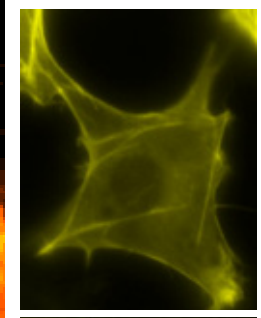
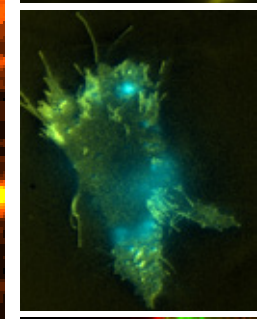
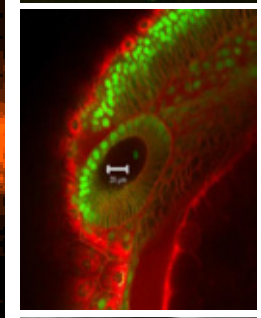
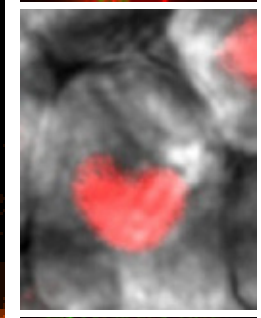
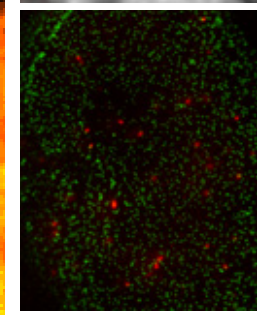
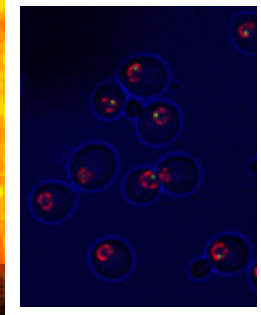

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15TH-17TH NOVEMBER 2017

BÂTIMENT DES COLLOQUES (338)
FACULTÉ DES SCIENCES, ORSAY

Paris-Saclay Conference PhysChemCell 2017

ADVANCED IMAGING METHODS FOR THE EXPLORATION OF LIVING MATTER

New probes and labeling strategies
Advanced biosensors and contrasts
Spatial and temporal analyses of cellular functions
Advanced methods for in vivo imaging
Histochemical analyses of cells and tissues

PLENARY SPEAKERS

Luke LAVIS (Ashburn, Janelia Research Campus)
Franck RIQUET (University of Ghent)
Peter DEDECKER (KU Leuven)
Francesco PAVONE (University of Florence)
Matthia KARREMAN (DKFZ, Heildelberg)

REGISTRATION AND INFORMATION: http://www.cpps.u-psud.fr/

15TH-17TH NOVEMBER 2017

BÂTIMENT DES COLLOQUES (338) - FACULTÉ DES SCIENCES, ORSAY

OBJECTIVES

Over the recent years, intensive research on the chemistry of probes, biomedical optics and innovative instrumentation have brought tremendous progress in cell imaging and microscopy. These advances open new strategies for the understanding of biological functions and pathologies at the molecular scale, with a major impact on drug discovery, therapeutic innovation and biotechnology. After its successful first edition held in 2015, the international symposium PhysChemCell 2017 will gather an interdisciplinary community (biologists, clinicians, chemists, physicists) within the Paris Saclay area and beyond, working on advanced analytical cell and tissue imaging, from the single cell to the living organism.

The objective of the conference is to favour interdisciplinary exchanges in the frame of a conference of international audience, to promote the translation of disruptive technologies appearing in chemistry and physics into new tools for the exploration of cell physiopathology, and ultimately into clinical or biotechnological applications.

The scope of the conference is unique at the national and even international level, by relying on basic research in chemistry and physics, while strongly targeting applicative fields. Latest advances in cell imaging, such as bio-orthogonal chemistry, super-resolution, correlative microscopies or functional in vivo imaging will be discussed.

The conference will last over 2 days and a half, and include 5 thematic sessions introduced by prominent international speakers :

– Developing and using new probes and labeling strategies
– Advanced biosensors and contrasts for analytical cell imaging
– Improving spatial and temporal analyses of cellular functions
– Advanced methods for deep 3D ex vivo/in vivo imaging
– Histochemical analyses of cells and tissues

ORGANIZING COMMITTEE

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SUMMARY

TIME TABLE

Wednesday 15 November

8:45 - Conference introduction

Session 1 - 9:00-12:30 - Developing and using new probes and labeling strategies

Chairs : Boris Vauzeilles, Florence Mahuteau Betzer

Designing brighter dyes for advanced fluorescence microscopy
Luke Lavis (Ashburn)

Metal complexes bio-imaging using a range of microspectroscopies —IR, AFM-IR, fluorescence and X-fluorescence
Clotilde Policar (Paris)

A Long-lived Triplet State is the Entrance Gateway to Oxidative Photochemistry in Green Fluorescent Proteins
Martin Byrdin (Grenoble)

11:00 - coffee-break

Chelating azides and mesionics, new tools for imaging and labeling applications
Frédéric Taran (Saclay)

Lanthanide complexes as prototypes for IRM-optical imaging of an enzyme activity
Philippe Durand (Gif-sur-Yvette)

12:30 - lunch & poster session

Session 2 - 14:00-18:30 - Advanced biosensors and contrasts for analytical cell imaging

Chairs : Fabienne Mérola, Oliver Nüsse

Paris-Saclay Chemistry Dept
Jean-Pierre Mahy, Director

Days of future past : Design and potential of genetically encoded biosensors for biomedical research
Franck Riquet (Ghent)

Fluorescent peptide biosensors for probing kinase activities : new tools for cancer diagnostics and drug discovery
May Morris (Montpellier)

Fluorescent proteins as probes to analyse protein complexes: from quantitative live cell imaging to structural model
Marie Erard (Orsay)

16:30 - coffee-break

Microtubules as platforms for visualizing and analyzing protein interactions in cells
David Pastre (Evry)

IR nanospectroscopy as a label-free imaging technique to characterize new antibiotics carriers
Jérémie Mathurin (Orsay)

STEM-cathodoluminescence, a promising novel approach for bioimaging
Marta De Frutos (Orsay)

Thursday 16 November

Session 3 - 9:00-13:00 - Improving spatial and temporal analyses of cellular functions

Chairs : Sandrine Lévêque-Fort, Laurent Combettes

Sub-diffraction imaging of biosensors using "smart" labels
Peter Dedecker (Leuven)

Combining complementary localization modalities for 3D imaging of biological samples
Clément Cabriel (Orsay)

Experimental phasing approaches of protein microcrystals structure determination inside the living cell
Pierre Montavillle (Saint-Aubin)

11:00 - coffee-break

The promises and challenges of high-density single molecule imaging : application to nanoscale organization of Rac1 signalling
Maxime Dahan (Paris)

Clustering of the Neurotrophin Receptor TrkB in the Membranes of SH-SY5Y Cells Detected by Super-resolution STED Microscopy
Angelina Angelova (Châtenay-Malabry)

Challenges in high spatial and temporal resolution imaging to study genome organization and function from bacteria to human cells
Karine Dubrana (Fontenay-aux-Roses)

13:00 - lunch & poster session

Session 4 - 14:30-18:30 - Advanced methods for deep 3D ex vivo/in vivo imaging

Chairs : Cedric Bouzigues, Sylvia Bruneau

The Strategic Research Initiative BIOPROBE at Paris Saclay
Fabienne Mérola

Multidimensional and multi-level imaging of tissue disease: towards the 3D digital histology
Francesco Pavone (Florence)

Probing cellular metabolism in living tissues by Fluorescence Lifetime Microscopy of intrinsic biomarkers
Chiara Stringari (Palaiseau)

16:30 - coffee-break

Fast 3D-Imaging of Neuronal Activity in Cortical Columns at Single Cell Resolution
Walther Akemann (Paris)

Towards in-vivo acousto-optic imaging of biological tissues
Maimouna Bocoum (Paris)

***In vivo* Reactive Oxygen Species imaging for complex pathology monitoring**
Antigoni Alexandrou (Palaiseau)

Friday 17 November

Session 5 - 9:00-12:30 - Histochemical analyses of cells and tissues

Chairs : Bertrand Cinquin, Larbi Amazit

Catch me if you CLEM: Imaging Single Tumor Cells at the Blood-Brain-Barrier using Multimodal Correlative Microscopy
Matthia Karreman (Heidelberg)

Biomineralisation and pathological calcifications :Beauty and the Beast
Dominique Bazin (Paris)

Infrared and Raman spectroscopy for the analysis of J774 macrophages
Sana Tfaili (Châtenay-Malabry)

11:00 - coffee-break

Last developments of TOF-SIMS for high spatial resolution molecular imaging and 3D-profiling
David Touboul (Gif-sur-Yvette)

3D high resolution imaging by X-ray Phase Contrast Tomography to study theranostic nanoparticles distribution in mice organs
Elena Longo (Palaiseau)

Closing remarks

15TH-17TH NOVEMBER 2017

BÂTIMENT DES COLLOQUES (338) - FACULTÉ DES SCIENCES, ORSAY

WEDNESDAY 15 NOVEMBER

SESSION 1: DEVELOPING AND USING NEW PROBES AND LABELING STRATEGIES

SPEAKERS :

Luke Lavis (Ashburn)
Clotilde Policar (Paris)
Martin Byrdin (Grenoble)
Frédéric Taran (Saclay)
Philippe Durand (Gif-sur-Yvette)

CHAIRMANS:

Boris Vauzeilles, Florence Mahuteau Betzer

Designing brighter dyes for advanced fluorescence microscopy

Luke D. Lavis

Janelia Research Campus, Howard Hughes Medical Institute, 19700 Helix Drive, Ashburn, Virginia 20147, USA

Specific labeling of biomolecules with bright, photostable fluorophores is the keystone of fluorescence microscopy. An expanding method to label cellular components utilizes genetically encoded self-labeling tags, which enable the attachment of chemical fluorophores to specific proteins inside living cells. This strategy combines the genetic specificity of fluorescent proteins with the favorable photophysics of synthetic dyes. However, intracellular labeling using these techniques requires small, cell-permeable fluorophores, thereby limiting utility to a small number of classic, unoptimized dyes. We discovered a simple structural modification to standard fluorophores that improves brightness and photostability while preserving other spectral properties and cell permeability. Inspired by computational experiments, we replaced the *N*,*N*-dimethylamino substituents in tetramethylrhodamine with a four-membered azetidine ring. This net addition of two carbon atoms doubles the quantum efficiency and improves the photon yield in living cells. The novel substitution is generalizable to fluorophores from different structural classes, yielding a palette of synthetically tractable chemical dyes with improved quantum efficiency and enabling multicolor single-molecule imaging experiments. These brighter versions of classic fluorophores can be further modified to fine-tune spectral and chemical properties for advanced imaging experiments in increasingly complex biological samples.

