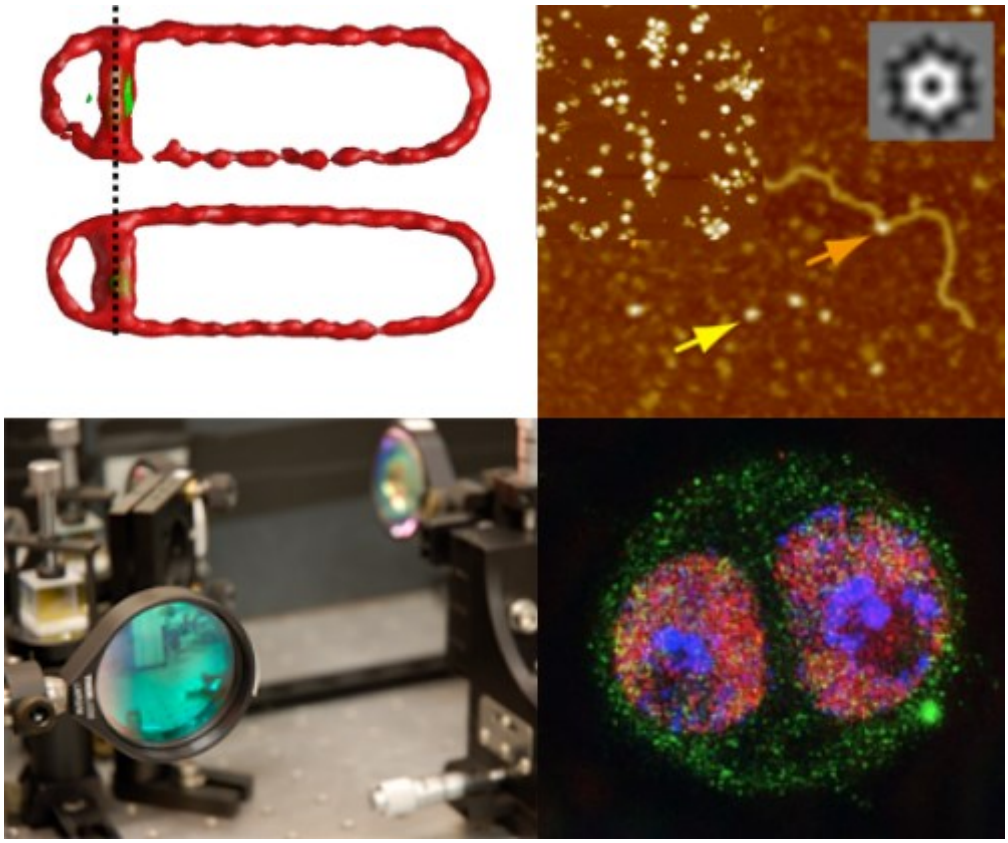
France BioImaging Advanced Training

FBIAT 2016



Montpellier, France
January 18th-22th 2016

We are very pleased to welcome you in Montpellier for the FBIAT 2016, and we hope that this event will foster your research and your use of quantitative cellular imaging techniques.

We would like to thank Dr. L. Journot for the support of the UMS BioCampus, and we are particularly indebted to Matthieu Richard for his help and availability during the organization of this event.

The organizers:

A. Abrieu; E. Bertrand; C. Favard; E. Margeat; P-E Milhiet; D. Muriaux; M. Nollmann

Program

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Sponsors

The organizing committee is grateful to the following sponsors for their financial support :

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Sponsors

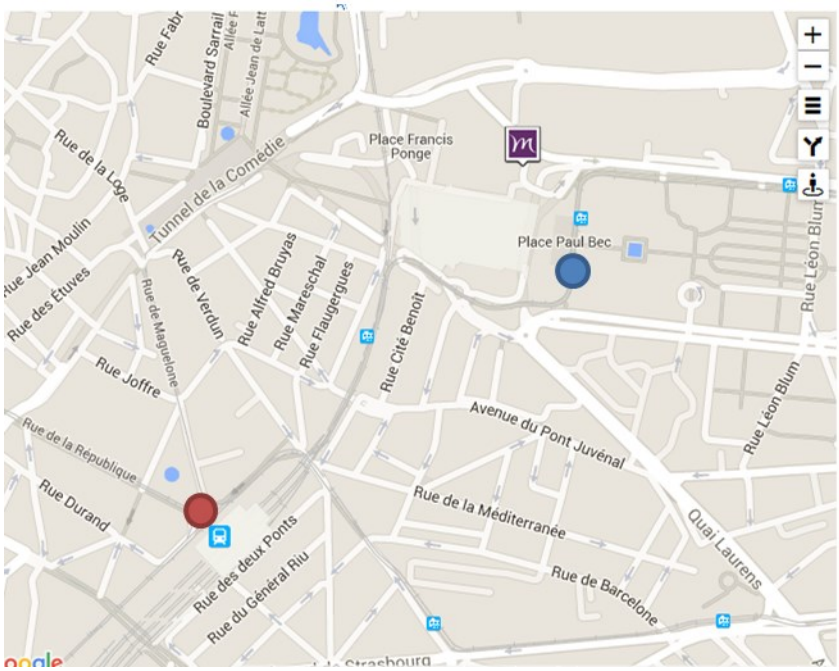
The organizing committee is grateful to the following companies for their contribution to the organization of the practical sessions and for lending microscopy set-ups:

Practical Sessions groups

MODULE	Practical Session Group					
	A		B		C	
1	Carlo	Beretta	Marta	Martin		
	Marie-Pierre	Blanchard	Marie	Pochitaloff		
	Patricia	Davidson	Benjamin	Lacroix		
	Théophile	Déjardin	Ruizhi	Tang		
	Simone	Granno				
2	Lydie	Boussicault	Mallory	Genest	Agnieszka	Płóciennikowska
	Dhiraj	Devidas Bhatia	Jia Hui	Li	Lucia	Rodríguez
	Valentin	Dunsing	Georgii	Nosov	Ton	Timmers
3	Laura	Breimann	Wenson David	Karunakaran	Ivanka	Kamenova
	Kaspar	Burger	Magdalena	Dziembowska	Alexandre	David
	Cyrille	Billaudeau	Carola	Fernandez	Miguel	Ruiz Torres
4	Julien	Burger	Vincent	Fraisier	Mickael	Lelek
	Nathalie	Campo	Mathieu	Ingouff	Antonios	Lioutas
	Kevin	Floc'h	Ksenia	Kudryashova	Kirti	Prakash
5	Nathalie	Barbier	Madlen	Luckner		
	Patricia	Bondia Raga	Fernando	Real		
	Abril	Escamilla Ayala	Béla	Varga		

Directions

City Center, Train Station, Hotel and Tram Line 1



Train station

Tram Line 1 stop : ANTIGONE

Hotel Mercure Montpellier Centre COMEDIE

Hotel Mercure Montpellier Centre COMEDIE
6 RUE DE LA SPIRALE
340000 MONTPELLIER - FRANCE
Téléphone : (+33)4/67998989 - Télécopie : (+33)0970064362
E-mail : H3043@accor.com

To reach your hotel from the train station or from the conference sites : Take the Tram Line 1, direction « Odysseum », and stop at « ANTIGONE ». After the stop, when the tracks turn right, you should turn left. Your hotel is just upstairs.

