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MiFoBio

Functional Microscopy for Biology

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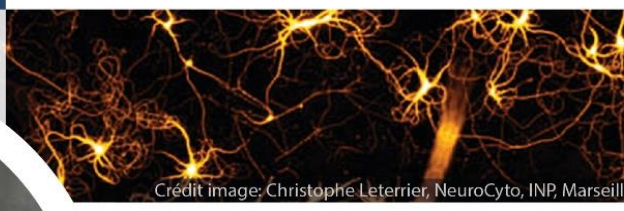

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Workshops

Fablab
Opticslab

Labelling, probes and constrasts.

New imaging for life

AI for bioimaging.

Molecular dynamics and interactions : *experiments and modeling.*

Quantification in nanoscopy.

Cellular signalization, Mecanobiology, Mecanotransduction.

Multicellular imaging : *organoids, tissue, embryos.*


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Super-resolved imaging in totale internal reflexion using random illumination microscopy

Presenter: Kévin AFFANNOUKOUÉ

Institut Fresnel - UMR 7249, Faculté des Sciences St Jérôme, 13013 Marseille, France

L'observation de structures biologiques au niveau de la membrane cellulaire en microscopie de fluorescence est difficile. Il s'agit d'observer des objets très fins, peu intenses et peu contrastés à cause de la fluorescence provenant du volume de la cellule. La microscopie TIRF (Total Internal Reflection Fluorescence) est une méthode qui consiste à exciter la fluorescence de l'échantillon par un éclairement évanescent sur seulement quelques centaines de nanomètres. La microscopie TIRF est ainsi largement utilisée pour étudier les phénomènes biologiques se passant au voisinage de la lamelle. Cependant, la qualité des images est limitée par une résolution transverse de l'ordre de 200 nm et la présence plus ou moins forte de fluorescence hors focus résiduelle provenant de la diffraction de l'onde évanescente par l'échantillon. Pour améliorer la résolution du TIRF, la configuration SIM (Structured Illumination Microscopy) a déjà été utilisée et permet de gagner un facteur deux sur la résolution. Néanmoins cette méthode est contraignante et difficile à mettre en œuvre car le SIM nécessite une illumination sinusoïdale parfaitement connue.

Or lorsque l'on travaille avec de grands angles, aux bords de l'ouverture numérique de l'objectif, ce dernier présente de fortes aberrations ce qui déforme la grille de lumière et rend la reconstruction des images très difficile. Le RIM (Random Illumination Microscopy) dans sa configuration TIRF permet d'atteindre une résolution équivalente au SIM (~100nm) ainsi que d'améliorer le sectionnement optique du TIRF sans avoir besoin de connaitre l'illumination grâce à des éclairements aléatoires.

MicroVIP: Microscopy image simulation on the Virtual Imaging Platform

Presenter: Ali AHMAD

Institut National des Sciences Appliquées de Lyon | INSA Lyon · Research Center for Medical Imaging (CREATIS - UMR 5220), France

MicroVIP is an open source software that assembles, in a unified web-application running on distributed computing ressources, simulators of the main fluorescent microscopy imaging modalities. MicroVIP provides realistic simulated images including several sources of noise (microfluidic blur effect, diffraction, Poisson noise, camera read out noise). MicroVIP also includes a module which simulates single cells with fluorescent markers and a module to analyse the simulated images with textural and pointillist feature spaces. MicroVIP is shown to be of value for supervised machine learning. It allow to automatically generate large sets of training images and virtual instrumentation to optimize the optical parameters before realizing real experiments.

Simultaneous estimation of 3D localization, 3D orientation and wobble for single emitters microscopy

Presenter: Luis Arturo Aleman CASTANEDA

Institut Fresnel - MOSAIC group, Aix-Marseille Université, 13397, Marseille, France

We present a new technique for fluorescence microscopy that enables orientation and wobbling characterization plus 3-D localization. The information is encoded in the PSF of the circularly polarized components using polarization-based Fourier filtering. The birefringent mask used is referred as stressed-engineered optic (SEO) that has been used previously for single-shot polarimetry. The method is compatible with normal fluorescent microscopy settings, even at low level photon counts, e.g. STORM experiments.