Metal complexes bio-imaging using a range of microspectroscopies —IR, AFM-IR, fluorescence and X-fluorescence

Clotilde Policar

François Lambert, Nicolas Delsuc, Hélène Bertrand, Anne-Sophie Bernard, Sylvain Clède, Sarah Hostachy, Emilie Mathieu, Lucas Henry

École Normale Supérieure - PSL Research University, Département de Chimie, Sorbonne Universités - UPMC Univ Paris 06, CNRS UMR 7203 LBM, 24 rue Lhomond, 75005 Paris, France.
clotilde.policar@ens.fr

Inorganic complexes are increasingly used for biological applications, as metallodrugs or metalloprobes.[1,2] Novel imaging techniques have emerged providing new information on sub-cellular distribution. The IR-energy range is particularly attractive for chemical imaging,[3] as IR vibrational excitations do not induce photo-bleaching and lead to little to no photodamage. The development of IR-probes is a challenge in the field of IR-imaging. Interestingly, biological media are almost transparent in the range 2200-1800 cm^{-1} where metal-COs show intense absorption bands. They can be imaged inside cells using a cutting edge technique coupling an AFM and an IR laser (AFMIR),[4] or synchrotron-based IR-light (μ -SR-FTIR).[4] An additional benefit of IR imaging is the ease with which it leads to direct quantification.[5] $(L)Re(CO)_3$ are luminescent when L is a ligand with low-lying π^*-orbitals: this led us design $(L)Re(CO)_3$ as multi-modal probes for IR and luminescent bio-imaging that we have called SCoMPI for Single Core Multimodal Probes for Imaging.[5,6] Recently we have explored X-fluorescence for imaging metal complexes and have shown that metal-COs[6] and other metal complexes[7] can be mapped in biological samples (cells, worms section) using synchrotron-based X-fluorescence (μ -SR-XAS). In this talk, imaging of metal-complexes in several examples will be presented. These approaches enabling direct imaging of metal-CO or, more generally with μ -SR-XAS, of metal-complexes are promising tools that will bolster the understanding of metal complexes in biological environments.

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A long- lived Triplet State is the Entrance Gateway to Oxidative Photochemistry in Green Fluorescent Proteins

Martin Byrdin[1], Chenxi Duan[1], Dominique Bourgeois[1], Klaus Brettel[2]

[1]Institut de Biologie Structurale, Université Grenoble Alpes, CEA, CNRS, 38044 Grenoble, France

[2]Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ. Paris Sud, Université Paris-Saclay, F-91198 Gif-sur-Yvette cedex, France

Though ubiquitously used as selective fluorescence markers in cellular biology, fluorescent proteins still have not disclosed all of their surprising properties. One important issue with their application for single protein counting is so called blinking (spontaneous fluorescence off- and on-switching), observed on timescales from submilliseconds to seconds. In a search for the underlying photophysics, we applied transient absorption spectroscopy to the prototypical enhanced green fluorescent protein (EGFP) and, for comparison, IrisFP, another green FP (chromophore identical to EGFP), but of anthozoan (coral) origin and a close relative (one single amino acid mutation) of the popular green-to-red switching EosFP

We identified a long-lived (approx. 5 milliseconds) “dark” state that is formed with low quantum yield (approx.1%) and has a pronounced absorption band around 900 nm.

Detection of phosphorescence emission with identical kinetics and excitation spectrum allowed identifying this state as the first excited triplet state of the deprotonated chromophore. This triplet state was further characterized by determining its phosphorescence emission spectrum, the temperature dependence of its decay kinetics and its reactivity towards oxygen and electron acceptors and donors. It is suggested that it is this triplet state that lies at the origin of the oxidative photochemistry leading to so called “oxidative redding”. We discuss the impact that the absorption characteristics of this newly identified state have on fluorescence microscopy applications, both “classical” and “super-resolution”.

CHELATING AZIDES AND MESOIONICS, NEW TOOLS FOR IMAGING AND LABELING APPLICATIONS

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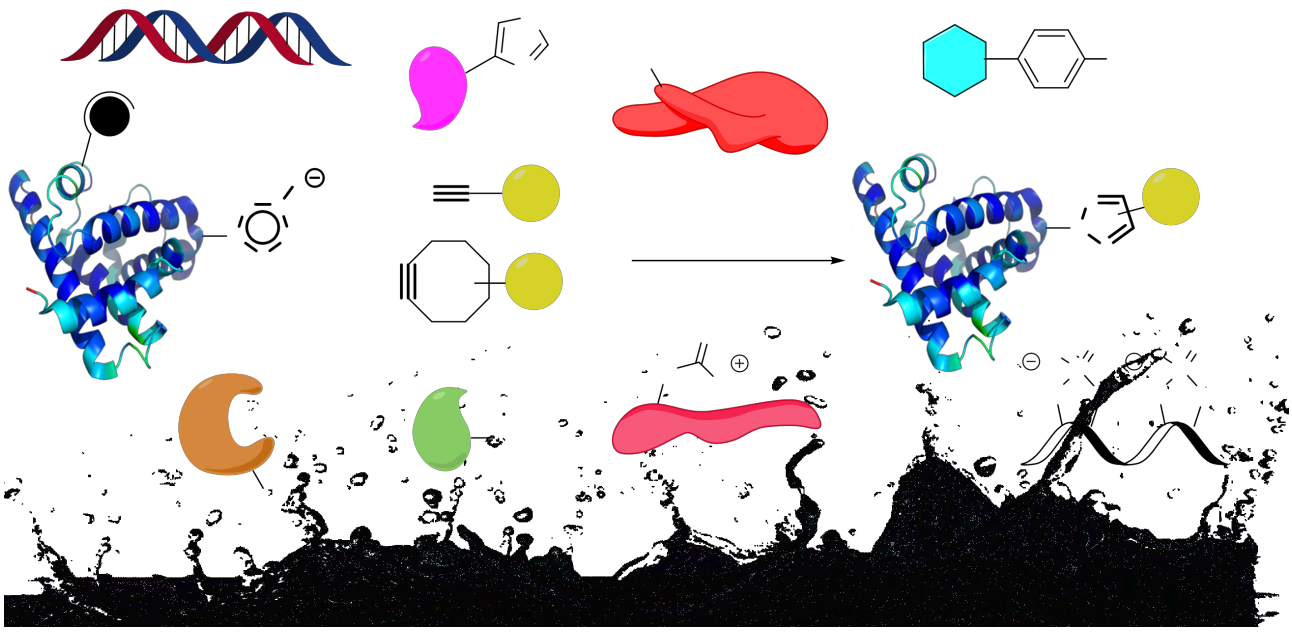
Summary:

The development of chemical reactions that can be performed in living systems (i.e. cells, model organisms) has long held unique fascination in the field of chemical biology. A bio-orthogonal reaction is characterized by the reaction of two functionalities, which will react under mild physiological conditions and are inert towards the biological environment. On the other hand, the discovery of chemical reactions fulfilling the criteria of the click chemistry concept continue to have a huge impact in many research fields including cell imaging and protein labeling. Quintessential example is the copper-catalyzed azide-alkyne cycloadditions (CuAAC) and its copper free version (SPAAC). Our laboratory is involved in the discovery and use of such reactions. For example, our group developed new copper-chelating azides[1] able to react very rapidly with alkynes allowing efficient ligation at low concentration and under milder conditions than with standard CuAAC conditions.[2] Recent work from our team identified several mesoionic compounds as new efficient dipoles for click reactions with terminal alkynes[3] and for bioorthogonal reactions with cyclic alkynes.[4] These reactions were used both for labeling and imaging applications.



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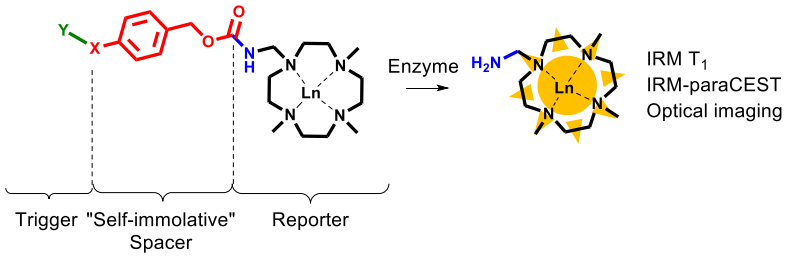
Lanthanide complexes as prototypes for IRM-optical imaging of an enzyme activity

Philippe DURAND, Institut de Chimie des Substances Naturelles (ICSN), Gif-sur-Yvette (UPR 2301), philippe.durand@cnrs.fr.

While enzyme activity is routinely determined using *in vitro* assays, its *in vivo* visualization remains a challenge. This is a subject of increasing interest in molecular imaging since dysregulation of these biological catalysts is associated with many diseases. In this context, we designed lanthanide-based probes that are responsive to a specific enzyme activity (β-galactosidase for instance). These probes can be monitored by one to three different modalities depending on the lanthanide used: T_1-MRI, paraCEST-MRI and optical imaging. The design and magnetic/optical properties of prototypes will be presented.



WEDNESDAY 15 NOVEMBER

SESSION 2: ADVANCED BIOSENSORS AND CONTRASTS FOR ANALYTICAL CELL IMAGING

SPEAKERS :

Franck Riquet (Ghent)
May Morris (Montpellier)
Marie Erard (Orsay)
David Pastre (Evry)
Jérémie Mathurin (Orsay)
Marta De Frutos (Orsay)

CHAIRMANS:

Fabienne Mérola, Oliver Nüsse

“Days of future past: Design and potential of genetically encoded biosensors for biomedical research.”