« Genopolys » Campus

To reach the « Genopolys » campus, you should take the Tram Line 1, Direction « Mosson », and stop at « Occitanie »

GENOPOLYS (G)

ENTRANCE : Use the small door (red arrow on the map) just across the « Occitanie » Tram Station, close to the « Institut de Genetique Humaine »

141 rue de la Cardonille, Montpellier

- Registration
- Plenary lectures (Monday to Thursday)
- Lunches (Tuesday to Thursday)
- Module Specific Lectures (Module 1, 2 and 5)
- Sponsor presentations

Centre de Biochimie Structurale (CBS)

29 rue de Navacelles, Montpellier

- Welcome Cocktail (Monday)
- Module Specific Lectures (Module 3)
- Practicals : 3.3, 4.2, 5.1, 5.2

MARS Research Platform (MARS)

INSERM, Delegation Regionale, 60 rue de Navacelles, Montpellier

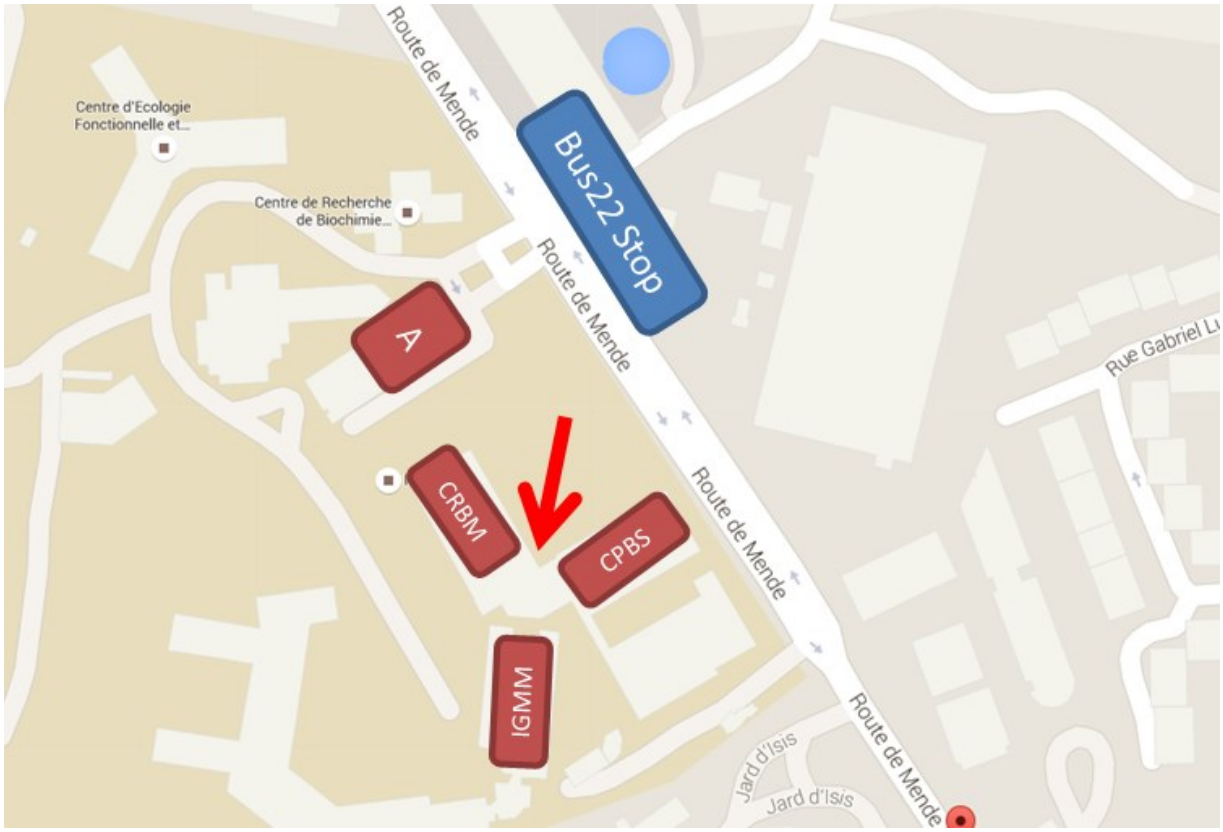
- Module Specific Lectures (Module 4)
- Practicals : 4.1, 4.3

MRI Platform (IGH)

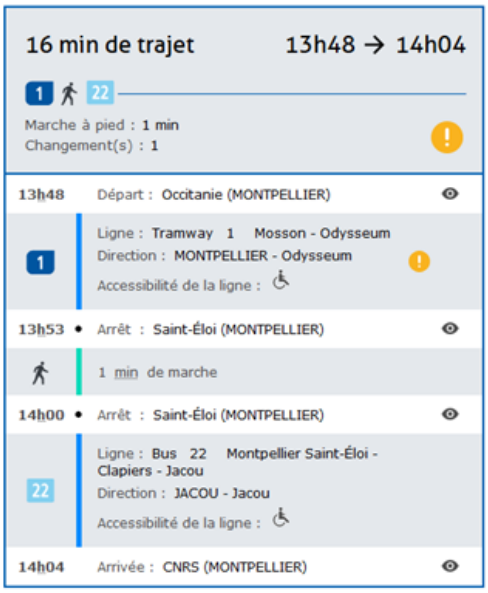
Institut de Génétique Humaine, Rue de la Cardonille, Montpellier

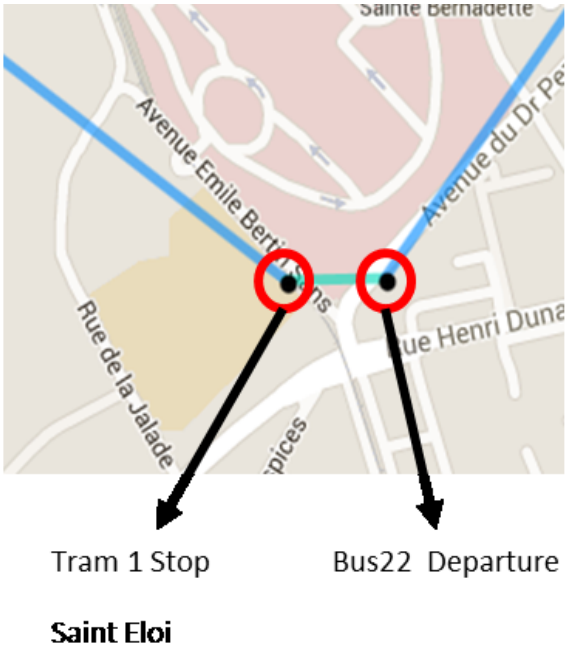
- Practical : 3.2

« CNRS » Campus



To reach the « CNRS » campus (1919 route de Mende, Montpellier), you should take the Tram Line 1, and stop at « Saint Eloi ». From there, take the bus 22 and stop after 4-5 minutes at « CNRS ». For those who need to be at the CNRS for the practical in the afternoon, here is the time table between Occitanie and CNRS (Tram + Bus) . Do not miss the bus at 2PM (14h) at Saint Eloi, since it runs only every 30 minutes.





CNRS Amphiteater (A)

- Plenary lectures (Friday)
- Lunch (Friday)

CPBS / CRBM building (entrance : red arrow)

- All Practical Sessions for Module 1 & 2
- Practical Sessions 3.1, 5.3, 5.4

Lunches and Cocktail :

Lunches, Opening Cocktail, and Coffee breaks will be provided by the catering service : "El Trovador", Montpellier

Dinner : “La Faluche” Restaurant

LA FALUCHE

40 Avenue Charles Flahault, 34090 Montpellier

04 67 04 81 16

Dinner at “La Faluche” is included in your registration from Monday to Thursday, for attendees and invited speakers.

However, **WE REQUEST THAT YOU SIGN-IN FOR DINNER AT THE REGISTRATION DESK. YOU SHOULD SIGN-IN BEFORE NOON, EVERY DAY YOU PLAN TO GO FOR DINNER AT LA FALUCHE**.

You have many other options for dinner in the City Center, closer to your hotel, but this is not included in the registration.

To reach “LA Faluche”, you should take the Tram Line 1, and stop at « Boutonnet ». From there, walk along the “Avenue Pierre d’Adhemar”, and turn right on the “Avenue Charles Flahault”. The restaurant is at 200m on your right, facing the “Faculté de Pharmacie”.