Label-free interferometric detection and characterization of single nanoparticles

Presenter: Samer ALHADDAD

Institut Langevin, ESPCI, Paris, France

At Langevin Institute, ESPCI Paris, we have developed a new, sensitive, interferometric, and non-destructive optical approach to detect label-free single nanoparticles (NPs) that allow us to count and sort different types of viruses[1] (Boccara et al, Biomed. Opt. Exp. 2016). In short, we measure the light scattered by the nanoparticles and obtain an interferometric signal; this measurement is complemented with single particle tracking analysis of their Brownian motion in order to determine the NPs size and nature, allowing us to distinguish between objects of the same size like for example virus and membrane vesicles. On one hand, thanks to the common-path and transmission configuration of our microscope, the ability to detect small virus and NPs is robust and insensitive to spherical aberrations produced by the index mismatch of the environment. This opens important perspectives to study virus infection and target-search strategies without the need of fluorescence labeling. On the other hand, we have developed an assay based on antibody recognition of targeted virus in which we associate changes in diffusion to antibody recognition and possibly aggregation.

We have applied this approach to different bacteriophages and mammalian viruses, with antibodies targeting different surface proteins, using only primary antibodies or a combination of primary and secondary antibodies. In all cases, we have observed a significant change in diffusion, an increase of the number of detected particles at the time mark of around one minute, and a decrease of the number of detected particles at a longer time scales indication aggregation (tens of minutes), validating thus our assay as a tool for rapid virus identification or to study antibody-antigen interactions.

BIAFLOWS: Benchmarking & Deploying Reproducible BioImage Analysis Workflows on the web

Presenter: Volker BÄCKER

INSERM, BioCampus Montpellier, France

BIAFLOWS (Rubens et al., Cell Patterns, 2020) is an open-source web platform extending Cytomine (Marée et al., Bioinformatics, 2016) that has been developed to package and benchmark bioimage analysis workflows by defining standard data formats for a broad range of problems and by storing image datasets, annotations, image analysis workflows and associated functional parameters in the same platform. Once integrated to BIAFLOWS, workflows written in any programming language or targeting any bioimage analysis software can be run remotely on the images of a dataset stored in the system, and all the results can be co-visualized through a user-friendly web interface.
Additionally, when ground truth annotations are available, a set of problem dependent benchmark metrics assessing the accuracy of the workflows is computed and available as consolidated statistics. All data is stored in unified databases and can be explored and reproducibly shared through the web for effective collaboration.

In vivo 3D-STED microscopy in the hippocampus

Presenter: Stéphane BANCELIN

Institut Interdisciplinaire de Neurosciences, IINS UMR5297, Bordeaux, France

In the past decade, STED microscopy has emerged as a powerful tool to visualized nanometric brain structures in various conditions. Based on the use of a donut or bottle shape depletion beam to break the diffraction barrier, STED microscopy crucially depends on the quality of the point spread function (PSF) of the depletion beam. However, maintaining a good PSF deep within brain tissue, to obtain sub-diffraction resolution, remains highly challenging, especially when imaging living acute brain slices and in vivo. Indeed, optical aberrations stemming both from the system and the specimen induce distortions on the laser wavefront that can drastically degrade the quality of the STED beam (especially its symmetry and the null in the center).

In this work we proposed several approaches to enable brain imaging beyond the diffraction limit in living mice. First, we demonstrated a simple approach to improve resolution in depth by using an a priori estimation of aberration as a function of depth, focusing on the main type of aberration induced by biological specimen, which is spherical. We imaged YFP-labelled pyramidal neurons in brain slices and show that, while this aberration correction does not provide the optimal PSF, it is still enough to significantly increase the image quality. Indeed, we observed a resolution down to 75 nm at a depth of 90 µm inside a living brain slice and, comparing corrected and uncorrected STED images, we observed a signal increase of up to 60%. We then developed and implemented a new hippocampal window to enable in vivo 3D-STED imaging of pyramidal neurons in a deep brain region.

Integrated microfluidic and microscopy set-up to measure oocytes mechanical properties and predict their fitness

Presenter: Lucie BARBIER

CIRB, Oocyte Mechanics and Morphogenesis (OMM), Collège de France, Paris

Oocyte production during meiosis in human females is error-prone and generates a basal rate of bad quality oocytes. They are associated with miscarriages and congenital diseases such as Down syndrome. Moreover, their rate increases with maternal age and leads to the need for medically assisted reproduction procedures such as in vitro fertilization (IVF). Therefore, it is crucial to move from qualitative to quantitative approaches and find new technologies to assess oocyte quality and increase the rate of successful IVF.

Recent works have shown that oocytes mechanical properties could be used as a biomarker of their development potential[1,2,3]. Indeed, oocytes with too high or too low cortical tension are not able to develop after fertilization[1]. Moreover, low cortical tension leads to chromosomes miss-segregation and aneuploidy[3]. However, current methods used in research laboratories to measure oocyte cortical tension are low throughput, require expensive equipment and manipulation skills, limiting their application in medical environment.