Franck. B Riquet[1,2 & 3]
[1]Molecular Signaling and Cell Death Unit, VIB Center for Inflammation Research, Ghent, Belgium
[2]Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium
[3]Unité de Glycobiologie Structurale et Fonctionnelle, CNRS-UMR 8576, Université Lille1, Villeneuve d’Ascq, France.

The team is mainly interested in the molecular signaling events that determine cell survival and cell death. A cell integrates a number of external cues through a complex network of intracellular signals which ultimately allows survival or triggers cell death. Among the many molecules implicated in this process, kinases (which represent 1/3 of all protein sequences) are key signal transduction effectors. We focus on these phosphorylation events to monitor the chain of events involved in cell fate decision.
Phosphorylation events can be monitored using genetically-encoded FRET biosensors of a generic design: such construct is made of a specific substrate of the kinase of interest flanked by a phospho-amino-acid binding domain (PAABD). When the substrate is phosphorylated by the kinase, the PAABD binds to it, changing the conformation of this sensor domain. This conformational change is reported by a change in FRET between a CFP/YFP pair of fluorophores placed on both ends of the construct. While this design has already been validated for a few kinases, we developed molecular tools to create and test biosensors for the kinases specifically involved in cell fate decision.
Studying the spatio-temporal regulation of unperturbed life and death cellular processes is a task easier said than done! For instance, even if the cell death process can be initiated experimentally at a well defined time, its execution proceeds at random times over a population of cells, each cell undergoing the process at its own pace. Such events are thus very hard to analyse with global approaches, whereas time-lapse imaging of biosensor provides a direct readout of when each cell underwent a critical phosphorylation event, and when cell death will occur after this event. Monitoring individual cells over long durations required specific hardware and software developments which will be described.
After introducing the seminal discoveries that have contributed and are still contributing to biosensing approaches, I will present what we have achieved so far, our findings related to cell cycle and programmed cell death, and discuss how the biological signal is encoded towards cell fate decision. To wrap up, I will open onto tangible applications in biomedical research.

FLUORESCENT PEPTIDE BIOSENSORS FOR PROBING KINASE ACTIVITIES : NEW TOOLS FOR CANCER DIAGNOSTICS AND DRUG DISCOVERY

May C. Morris[1]

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Keywords: *Fluorescent Biosensor, Peptide, Protein Kinase Activity, Cancer*

Cyclin-dependent kinases (CDK/cyclins) are heterodimeric kinases that play a central role in coordination of cell cycle progression and participate in several essential biological processes [1]. These kinases are frequently hyperactivated in cancer cells and constitute established cancer biomarkers and attractive pharmacological targets for anticancer therapeutics [2, 3]. However, despite their oncological relevance, there are very few means of quantifying their relative activities to identify their hyperactivation.

In order to monitor the activity of these kinases in complex biological samples, such as cell extracts, tissue or tumour biopsies, and develop sensitive tools for diagnostic purposes, we have developed a toolbox of fluorescent biosensors through conjugation of environmentally-sensitive probes to synthetic modular polypeptides. These non-genetic biosensors offer a straightforward means of sensing subtle alterations in kinase activity in real time, *in vitro* and in living cells following facilited delivery by cell-penetrating peptides [4]. These selective chemical probes allow to quantify differences between healthy and cancer cell lines, and in response to therapeutics. This technology is further suitable for probing and alterations in kinase activities in living cells, as well as in tissue samples and tumour biopsies. In particular, we have engineered a CDK4/Cyclin D-specific biosensor which we have implemented to quantify CDK4/Cyclin D activity in healthy and pathological skin biopsies [5], and a CDK5/p25-specific biosensor which provides means of monitoring this kinase in neuronal cells and assessing its hyperactivation in neuronal disorders. Taken together, these fluorescent biosensors constitute attractive tools for cancer diagnostics, for monitoring cancer progression and evaluating response to therapeutics, whilst also enabling development of sensitive assays for drug discovery purposes [6, 7].

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Fluorescent proteins as probes to analyse protein complexes: from quantitative live cell imaging to structural model

Cornelia S. Ziegler[a], Marc Tramier[b], Dominique Durand[c], Franck Fieschi[d] Sophie Dupré-Crochet[a], Fabienne Mérola[a], Oliver Nüße[a], **Marie Erard[a]**

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[c] Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS UMR 9198, Université Paris-Sud, Université Paris-Saclay, Gif-sur-Yvette, France
[d] Université Grenoble Alpes, CNRS, CEA, Institut de Biologie Structurale, F-38044 Grenoble, France

We developed a general analytical strategy based on FRET-FLIM and FCCS for structural and quantitative characterization of protein-protein interactions in their native cellular environment. This strategy was applied to the phagocyte NADPH oxidase. This protein complex produces superoxide anions, a precursor of reactive oxygen species (ROS), critical for the host response to microbial infections. Uncontrolled ROS production contributes also to inflammatory diseases making the oxidase a major drug target. This oxidase consists of two membranous (Nox2, $p22^{phox}$) and three cytosolic subunits ($p40^{phox}$, $p47^{phox}$, $p67^{phox}$) undergoing structural changes during activation. Using our strategy, we characterized the inter- and intra-molecular interactions of all three cytosolic subunits with regard to their conformation, stoichiometry, interacting fraction, and affinity in live cells. This study revealed for the first time in a living cell a 1:1:1 stoichiometry and nearly 100% of protein in complex. Furthermore, we built a new 3D-model of the entire cytosolic complex by uniting our FRET-FLIM and FCCS data with SAXS models, HDX-MS experiments and published crystal structures of isolated domains and subunits.

Microtubules as platforms for visualizing and analyzing protein interactions in cells

David Pastré

SABNP Laboratory, Univ Evry, INSERM U1204, Université Paris-Saclay, 91025, Evry, France

Abstract:

We are currently developing an original method to probe protein interactions along microtubules in specifically engineered mammalian cells by fluorescence microscopy. The principle is simple: A bait protein is brought onto microtubules and the presence of a putative molecular partner, attracted by the bait protein, is then detected on microtubules by fluorescence microscopy. Besides visualizing interactions in cells, this technology presents potential advantages in terms of sensibility and quantification at the single cell level. The domain of applications are broad spanning from discovery of new drugs that target protein interactions, identifying molecular partners, exploring the consequences of mutations.

IR NANOSPECTROSCOPY AS A LABEL-FREE IMAGING TECHNIQUE TO CHARACTERIZE NEW ANTIBIOTICS CARRIERS

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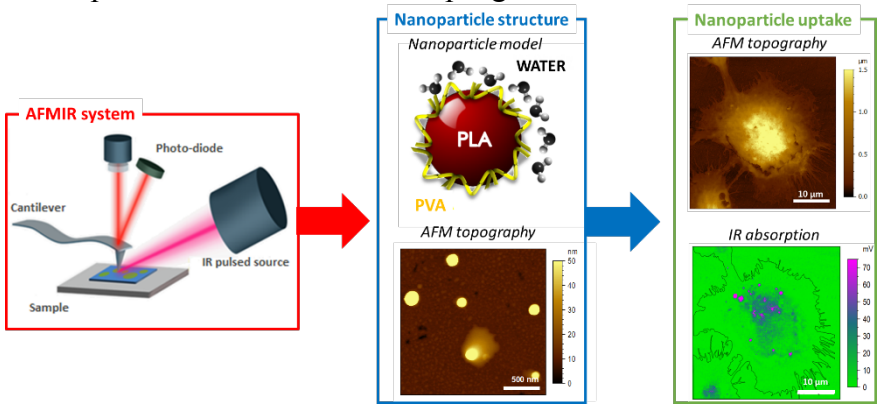
KEY SENTENCE: The main goal of the project is to characterize the structure of the free new antibiotic carriers and their detection after cellular uptake using IR nanospectroscopy as a label-free imaging technique.

KEYWORDS: IR nanospectroscopy, label free imaging, polymeric nanoparticle, macrophage

With the increase of antibiotic resistance significant effort is focused on research towards higher efficiency and better selectivity solutions. However, the side effects of high efficacy antibiotics can influence the general condition of patients, therefore using first generation compounds with nanoparticles as delivery vehicles may be a good solution to specifically target bacteria with higher doses of antibiotics. For such developments it is mandatory to understand the structure and internal chemistry of nanoparticle/antibiotic systems. To this end the most effective studies should involve label-free techniques capable of sub-micrometer spatial resolution**.**
AFM-IR microscopy is a well-established technique first demonstrated in 2005 combining the spatial resolution of an AFM microscope and a tunable IR sources to reach optical resolutions smaller than the far field diffraction limit by measuring molecular expansion [1]. Recent developments focusing on resonant excitation of the AFM cantilever (Resonance Enhanced AFM-IR or RE-AFM-IR) by a tunable pulsed source led to significant increase in sensitivity and spatial resolution up to 10 nm [2].
We will discuss the possibilities to apply RE-AFM-IR as a label free technique for the study of polymeric nanoparticles. In the first part, we will show how the method can be used to reveal new insight about the structure of polymeric nanoparticles compared with low resolution methods. In the second part, we will demonstrate the use of RE-AFM-IR as a new tool to follow the localization of nanoparticles inside fixed macrophages at the subcellular scale.



From left to right: Principle of the technique - Polymeric nanoparticle (NPs): theoretical scheme and topographic image - IR nanospectroscopy of Nps within macrophage: topographic image and chemical map.

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STEM-cathodoluminescence, a promising novel approach for bioimaging

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Correlative microscopy (CLEM) has turned an essential approach for investigations on biological systems that combines the advantages of fluorescence microscopy and electron microscopy. Classically, experimental procedures are often hampered by the use of two separate microscopes and an important limitation comes from the accuracy of the pinpointing location between light and electron microscopy data. Integrated setups allowing simultaneous light and electron imaging in the same microscope offer an interesting alternative. We proposed a different approach based on a scanning transmission electron microscope (STEM): the electron beam is used to excite the luminescence of biolabels (cathodoluminescence). Cathodoluminescence can be tracked with sub-10 nm resolution and morphological images with sub-nanometer resolution can be acquired in parallel. This method relies on the choice of luminescent nanoparticles well adapted for biolabelling. The proof of principle was established using fluorescent nanodiamonds of size ≈150 nm for cell labelling. The technique offers the possibility to use two types of labels with a distinct spectral signature. To go further, the main issue is to identify biolabels allowing molecule detection and imaging at the nanometer scale. Experiments were performed using rare-earth nanoparticles. The emission spectra of $YVO4:Eu^{3+}$ nanoparticles obtained by cathodoluminescence were compatible with the spectra measured in photon experiments. Single nanoparticles were detected on carbon film but also on resin substrates ensuring the applicability to biological samples.