Monday January 18th

Venue : Genopolys

1PM : Registration

2:00 PM : Welcome address (E. Bertrand)

2:15 PM : Plenary Session

Chair : Delphine Muriaux (CPBS, Montpellier)

2:15-3:00PM **Dominique Bourgeois** *Institut de Biologie Structurale, Grenoble*

Fluorescent markers for superresolution microscopy

3:00-3:45PM **Lothar Schermelleh** *University of Oxford, UK*

Quantitative 3D super-resolution imaging of functional chromatin topology

3:45-4:30PM **Cendrine Faivre-Moskalenko** *Ecole Normale Supérieure de Lyon &* **Pierre-Emmanuel Milhiet** *Centre de Biochimie Structurale, Montpellier*

"Introduction to Atomic Force Microscopy and recent developments"

4:30 PM : Coffee and refreshments break

5:00 PM : Plenary Session

Chair : Cyril Favard (CPBS, Montpellier)

5:00-5:45PM **Christian Eggeling** *University of Oxford, UK*

Super-Resolution STED Microscopy

5:45-6:30PM **Michelle Digman** *University of California Irvine, USA*

Mapping and quantitatifying the oligomeric state of proteins in real-time

6:30-7:15PM **Edouard Bertrand** *IGMM, Montpellier*

Lighting up you protein of interest using genome-editing: a practical guide to the CRISP/Cas9 system

Venue : Centre de Biochimie Structurale

7:15PM : Cocktail Dinner

Tuesday January 19th

Venue : Genopolys

9:00 AM : Plenary Session

Chair : Emmanuel Margeat, (CBS Montpellier)

9:00-9:45AM **Maxime Dahan** *Institut Curie, Paris*

3D imaging in single molecule microscopy

9:45-10:30AM **Don Lamb** *LMU Munich, Germany*

Single particle tracking in the 21st century

10:30 AM : Coffee and refreshments break

11:00 AM : Module-specific session 1 (see below)

12:00 AM – 1:30 PM: Lunch

2:00 PM : Practical Session 1 (see below)

8:00 PM : Dinner at La Faluche (please register before noon)

Wednesday January 20th

Venue : Genopolys

9:00 AM : Plenary Session

Chair : Edouard Bertrand (IGMM, Montpellier)

9:00-9:45AM	**Maria Carmo-Fonseca** *Universidade de Lisboa, Portugal*
	Single-molecule imaging of mRNA biogenesis
9:45-10:05AM	**Commercial Presentation : Imagine Optics**
10:00-10:15AM	**Commercial Presentation : Physik Instrumente (PI)**

10:15 AM : Coffee and refreshments break

10:45 AM : Module-specific session 2 (see below)

12:30 AM – 1:30 PM: Lunch

2:00 PM : Practical Session 2 (see below)

8:00 PM : Dinner at La Faluche (please register before noon)

Thursday January 21th

Venue : Genopolys

9:00 AM : Plenary Session

Chair : Ariane Abrieu (CRBM, Montpellier)

9:00-9:45AM **Jeffrey Moffitt** *Harvard University, USA*

Imaging-based methods for single-cell transcriptomics

9:45-10:30AM **Laurent Blanchoin** *iRTSV, Grenoble*

Actin architecture and connectivity govern myosin contractile response

10:30 AM : Coffee and refreshments break

11:00 AM : Module-specific session 3 (see below)

12:00 AM – 1:30 PM: Lunch

2:00 PM : Practical Session 3 (see below)

8:00 PM : Dinner at La Faluche (please register before noon)

Friday January 22th

Venue : Amphitheatre du CNRS

9:00 AM : Plenary Session

Chair : Marcelo Nollmann (CBS, Montpellier)

9:00-9:45AM **Mathew Stracy** *University of Oxford, UK*

Single-molecule imaging of transcription, chromosome organization, and DNA repair in live bacteria.

9:45-10:30AM **Davide Mazza** *Milano, IT* – to be confirmed

TBA

10:30 AM : Coffee and refreshments break

11:00 AM : Plenary Session

11:00-11:45AM **Giacomo Cavalli** *Institut de Genetique Humaine, Montpellier*

3D Genome regulation by Polycomb proteins

11:45-12:30AM **ROUND TABLE**

12:30 AM – 1:30 PM: Lunch

2:00 PM : Data analysis session

Module 1 : Cytoskeleton dynamics

Specific sessions : Genopolys Amphiteater

Tuesday 11:00 AM : Module-specific session 1

11:00-11:45AM **Laurent Blanchoin** *iRTSV, Grenoble*

Imaging cytoskeleton dynamics. From single molecule to macroscopic organization

Wednesday 10:45 AM : Module-specific session 2

10:45-11:30AM **Laetitia Kurzawa** *iRTSV, Grenoble*

Investigating cellular force scaling

11:30-12:15AM **Sebastian Maurer** *CRG, Barcelone, Spain*

Microtubule plus end tracking and dynamic instability are mechanistically linked

Thursday 11:00 AM : Module-specific session 3

11:00-11:45AM **Dimitris Liakopoulos** *CRBM, Montpellier*

Analysis of microtubule regulation by kinesin

Practicals

Meeting point : Module 1 participants could meet at 13.45 at Genopolys to commute to the CRBM by car. Talk to Ariane Abrieu or Dimitris Liakopoulos. Alternatively, you can use the Tram Line (see p.8-9)

	GROUP	
	1A	1B
Tuesday	Practical 1.1	Practical 1.3
Wednesday	Practical 1.3	Practical 1.2
Thursday	Practical 1.2	Practical 1.1
Friday	Data analysis	

Practical 1.1 : Simultaneous reconstitution of actin and microtubule dynamics onto micropatterns – TIRF

CNRS Campus, (CRBM Basement - MRI Platform - Room 011A)

Practical 1.2 : Traction force microscopy (widefield) and Photoconversion (TIRF)

CNRS Campus, (CRBM Basement - MRI Platform - Room 011A)

Practical 1.3 : Single kinesin motily - TIRF

CNRS Campus, (CRBM Basement - MRI Platform - Room 011A)

Module 2 : Membrane dynamics

Specific sessions : Genopolys salle ESPACE

Tuesday 11:00 AM : Module-specific session 1

11:00-11:45AM **Erdinc Sezgin** *University of Oxford, UK*

Biophysical tools to investigate membrane domains

Wednesday 10:45 AM : Module-specific session 2

10:45-11:30AM **Christian Eggeling** *University of Oxford, UK*

Determining molecular mobility - from ensemble to super-resolution experiments

11:30-12:15AM **Michelle Digman** *University of California Irvine, USA*

Mapping Spatio-temporal proteins dynamics using the Raster Image Correlation Method (RICS)

Thursday 11:00 AM : Module-specific session 3

11:00-11:45AM **Jean-Baptiste Sibarita** *IINS, Bordeaux*

Deciphering Molecular Organization and Dynamics using Single-Molecule Localization Microscopy

Practicals

<u>Meeting point</u> : CRBM Hall, CNRS campus. Transfer by Tramway + Bus

	GROUP		
	2A	2B	2C
Tuesday	Practical 2.1	Practical 2.2	Practical 2.3
Wednesday	Practical 2.2	Practical 2.3	Practical 2.1
Thursday	Practical 2.3	Practical 2.1	Practical 2.2
Friday	Data analysis		

Practical 2.1 : spt-PALM on integrins proteins. (JB Sibarita, A. Beghin)

CNRS Campus, CEMIPAI underground floor, spt-PALM Nikon Microscope

Practical 2.2 : FCS and line FCS on model membranes and GPi proteins. (E. Sezgin, N., Yandrapalli, C. Favard)

CNRS Campus, CRBM Basement, MRI plateform, Leica TCS sp5 microscope

Practical 2.3 : Evaluation of Focal Adhesion dynamics with the Raster Image Correlation Spectroscopy (RICS) Analysis and N&B method (M. Digman)