Thus, we aim to develop a simple minimally invasive microfluidic device able to sort oocytes depending on their cortical tension to implement its use as a predictive biomarker in clinical practice. We based our approach on micro-metric constriction as cells mechanical properties can be linked to different transit score parameters[4]. We are implementing an integrated environment to control both the flow in the microfluidic device and image recording of oocyte deformation to allow their measurement by non-trained users on a standard video microscope. By recovering oocyte after measurement, we have shown that oocyte transit in the microfluidic device did not alter their development. Moreover, we found significant variability in passage time within a wild-type oocyte population reflecting the diversity in cortical tension in murine oocytes. These values are currently being coupled with tension measurements to confirm that the microfluidic device can discriminate oocytes by their mechanical properties. These preliminary experiments show the potential use of our comprehensive set-up to assess oocytes mechanical properties in a user friendly and non-invasive manner.

1. Yanez LZ, Han J, Behr BB, Pera RA, Camarillo DB. Human oocyte developmental potential is predicted by mechanical properties within hours after fertilization. Nat Commun. 7:10809 (2016)
2. Chaigne A, Campillo C, Gov NS, Voituriez R, Sykes C, Verlhac MH and Terret ME. A narrow window of cortical tension guides asymmetric spindle positioning in the mouse oocyte. Nat Commun. 6:6027 (2015).
3. Bennabi I, Crozet F, Nikalayevich E, Chaigne A, Letort G, Manil-Segalen M, Campillo C, Cadart C, Othmani A, Attia R, Genovesio A, Verlhac MH, Terret ME. Increase in cytoplasmic myosin-II activity impairs chromosome capture in mammalian oocytes generating Aneuploidy. Nature Commun. 11: 1649 (2020)
4. Luo Z, Guven S, Gozen I, Chen P, Tasoglu S, Anchan RM, Bai B, Demirci U. Deformation of a single mouse oocyte in a constricted microfluidic channel. Microfluid Nanofluidics. 19(4):883-890. (2015)

Bacteria's BioMass measurements using 4 wave interferometry

Presenter: Maëlle BÉNÉFICE

Institut Fresnel, UMR 7249, Marseille, France

4 wave interferometry is an optical phase imaging technique based on the simple use of a 2D diffraction gratting placed a few millimeters in front of a regular camera. This combination creates quantitative phase images allowing to image live specimens quantitatively, non-invasively, without labelling and with good contrast. We show here all that this technique can provide for the imaging of bacteria, in particular the monitoring of growth by the measurements of dry mass.

Large volume multicolor imaging with chromatic multiphoton serial (ChroMS) microscopy

Presenter: Hugo BLANC

Laboratoire d'Optique et Biosciences (LOB), Ecole Polytechnique, Palaiseau, France

Large-scale microscopy approaches lack efficient multicolor / multicontrast modalities. At LOB and in collaboration with IDV, we recently introduced chromatic multiphoton serial (ChroMS) microscopy, a novel method combining multicolor two-photon excitation through wavelength mixing and serial block-face acquisition. This approach enables organ-scale imaging of spectrally distinct fluorescent proteins with intrinsic submicron channel registration and constant diffraction-limited resolution.

We applied ChroMS microscopy on mouse brains expressing Brainbow transgenic markers and viral- or electroporation-based multicolor labels, and demonstrated continuous 3D multicolor imaging over cubic millimeters of neural tissue as well as brain-wide serial 2D multichannel imaging.

We now work on novel applications and technological improvements of ChrOMS.

Abdeladim, Nat Commun 2019, Mahou, Nat Meth 2012, Livet, Nature 2007

Imaging genome reorganization upon transcriptional activation using Oligopaints

Presenter: Kyra BORGMAN

Nuclear Dynamics, Institut Curie, UMR3664, Paris, France

The genome of higher eukaryotes is distinctly organized and its spatial 3D distribution critically affects transcription. When looking at different nuclear elements, a tendency emerges in which nuclear components that are functionally related frequently cluster together. Different regulatory elements such as transcription factors and RNA pol II assemble into nuclear foci that can be clearly observed using fluorescence microscopy, and the degree of clustering is often linked to transcriptional activity in the cell. Moreover, recent ensemble chromatin-conformation-capture methods such as Hi-C reveal a clear pattern of preferential interaction between genomic loci across different scales. In addition, dramatic changes in transcription, for example during cell differentiation, go in many occasions hand in hand with large changes in chromatin conformation and genomic contacts. However, important changes in transcriptional activity are not always accompanied by conformational changes, and contacts important for induced transcription might be pre-established. Many questions thus remain as to how 3D organization and gene activity are intertwined, especially at the single cell level with transcription being extremely heterogeneous.

