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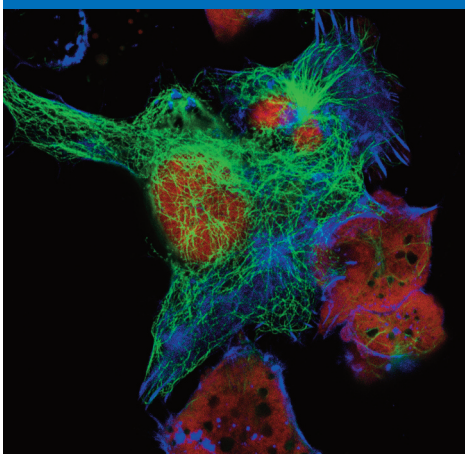
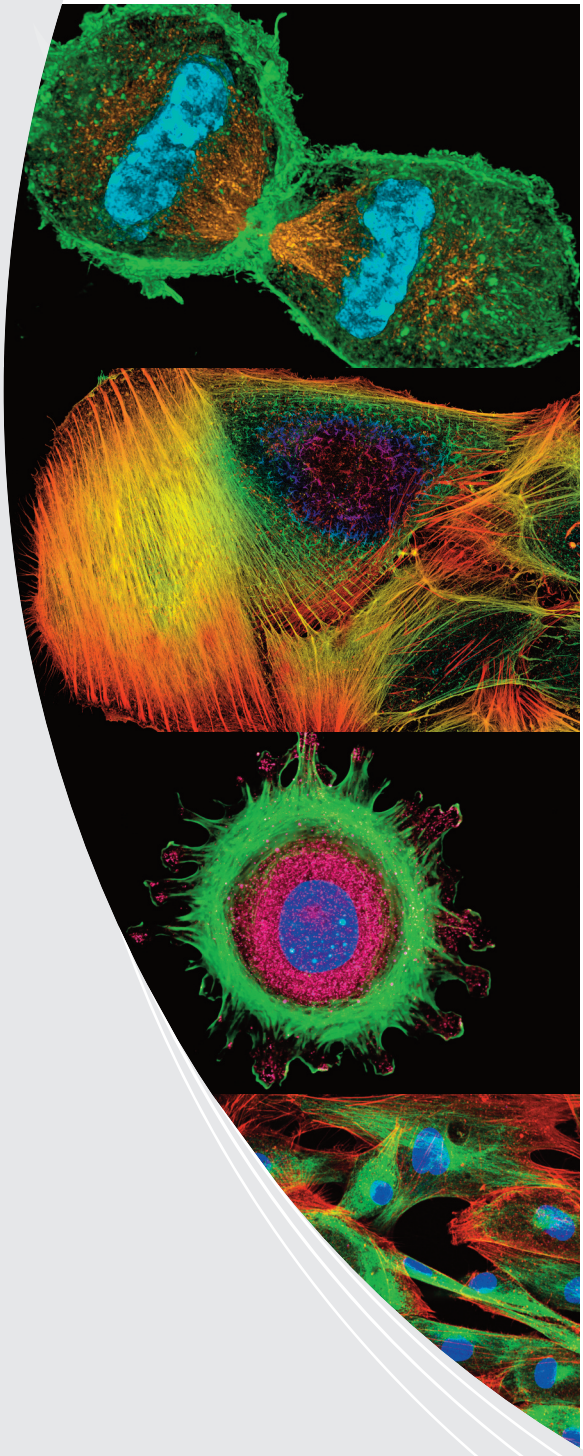