CNRS Campus, CRBM Basement, MRI Plateform, Zeiss LSM 780 Microscope

Module 3 : RNA imaging and gene expression

Tuesday 11:00 AM : Module-specific session 1 - Centre de Biochimie Structurale

11:00-11:45AM **Edouard Bertrand and Emmanuel Margeat** *Montpellier*

Overview of the practical + Participants presentation

Wednesday 10:45 AM : Module-specific session 2

10:45-11:30AM *COMMON PRESENTATION WITH MODULE 4 – INSERM / MARS building*

Melike Lakadamyali *ICFO-Institute of Photonic Sciences, Spain*

Super-resolution imaging of nucleosome organization

11:30-12:15AM *Centre de Biochimie Structurale*

Nathalie Declerck *Centre de Biochimie Structurale, Montpellier*

Monitoring stochastic gene expression in live bacterial cells using two-photon fluorescence fluctuation microscopy

Thursday 11:00 AM : Module-specific session 3 - Centre de Biochimie Structurale

11:00-11:45AM **Florian Müller** *Institut Pasteur, Paris*

Studying transcriptional heterogeneity by single molecule imaging

Practicals

Meeting point : **Practical 3.1 : CRBM Hall, CNRS Campus**
Practical 3.2 & 3.3 : Genopolys lunch room

	GROUP		
	3A	3B	3C
Tuesday	Practical 3.1	Practical 3.2	Practical 3.3
Wednesday	Practical 3.2	Practical 3.3	Practical 3.1
Thursday	Practical 3.3	Practical 3.1	Practical 3.2
Friday	Data analysis		

Practical 3.1 : Fluctuations of transcriptional activity by live cell RNA imaging
CNRS Campus

Practical 3.2 : Counting of nascent and mature single mRNA by WF and SIM microscopy
Genopolys campus, Institut de Génétique Humaine

Practical 3.3 : Protein counting by N&B (C. Clerte)
Centre de Biochimie Structurale (CBS)

Module 4 : DNA organization in eukaryotes and bacteria

Specific sessions : INSERM/MARS building across the street from Genopolys

Tuesday 11:00 AM : Module-specific session 1

11:00-11:45AM **Maxime Dahan** *Institut Curie, Paris*

Finding a needle in a haystack: the target search of DNA-binding proteins in mammalian cells

Wednesday 10:45 AM : Module-specific session 2

10:45-11:30AM **Melike Lakadamyali** *ICFO-Institute of Photonic Sciences, Spain*

Super-resolution imaging of nucleosome organization

11:30-12:15AM **Lothar Schermelleh (Oxford University, UK) & Antoine Le Gall (CBS, Montpellier)**

Nitty-gritty of 3D-SIM : Short talks and round table

Thursday 11:00 AM : Module-specific session 3

11:00-11:45AM **Mathew Stracy (Oxford University, UK), Jeff Moffitt (Harvard) & Jean-Bernard Fiche (CBS Montpellier)**

Nitty-gritty of SMLM : Short talks and round table

Practicals

Meeting point : **INSERM/MARS building across the street from Genopolys**

	GROUP		
	4A	4B	4C
Tuesday	Practical 4.1	Practical 4.2	Practical 4.3
Wednesday	Practical 4.2	Practical 4.3	Practical 4.1
Thursday	Practical 4.3	Practical 4.1	Practical 4.2
Friday	Data analysis		

Practical 4.1 : 3D-SIM imaging
INSERM Delegation – MARS Platform

Practical 4.2 : 3D STORM/PALM imaging using adaptive optics
Centre de Biochimie Structurale

Practical 4.3 : spt-PALM
INSERM Delegation – MARS Platform

Module 5 : Structure and dynamics of viral components

Specific sessions : Genopolys salle HORIZON

Tuesday 11:00 AM : Module-specific session 1

11:00-11:45AM **Pierre-Emmanuel Milhiet** *CBS, Montpellier*

Introduction to atomic force microscopy on virus

Wednesday 10:45 AM : Module-specific session 2

10:45-11:30AM **Delphine Muriaux** *CPBS, Montpellier*

Introduction of the biological topic and description of viral sample preparations dedicated to fluorescence imaging and atomic force microscopy

11:30-12:15AM **Hugues de Rocquigny** *Laboratoire de Biophotonique et Pharmacologie, Illkirch*

Protein-protein interaction followed by FRET using Fluorescent Lifetime Imaging Microscopy

Thursday 11:00 AM : Module-specific session 3

11:00-11:45AM **Christian Sieben** *EPFL, Lausanne, Switzerland*

Introduction into Single Molecule Localization Microscopy

Practicals

Meeting point : - Tuesday, Wednesday : Centre de Biochimie Structurale, Genopolys Campus
- Thursday, Friday : CRBM Hall, CNRS Campus

	GROUP	
	5A	5B
Tuesday	Practical 5.1	Practical 5.2
Wednesday	Practical 5.2	Practical 5.1
Thursday	Practical 5.3	Practical 5.4
Friday	Practical 5.4	Practical 5.3

Practical 5.1 : Morphogenesis of viruses by AFM imaging (by P.E. Milhiet and C. Godefroy))

Centre de Biochimie Structurale

Practical 5.2 : Nanomechanical properties of Gag-VLP by AFM: imaging and force spectroscopy (by C. Moskalenko and D.Muriaux)

Centre de Biochimie Structurale

Practical 5.3 : Budding of HIV-1 Gag in intact cells using TIRF/PALM microscopy (by C. Marini and C. Sieben)

CNRS Campus, CRBM

Practical 5.4 : Assembly and budding of HIV-1 Gag VLP in intact cells using TIRF/PALM microscopy (by H. de Rocquigny and D.Muriaux)

CNRS Campus, CRBM

LIST OF ABSTRACTS

ABSTRACTS

Monday January 12th : Plenary Lectures

Fluorescent markers for superresolution microscopy

Dominique Bourgeois

Institut de Biologie Structurale J-P. Ebel, 71 Avenue des Martyrs, 38044 GRENOBLE

Super-resolution fluorescence microscopy ("nanoscopy") has emerged as a revolutionary tool to investigate biological samples at the nanoscale by surpassing the diffraction barrier. When setting up a super-resolution project, a first highly critical point is the choice of suitable fluorescent markers. Indeed, beyond live-cell compatibility, labeling specificity, preservation of target biological function, minimization of cyto- and photo-toxicity, suitable emission color and maximization of fluorescence brightness, all nanoscopy techniques fundamentally rely on adequate manipulation of the highly complex photophysical properties of the markers. In this lecture, I will review the different types of fluorescent markers available for super-resolution microscopy, and list their main advantages and drawbacks. I will put particular emphasis on the so-called "phototransformable" fluorescent proteins which in a large number of cases remain the markers of choice since they allows genetic encoding and thus extreme specificity of labeling, notably in live cells. I will discuss the molecular mechanisms of these fluorescent proteins that drive critical phenomena such as photoactivation, photoconversion, photoswitching, photoblinking and photobleaching.

Quantitative 3D super-resolution imaging of functional chromatin topology

Lothar Schermelleh

Micron Oxford Advanced Bioimaging Unit, Department of Biochemistry, University of Oxford

Genome function occurs within the context of a polymorphic three-dimensional (3D) chromatin landscape, composed of macromolecular structures ranging from tens to hundreds of nanometers in size – below the resolution limit of conventional light microscopy. Super-resolution 3D structured illumination microscopy (3D-SIM) allows multicolour imaging of entire cells with eight-fold increased volumetric resolution, and is uniquely well-suited to study the topological organization of mammalian cell nuclei.

In my talk I will present our recent studies using 3D-SIM, which reveal organizational principles of active versus inactive X chromosomes, and the spatial relationships of both established and novel interactors of the long non-coding Xist RNA, the main effector of X inactivation. Furthermore, I will introduce our current efforts in systematic analysis of functional chromatin topology on the scale of Mb-sized domains. These data highlight the power of 3D-SIM to both analyse the spatial and temporal relationships of nuclear factors in single cells, and to map these relationships onto the context of the 3D chromatin landscape.