We use estrogen induction in the human breast cancer cell line MCF-7 as a model system to study the organization of a subset of estrogen responsive genes. Using Oligopaints to stain 30 responsive genes and 30 constitutively active genes simultaneously in a dual color DNA FISH experiment, we are able to determine their respective spatial (re)distribution at the single cell level upon hormone stimulation. Combining this labeling strategy with microfluidics and multiple rounds of fluorescent oligo-based staining of DNA as well as RNA of the same selection of loci will allow us to identify the position of each locus individually, and will tell us for each locus whether its actively transcribing or not in that specific subnuclear location. Colocalization studies including estrogen receptor ERa into the picture will eventually give more insight into the regulatory mechanisms behind the 3D organization of the genome. This will give us an unbiased picture of genomic redistribution upon transcriptional activation, and shed a new light on the interplay between 3D organization and transcription in the nucleus, with increased resolution up to the single locus level.

Fast and robust detection of calibration objects for in-line holography

Presenter: Dylan BRAULT

Laboratoire Hubert Curien, UMR 5516, Saint-Étienne, France

Optical microbiology allows to directly observe bacteria, cells, etc. and realize tests to characterize pathogenic agents or the biological material of the patient in order to help the medical diagnosis. Most of the biological objects are transparent when observed in white light and the classic procedure consist in staining them to increase their contrast. Phase microscopy, like in-line holography, allows to avoid this staining step by characterizing the objects using their refractive and morphological properties.

In-line holography, is a simple and low-cost setup, consisting in recording the intensity of the diffraction patterns of a biological sample illuminated by a coherent light. Since only the intensity of the diffracted wave is recorded on the sensor, dedicated algorithm are needed to reconstruct the phase information. These algorithms need the accurate knowledge of the defocus distance z of the sample. To estimate z, known objects, as calibration beads, can be added to the biological material and reconstructed using parametric inverse problem approaches. From the reconstructed parameters of the beads (3D coordinate and size) the defocus distance z can be deduced. In order to estimate the defocus distance with well known objects, a first step consists in detecting them on the hologram. Due to inter-object interference and contrasted fringes caused by the biological sample, this first detection step can lead to false detections and introduce a bias in the estimation of z.

Usually, the least square penalization function is used to reduce the computational time of the detection by using fast Fourier-transform based correlation. However robust detection techniques are needed to avoid false detection in the detection step but their complexity can be prohibitive. In this work, we propose to approximate various robust loss functions to detect and locate objects at moderate computational cost.

Analysis and quantification of M2 macrophages in light sheet 3D heart mice images

Presenter: Marine BREUILLY

Lyon Multiscale Imaging Center (LyMIC), Lyon, France

A mouse model of ischemia reperfusion was set up by the IRIS team from the CarMeN laboratory with the aim of characterizing the dynamic of anti-inflammatory macrophage response in the acute phase of myocardial infarction. A large number of hearts have gone through the process of preparation and imaging. This includes "healthy area" and "area-at-risk zone » (=ischemic area) slicing, clarification, immunostaining of M2 macrophages and imaging with the Light Sheet microscope of the Ciqle platform (LYON), producing an enormous amount of data.
Final size of the image stacks varies between 40GB and 130GB for a half-core. Such an image requires a specific analysis pipeline, adapted to the size of the image, robust and reproducible over the entire dataset.

In this poster, we will present the pipeline that has been set up in order to identify and quantify M2 macrophages located in the vascular system and / or in the lymphatic system from those infiltrated in the interstitial tissue during the post-ischemic reperfusion phase.
The preliminary result will be given on a subset of images.

Quantification of virus-driven plaque expansion in real time

Presenter: Julián BULSSICO

Laboratoire de Chimie Bactérienne, UMR7283, Centre National de la Recherche Scientifique, Aix-Marseille Université, Marseille, France

Bacterial cell lysis due to the propagation of viral infection caused by (bacterio)phages is commonly observed as plaques, or clear areas, on a bacterial lawn. Plaque formation involves phage outward diffusion from an initial infection spot, and its expansion is fed by new infection events of the cells situated at the edges of the expanding plaque. Many determinants influence plaque expansion dynamics and several models have been proposed to characterize it. However, a comprehensive imaging analysis has never been performed to visualize and quantify the development of plaque formation.

In this project, we present a multiscale (micro and macroscopic) tracking of lysis plaque expansion in real time. We developed several image analysis techniques to tackle the parameters involved in this phenomenon in the context of synergistic contribution of sublethal antibiotic concentrations to phage virulence. Importantly, the methods developed can be used for any plaque forming virus.