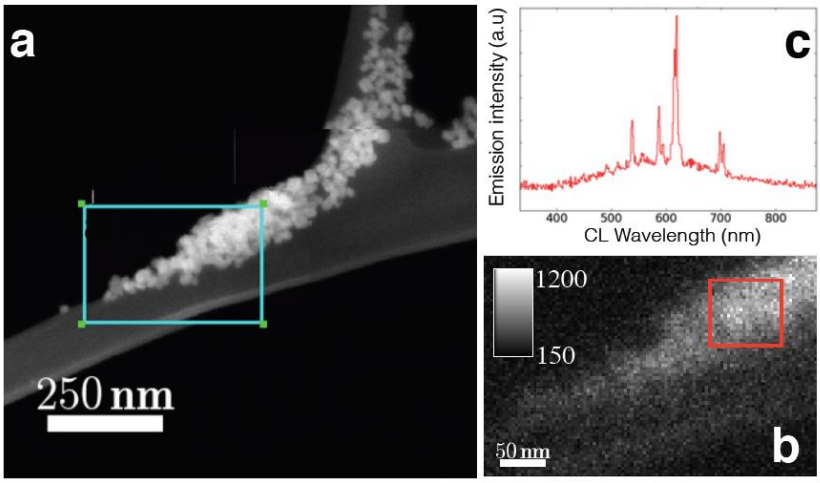

Figure: (a) STEM-HADF image of $YVO4:Eu^{3+}$ nanoparticles; (b) Cathodoluminescence image corresponding to the blue region in (a); (c) Cathodoluminescence spectrum averaged over the area delimited in red in (b)

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THRUSDAY 16 NOVEMBER

SESSION 3: IMPROVING SPATIAL AND TEMPORAL ANALYSES OF CELLULAR FUNCTIONS

SPEAKERS :

Peter Dedecker (Leuven)
Clément Cabriel (Orsay)
Pierre Montavillle (Saint-Aubin)
Maxime Dahan (Paris)
Angelina Angelova (Châtenay-Malabry)
Karine Dubrana (Fontenay-aux-Roses)

CHAIRMANS:

Sandrine Lévêque-Fort, Laurent Combettes

Sub-diffraction imaging of biosensors using “smart” labels

Peter Dedecker

Laboratory for Nanobiology, Department of Chemistry
KU Leuven

It is generally accepted that living cells make extensive use of spatial compartmentalization and structuring to limit the interactions that can occur, giving rise to e.g. signaling specificity. In this presentation I will discuss our recent work on trying to visualize biosensor responses in live cells with a spatial resolution below the diffraction limit. Our efforts thus far have centered on the use of stochastic optical fluctuation imaging (SOFI) combined with engineered sensor variants that possess appropriate single-molecule fluorescence dynamics. After introducing the fundamentals of the imaging and the fluorophores, I will discuss two recent results. In the first study, we developed an interaction sensor based on bimolecular fluorescence complementation (BiFC), by creating variants of a photochromic fluorescent protein. Using this label we could visualize protein interactions at a spatial resolution of about 100 nm. In the second study, we developed a variant of AKAR (PKA activity reporter) that encoded the activity of PKA into changes in fluorescence dynamics. Using SOFI, we could read out this kinase activity with about 100 nm spatial resolution and a temporal resolution of a few second. In this way we observed dynamic PKA activity microdomains or ‘hot spots’, whose existence was corroborated with STORM-based experiments using antibodies selective for PKA phosphorylation sites.

Combining complementary localization modalities for 3D imaging of biological samples

Clément Cabriel[1], Nicolas Bourg[1], Guillaume Dupuis[2], Aurélie Baron[3], Boris Vauzeilles[3,4], Emmanuel Fort[5], Sandrine Lévêque-Fort[1]

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Although single molecule localization microscopy (SMLM) is a well-established method for 2D super-resolution imaging of biological samples, retrieving 3D SMLM information remains a challenging task. Point spread function (PSF) engineering enables the detection of the axial positions of fluorescent molecules, but this comes at the cost of a compromise between axial capture range, axial localization precision, lateral localization precision and density of molecules [1].

To minimize the loss of lateral information when improving the axial resolution and detection range, we developed a dual-view optical setup that combines strong astigmatism PSF shaping with supercritical angle fluorescence (SAF) detection [2]. As SAF information arises from intensity measurements of the fluorophores' near-field emission coupled into propagative waves at the sample/coverslip interface, it yields a complementary absolute axial measurement, that gives a reference to the astigmatism approach. This technique, called depth astigmatic imaging with SAF yield (DAISY), provides 3D absolute information over a 1.2 µm capture range above the glass coverslip and an axial localization precision down to 15 nm with minimal loss of lateral resolution and little sensitivity to field aberrations.

We will discuss the implementation and the calibration as well as the optimal merging of the axial information sources and the performances. After presenting dual-color images on cytoskeletal networks, we will show that DAISY enables the imaging of whole living *E. coli* bacteria in the framework of the study of new click chemistry labelling techniques.

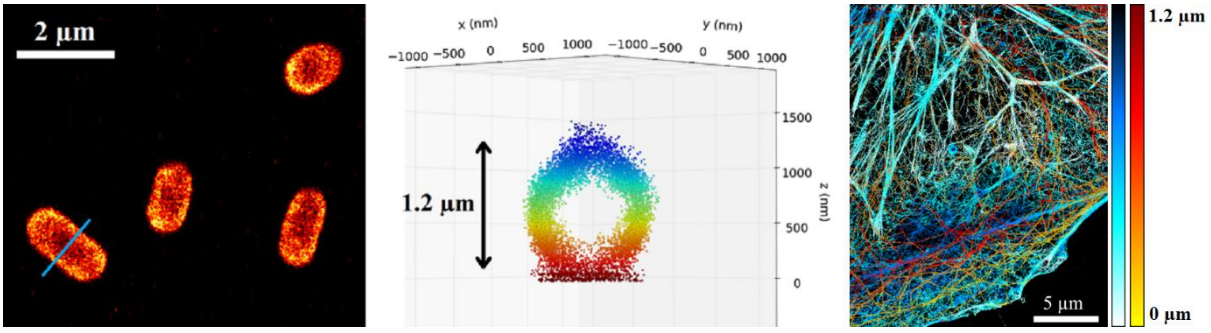

Figure 1. (left) 2D image of *E. coli* bacteria labelled with AF647. (center) 3D profile of a bacterium along the displayed blue line. (right) Dual color 3D image of the cytoskeleton of a COS-7 cell (cyan-blue: actin, yellow-red: tubulin).

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Experimental phasing approaches for in-vivo grown protein microcrystals structure determination.

Pierre Montaville, Synchrotron SOLEIL

The combined use of modern microfocus synchrotron beamlines in conjunction with serial crystallography approaches allows the exploitation of a reasonably low number of micron-sized protein crystals for structure determination. Such micro-crystallography techniques open the window towards the investigation of the full potential of in-vivo crystallography.
In-vivo crystallography is a phenomenon in which protein crystals occur within the cell or living bodies, naturally or induced by heterologous expression, in the cytoplasm or in specific subcellular compartments. Such crystals are limited in size but are produced in a native like environment. Anticipated benefits of such system are the possibility to obtain crystals of proteins for which classical in vitro crystal growth revealed unsuccessful and the opportunity to study functionally important post-translational modifications potentially providing a new tool in the protein crystallography area. Additionally, when properly understood, in vivo crystallography could minimize the amount of efforts provided in optimizing purification of samples prior to in vitro crystallization, currently presenting a very large bottleneck in the technique.

Whereas subsequent crystals purification can be performed for diffraction experiments, the more delicate ones are prone to dissolution upon cell lysis. Nevertheless in cellulo diffraction studies of such crystals have proved to be feasible and led to protein structure determination. Further characterization of properties of in cellulo diffraction is of great interest for several reasons.

Beside the fact of dealing with protein crystals grown in a native-like environment (presence of co-factors, ligands, proteolytic processing…), established advantages of in cellulo protein crystals diffraction represent a simplified and time-saving workflow for sample obtention and preparation, as the intracellular medium might act as a cryoprotectant for example, without trading for diffraction pattern quality due to the minimal crystal handling. It gives also the possibility to deal with sensitive protein crystals which revealed not stable upon crystals purification, as well as it might help for increasing the hit rate during diffraction experiment via cell staining and simplified sample identification.

However, on the road to de novo crystal structure determination, little is known about the possibility to achieve the limiting step of phase determination. As in vivo crystallography stands, it may represent a clear challenge for phase determination without experimental phases. Further to the native nature of the crystals and proteins within, which eventually include post-translational modifications currently unseen in most of the in vitro prepared samples, the requirement to perform serial crystallography measurements adds to the complexity of the process. Our preliminary results tend to indicate that, although not undocumented, structure determination of in vivo grown crystals without experimental phasing may benefit from an iterative approach combining new methods to the latest advances in structure determination from native data sets and weak anomalous scatterers.

On one hand, it is necessary to measure the effect of intracellular medium scattering on extracting the weak anomalous signal from native sulfur atoms for SAD or MAD approaches. On the other hand in order to exploit the advantage of the straightforward recording of strong anomalous signal from synchrotron radiation sources, establishing simple protocols to incorporate heavy atoms derivatives in cytoplasmic and/or compartmentalized in-cellulo grown crystals appears essential to keep the benefit of the in-vivo crystallization workflow. Therefore we propose to explore strategies to promote cellular uptake of heavy atoms and heavy atom derivatives within the cytoplasm and subcellular compartments in order to perform in-cellulo isomorphous replacement for MIR, MAD or SAD experiments.

The promises and challenges of high-density single molecule imaging : application to nanoscale organization of Rac1 signalling

Maxime Dahan

Institut Curie, CNRS UMR 168

Novel imaging techniques such as sptPALM now allow the acquisition of high-density single molecule imaging data in live cells, with up to millions of localization in a few minutes. This opens new prospects for the analysis of single molecule data and enables the implementation of advanced statistical tools and computational methods. Here I will present our effort (in collaboration with J.B. Masson, Pasteur Institute) to implement novel tracking algorithms for high density detection and to apply bayesian inference tools to these high-density tracking data in order to map the membrane environment at the subcellular scale. I will describe the principles of these methods and show their application in the case of the spatiotemporal mapping of Rac1 for which we identified a novel level of supramolecular organization.