La microscopie à super résolution

colloque en anglais

8 novembre 2016 à 14h30
Grande salle des séances de l'Institut de France

23, quai de Conti, 75006 Paris

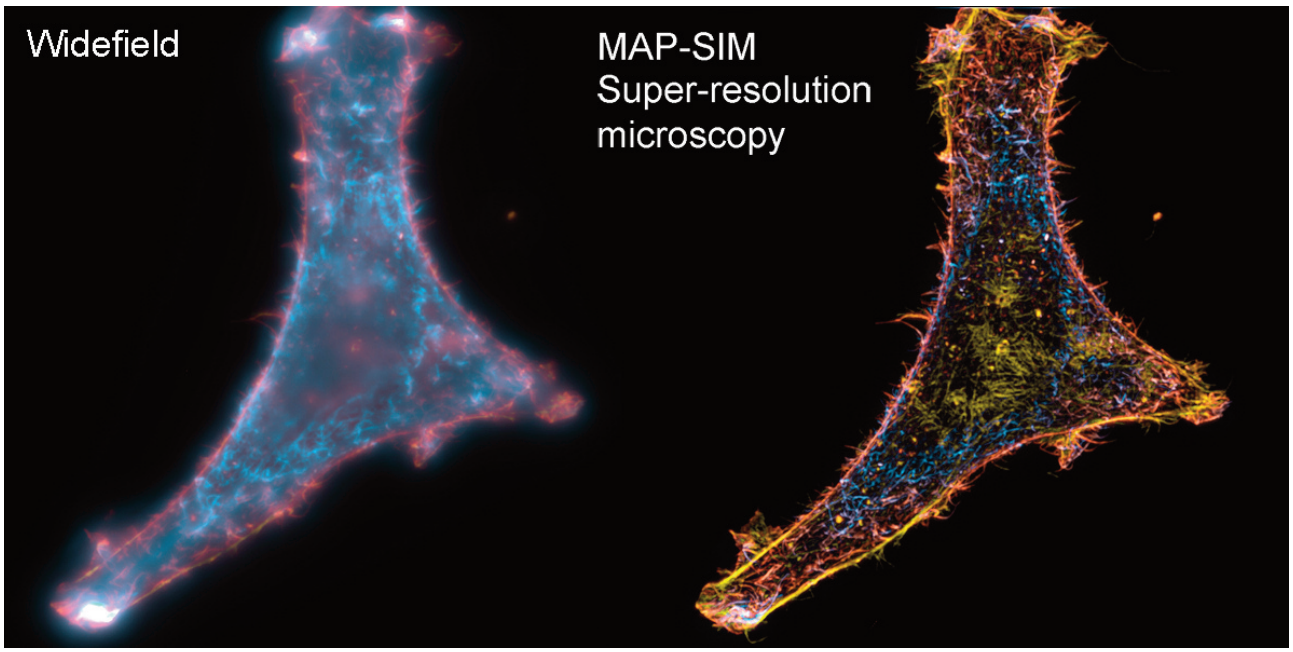
Le principe d'incertitude d'Heisenberg nous dit qu'il n'est pas possible de localiser une particule dans une expérience impliquant un photon unique, avec une précision supérieure à la longueur d'onde de la radiation utilisée. Si l'on réalise un nombre N d'expériences indépendantes pendant que l'objet ne bouge pas on peut améliorer la résolution par un facteur $\sqrt{N}$ et avec quelques millions de photons optiques obtenir des résolutions de l'ordre de quelques nanomètres. Facile à dire mais pas facile à réaliser. Au cours de ces quelques dernières années un certain nombre de techniques astucieuses sont apparues et ont révolutionné la microscopie optique. Les images en haute résolution de systèmes complexes tels que des cellules ou des axones ont révélé des structures inattendues et on a pu suivre le comportement de molécules individuelles à des échelles de temps pertinentes en biologie. Ces expériences sur molécules individuelles ont permis d'éviter les moyennes d'ensemble, de capturer des intermédiaires de réactions nouveaux, de caractériser l'hétérogénéité cellulaire, de sonder l'ergodicité des systèmes, etc. Il s'agit d'un domaine de recherche en explosion que ce colloque tentera d'illustrer avec des exemples issus essentiellement des systèmes biologiques.