Mechanical characterization with AFM of murine oocytes to predict their fitness

Presenter: Rose BULTEAU

Laboratoire Analyse, Modélisation, Matériaux pour la Biologie et l'Environnement, LAMBE CNRS UMR8587, Evry, France

Oocyte production during meiosis in human females is essential for sexual reproduction. However, this process is error-prone and generates a basal rate of bad quality oocytes, impacting their developmental potential and having deleterious consequences for fertility and offspring development. It has been shown that aberrant oocyte stiffness, a common defect in murine and human oocytes, alters the developmental capacity of the oocyte. Thus, mechanical properties could be used to predict developmental potential of oocyte and thus guide their selection for assisted reproductive technologies. The mechanical properties of such big cells have been studied in our labs using the micropipette aspiration method. However, this method has a low yield and is not adapted to follow oocyte development. AFM (Atomic Force Microscopy) has a much higher throughput (less than a minute per cell) and provides robust measurement of cell elasticity and cortical tension (Chaigne et al., 2013, 2015, 2016; Yanez & Camarillo, 2017).

Hence, we have designed a protocol to measure with AFM the evolution of the mechanical properties of the murine oocyte during its development. This was challenging, since oocytes are big non-adherent cells surrounded by an extracellular matrix called the zona pellucida. Using a Sneddon model combined with a linear model, we managed to extract elasticity and cortical tension values from force indentation for oocytes at different stages of development. Our results have shown that elasticity and cortical tension decrease concomitant to oocyte development. They confirm our previous micropipette measurements and indicate that we can use this technique in a high throughput manner to classify oocytes according to their cortical tension and set threshold values of cortical tension correlating with a good developmental potential.

The next step is to measure the mechanical parameters of mouse oocytes engineered to be extra-soft and stiff. Finally, our long-term goal is to adapt our measurement on human oocyte. The latest will provide information about oocyte development applicable for medical use to select human oocytes in In vitro Fertilization clinics where up to now the selection consists of subjective morphological assessments.

Dynamic single particule orientation detection

Presenter: Marie-Charlotte CHANDECLERC

Laboratoire Lumière Matière Interaction, LUMIN, Gif sur Yvette, France

Our team is developing a microscope to mesure intraneuronal transport parameters thanks to non linear nanoparticules and in a super localisation regim [1]. This microscope uses a holographic technic to change the excitation position (presented by the poster of Florian Semmer another member of LUMIN).

We want to complete the intraneuronal transport parameters with the measurement of the rotational movement of vesicules. This additional parameter is useful to understand how the molecular motors are driven along the microtubules. We use second harmonic generation particules with different non linear coefficients along their axis to determine the rotation. These measures have already been realised with fixed KTP [2] and we want to couple them to the translational measurement in neurons in real time. To do so we add a rotation of the incident polarization and two orthogonal polarization detection. We will be able to track the motion and the rotation of the particule in 3D neurons and in real time.

[1] Haziza et al. Nature Nanotechnology. 12(4), 322-328, 2017
[2] Hsieh et al. Optics Express. 18, 11,2010

Deformable mirrors optimized for focus, astigmatism and spherical aberration

Presenter: Julien CHARTON

ALPAO, 727 Rue Aristide Berges, 38330, Montbonnot, France

Deformable mirrors are used in many advanced microscopy applications to correct for aberrations or modify the 3D point spread function (PSF) shape. When only a few wavefront deformation modes (i.e Zernike modes) are required, using conventional deformable mirrors with large number of actuators may add significant complexity and cost to both software and hardware implementations. This poster describes a new type of “modal deformable mirror” specially optimized for common aberrations such as focus, astigmatism, spherical… The simplicity and cost of this mirror, drive electronics and software open the path to a broader use of adaptive optics in microscopy.

Development of models to study brain metastasis of breast cancer

Presenter: Julien CICERO

CANTHER - UMR 9020 CNRS - UMR 1277 Inserm, CANTHER - Team "Cell Plasticity and Cancer", Villeneuve d'Ascq, France

With nearly 2.1 million cases diagnosed worldwide each year and approximately 625,000 deaths in 2018, breast cancer is the leading cause of cancer-related death in women. Despite significant improvements in patient management and therapy, metastatic disease is a particularly damaging development for this type of cancer. In addition to death, brain metastases lead to severe cognitive complications that severely impair quality of life. The incidence of brain metastases varies according to breast cancer subtypes: from 14% for ER+ to more than 33% for HER2-positive and even 50% in triple-negative (TN). It is therefore crucial to know the molecular players that promote the metastatic dissemination of TN breast cancer to the brain.
My work focuses on the development of different in vitro and in vivo models to study: 1) the interaction of breast cancer cells with the BBB (Blood Brain Barrier) and 2) the persistence and development of these cells in the brain parenchyma.