Clustering of the Neurotrophin Receptor TrkB in the Membranes of SH-SY5Y Cells Detected by Super-resolution STED Microscopy

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We employed super-resolution stimulated emission depletion (STED) microscopy to study the nanoscale organization of the tropomyosin-related kinase receptor type B (TrkB), which is a promising therapeutic target for Alzheimer's (AD) and Huntington's (HD) diseases, amyotrophic lateral sclerosis, epilepsy, and severe neuropsychiatric disorders (schizophrenia, anxiety, and bipolar disorder). The human neuroblastoma SH-SY5Y cell line was differentiated and treated by neurotoxins in order to induce neurodegeneration. The performed imaging and subdiffraction quantification of the TrkB receptor oligomerization suggested that membrane receptor clustering may impede specific ligand-receptor binding, and thus diminish the molecular recognition by neurotransmitters acting as drugs.

Acknowledgment

Dr. V. Nicolas and the platform MIPSIT of Paris-Saclay Institute of Therapeutic Innovation is thanked for granting access to the super-resolution microscopy setup.

Challenges in high spatial and temporal resolution imaging to study genome organization and function from bacteria to human cells.

Karine Dubrana for the 3D-CHROME consortium

The 3D_CHROME consortium gathered a multidisciplinary scientific community in the Paris-Saclay area with strong and convergent interest in three-dimensional (3D) organization of genomes, which has emerged as a major player in controlling chromosome dynamics and biological processes in living cells. Genome stability, plasticity and gene expression depend upon 3D organization, which in turn may impose evolutionary constraints on genome evolution. The 3D_CHROME teams study 3D genome organization and its dynamics in living cells in a wide array of model organisms from all kingdoms of life (bacteria, archaea, uni- and pluri-cellular eukaryotes, including plant and mammalian cells). Their common aims are to identify the factors that drive the dynamics of 3D genome organization, the impact of this organization on genome functions and to evaluate the reciprocal impact of 3D organization on genome evolution. We will present some of the recent results and the challenges faced by the consortium teams that implement high spatial and temporal resolution live-cell imaging to study chromosome choreography and its consequences on genome function.

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SESSION 4: ADVANCED METHODS FOR DEEP 3D EX VIVO/IN VIVO IMAGING

SPEAKERS :

Francesco Pavone (Florence)
Chiara Stringari (Palaiseau)
Walther Akemann (Paris)
Maimouna Bocoum (Paris)
Antigoni Alexandrou (Palaiseau)

CHAIRMANS:

Cedric Bouzigues, Sylvia Bruneau

Multidimensional and multilevel imaging of tissue disease: towards the 3D digital histology

Francesco S. Pavone

European Laboratory for Non Linear Spectroscopy
University of Florence
Via N. Carrara 1

Modern optics and spectroscopy are offering promising non-invasive solutions to potentially improve diagnostic capability on tissues, as demonstrated by the extensive use of non-linear laser scanning microscopy for tissue imaging in the past decade.

The recent development and integration of multiple non-linear microscopy techniques in a single instrument has provided new opportunities for integrating morphological and functional information and for correlating the observed molecular and cellular changes with disease behaviour. In particular, multimodal non-linear/linear imaging is able to perform a morpho-chemical quantitative analysis in tumour cells and tissue specimens, providing a high-resolution label-free alternative to both histological and immune-histochemical examination of tissues. Although up to now limited to optical research labs, multimodal non-linear imaging is becoming increasingly popular among medical doctors and has the potential to find a stable place in a clinical setting in the near future.

In this talk, a brief overview on the non linear and linear laser brain imaging techniques will be displayed both for label free and specific labeling detection.

Morpho-functional characterization of tissue will be displayed as an interesting tool for early diagnosis of pathologies: different kind of approaches will be shown for in vivo imaging assisted surgery operation or as tools to support anatomo-pathologists decision.

For example, several techniques, useful to create a new 3D histological analysis also on cleared tissue will be shown, and a fiber sensors based on multidimensional spectral will be described with particular applications to tumor detection.

Probing cellular metabolism in living tissues by Fluorescence Lifetime Microscopy of intrinsic biomarkers

Chiara Stringari

Laboratory for Optics and Biosciences, École Polytechnique, Paris-Saclay, France

Cellular metabolism plays a crucial role in several physiological processes such as stem cell differentiation, cell migration, embryo morphogenesis, neurodegenerative diseases and cancer progression. A non-invasive and high resolution mapping of cell metabolism *in vivo* is fundamental for the understanding of tissue development and for testing effective treatments. We established a sensitive and efficient method for metabolic imaging of living tissues by combining non-linear optical microscopy and the use of endogenous fluorophores. Functional images of tissues autofluorescence are provided by two-photon-excitation, wavelength mixing, fluorescence lifetime microscopy (FLIM) and the metabolic coenzymes nicotinamide adenine dinucleotide (NADH) and Flavin adenine dinucleotide (FAD). NADH and FAD are the principal electron acceptor and donor in oxidative phosphorylation and they are very informative intrinsic biomarker for metabolism. We perform simultaneous imaging of NADH and FAD by using wavelength mixing, achieving efficient ratiometric redox imaging and simultaneous efficient two-photon FLIM of NADH and FAD. We are able to quantify different rates of oxidative phosphorylation and glycolysis in single cells within the tissue microenvironment and to measure lifetime gradients associated with different cellular metabolic and differentiation states *in vivo*. In conclusion, our method represents a promising tool for measuring single-cell metabolic phenotype in intact tissues with minimal phototoxicity and can be widely applied non-invasively for longitudinal studies *in vivo*.

Fast 3D-Imaging of Neuronal Activity in Cortical Columns at Single Cell Resolution

Walther Akemann, Jean-François Léger, Vincent Villette, Cathie Ventalon,
Benjamin Mathieu, Stéphane Dieudonné and Laurent Bourdieu

Institut de la Biologie, École Normale Supérieure, 46 rue d'Ulm, 75005 Paris

We developed a new type of 3D two-photon microscope able to acquire electrical activity from small networks of cortical neurons in awake animals with kilohertz sampling speed. Infrared laser pulses (200 fs) of high energy (~5 μJ) are spatially modulated in such a way so that consecutive pulses are addressing the somata of neurons at various locations in the cortical 3D volume, without addressing the in-between neuropil space. Fast modulation is obtained from a dispersion-compensated and fully programmable two-axis acousto-optic spatial light modulator (AO-SLM) [1] of large aperture (15 mm) operating at 40 kHz (full refresh cycle). To fully control the phase of the AO-SLM-diffracted laser beam, laser pulse emission is locked to the AO-SLM write-cycle and thereby fixed to a 40-kHz repetition rate. To partially compensate for the low repetition (relative to common infrared laser oscillators) and to enhance the single pulse emission yield of the indicator, laser pulses are amplified in a regenerative amplifier to high pulse energy (~10 μJ). Subsequently, the amplified pulses (1030 nm) are parametrically converted to match the peak wavelength of two-photon absorption of the indicator. For further increase in single pulse photon yield, while avoiding indicator excitation saturation as well as other unwanted consequences of high laser peak power, we added further phase modulation to the excitation beam, in addition to tilt and defocus, replacing the single spot focus by a multi spot array of up to 25 foci, all closely enough spaced to target the cell body of the same neuron.

We tested this microscope by recording single unit responses of neurons in V1visual cortex to visual stimuli of moving gratings with different orientations in anesthetized and awake mice using the calcium indicator GCaMP6f. In agreement with the known properties of V1 neurons in layer 2/3 of V1 in mice we find neurons with spiking tuned to a preferred orientation as well as neurons lacking orientation preference in their spike response. We demonstrate how holographic modulation of the excitation beam can help to prevent motion artefacts in recordings from awake mice during locomotion. We are currently trying to map out evoked and spontaneous activity throughout the cortical column by simultaneously targeting neurons in different cortical layers.

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Towards *in-vivo* acousto-optic imaging of biological tissues

Maïmouna Bocoum[1*], Jean-Baptiste Leauderau[1], Jean-Luc guenisson[1], Clément Dupuis[1], Jean-Pierre Huignard[1], Caroline Venet[1], François Ramaz[1]

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Accessing optical properties inside biological tissues is challenging because of multiple scattering. However, local measurements of the absorption and scattering coefficients is important for multiple medical applications. For instance, malignant *ductal carcimona* breast tumors absorb two to four times more, and diffuse nearly 20% more than sane breast tissues in the Near Infrared [1]. Today, techniques based on Diffused Optical Tomography can provide substantial quantitative information on tissues, however, they rely on inversion algorithms to provide actual images. Acousto-Optics (AO) imaging [2] is a bimodal imaging technique which couples ultrasounds (US) to Infrared (IR) light inside a scattering medium. The photons which paths cross with a controlled MHz-ultrasound pulse undergo the acousto-optic effect [3], resulting in the frequency shift of ω_{US} that can be selectively detected using self-adaptive wavefront holography [4]. By scanning the medium using the US, we perform direct imaging combining the spatial resolution of US to the optical sensitivity of IR light. To perform AO *in-vivo* imaging, we need to account for the fast speckle decorrelation induced by living tissues (ms). We will discuss the current limitations of our filtering technique in this regard, and what alternative solutions can be implemented to overcome this difficulty.

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***In vivo* Reactive Oxygen Species imaging for complex pathology monitoring**

Nicolas Pétri[1], Mouna Abdesselem[1], Valérie Rouffiac[2], Rivo O. Ramodiharilafy[1], Thierry Gacoin[3], Corinne Laplace-Builhé [2], Cedric I. Bouzigues[1] and Antigoni Alexandrou[1]

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Reactive Oxygen Species (ROS) are involved in numerous pathologies and, in particular, in inflammation-related conditions. Their role in the control of complex pathophysiological processes, such as some cancers, has thus has received considerable attention and different studies have yielded contradictory results. Indeed, in some cases, ROS inhibitors or scavengers were proposed to alleviate the cancer symptoms and evolution, whereas, in others, the presence of ROS leads to inhibition of the tumor metastatic potential. This highlights the importance of accurate measurements of ROS *in vivo* in order to precisely characterize their effects and determine their role in pathologies.