"Introduction to Atomic Force Microscopy and recent developments"

Cendrine Faivre-Moskalenko(1) and Pierre-Emmanuel Milhiet(2)

(1) : Laboratoire de Physique, Ecole Normale Supérieure de Lyon, CNRS UMR 5672, Université de Lyon; (2): Centre de Biochimie Structurale, INSERM U1054, CNRS UMR5048, Université Montpellier;

In this talk we review basic principles of Atomic Force Microscopy imaging and nano-mechanics. We present examples of high resolution AFM imaging in membrane biophysics, chromatin structure and dynamics, as well as mechanical measurements on cells and viruses. In addition, we highlight some recent developments in AFM imaging techniques such as high speed AFM, or coupling AFM with single molecule localization microscopy.

Super-Resolution STED Microscopy

Christian Eggeling

MRC Human Immunology Unit and Wolfson Imaging Centre Oxford, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, Headley Way, OX3 9DS, United Kingdom

Far-field fluorescence microscopy is a non-invasive and very sensitive analysis technique, allowing for the disclosure of complex biological systems. However, prominent problems in biology or elsewhere can often not be solved due to the limited resolution of conventional optical microscopy. A remedy to this physical limit is the reversible inhibition of fluorescence, ensuring that the measured signal stems from a region of the sample that is much smaller than these 200 nm. Examples of such optical super-resolution microscopes or nanoscopes include STED, RESOLFT or STORM/PALM microscopy, where the inhibition of fluorescence is realized by stimulated emission, photoswitchable fluorophores, or dark state transitions. These developments in fluorescence far-field microscopy have realized imaging of cells or other materials with a spatial resolution far below the diffraction limit. We present the implementation of optical nanoscopy for novel insights into cellular mechanisms.

Mapping and quantitatifying the oligomeric state of proteins in real-time

Michelle A. Digman

Michelle A. Digman, University of California Irvine, Department of Biomedical Engieering

The separation of the brightness and the number of molecules in every pixel of a raster scan image is shown as well as example of determination of brightness of protein aggregates. In microscopy images a pixel with a given intensity could contain few bright molecules or many dim molecules. We developed an analysis, termed the Number and Molecular Brightness (N&B) method, that is capable of distinguishing between these two scenarios. This analysis is based on the intensity fluctuations measured at each pixel of an image sequence. The N&B (molecular number and brightness analysis) is complementary to the RICS (Raster-scan Image Correlation Spectroscopy) analysis. It provides pixel resolution information about the number and size of protein aggregates in images of live cells independently of the diffusion of the molecule. We used this analysis to show that Paxillin, a protein found at focal adhesions, exists as a monomer in the cytosol and forms relatively large aggregates at focal adhesions. At these adhesions, paxillin is relatively immobile, and it slowly exchange with the surrounding population. The N&B analysis of growing adhesions show that paxillin adds to the adhesion in the form of monomers. The B (brightness) map obtained during this phase is relatively constant except at the adhesions where B tends to the limiting value of 1, as expected for

the immobile fraction. When the adhesion disassembles, large fluctuations are detected. The N&B analysis shows that at the disassembling edge of the adhesions there are fluctuations due to paxillin clusters of about 5 to 12 monomers. The observation of these clusters is transient and it is predominant in the initial phase of disassembling. The focus of this talk will be to encourage researchers imaging on commercial laser scanning microscopes and who are interested in protein aggregation to exploit this type of analysis.

Lighting up you protein of interest using genome-editing: a practical guide to the CRISP/Cas9 system

Marie-Cécile Robert, Viviana Lamberti, Serena Capozi, Frédéric Lionneton and Edouard Bertrand

Institut de Génétique Moléculaire de Montpellier; CNRS and University of Montpellier, France

Genome editing has been revolutionized with the advent of CRISPR/Cas9 and related systems, and these techniques indeed provide a very convenient way to tag your protein of interest directly at the genomic level. In this talk, we will provide a set of practical guidelines to help designing and performing a knock-in experiment in mammalian cell lines.

Tuesday January 13th : Plenary Lectures

3D imaging in single molecule microscopy

Maxime Dahan

Laboratoire Physico Chimie, Institut Curie

Only a few years after its inception, single molecule imaging has become widely employed in biological studies, with applications ranging from dynamic tracking to localization-based super-resolution microscopy. Traditionally, the detection of individual emitters in vitro or in vivo is achieved with total internal reflection microscopy (TIRF). Yet, TIRF is limited to 2D imaging of molecules in the cell membrane. Since most biological systems are three-dimensional, accessing the 3D localization of single molecules is an important goal but it still raises many challenges. To address these challenges, optical and computational techniques for 3D single molecule imaging have been developed. They include engineering of the point-spread function, multiplane imaging, interferometric detection or light-sheet imaging. Here I will review the principles of these techniques, illustrate their results, and discuss their benefits and limitations.

Single particle tracking in the 21st century

Don Lamb

Physical Chemistry, Department of Chemistry, LMU Munich, Germany

The ability to follow individual objects provides a myriad of information with respect to that object. If we can follow a particle with high temporal and spatial resolution, we know its location with high precision and hence can determine its mobility, interaction partners etc., as it goes about performing its daily

routine. For hundreds of years, single particle tracking (SPT) was used to follow the planets leading to the laws of gravitation. Today, it is possible to follow single viruses and even individual proteins in biological systems with nm-accuracy and ms-time resolution.

In this lecture, I will discuss the basic principles of SPT. The first step is to follow the particle in two or three dimensions, which can be done using different microscopy methods. From these measurements, we can extract a trajectory of the particle as a function of time. From the trajectory, we can determine what type of diffusional behavior the molecule is undergoing via, for example, determination of the mean-squared-displacement (MSD).

To exemplify what can be done via SPT, I will give three from research within my group:

As a first example, I will discuss the tracking of individual nascent HIV particles during the assembly process. Using a combination of wide-field and TIRF microscopy, we could follow the assembly of viruses from initiation to release in two-dimensions.

In a second example, we have used z-stacking of confocal images to follow the fusion process in foamy viruses in three dimensions. Using analysis methods developed in my group, we could detect a novel intermediate state during this fusion process where the envelope and capsid separate but are still attached.

As a last example, I will discuss the principle of the orbital tracking microscope and show how we use it to follow the transport of mitochondria in living zebra-fish embryos. Thereby, we could detect transport processes that were not observable previously. In the 21st century, it is now possible to follow individual objects with nm-accuracy in three dimensions in real time in living organisms, which opens a whole new world for scientific investigation.

Wednesday January 14th: Plenary Lecture

Single-molecule imaging of mRNA biogenesis

Maria Carmo-Fonseca

Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Portugal

Expression of genetic information in eukaryotes involves a series of interconnected processes that ultimately determine the quality and amount of proteins in the cell. Many individual steps in gene expression are kinetically coupled, but tools are lacking to determine how temporal relationships between chemical reactions contribute to the output of the final gene product. In our lab, we developed a strategy that permits direct imaging of single pre-mRNA molecules in live cells. The approach consists in genetically inserting the binding sites for bacteriophage proteins in the RNA of interest. Insertion of the binding sites in the terminal exon of reporter genes reveals kinetic properties of the entire mRNA life cycle, from transcription to transport in the nucleus and export to the cytoplasm. Using intronic insertions to track the dynamics of splicing, we found that splicing can occur much faster than previously proposed (1, 2).