I. Understanding the mechanisms of extravasation of breast cancer cells: Human BBB model
The LBHE (Blood Brain Barrier Research Center) has developed an in vitro model of the human BBB. This system allows the study of the influence of different cell types on BBB properties (adhesion, transmigration...) but also the evaluation of the passage of molecules and cells through the BBB endothelium.

II. Persistence and tumor development in the brain parenchyma: Human 3D organotypic matrix model
There are many interactions between cancer cells and their microenvironment. The brain parenchyma offers particular conditions for cancer cell metastasis due to the presence of the BBB but also to its specialised microenvironment. Therefore, in order to mimic the microenvironment of cancer cells, they can be grown in different organotypic 3D matrices that have properties equivalent to those found in the brain parenchyma.

III. Validation in in vivo models
With the collaboration of the INSERM U1172 laboratory, we have developed an advanced iDISCO (immunolabeling 3D imaging of solvent-cleared organs) model on thick sections (about 1 mm) of the brains and on whole brains of xenografted mice. The organ will thus be transparent, which will make it possible to determine the precise location of metastases in the various brain structures (blood vessels, BBB and parenchyma), but also to assess tumorigenesis and angiogenesis.

Technological platform for microfluidics at Institut Pierre Gilles de Gennes

Presenter: Bertrand CINQUIN

IPGG, UMS3140, Paris, France

La plateforme technologique de l'IPGG est un ensemble de salles d'une superficie de 550 m² qui offre toutes les technologies nécessaires à la réalisation de dispositifs microfluidiques, à leur caractérisation et à leur utilisation. Elle est une unité mixte de service du CNRS (UMS 3750) depuis le 1 janvier 2016 et fait partie du réseau SBPC qui fédère l'ensemble des salles blanches du centre parisien.

Plus d'informations: https://www.plateformeipgg.fr/en/

Protein-like particles through nanoprecipitation of mixtures of polymers of opposite charge

Presenter: Antoine COMBES

Laboratoire de Bioimagerie et Pathologies (LBP), Illkirch-Graffenstaden, France

Fluorescent nanoparticles are an attractive alternative to organics dye for bioimaging, due to their superior brightness. However, they often suffer from nonspecific interactions in biological media. Tuning polymer chemistry to overcome this limit while enhancing dye encapsulation and fluorescence is a challenging but rewarding approach.
We present here an approach using co-precipitation of two oppositively charged polymers and a hydrophobic dye to obtain small and bright nanoparticles. Zeta potential of these particles is pH dependant and can be reversed after nanoparticle formation.

Study of pneumococcal division during the competent state using 3D-PALM

Presenter: Elise COURTAIS

Laboratoire de Microbiologie et de Génétique Moléculaires, LMGM UMR5100, Toulouse, France

Streptococcus pneumoniae (the pneumococcus) is an important human pathogen. A particularly interesting characteristic of pneumococcus is its ability to naturally transform. Natural genetic transformation, in which cells internalize exogenous DNA and integrate it into their chromosome, is widespread in the bacterial kingdom. It involves a specialized membrane-associated machinery, the transformasome, for binding double-stranded (ds) DNA and uptake of single-stranded (ss) fragments. In the human pathogen Streptococcus pneumoniae, this machinery is specifically assembled at competence, a state of transient differentiation state. In this species, competence develops in all cells of an exponentially growing culture and causes a delay of the cell division process. Moreover, it has been shown in the laboratory that cell division process is delayed during competence. Interestingly, fluorescence microscopy revealed a localization at the septum for both transformasome and division machinery.

The aim of this project is to understand how competence interferes with the division process. For this, we seek to determine if the presence of the transformasome machinery at the division site can modify three-dimensional structures formed by other key proteins of the division such as FtsZ. We will present the adaptation of the PhotoActivated Localization Microscopy (PALM) technology in three dimensions to analyse the nanostructure of the FtsZ-ring in S. pneumoniae.

Lagon Team : fluorescent dyes and molecular-based nanoparticles as powerful optical toolbox for biology

Presenter: Jonathan DANIEL

Institut des Sciences Moléculaires, UMR5255, Talence, France

The continuous refinement of fluorescence microscopies (including two-photon excited fluorescence,(1) super-resolution ….) opens new opportunities for biologists for better monitoring and understanding of complex biological phenomena. Yet, with the incoming of these technologies, new limitations arose, such as the insufficient brightness and/or photostability of commercially available dyes. In this respect, lot of efforts has been conducted in the last decade to optimize fluorescent dyes. In parallel, fluorescent organic nanoparticles (FONs) emerged as a new class of bright nanotools which represent highly promising alternatives to quantum dots that have become highly popular for bioimaging, including in vivo imaging.