We have developed a highly efficient ROS sensor based on Eu-doped vanadate nanoparticles relying on oxido-reduction processes modulating the 617-nm nanoparticle emission intensity [1,2] and demonstrated efficient quantitative, time- and space-resolved intracellular ROS detection down to 1 µM [1,3]. Transposing these nanoparticles to *in vivo* ROS measurements, however, represents a challenge. We have addressed these challenges in a mouse model of inflammation. In particular, we subcutaneously injected them into anesthetized mouse ears, and imaged them at an ensemble level using a direct Eu^{3+}-ion 466-nm laser excitation and a macroscope.

After nanoparticle injection, we triggered a local inflammation through topical application of methylsalicylate to the mouse ear. Visualizing the diffusion of TRITC-Dextran injected into the blood circulation showed the characteristic increase in blood vessel diameter and in tissue perfusion expected in the case of inflammation. We could detect a specific rise of the nanoparticle luminescence due to the local production of oxidants, whereas, in the absence of the irritant, no significant luminescence change was observed. We thus revealed a robust and reproducible ROS production in different mice, but with a kinetics presenting an important inter-individual variability. However, a general pattern can be distinguished showing a rapid ROS increase probably due to resident immune cells followed by a slower rise that can be attributed to recruited immune cells. Calibration experiments should enable quantitative information on the local ROS production, not necessarily measuring a specific species but rather a total oxidant content. These results provide a framework to dissect molecular/cellular mechanisms of inflammation: further experiments including pharmacological interventions inhibiting different contributors of the reactive oxygen (ROS) and reactive nitrogen species (RNS) will shed light onto their relative importance in inflammatory signaling process.

This work paves the way to precise oxidant measurements in various pathologies which may lead to establishing correlations between reactive oxygen and nitrogen species measurements and disease severity, evolution, as well as treatment efficiency.

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FRIDAY 17 NOVEMBER

SESSION 5: HISTOCHEMICAL ANALYSES OF CELLS AND TISSUES

SPEAKERS :

Matthia Karreman (Heidelberg)
Dominique Bazin (Paris)
Sana Tfaili (Châtenay-Malabry)
David Touboul (Gif-sur-Yvette)
Elena Longo (Palaiseau)

CHAIRMANS:

Bertrand Cinquin, Larbi Amazit

Catch me if you CLEM: Imaging Single Tumor Cells at the Blood-Brain-Barrier using Multimodal Correlative Microscopy

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The main cause of mortality in cancer patients is metastasis, the spreading of tumor cells (TCs). Following release from the primary tumor, the tumor cells enter the bloodstream and arrest at a distant site. There, they exit the bloodvessel (extravasation) and a small subset of these cells grows out into metastases [1]. Since extravasation is a rare and transient event, how this process occurs *in vivo* is still largely unknown. Intravital microscopy (IVM) enables studying crucial steps of the metastatic process [1,2], but it is limited in resolution and it fails to reveal the structural context. Combining IVM to 3D Electron Microscopy (3DEM) enables to correlate functional and dynamic in vivo imaging to high-resolution of the tumor cells and their microenvironment. However, keeping track of single tumor cells when moving from IVM to EM imaging is highly challenging in complex tissue samples [3].

In this talk, I will demonstrate Multimodal Correlative microscopy, our newly developed approach that combines x-ray microscopic computer tomography (microCT) [4] to correlate IVM to EM [5,6]. We used this workflow to study extravasation of TCs in mice brain *in vivo*, to gain more insight in how metastatic cells cross the blood-brain-barrier. Here, *in vivo* FM imaging was performed of intracardially-injected fluorescent tumor cells, arrested in the brain vasculature of a living mouse. Subsequently, brain biopsies containing the tumor cells were processed for EM and embedded in a resin block. Next, microCT scans were obtained and correlated to the FM volumes, based on structural features of the sample visible in both datasets. 3D registration of both datasets enabled to predict the position of the tumor cell inside the resin block, allowing to accurately approach this area and studying it at high resolution with EM [5,6]. Significantly speeding up the correlative workflow, this method allows performing high-resolution imaging of a statistically relevant number of samples.

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BIOMINERALISATION AND PATHOLOGICAL CALCIFICATIONS :BEAUTY AND THE BEAST

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Recent advances in the characterization of microcrystals in tissue samples based on physico-chemical techniques are reviewed. It is a new opportunity for the physician to access early a diagnosis of rare but severe pathologies and to start a specific therapy able to delay or to stop an irreversible alteration of the organ, for example to avoid dialysis and transplantation when kidney is mainly affected. To date, more than 400 biopsies of kidneys containing crystals were performed and characterized using such techniques. The data collected on crystals, tissue alteration, clinical and biological investigations are of prime importance to help a better understanding of biochemical process involved in crystal formation. Such techniques may be applied to microcrystalline pathologies affecting other organs than kidney, namely prostate, pancreas, thyroid, breast. Multicentric and international collaborations were developed, thus offering new opportunities in studying pathophysiology of deposited microcrystals and their consequences. In fact, crystals may be the consequence of various pathologies, but they are also involved in the dysfunction of the tissue where they accumulate. Recent results will be presented.

Infrared and Raman spectroscopy for the analysis of J774 macrophages.

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Résumé/abstract

Atherosclerosis is an inflammatory disease of the arterial wall caused by the formation of an atheroma plaque in the vessels wall. The absorption of the LDL lipoproteins by sub-endothelial macrophages induces the latter's transformation into foam cells, which is the key step of the atheroma plaque formation. The modifications of neutral lipids caused by foam cells formation can be marked by the appearance of lipid droplets. A comparative study is conducted on murine macrophages J774, loaded or not with cholesterol, enriched or not with Eicosapentaenoic acid EPA. The J774 in the four different cell medium conditions are analyzed by Raman and infrared vibrational spectroscopies. Data analysis of single point spectra and images led to the identification of some spectral changes due to the murine macrophages J774 enrichment with fatty acids.

Last developments of TOF-SIMS for high spatial resolution molecular imaging and 3D-profiling

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MS imaging (MSI) has already become a very popular analytical tool for visualizing the local chemical composition on surfaces and to determine the distribution of each ion detected on the collected mass spectra. Four main methods are now used for this purpose: MALDI, focused ion beams (secondary ion MS, SIMS), DESI and inductively coupled plasma MS (ICP-MS). Whereas SIMS imaging was first described in the 60s, it is often considered as a physicist tool to scrutinize inorganic surfaces.

With the development of cluster ion sources (Au_n^+, Bi_n^+ with $n < 5$, $C60^+$ and Ar_n^+ with $n > 250$), TOF-SIMS is regaining of interest especially for the detection of small biomolecules. It is thus interesting to shortly compare the capability of each MSI technique toward TOF-SIMS to understand why the potential of the latter is still largely underestimated for biological applications.[1]

Two recent significant improvements of TOF-SIMS imaging, *i.e.* the capacity of performing both high mass and spatial resolutions using a delayed extraction[2] and the ability to get 3D-profiling using dual ion beam technologies,[3] will be introduced through experimental developments and biological applications (distribution of lipids on tissue section[2], mapping wood constituents,[4] and 3D-profile of natural products in leaves[5]).

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3D high resolution imaging by means of X-ray Phase Contrast Tomography to study theranostic nanoparticles distribution in mice organs

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X-ray Phase Contrast Tomography (XPCT) allows imaging anatomical details with great accuracy by achieving the 3D reconstruction of a specimen in a non-destructive manner. It is widely used at micro-scale to detect abnormalities of the vessels, which are associated to the tumor growth or to the development of neurodegenerative diseases[1],[2]. XPCT represents also a promising and complementary tool, next to other well-established techniques such as histology or LIBS, for studying the biodistribution of theranostic nanoparticles (NPs) in healthy and cancer tissues [3],[4]. High resolution images with a spatial resolution of 3 µm of mice organs injected with gadolinium-based NPs and gold nanoclusters were acquired at the biomedical beamline ID17 of European Synchrotron Radiation Facility (ESRF), France. In this work, we will show different applications for XPCT: from the imaging of the 3D vascular network in different mice model organs up to tiny vessels, to the biodistribution of theranostic NPs in different biological tissues. In particular, we present the capability of deep tumor targeting, such as melanoma metastasis in mouse model brain, exerted by gadolinium-based NPs. These compounds revealed excellent diagnostic agents thanks to their strong contrast properties with respect to soft tissue.

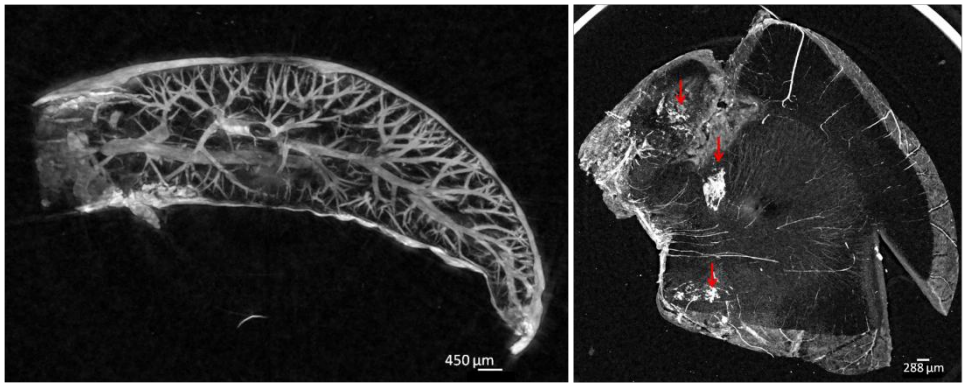

Figure 1. On the left, 3D vascular network of the liver of a mouse model. On the right, 765 µm thickness of a mouse brain showing multiple melanoma metastasis targeted by gadolinium-based NPs (red arrows).

