have demonstrated that a “bar-coded” nanofluidic biochip can identify analytes in a matter of minutes by means of a unique spatio-temporal mapping of concentration focal points detected by fluorescence.

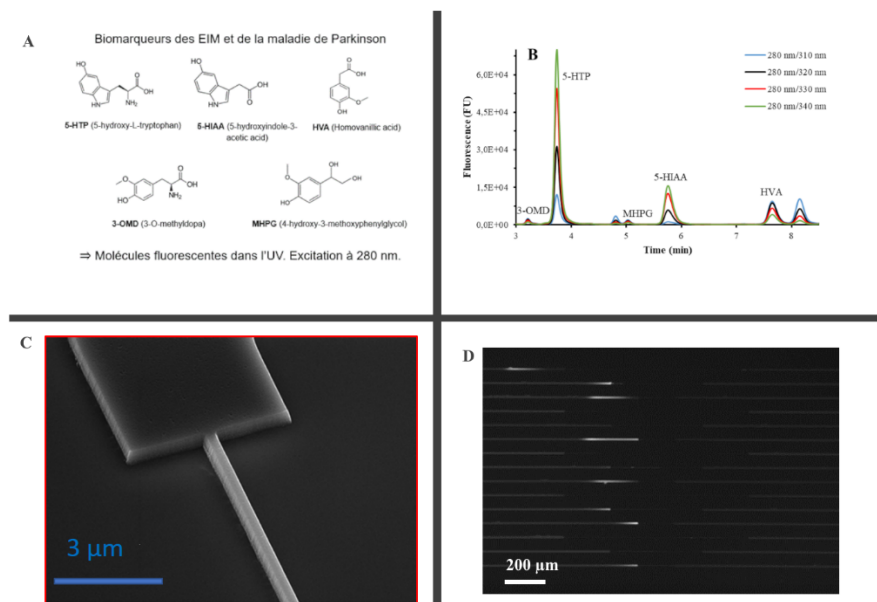


Figure: [A] biomarker structures [B] optimization of biomarker emission wavelengths [C] electron microscopy image of the mask bearing the nanochannels [D] UV-domain fluorescence image during electro-preconcentration of biomarkers spatio-temporal focal points of concentration.

**Location (address and meeting point):** ICP, bat 350, campus vallée

**Contact :** Antoine Pallandre [antoine.pallandre@universite-paris-saclay.fr](mailto:antoine.pallandre@universite-paris-saclay.fr)

**Website :** <https://www.icp.universite-paris-saclay.fr/capri/en/analytical-physical-chemistry/microfluidic-techniques-for-analysis/>

## **ChemPhysBio2025 - Interdisciplinary Summer School**

### **Imaging Biology: Interdisciplinary Tools and Techniques to Unravel Cellular Dynamics**

**Hands-on Title: Characterization of spectroscopic properties of a probe**

**A few keywords on methods :**

- **Probes : Fluorescent proteins**
- **Biological question: none**
- **Instrument : Spectrometer, time resolved fluorescence, wide field microscope**
- **Data Analysis : none**

**Objective and short description :**

- How to choose the right fluorescent protein for your experiment? What are the important parameters?
- Spectra acquisition et properties (absorption, fluorescence)
- Lifetime measurements
- Photobleaching observation and characterization (if possible)

**Location (address and meeting point): ICP, bat 350, campus vallée**

**Contact : Marie Erard [marie.erard@universite-paris-saclay.fr](mailto:marie.erard@universite-paris-saclay.fr)**

**Website :**

<https://www.icp.universite-paris-saclay.fr/cpsysbio/en/scientific-axes/fluorescent-proteins/>

## ChemPhysBio2025 - Interdisciplinary Summer School

### Imaging Biology: Interdisciplinary Tools and Techniques to Unravel Cellular Dynamics

**Hands-on Title: mesoSPIM (mesoscale selective plane illumination microscopy), an open-hardware microscopy platform dedicated to imaging of large cleared tissues**

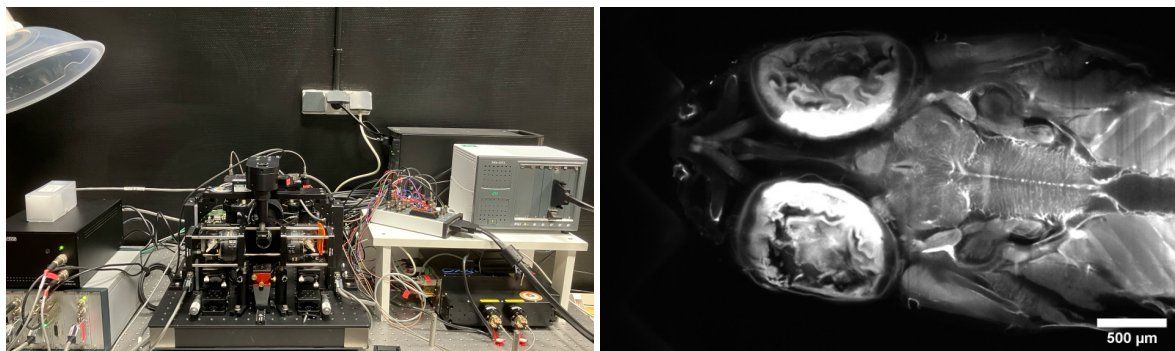
**A few key words on methods:**

- **Probes:** fluorescent proteins or organic dyes
- **Sample preparation:** clearing and expansion of biological tissue
- **Biological question:** Infectiology, inflammation, developmental and reproductive biology, as well as the study of metabolic, neurodegenerative, neuromuscular diseases, and cancer
- **Instrument:** open-source light sheet microscope
- **Data Analysis:** 3D reconstruction of the biological sample

**Objectif and short description :**

**The goals of this labwork are**

- Explain and show how mesoSPIM works
- Explain how to prepare biological samples (clearing and expanding)
- Acquire data from basic samples
- Show the 3D reconstruction and the cellular resolution of centime-sized samples



Left: mesoSPIM set-up. Right: image of a trout brain

**Location (address and meeting point):** ENS Paris-Saclay 4 avenue des Sciences 91190 Gif sur Yvette – wait in the main lobby or INRAE Jouy-en Josas

**Contact :** [karen.perronet@ens-paris-saclay.fr](mailto:karen.perronet@ens-paris-saclay.fr) +33 1 81 87 55 87 (if ENS)  
[Maxence.fretaud@inrae.fr](mailto:Maxence.fretaud@inrae.fr) +33 1 34 65 26 47 (if INRAE)