1 Martin, Rino et al. Cell Rep. 2013

2 Rino et al Methods Enzymol. 2015

Thursday January 15th : Plenary Lectures

Imaging-based methods for single-cell transcriptomics

Jeffrey R. Moffitt (1), Kok Hao Chen (1), Alistair N. Boettiger (1), Siyuan Wang (1), and Xiaowei Zhuang (1,2)

(1): Howard Hughes Medical Institute, Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA USA 02138;

(2): Department of Physics, Harvard University, Cambridge, MA USA 02138;

The spatial organization of the transcriptome, where individual mRNAs reside within the cell, has emerged as a powerful modulator of post-transcriptional fate. Single-molecule fluorescence in situ hybridization (smFISH) has been an instrumental tool in the study of both the spatial distribution and copy number of individual RNAs, yet the application of this technique to systems-level questions has been limited by its extremely low throughput. Here I present two approaches to extend smFISH to the transcriptome scale in both bacteria and mammalian systems by exploiting recent advances in massively parallel DNA synthesis. The later approach—multiplexed, error-robust FISH (MERFISH)—can accurately measure the number and spatial localization of hundreds to thousands of mRNA species simultaneously in hundreds of single cells in culture or tissue. This technique promises to be an exciting complement to sequencing-based approaches to single-cell transcriptomics.

Actin architecture and connectivity govern myosin contractile response

Hajer Ennomani1, Gaëlle Letort1, Christophe Guérin1, François Nédélec3, Enrique De La Cruz4, Manuel Théry1,2 and Laurent Blanchoin1

1 Institut de recherches en Technologies et Sciences pour le vivant, Laboratoire de Physiologie Cellulaire et Vegétale, CNRS/CEA/UJF/INRA Grenoble, France

2 Unité de thérapie Cellulaire, Hopital Saint-Louis, Avenue Claude Vellefaux 75010 Paris

3 Cell Bi

Cellular contractility – the internal generation of force or tension by a cell orchestrated by the actomyosin machinery – has emerged as a critical regulator of a wide range of processes during development. Tremendous efforts have deepened our understanding of the biochemical and mechanical properties of the actomyosin contractile units encountered in the complex cell environment. Yet, how the structural organization of actin filaments regulates myosin-driven contraction remains to be established.

We used the micropatterning method that enables the spatial control of actin nucleation sites for in vitro assays developed by Reymann et al., 2010. These actin templates were used to evaluate the response of oriented actin structures to myosin-induced contractility. We demonstrated that crosslinking level modulates the myosin-induced deformation of actin networks according to their architecture. We showed also that crosslinkers are necessary to sustain myosin-driven deformation and force production of dynamic actin networks. In addition, we developed numerical simulation in order to relate the observed myosin-driven actin deformation with the underlying microscopic mechanism.

The fact that we were able to tune myosin-driven deformation of minimal reconstituted actin networks,

for which we control the actin organization and dynamics, demonstrates that our study captures fundamental rules coordinating myosin-mediated contractility and force production.

Friday January 16th : Plenary Lectures

Single-molecule imaging of transcription, chromosome organization, and DNA repair in live bacteria.

Mathew Stracy (1), Pawel Zawadzki (2), Achillefs N. Kapanidis (3)

(1) Biological Physics Research Group, Clarendon Laboratory, Department of Physics, University of Oxford, Oxford OX1 3PU, United Kingdom.

(2) Department of Biochemistry, University of Oxford, Oxford OX1 3QU, United Kingdom

Protein-DNA interactions are critical to many important biological functions, from transcription and chromosome organization, to DNA replication and repair. Using photoactivated single-molecule tracking in live bacteria allows proteins specifically bound to DNA to be distinguished from mobile proteins, offering a new perspective on the behaviour of DNA-binding proteins, from the molecular level to the length scale of whole bacterial cells. We demonstrate how we have applied this technique to answer key questions in transcription, chromosome organisation, and DNA repair in Escherichia coli.

Discriminating transcribing RNA polymerase (RNAP) molecules from the rest of the population showed that low levels of transcription can occur throughout the nucleoid, but clustering analysis and 3D Structured Illumination Microscopy (SIM) showed that dense clusters of transcribing RNAPs form at the nucleoid periphery, indicating a movement of gene loci out of the bulk of DNA as levels of transcription increase.

We further study the formation and DNA-binding behaviour of the proteins initiating the nucleotide excision repair pathway, UvrA and UvrB. Analysing the mobility of UvrA in vivo showed that it interacts with DNA to locate lesions independently of UvrB. UvrA is rarely complexed to UvrB in solution; instead UvrA recruits UvrB to DNA only after initial damage recognition.

TBA

Davide Mazza

Milano, IT

TBA

3D Genome regulation by Polycomb proteins

Giacomo Cavalli

Institute of Human Genetics, CNRS. 34396 Montpellier Cedex 5, France and Chromatin and Cell Biology lab, University of Montpellier, Montpellier, France

Polycomb Group (PcG) and trithorax group (trxG) proteins form multimeric protein complexes that regulate chromatin via histone modifications and modulation of nucleosome remodeling activities, targeted to specific cis-regulatory elements named PcG response elements (PREs). However, they can also dynamically bind to other genes and affect cell proliferation and differentiation in a wide variety of biological processes.

In addition to silencing the genes flanking their genomic target sites, PcG proteins play a role in nuclear organization by forming large chromosomal domains and nuclear foci which are the physical sites of Polycomb-mediated silencing. Moreover, endogenous PcG target loci form chromosomal domains that can frequently colocalize in the cell nucleus. These contacts depend on Polycomb proteins and stabilize epigenetic gene silencing. The ability to form chromosomal domains, also defined as TADs, is a fundamental feature of the genome and involves not only Polycomb but also other chromatin components. Using Polycomb as a paradigm, we are currently studying the determinants of chromosome architectural features. Here, I will present our progress in this direction.

Module 1 : Cytoskeleton dynamics

Imaging cytoskeleton dynamics. From single molecule to macroscopic organization

Laurent Blanchoin

Cytomorpho lab, Laboratoire de Physiologie Cellulaire et Végétale UMR 5168, CEA Grenoble, 17 Rue des Martyrs, Grenoble, France

The organization of cytoskeleton elements into higher-ordered structures governs eukaryotic cell shape and movement. Global cytoskeleton network size and architecture is maintained in a dynamic steady state through regulated assembly and disassembly. We have developed a micropatterning method that enables the spatial control of assembly sites for in vitro assays (Reymann et al., Nat Mat, 2010; Portran et al., Cytoskeleton, 2013). Recently, we updated this method using laser micropatterning to geometrically-control actin assembly in both 2D and 3D (Galland et al., Nat Mat 2013). We will present how these new techniques can be used in parallel with microfluidics (Schaedel et al., Nat Mat 2015) and/or total internal reflection microscopy (Gressin et al., Curr. Biol., 2015) to study the dynamic of the cytoskeleton from the single molecule to the formation of macroscopic organization.

Investigating cellular force scaling

Laëtitia Kurzawa (1), Timothée Vignaud (2), Ben Fogelson (3), Alex Mogilner (4), Laurent Blanchoin (5), Manuel Théry (6)

(1), (2), (5), (6): Cytomorpholab, BIG, CEA (3) et (4): Courant Institute

and Department of Biology, New York University

Cells have the remarkable ability to sense geometrical and physical cues from their environment and adapt their architecture accordingly. This process requires a tight regulation of the permanent remodeling actomyosin network, that can both transmit and generate intra-cellular forces. An important challenge in understanding cell mechanics is to establish the mechanisms controlling the interplay between actin dynamics, cytoskeleton architecture and mechanical properties of the cells. In this context, we are studying the scaling of contractile force magnitude in stress fibers and investigating the role of actin network dynamics and architecture in this process.

By combining the use of micropatterned substrates and traction force microscopy, we were able to control the length and spatial organization of stress fibers in adherent cells and measure the traction forces they produce on deformable substrates. Thereby, we demonstrated that force scaling exhibit a biphasic behavior. We were also able to retrieve the contractile energy contained in stress fibers following photoablation experiments with a UV-pulsed laser and to study the dynamics and turnover of molecular components of stress fibers by performing targeted illumination experiments. This presentation will emphasize how we can combine all this imaging tools toward a molecular understanding of cell mechanics.