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POSTERS ABSTRACTS

15TH-17TH NOVEMBER 2017

BÂTIMENT DES COLLOQUES (338) - FACULTÉ DES SCIENCES, ORSAY

Glycan metabolic engineering, a powerfull tool for cell labeling

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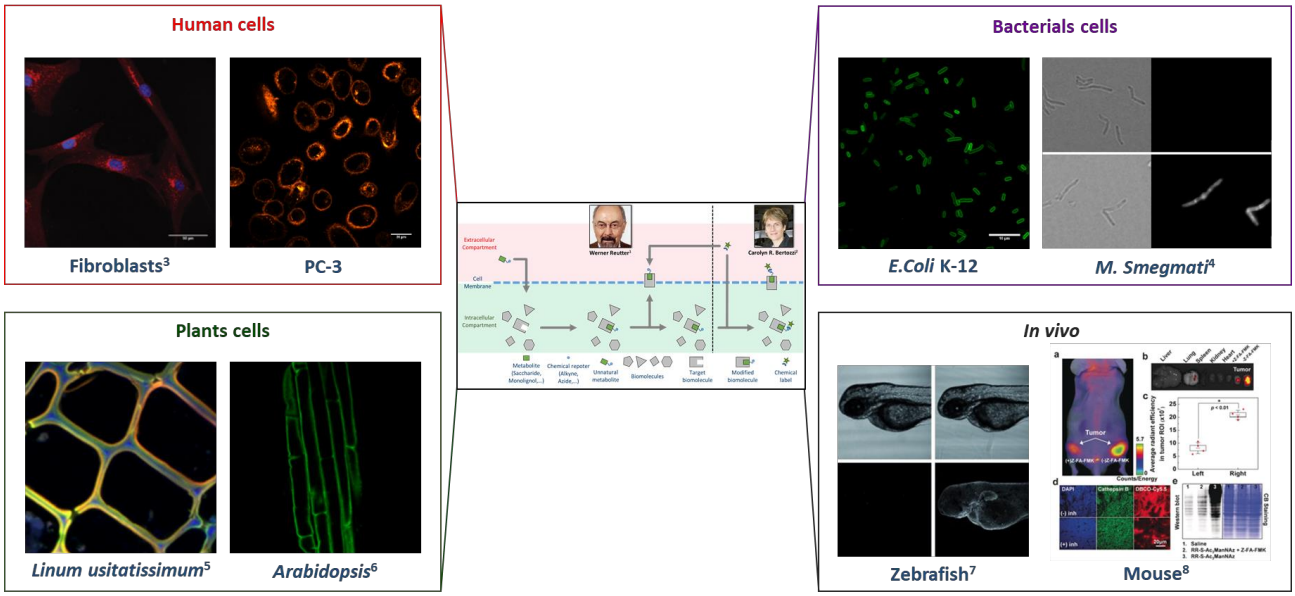
Glycan metabolic engineering is a powerfull tool for cell labeling. This method uses a synthesized unnatural saccharide bearing a chemical reporter. This sugar is metabolically incorporated into glycans and may be labeled by fluorescent probes.[1,2] The fluorescent probes can be detected by flow cytometry or visualized by confocal microscopy to validate the labeling of modified glycans *in cellulo*.



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Why do proteins remain well dispersed in membranes?

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Cell membranes contain many inclusions (such as protein membranes) which deform the underlying lipid bilayer, by thinning or thickening it to match the thickness of the transmembrane domain of the protein. This deformation engenders between the included objects an interaction, which simple elastic theories predict to be attractive. Combined with the high concentration of inclusions, such an attraction should lead to their aggregation. However, a large majority of membrane proteins remain well dispersed.

To solve this long-standing puzzle, we use a complete elastic theory for the deformation [1] in order to determine the interaction potential between gramicidin channels in membranes by fitting small-angle X-ray scattering data recorded at varying channel concentration [2]. We show [3] that the essential ingredient is the preferred slope at contact, which induces a short-range repulsion. In phospholipid (DLPC) bilayers, the membrane thickness decreases with an angle of about 30° away from contact.

We confirm our results by predicting (with no adjustable parameters) numerical simulations for the interaction of gramicidin channels in other types of membranes as well as experimental conductivity data for the lifetime of the channels in DOPC, bringing together three completely different experimental techniques within one theoretical framework.

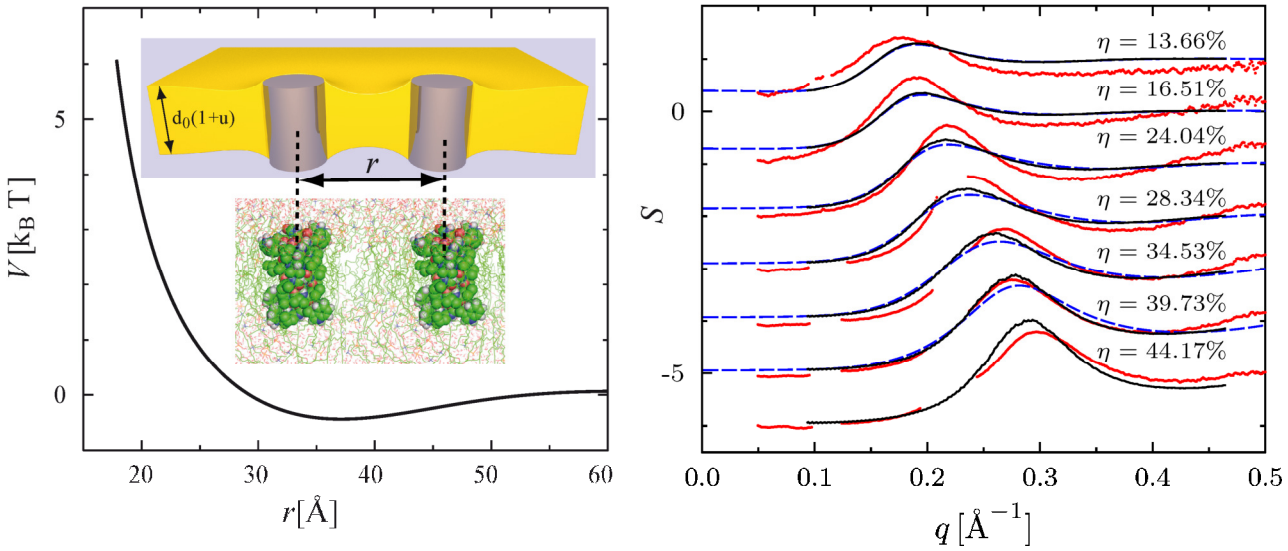

Figure 1 Left: Interaction potential $V(r)$ between two gramicidin channels in a DLPC membrane. The curve corresponds to our best fit of the experimental data. Inset: elastic model of the membrane, with the channels described as rigid cylinders; a more realistic representation is given below. Right: Structure factors $S(q)$ for the same system at different surface fractions η of inclusions (red solid lines) and best fits using the interaction potential on the left. The fits are obtained via Monte Carlo simulations (solid black lines) or using the HNC approximation (dashed blue lines).

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Multiscale, dynamic, *in vivo* imaging of *Arabidopsis* roots

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A recently developed microfluidic platform, the Rootchip, demonstrated a new technology for *in vivo* imaging of plant roots [1a, b]. We have expanded on the use of PDMS microfabrication on coverslips to create a wide variety of microfluidic devices for *in vivo* dynamic imaging of model *Arabidopsis*, and potentially other, plant roots. These microponic chips offer access to detailed imaging, measurement and analysis of multiscale 4D cellular processes and events that may be unravelled thanks to the concomitant development of plant lines containing targeted, genetically encoded, fluorescent nanosensors. Some of the current uses of these microponic chips combined with nanosensor imaging include, for example:

- elucidating key cell cycle and division events by imaging membrane, cytoskeleton, and cell-cycle markers, and finely mapping cellular parameters relevant to the temporal/spatial control of cell divisions in the root meristem [2],
- study of cell elongation through the use of biosensors such as pH, calcium and ROS, and inhibition of cell elongation by drug application,
- elucidating early cellular events involved in autophagosome formation, and controlling induction of autophagy through stress [3],
- study of ion and metal trafficking, concentration, localization and transport [4].

Further microponic chips under development include mechanosensing chips, for studying the roles of mechanosensitive channels in response to stress [5] through the use of integrated features and dynamic valves for inducing passive or dynamic mechanical constraints on the root, and regeneration chips, for studying the control of plant regeneration from single undifferentiated cells. Our microponic chips offer a catalogue of flow layouts to suit the application, including parallel imaging of multiple roots in the same device, array layouts, different channel sizes to suit the root model under study, as well as easily adjusted flow setups for perfusion of media or addition of drugs or treatments.

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Receptor-based artificial metalloenzymes on living human cells

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Artificial metalloenzymes are known to be promising tools for biocatalysis, but their recent compartmentalization has led to compatibly with cell components thus shedding light on possible therapeutic applications.

We prepared artificial metalloenzymes based on the A_{2A} adenosine receptor embedded in the cytoplasmic membranes of living human cells. The wild type receptor was chemically engineered into metalloenzymes by its association with strong antagonists that were covalently bound to copper(II) catalysts. Their characterization was performed by competition with a radio ligand, by a functional assay and by microscopic imaging of the surface of the cells. The artificial metalloenzymes at the surface of living cells enantioselectively catalyzed the abiotic Diels-Alder cycloaddition reaction of cyclopentadiene and azachalcone.

The prospects of this strategy lie in the organ confined *in vivo* preparation of receptor-based artificial metalloenzymes for the catalysis of reactions exogenous to the human metabolism. These could be used for the targeted synthesis of either drugs or deficient metabolites and for the activation of prodrugs, leading to therapeutic tools with unforeseen applications.

Pipet-based Scanning Probe Microscopy Tip-Enhanced Raman Spectroscopy: A Novel Approach for TERS in Liquids

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In the recent years, a set of very powerful Scanning Probe Microscopy (SPM) techniques that employ pulled glass pipets as probe have demonstrated its potential in key fields of science and technology such as material science, electrochemistry and biology.[1] Despite the inherent simplicity of the probe and its feedback mechanism, based on ion flow through the open barrel(s) of the pipet, pipet-probe SPM (pb-SPM) such as Scanning Ion Conductance Microscopy (SICM) are renowned as strong alternatives to AFM due to the truly non-contact nature of its imaging mode.[2]

Beyond the topographic imaging capability at nanometer resolution, the potential of pb-SPM techniques resides in the ability of functional imaging, employing an extended variety of multifunctional probes that allows relevant complementary information to be obtained simultaneously during imaging, such as local sensing (e.g. pH, electroactive species, ion selective).[3]

We are expanding further the capabilities of the functional imaging capabilities of SICM with chemical and structural information at the interface via Tip Enhanced Raman Spectroscopy (TERS), a label-free non-destructive optical technique. This unprecedented imaging and nanospectroscopy platform will be capable to perform experiments in liquids, on life cells, bringing insight into interfacial phenomena such as membrane trafficking.