We published the design of hyperbright FONs - named HiFONs - built from specifically engineered dipolar dyes. HiFONs combine NIR emission, of major interest for imaging in thick tissues, as well as biocompatibility, low toxicity, remarkable stability in cellular environment and improved photostability. Thanks to these features, these FONs were found to be more stable and brighter that commercially NIR emitting dyes and were used in real-time single nanoparticle tracking.(2) Our next goal was to propose a robust approach towards a library of hyperbright FONS that would also show giant two-photon brightness - thus allowing for excitation in the NIR – and tunable emission spanning the whole visible region to the near IR. In this presentation, I give on overview of different applications of our dyes and nanoparticles as fluorescent tracers in live cell imaging (confocal, multiphotonic, SHG, single particle tracking..) or as therapeutic nanotools.

(1) W. R. Zipfel, R. M. Williams, W. W. Webb, Nat. Biotechnol. , Vol. 21, No. 11, pp. 1369-1377, 2003. (2) E. Genin, Z. Gao, J. A. Varela, J. Daniel, T. Bsaibess, I. Gosse, L. Groc, L. Cognet, M. Blanchard-Desce, Adv. Mater., Vol. 26, No. 14, pp. 2258-2261, 2014.

Inducing cell contraction and protrusion with a single optogenetic activator

Presenter: Jean DE SEZE

Laboratoire Physico Chimie Curie, UMR168, Paris, France

Cell migration is a complex process that takes place in many biological contexts like development or cancer. It relies on a equilibrium between contraction and protrusion of the cytoskeleton, which is itself mainly regulated by small proteins, the GTPases. Among them, RhoA has a mysterious behaviour, being active both in protrusions and contractions.

Supporting this dual role, we recently observed that local activation of RhoA through optogenetics can lead to both protrusion or retraction (unpublished data). How can the same protein be directly responsible for two antagonist effects?

This is a striking example of multiplexing: one protein is used by the cells for two different outcomes. Without having any clear conclusion for the moment, the poster describes our main hypothesis and results explaining how the cell chooses one outcome depending on the cellular context.

3D High-Throughput Screening of spheroids development using soSPIM

Presenter: Tom DELAIRE

Institut Interdisciplinaire de Neurosciences (IINS), Bordeaux, France

The recent breakthrough of organoids in the biological research field has offered the possibility to recreate in vitro complex 3D structures closely reproducing morphologies and functions of human organs. Organoids have a strong potential in many fields such as drug screening, toxicity assay, or personalized and regenerative medicine. However, their systematic use still requires to address several challenges in terms of culture standardization and 3D high throughput live imaging. In particular, their 3D morphologies and high sensitivity requires low-phototoxic 3D imaging methods to assess their development, as well as culture support compatible with advanced imaging methods. In this regard, light-sheet microscopy seems to be the most appropriate technology to image live organoids in 3D, but their multi-objective architecture prevents high throughput screening approaches due to cumbersome sample mounting processes.

Here we propose to use a single-objective light-sheet microscopy technique, called soSPIM [1], to overcome these limits. The soSPIM technology relies on the use of dedicated micro-fabricated devices integrating 45° mirrors allowing for the creation of a light-sheet perpendicular to the optical axis of the objective. To perform high content imaging of 3D cell cultures, we designed new devices composed of arrays of truncated pyramidal shaped microcavities, called JeWells, compatible with organoids culture and soSPIM imaging. The combination of the JeWells and the soSPIM technology makes now possible to both standardize and parallelize the culture and the 3D live imaging of hundreds of organoids in a single imaging devices.

We will describe how we transform the soSPIM technology into a high content 3D acquisition platform, and demonstrate its capacity to monitor live and fixed 3D cellular cultures with unprecedented throughput and limited photobleaching and toxicity. In particularly, we will highlight the tools we implemented to help for the automation of the acquisition processes of hundreds of organoids over time.

[1] Galland et al, “3D high- and super-resolution imaging using single-objective SPIM”, Nature Methods, 2015

Localizing microtubule associated protein 6 (MAP6) in neurons using expansion microscopy

Presenter: Eric DENARIER

Grenoble Institut des Neurosciences, U1216, La Tronche, France

The shaft of neuron extensions contains bundles of microtubules (MT) allowing the continuity of cellular space to reach a length of one meter in humans. The regulation of MT function is mediated by a combination of tubulin (the MT's subunit) post-translational modifications such as acetylation, de-tyrosination etc... and the binding of microtubule associated proteins (MAPs). Our studies focus on MAP6 that is highly expressed in neurons: MAP6 has the unusual property of protecting microtubules against drug- and cold-induced depolymerization and has been recently shown to localize inside the MT lumen. This protein is implicated in the normal maintenance and function of neurons: MAP6-deficient mice suffer severe deficits in synaptic plasticity similar to those seen in schizophrenia. As the functions of MAP6 is intimately related to microtubules we are interested to establish whether it presents a preferential localization for microtubules harboring specific tubulin modifications.
Using expansion microscopy with "high-resolution" microscopy techniques (Airyscan), we multiply the resolution gain of two techniques in order to address the following problems on a nanoscale:

1) Do the different tubulin post-translational modifications occur in a subpopulation of microtubule in the neuronal extensions (axon or dendrite) and/or at specific region (e.g. axon initial segment)?
2) Does MAP6, prefer a subpopulation of microtubule harboring a peculiar post-translational modification (de-tyrosination, acetylation)?