Microtubule plus end tracking and dynamic instability are mechanistically linked

Sebastian Maurer

(1)Centre for Genomic Regulation (CRG), Dr. Aiguader 88, 08003 Barcelona, Spain

(2)Universitat Pompeu Fabra (UPF), Barcelona, Spain

The microtubule cytoskeleton is of crucial importance for many essential cellular functions such as establishing cell morphology, intracellular transport, chromosome segregation during cell division, and cell motility. Failures in these processes can result in promotion of cancer and developmental diseases.

During microtubule assembly GTP is hydrolysed; this reaction is ultimately responsible for the property of microtubules to randomly switch between phases of growth and shrinkage, a behaviour termed dynamic instability. It is essential for the ability of microtubules to explore intracellular space, to rapidly reorganize their distribution and to contribute to generating the pushing and pulling forces that move chromosomes during cell division. Critical decisions between microtubule assembly and disassembly take place at microtubule ends. End-Binding proteins (EBs) bind there to a specialised region. EBs recruit a variety of other factors, thereby constituting the core of a versatile protein interaction network, which allows the cell to shape and control their microtubule cytoskeleton.

To better understand how EBs interact with microtubule ends, we examined the structural characteristics of this interaction. Using cryo-electron microscopy, sub nanometre single-particle reconstruction, and high-precision fluorescence microscopy imaging, we discovered that EBs bridge microtubule protofilaments at a position that is right next to the beta-tubulin GTPase site. That is of interest, because this binding site positions them ideally to sense GTP hydrolysis. We further found that the region recognized by EBs functions as a stabilizing structural cap which protects microtubules from disassembly, thus allowing them to have extended episodes of continuous growth. Finally we show that EBs catalyse the formation of the mature GDP-lattice which leads to a reduction of the structural cap. Taken together, our findings establish a structural link between two important biological phenomena, microtubule dynamic instability and microtubule end tracking by EBs.

Analysis of microtubule regulation by kinesin

Anneke Hibbel (1), Hauke Drechsler (2), Erik Schäfer (3), Dimitris Liakopoulos (4), Jonathon Howard (5)

(1): Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany; (2): Mechanochemical Cell Biology Building, Warwick Medical School, The University of Warwick, UK; (3): ZMBP, Eberhard-Karls-Universität Tübingen, Germany; (4): CRBM/CNRS

Regulation of microtubule length requires feedback to set the balance between growth and shrinkage. Whereas negative feedback mechanisms for microtubule length control, based on depolymerizing kinesins and severing proteins, have been studied extensively, positive feedback mechanisms are not known.

Here we use single molecular TIRF imaging and show that the budding yeast kinesin Kip2 is a microtubule polymerase and catastrophe inhibitor in vitro that uses its processive motor activity as part of a feedback loop to further promote microtubule growth. Positive feedback arises because longer microtubules bind more motors, which walk to the ends where they further reinforce growth and inhibit catastrophe. We show that this mechanism is reinforced by the increase of Kip2 processivity provided by the interaction of the kinesin with Bim1/EB1 and is downregulated by GSK-3 phosphorylation. Using this circuit, Kip2 facilitates spindle positioning through transport of microtubule-associated proteins, such as dynein and the Adenomatous Polyposis Coli-related protein Kar9 to the plus ends of astral microtubules.

Module 2 : Membrane dynamics

Biophysical tools to investigate membrane domains

Erdinc Sezgin

University of Oxford

The cellular plasma membrane has long been pictured as two parallel layers of lipid components which creates a passive barrier between the inner cell and the outside. However, this idea has been replaced after the proteins has been found as important elements of the plasma membrane. Moreover, this picture was revolutionized by the introduction of heterogeneity in the membrane, the raft hypothesis. According to this concept, there are nanodomains in the cell membrane enriched in saturated lipids, cholesterol and certain proteins such as GPI-anchor proteins. Although the raft concept is largely accepted among the scientific community, there are still several questions to be answered and contradicting data to be unraveled concerning the nature of rafts (composition, dynamics, size etc.). The main issue in raft research is to apply the right tools (which include the techniques (microscopy, spectroscopy etc.), molecular probes and model membrane systems) for certain kind of questions. Each tool has certain advantages and disadvantages. They produce their own facts and artifacts.

Here, we will give a general introduction to the question of membrane heterogeneity. The history, evolution, recent findings and open questions will be addressed. This part is followed by the introduction to the model membranes and fluorescent probes to investigate the membrane heterogeneity.

Determining molecular mobility - from ensemble to super-resolution experiments

Christian Eggeling

MRC Human Immunology Unit and Wolfson Imaging Centre Oxford, Weatherall Institute of Molecular Medicine, University of Oxford, Headley Way, Oxford, OX3 9DS, United Kingdom

Molecular interactions are key in cellular signalling. They are often ruled or rendered by the mobility of the involved molecules. We present different tools that are able to determine such mobility and potentially extract interaction dynamics. Specifically, the direct and non-invasive observation of the interactions in the living cell is often impeded by principle limitations of conventional far-field optical microscopes, specifically with respect to limited spatio-temporal resolution. We depict how novel details of molecular membrane dynamics can be obtained by using advanced microscopy approaches such as the combination of super-resolution STED microscopy with fluorescence correlation spectroscopy (STED-FCS), of fast beam-scanning with FCS (scanning (STED-)FCS), of fluorescence recovery after photobleaching (FRAP), or of single-particle tracking. Their performance on investigating different diffusion modes of plasma membrane proteins and lipids in the living cell are compared, and we highlight how these modes give novel details of membrane bioactivity. It is often optimal to gather complementary information from all techniques.

Mapping Spatio-temporal proteins dynamics using the Raster Image Correlation Method (RICS)

Michelle A. Digman

University of California Irvine

For biological reaction-diffusion systems, live single cell spatial-temporal analysis of protein dynamics provides a mean to observe stochastic biochemical signaling in correlations with intracellular environments which may lead to better understanding of cancer cell invasion, stem cell differentiation and other fundamental biological processes. This talk will describe methodologies in fluctuation analysis to answer significant biological questions in cell migration. Using the Raster Image Correlation Spectroscopy (RICS) and the complementary cross-RICS approach, we can answer the following questions: for how long, how many and how fast do proteins respond in live cells and how they interact with their binding partners. Images taken on a commercial laser scanning microscope can be used for the RICS analysis provided that specific parameters and conditions are met on the scanning system including the pixel dwell time, pixel size and the volume of excitation. Given that pixels smaller than the volume of excitation are contiguous in space while scanning along the x-axis, we can correlate in time and space the intensities along the x and y axis for every pixel and shifted by one pixel for a total of the maximum shifts allowed that equals half of the size of the image. In addition several algorithms are needed to subtract static structures as well as slow moving features such as cell motion and photobleaching. In this lecture, I will that molecular interactions and mobility can be studied in live cells. This methodology is not only valuable to the study of cell migration but may be of interest to researchers studying biochemical signaling pathways.

Deciphering Molecular Organization and Dynamics using Single-Molecule Localization Microscopy

Jean-Baptiste Sibarita

Interdisciplinary Institute for Neuroscience,

University of Bordeaux, UMR5297 CNRS

Module 3 : RNA Imaging and gene expression

Overview of the practical

Edouard Bertrand and Emmanuel Margeat

Institut de Genetique Moléculaire du CNRS and Centre de Biochimie Structurale, CNRS, INSERM and University of Montpellier

We will introduce the practicals of Module 3.

Super-resolution imaging of nucleosome organization

Melike Lakadamyali

ICFO-Institute of Photonic Sciences

Nucleosomes help structure chromosomes by compacting DNA into fibers. Chromatin organization likely plays an important role for regulating gene expression; however, due to the nanometer length scales involved, it has been very difficult to visualize chromatin fibers in vivo. To gain insight into how nucleosomes are arranged in vivo, we combined quantitative super-resolution nanoscopy with computer simulations to visualize and count nucleosomes along the chromatin fiber in single nuclei. Nucleosomes assembled in heterogeneous groups of varying sizes, which we named "clutches," in analogy with "egg clutches". Despite the heterogeneity in clutch size in a given nucleus, strikingly, the median number of nucleosomes and their compaction inside clutches were highly cell type specific. Ground-state pluripotent stem cells had, on average, less dense clutches containing fewer nucleosomes and clutch size strongly correlated with the pluripotency grade of induced pluripotent stem cells. RNA polymerase II preferentially associated with the smallest clutches while the large clutches were enriched in heterochromatin. Our results reveal how the chromatin fiber is formed at nanoscale level and link chromatin fiber architecture to stem cell state.