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   Zhang et al *ACS Nano*, **2016**, 10 3214–3221

Cell-dependent EGFR membrane nano-organization revealed by single-particle imaging

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Membrane organization in microdomains is considered to be essential for the optimization of signaling processes. However, little direct experimental evidence is available to support this hypothesis. We here focus on epidermal growth-factor receptors (EGFR), which are implicated in numerous signaling pathways and involved in widespread diseases like some cancers or rapidly progressive glomerulonephritis (RPGN), a severe disease leading to kidney failure. Experiments on mouse models have shown that mice in which EGFR or its ligand, HB-EGF, are inhibited [1], or in which tetraspanin CD9, a protein responsible for the formation of tetraspanin-enriched microdomains, is not expressed, are partially protected from the disease.

We label EGFRs which are usually confined in microdomains with luminescent, highly photostable $Y_{0.6}Eu_{0.4}VO_4$ nanoparticles and use single-molecule tracking to examine the kind of motion they are undergoing [2]. In MDCK (Madin-Darby canine kidney) epithelial cells, we found that EGF receptors are confined in cholesterol- and sphingomyelin-rich microdomains. In contrast, in cells involved in RPGN (Parietal Epithelial Cells or PEC), EGFR are mostly confined but the characteristics of the confinement domains are different from those in MDCK cells. This observation, combined with the *in vivo* data, indicates that EGF receptors may be confined in tetraspanin-enriched microdomains in these cells. This suggests that EGF receptors may be confined in different microdomains depending on the cell type. Further single-molecule tracking experiments in cells with and without treatment with different pharmacological substances targeting specific actors involved in the membrane micro-organization should provide a quantification of the differential nature of EGFR confining microdomains.

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METROLOGY IN NONLINEAR MICROSCOPY USING HARMONIC GENERATION NANOPROBES

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Over the last decades the range of excitation wavelengths in nonlinear microscopy has continuously shifted toward the near infrared window. Nowadays, excitation wavelengths ranging for example from 750nm up to 1300nm are routinely used to perform multi-parametric imaging [1,2]. This new trend opens new perspectives and applications but it also raises new technical issues and calls for new standards for quantifying and comparing the performance of nonlinear microscopes. In particular, microscopes equipped with multiple femtosecond sources spanning the entire near-infrared wavelength range are often difficult to characterize with current approaches based on fluorescent probes.

In this study, we present a new and straightforward method to calibrate the imaging properties of nonlinear microscopes over a broad range of excitation wavelengths [3]. We show that harmonic generation nanoprobes are a unique tool to map the spatial resolution, field curvature and chromatic aberrations of nonlinear microscopes with a precision below the diffraction limit and across the whole field of view. We analyze and compare measurements obtained with several microscope objectives designed for multiphoton microscopy over the 850-1100nm wavelength range. Finally, we discuss strategies to minimize the impact of chromatic aberrations for multicolor two-photon acquisitions and we show how our method can be used for the post-acquisition correction of chromatic aberrations.

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Gold Nanoparticles for Plasmonics and Medicine

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Abstract:

Gold-bioconjugates are studied worldwide and promising for new technologies for health. In the long term, biodegradable gold nanoparticles (NPs) are expected to have a large impact on diagnosis through the development of new contrast agents for imaging or new ultrasensitive sensors. Gold-NPs have a high potential as contrast agents for several bioimaging modalities such as computed tomography, photoacoustic imaging, dark field scattering, multiphoton luminescence, high frequency ultrasound, quantitative phase contrast. New therapies and surgeries will develop because these gold-nano are also able to generate Reactive Oxygen Species (ROS) and heat.

Our research activities concentrate on the synthesis and assembly of gold nanoparticles of **high quality** to provide **original materials** for plasmonics since 2008. The irradiation of gold NPs by **short laser pulses** sets off a cascade of complex transient phenomena. Following this irradiation, one observe exaltation and confinement of the incident light at the surface of the NP but also singlet oxygen generation and ROS through the injection of hot electrons (or hot holes) to a nearby molecule. In short, gold nanoparticles are **nanosources of light, heat and hot carriers** and the **morphology of the NP is a key point** for these three characteristics because competitive relaxation processes depend on the size, shape and aspect ratio of NP.

We use colloidal chemistry to synthesize **gold nanoparticles of controlled shape and size**. The figure illustrates some of the gold NPs we propose. Contrary to spherical and rod-shaped NPs that are commercially-available, other shapes such as cubes, triangles .. and plates with tunable sizes are only produced in our lab and in few laboratories worldwide. In literature, triangular-gold nanoplates are already used for ultrasensitive sensing, and the construction of original plasmon-based optical devices**.** The recent discovery of a third and fourth biological transparency windows centered respectively at 1.8µm and 2.2µm and the recent commercialization of new NIR-lasers make gold-nanoplates attractive for biomedicine in this still unexplored spectral domain. The synthesis of highly uniform shapes relies on trial and error procedure because it is very hard to predict the right recipe and additional purification steps are also often required. **In the future**, we are eager to widen the application range of these NPs to medicine through **new collaborations either with biochemists for appropriate biofunctionalization, biophysicists for imaging and physicians for therapy.** As a first step towards this objective, we are developing recipes to produce Au@SiO_2 core-shell NP while waiting for precise specifications.

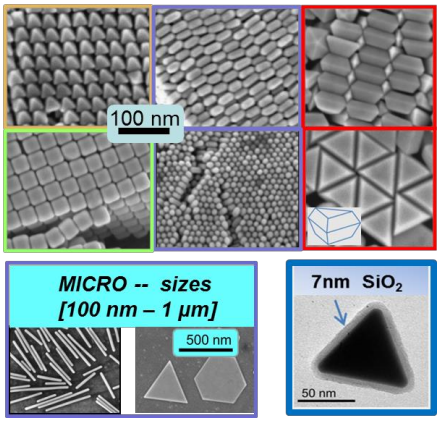

Figure 1: illustration of some of the monodisperse gold particles (nano- and micro-) and gold hybrids (Au@SiO2) that have been synthesized, with tunable sizes and thicknesses.

Caractérisation de la réponse immune à l'infection et à la vaccination par microscopie biphotonique chez le primate non humain

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L'infrastructure IDMIT (Infectious Disease Models and Innovative Therapies), située sur le site du CEA de Fontenay-aux-Roses, est un centre de recherche préclinique dédié à l'étude des maladies infectieuses humaines chez le primate non humain.

La plateforme d'imagerie du centre IDMIT est un complexe unique proposant des technologies d'imagerie multimodale (fluorescence et TEP-TDM) installées dans des laboratoires et des animaleries en niveaux 2 et 3 de confinement biologique pour l'observation *in vivo* et *ex vivo* d'animaux infectés ou traités expérimentalement.

Parmi les différents équipements, nous disposons d'un microscope multiphoton. Ce système étant couramment utilisé en recherche préclinique chez la souris, nous avons dû l'adapter au macaque et à l'utilisation dans un environnement confiné. Des résultats ont déjà été obtenus *ex vivo* notamment sur des biopsies de poumons issus de babouins infectés par la coqueluche et sur des ganglions trigéminés de souris infectées par le virus HSV. Notre objectif à présent est d'étudier *in vivo* les interactions hôte / pathogène et de caractériser la réponse immune à l'infection et à la vaccination chez le macaque au niveau de la peau et des ganglions par exemple. Ces études sont actuellement en cours de développement.

L'imagerie *in vivo* : une méthode alternative pour l'étude des maladies infectieuses chez le primate non humain

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Les approches utilisant l'imagerie *in vivo* pour l'étude des maladies infectieuses chez le primate non humain (PNH) ne sont pas encore très répandues. Ceci s'explique en partie par l'accès limité à des structures et des équipements adéquats pour l'imagerie chez le gros animal en laboratoire de confinement biologique pour les pathogènes humains de classe 2 et 3.

Le centre IDMIT est une infrastructure nationale dont l'objectif est de fournir à la communauté scientifique des ressources pour l'étude des maladies infectieuses chez le PNH. IDMIT est constitué de laboratoires de recherche et de plateformes technologiques (cytométrie de flux et masse, immunomonitorage, expérimentation et bien-être animal, imagerie *in vivo*). Les principaux objectifs du Laboratoire d'Imagerie de l'Infection et de l'Immunité sont de développer des approches permettant le suivi longitudinal et minimalement invasif de l'infection, de la réponse immune de l'hôte à l'infection et aux traitements, et de l'impact de ces traitements sur la persistance des pathogènes. L'imagerie *in vivo*, en réduisant les procédures invasives, peut contribuer au raffinement de l'utilisation des PNH. Les différentes modalités d'imagerie (TEP, imagerie optique) accessibles au sein de cette plateforme sont installées dans des laboratoires en niveau 2 et 3 de confinement biologique pour permettre l'observation d'animaux infectés ou traités expérimentalement.

Far infrared micro-spectroscopy: an innovative method to detect individual Metal-Organic Framework particles

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The AFMIR method of micro-spectroscopy measures directly the transient local dilatation absorption due to light absorption [1] and is a standard method of the CLIO infrared free-electron laser (FEL) facility. We extended it to far-infrared (10 to 50 μm typically) by using the combination of diamond substrates and the FEL as the tunable source. The purpose of this extension is to establish a differential method of infrared micro-spectroscopy in order to allow the accurate detection of nanoparticles of interest for biomedical applications. We take then advantage of the presence of specific adsorption bands in far infrared in order to individually discriminate them from those of ordinary biological materials, possessing broad bands in the 2 -10 μm rang. Among these, Metal-Organic Framework (MOF) nanoparticles attracted increasing interest due to their capacity to incorporate high drug payloads, biodegradability and possibility to tailor their surface by grafting specific ligands. However, MOF particle detection in biological media without grafting or incorporating fluorescent molecules is challenging. Here we show that single MOF nanoparticles can be imaged with a spatial resolution of a few tens of nanometers[2].

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