Presentation

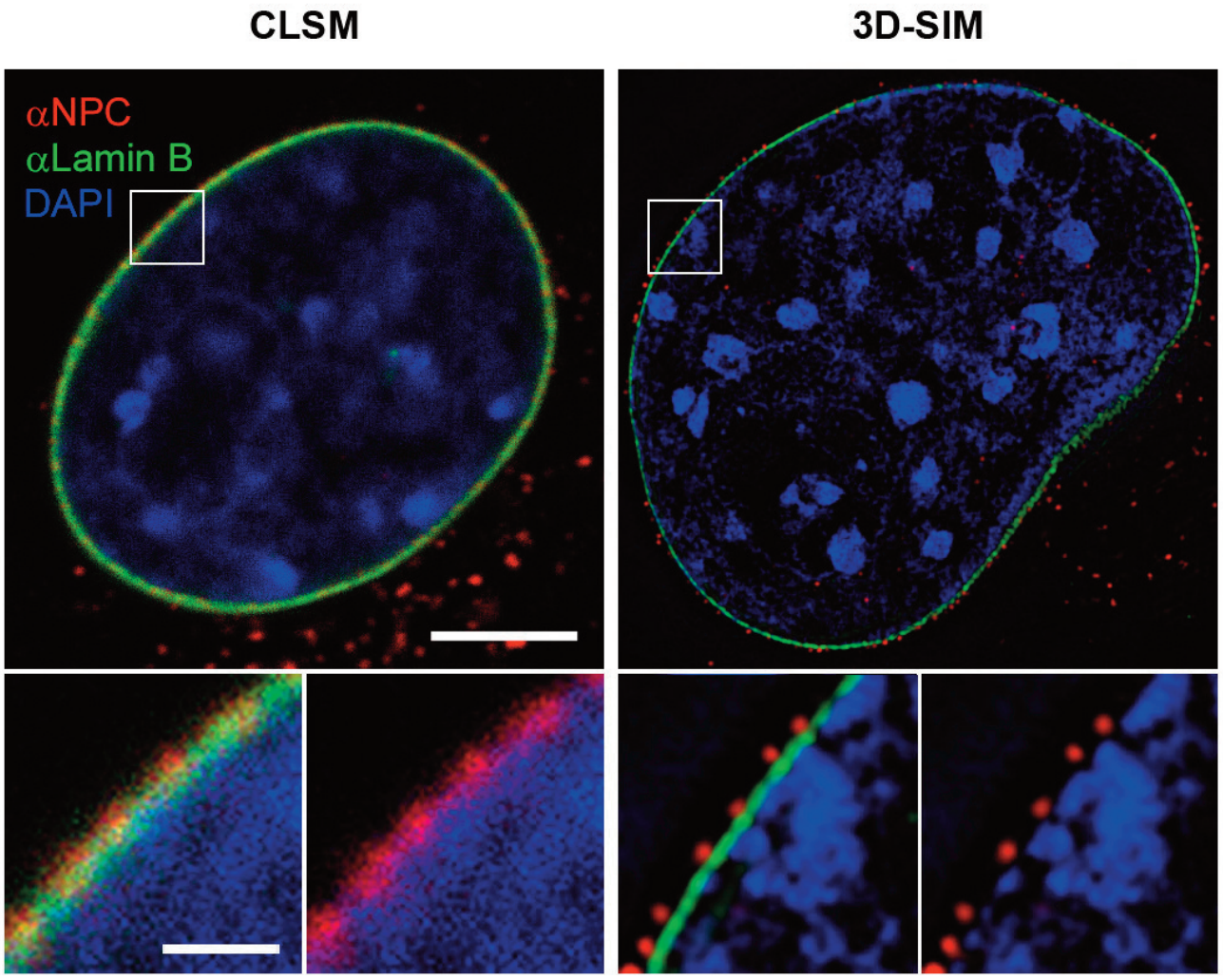
Heisenberg uncertainty principle tells us that it is not possible to locate a point like object in a scattering experiment involving a single photon, with an accuracy better than the wavelength of the used radiation. If one can make N independent experiments, while the object does not move, one can then improve the resolution by a factor $\sqrt{N}$ and with a few million optical photons obtain resolutions on the order of nanometers. Easy to say, not so easy to achieve. Over the last few years a number of clever techniques have emerged and brought a real revolution in optical microscopy. High resolution images of complex systems such as cells or axons have revealed unexpected structures and tracking of single molecules has been possible on time scales relevant to biological phenomena. This allowed for experimentations avoiding ensemble averages, capturing transient intermediates, characterizing heterogeneous behavior, probing ergodicity etc… This is an exploding new field of research, which this colloquium intends to illustrate with examples essentially issued from biological systems.

Program

2:30 pm	Opening
2:35 pm	Presentation
2:50 pm	Single Molecules and Nanoparticles as Windows on Soft Matter at Nanometer Scale **Michel Orrit,** Leiden University, Leiden, The Netherlands
3:20 pm	Questions
3:30 pm	Advanced methods of high and superresolution fluorescence microscopy **Joerg Enderlein,** Göttingen University, Göttingen, Germany replaced by **Narain Karedla**, Göttingen University, Göttingen, Germany
4:00 pm	Questions
4:10 pm	Dissecting the structure and assembly of nuclear pores and mitotic chromosomes by super-resolution microscopy **Jan Ellenberg,** EMBL, Heidelberg, Germany
4:40 pm	Questions
4:50 pm	*In situ* biochemistry with single molecule imaging and super-resolution microscopy **Maxime Dahan,** Institut Curie, Paris, France
5:20 pm	Questions
5:30 pm	Discussion and conlusion



lukes *et al*, optics express, 2014



Schermelleh *et al*, Science, 2008

Organising comittee

Antoine TRILLER

Member of the Académie des sciences, Ecole Normale Supérieure, Institut National de la Santé et de la Recherche Médicale