Monitoring stochastic gene expression in live bacterial cells using two-photon fluorescence fluctuation microscopy

Nathalie Declerck

Centre de Biochimie Structurale, Montpellier, France

Gene expression and regulation exhibit a high degree of stochasticity when studied at the level of individual cells. Even in genetically identical cell populations exposed to a uniform environment, gene activity levels are subject to random fluctuations that generate cell-to-cell variations and eventually lead to alternative cell fates. This "noise" in gene expression is crucial in many cellular processes with important fundamental and medical consequences, e.g. cell survival and adaptation in rapidly changing environments, differentiation during normal and pathological development, cellular response to stimuli or medical treatments, resistance of microbes or cancer cells to drugs… With the advent of fluorescence-based technologies in single cells, gene expression noise can be quantified with unprecedented precision. We are using a highly sensitive and quantitative fluorescence fluctuation-based method, specifically two-photon scanning number & brightness (2psN&B) analysis, to measure directly, at the single cell level and with single molecule sensitivity, the absolute concentration of fluorescent proteins produced in individual live bacterial cells. Our studies focus on a highly regulated gene network allowing the degradation or synthesis of glucose in Bacillus subtilis, a Gram positive soil bacterium able to rapidly adapt its metabolism depending on the nutrients available in the

environment. We record very rapid scans of immobilized bacterial cells producing fluorescent reporter proteins (GFP) expressed either alone from different gene promotors (promotor fusions), or fused to regulatory proteins implicated in the adaptive response (protein fusions). 2psN&B analysis of the fluorescence fluctuations at each pixel over 50 scans allows for the direct measurement of the concentration and molecular brightness of the fluorescent proteins, providing information on the expression level and oligomerization state of the proteins. Cell-to-cell variations in GFP concentrations can be determined even for very weakly transcribed genes under strong catabolite repression. Analysis of the protein number distributions in the cell population yields the statistical parameters associated to the intrinsic noise in gene expression. Distinct changes in noise patterns were observed upon a switch in carbon source, reflecting distinct underlying molecular mechanisms of regulation.

Studying transcriptional heterogeneity by single molecule imaging

Florian Müller

Institut Pasteur, Département Biologie Cellulaire et Infections, Computational Imaging & Modeling Unit

Transcription, the synthesis of mRNA molecules from a DNA template, is a heterogeneous process. This heterogeneity occurs not only for mRNA production, which is often stochastic, but also for mRNA localization, which is non-random for many genes. A crucial technique in the discovery and quantification of these phenomena is single molecule FISH (smFISH), which allows visualizing individual mRNAs as diffraction-limited spots. I will discuss the technical basics of smFISH from experiment to data analysis based on some application examples. I will finish with an overview of recent, advanced smFISH methods which opened the door for image-based transcriptomics.

Module 4 : DNA organization in eukaryotes and bacteria

Finding a needle in a haystack: the target search of DNA-binding proteins in mammalian cells

Maxime Dahan

Laboratoire Physico Chimie, Institut Curie

For many cellular functions, DNA-binding proteins (DBPs) need to find specific target sites in the genome. Facilitated diffusion (FD), namely the combination of one-dimensional motion along non-specific DNA and three-dimensional exploration, is the dominant model for the target search (TS) of DBPs. Yet, this model has hardly been tested in vivo, particularly in the complex environment of a mammalian nucleus, and it is still controversial whether it accelerates association to specific DNA binding sites. To address that question, we have implemented a TS assay using human cells with a unique target locus for an inducible exogenous searcher, the tetracycline repressor (TetR). Using single-molecule tracking and in situ biochemical measurements of association kinetics, we directly characterize the mobility of TetR, its transient interaction with non-cognate DNA and the kinetics of binding to the specific locus. Overall, we find that the searcher follows a FD strategy but that the search kinetics is not limited by the diffusive motion but by the low association efficiency to non-specific DNA sites. Importantly, we observe a lack of delimitation between specific and non-specific binding kinetics, with a broad, power-law distribution of off-target binding times. Similar results are obtained for other DNA-binding proteins (TALE, CRISPR Cas9, LacI,…) and we will present a simple model to account for this general observation.

Super-resolution imaging of nucleosome organization

Melike Lakadamyali

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Nucleosomes help structure chromosomes by compacting DNA into fibers. Chromatin organization likely plays an important role for regulating gene expression; however, due to the nanometer length scales involved, it has been very difficult to visualize chromatin fibers in vivo. To gain insight into how nucleosomes are arranged in vivo, we combined quantitative super-resolution nanoscopy with computer simulations to visualize and count nucleosomes along the chromatin fiber in single nuclei. Nucleosomes assembled in heterogeneous groups of varying sizes, which we named “clutches,” in analogy with “egg clutches”. Despite the heterogeneity in clutch size in a given nucleus, strikingly, the median number of nucleosomes and their compaction inside clutches were highly cell type specific. Ground-state pluripotent stem cells had, on average, less dense clutches containing fewer nucleosomes and clutch size strongly correlated with the pluripotency grade of induced pluripotent stem cells. RNA polymerase II preferentially associated with the smallest clutches while the large clutches were enriched in heterochromatin. Our results reveal how the chromatin fiber is formed at nanoscale level and link chromatin fiber architecture to stem cell state.

Nitty-gritty of 3D-SIM : Short talks and round table

Nitty-gritty of SMLM : Short talks and round table

Module 5 : Structure and dynamics of viral components

Introduction to atomic force microscopy on virus

Pierre-Emmanuel Milhiet

Centre de Biochimie Structurale, U1054 INSERM, UMR5048 CNRS, Montpellier University

This talk will be dedicated to AFM characterization of viruses, from the sample preparation to the modes of imaging. I will further illustrate the recent developments in AFM shown during the plenary lecture (Correlative Atomic Force/Single Molecule Localization microscopies and High-Speed AFM) in the virus field.

Introduction of the biological topic and description of viral sample preparations dedicated to fluorescence imaging and atomic force microscopy

Delphine Muriaux

CPBS, Montpellier

Protein-protein interaction followed by FRET using Fluorescent Lifetime Imaging Microscopy

Hugues de Rocquigny

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FRET is a non-radiative energy transfer between fluorescent donor and acceptor when fluorophores are less than ~10 nm apart, corresponding to typical distances observed in protein-protein interactions. FRET can be measured by fluorescence intensity changes of the donor before and after acceptor photobleaching, but FRET values can then be affected by local fluorescence intensity or by the efficiency and/or specificity of the acceptor photobleaching. Alternatively, FRET can be measured from changes in the fluorescence lifetime (◎) of the donor, as FRET results in shortening the donor fluorescent lifetime. ◎ is an intrinsic parameter that does not depend on the instrument or on the concentration of the donor. Fluorescence lifetime images (FLIM) are acquired measuring the fluorescence decay◎at each pixel (or group of pixels) by scanning the sample. Then, FLIM images are constructed through an arbitrary colour scale, corresponding to the different lifetimes of the donor. FLIM applications will be illustrated by the characterization of Gag-Gag or Gag-RPL7 interaction required along the assembly of HIV-1 particles.

Introduction into Single Molecule Localization Microscopy

Christian Sieben

Laboratory of Experimental Biophysics, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

In this lecture I will give a general theoretical introduction into the topic as well as provide some practical considerations. In the last part of the presentation I will then highlight and discuss some key applications.

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