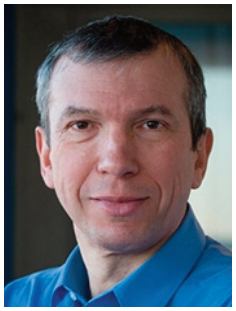
Antoine Triller is director of research at Inserm. He is the head of the *Institut de Biologie* of the *Ecole Normale Supérieur*e. His research focuses on molecular and cellular biology and neural communication. Recently, when developing single molecule tracking approaches, he along with Daniel Choquet, discovered that synaptic receptors diffuse continuously in the membrane plan and are transiently stabilized at synapses. This phenomenon, involved in learning processes, is regulated by neuron activity. This was at the origin of a new framework for synaptic plasticity. He later showed that this phenomenon is altered in neuro-degenerative diseases such as Alzheimer and Parkinson ones, thus opening new therapeutic perspectives ■

Jacques PROST

Member of the Académie des sciences

Jacques Prost developed a research activity on Soft Condensed Matter and Statistical Physics with a strong emphasis on Liquid Crystals before shifting his interest to the physics-biology interface. He has been successively involved in the description of molecular motors, hearing, cell and tissue dynamics for which he introduced the concept of "active gels". He develops his research activity at the Curie Institute as an emeritus member of CNRS, and simultaneously in the Mechanobiology Institute of the National University of Singapore ■

Abstracts and biographies



Michel Orrit

Leiden University, Leiden, The Netherlands

Michel Orrit's observed the first fluorescence signal from a single molecule in 1990. His method was quickly adopted in several groups worldwide, and was extended to room temperature in 1993. Since then, Orrit's group first in Bordeaux and later in Leiden (Netherlands) since 2001, has applied single-molecule spectroscopy to molecular photophysics, solid-state dynamics, and nonlinear optics. His current interests include the properties of individual nano-objects (organic (bio)molecules, gold nanoparticles), and their uses to probe structure and dynamics of soft condensed matter at nanometer scales.

Single Molecules and Nanoparticles as Windows on Soft Matter at Nanometer Scales

The optical detection and study of single molecules and other nano-objects provides unique insights into the dynamics of these small systems and their surroundings. They can also be applied in several classes of materials, as I shall illustrate with some recent experiments. The lifetime-limited lines of single molecules at cryogenic conditions probe acoustic and electric perturbations, making them attractive for quantum optomechanics. Rotational diffusion of single molecules can be followed in glass-forming supercooled liquids and reveal the surprisingly large extent of dynamical heterogeneity. We recently studied the dynamics of vapor nanobubbles created in a liquid surrounding a single immobilized gold nanosphere. These nanobubbles form in an instable, explosive process before collapsing and can generate, as well as react to, sound waves. Photothermal microscopy opens the study of non-fluorescent absorbers, down to single-molecule sensitivity. The high signal-to-noise ratio of this absorption method enables local plasmonic and chemical probing. Fluorescence enhancement in excess of thousand-fold can be observed in the near field of a gold nanorod at its plasmon resonance. This phenomenon enables the observation of individual weak emitters, a generalization of single-molecule experiments to a much broader class of molecules ■

Jeorg Enderlein

Göttingen University, Göttingen, Germany

Jeorg Enderlein received his PhD in 1991 at the Humboldt University in Berlin. He worked for five years with PicoQuant (Berlin) developing pulsed laser systems and high-speed electronics for ultrasensitive fluorescence detection. In summer 2000, he finished his Habilitation at Regensburg University. From 2001 through 2006, he was head of the Single Molecule Spectroscopy group at the Research Center in Jülich. In 2007/2008, he was Professor for Biophysical Chemistry at the Eberhard Karls Tübingen in Germany, and since 2008 he is professor for Biophysics at the Georg August University in Göttingen.

Advanced methods of high and superresolution fluorescence microscopy

Classical fluorescence microscopy is limited in resolution by the wavelength of light (diffraction limit) restricting lateral resolution to ca. 200 nm, and axial resolution to ca. 500 nm (at typical excitation and emission wavelengths around 500 nm). However, recent years have seen a tremendous development in high and super-resolution techniques of fluorescence microscopy, pushing the spatial resolution much beyond its diffraction limit. My presentation will focus on several on recent advances in this field, starting with a discussion of the recently introduced Image Scanning Microscopy (ISM). Then, I will turn to the recently developed true superresolution microscopy approaches, which really circumvent the classical diffraction-induced resolution limit, specifically the currently most widely employed superresolution techniques of Photactivation Localization Microscopy (PALM) and Stochastic Optical Reconstruction Microscopy (STORM). In this context, I will present our own efforts for pushing the resolution limit with enhancing fluorophore photostability by cooling samples to cryogenic temperatures. Finally, I will focus on several lesser known techniques which are particularly interesting methods due to their exceptional simplicity and broad applicability. Special emphasis will be put on bridging the gap between the physical principles behind all these methods, their practicability, and the gain in information when applied to bioimaging■

Jan ELLENBERG
EMBL, Heidelberg, Germany

Jan Ellenberg heads the Cell Biology & Biophysics unit and is senior scientist at EMBL Heidelberg. For over 20 years, he has been interested in cell division and nuclear biogenesis, including systematic analysis of mitosis, NPC assembly, and formation of mitotic and meiotic chromosomes. His goal has been to obtain structural and functional measures of the required molecular machinery inside cells using quantitative 4D imaging, single molecule spectroscopy, as well as super-resolution microscopy. His research group played a key role in large EU-wide efforts on systems biology of mitosis, as well as microscopy automation and unbiased computational image analysis. He has coordinated European efforts to make imaging technologies more accessible to researchers via his role as Coordinator of EuBI Preparatory Phase II and as EMBL delegate in the EuBI Interim Board

Dissecting the structure and assembly of nuclear pores and mitotic chromosomes by super-resolution microscopy

How the nuclear pore complex (NPC) assembles into the double membrane boundary of the nucleus remains enigmatic. To capture assembly intermediates we correlated live cell imaging with high-resolution electron tomography and super-resolution microscopy. Surprisingly, assembly intermediates are dome-shaped evaginations of the inner nuclear membrane, which grow in diameter and depth until they fuse with the flat outer nuclear membrane. Super-resolution microscopy revealed the molecular maturation of assembly intermediates, which initially contain nuclear ring, and only later cytoplasmic filament proteins. NPC assembly thus proceeds by an asymmetric inside-out fusion of the inner with the outer nuclear membrane. Combining 3D super-resolution imaging with computational single particle averaging now allows us to unravel the molecular architecture of the NPC at nano-scale resolution. What the physiological structure of chromosomes inside the nucleus is and how they compact during cell division is not understood. To address this, we have labeled replication domains (RD) with fluorescent nucleotides and performed correlative confocal and super-resolved imaging in human cells. We find that RDs are stable units of chromosome structure that on average contain four co-replicating origins, measure 150 nm in diameter and are spaced 270 nm from each other along the chromosome. Surprisingly, during mitotic compaction the internal organization of RDs remained unchanged while they cluster into large megadomains that build the metaphase chromosome ■

Maxime DAHAN
Institut Curie, Paris, France

Maxime Dahan is Directeur de Recherche (DR1) at CNRS and director of the laboratoire Physico-Chimie (CNRS UMR168) at Institut Curie, and associate professor at Ecole Polytechnique. His research activities are centered on the development and applications of novel methods for imaging and manipulation of molecular systems in living cells. He has been awarded the Grand Prix Jacques Herbrand of the French Academy of Sciences and the Bronze medal from CNRS.

In situ biochemistry with single molecule imaging and super-resolution microscopy

Single molecule methods have started revolutionizing the way we investigate the properties of living systems. Thanks to different experimental modalities, such as localization PALM/STORM microscopy, single molecule counting and tracking, it is now possible to determine the dynamic architecture of supramolecular assemblies in their endogeneous cellular habitat, down to their most elementary molecular constituents. Here, I will describe our approach to develop the next generation of single molecule assays in living cells, that combine novel imaging approaches, such as multifocal imaging and adaptive optics, and advanced computational methods inspired by data sciences. The methods will be illustrated by results on the dynamic organization of membrane proteins or of proteins involved in the regulation of gene expression ■

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