We will present our preliminary data towards achieving these goals using expansion microscopy coupled with high-resolution techniques.

Super resolution microscopy: an eye to explore the role of F-actin in HIV-1 assembly

Presenter: Rayane DIBSY

IRIM, CNRS UMR9004, CNRS and Montpellier University, Montpellier, France

Human immunodeficiency virus type 1 (HIV-1) assembles and buds at the plasma membrane of host CD4 T cells. Distinct cortical actin nanostructures have been visualized at viral buds by cryoEM[1], however the functional role of cortical actin in this process remains unclear. Because HIV-1 is an enveloped virus of 100-150 nm of diameter, super-resolution microscopy is a tool of choice to study host cell factors at assembly sites. In this study, we investigate the role of F-actin in HIV-1 assembly as we have shown before that some cortical actin regulatory factors are required for HIV-1 particle production and membrane curvature [2,3].

Total Internal Reflection Fluorescence (TIRF) microscopy mode is used to investigate virus assembly platforms at the cell plasma membrane. When coupled to Photo-activated localization microscopy (PALM)[4] and Stochastic Optical Reconstruction Microscopy (STORM) it is then used to gain more resolution and observe HIV-1 single viral clusters with single Gag localization. In this study, infectious HIV-1(i)GFP□Env/VSVg and HIV-1Gag(i)mEOS2 were used to label and observe viral assembly platforms. Then HIV-1 Gag expressing cells were treated with actin interfering drugs, and the assembly site size and density were studied using TIRF, STORM/TIRF microscopy and corresponding particle release was monitored using p24alphaLISA and immunoblots. Furthermore, dSTORM was coupled to PALM/TIRF, in order to observe F-actin structures at HIV-1 Gag(i)mEOS2 assembly platforms. PALM/STORM image analysis reveal that half of HIV-1 buds are surrounded by F-actin. In the other hand, Stimulated emission depletion (STED) microscopy was also used and revealed changes in cellular F actin structures following HIV-1 infection. Our results indicate the requirement of a finely tuned actin dynamic process during HIV-1 particle formation.

Altogether, our data support a role for F-actin in HIV-1 assembly and release, suggesting a regulation of F-actin polymerization and debranching during HIV-1 particle formation in host CD4 T cells.

1. Cryo Electron Tomography of Native HIV-1 Budding Sites. Lars-Anders Carlson, Alex de Marco, Heike Oberwinkler, Anja Habermann, John A. G. Briggs, Hans-Georg Kräusslich, Kay Grünewald. PLOS Pathogens, 2010.
2. Involvement of the Rac1-IRSp53-Wave2-Arp2/3 Signaling Pathway in HIV-1 Gag Particle Release in CD4 T CellsAuthors: Audrey Thomas, Charlotte Mariani-Floderer, Maria Rosa López-Huertas, Nathalie Gros, Elise Hamard-Péron, Cyril Favard, Theophile Ohlmann, José Alcamí, Delphine Muriaux. Jornal of Virology, 2015
3. Full assembly of HIV-1 particles requires assistance of the membrane curvature factor IRSp53. Kaushik Inamdar , Feng-Ching Tsai , Rayane Dibsy , Aurore de Poret , John Manzi , Peggy Merida , Remi Muller , Pekka Lappalainen , Philippe Roingeard , Johnson Mak , Patricia Bassereau , Cyril Favard , Delphine Muriaux . eLife, 2021.
4. Single molecule localisation microscopy reveals how HIV-1 Gag proteins sense membrane virus assembly sites in living host CD4 T cells. Floderer C, Masson JB, Boilley E, Georgeault S, Merida P, El Beheiry M, Dahan M, Roingeard P, Sibarita JB, Favard C, Muriaux D. Sci Rep, 2018.

Nanoscale distribution of Calreticulin, Phosphatidylserine and CD47 enlightens on how plasma membrane modifications shape apoptotic cell recognition

Presenter: Samy DUFOUR

Institut de Biologie Structurale, I2SR, Grenoble, France

Ecto-Calreticulin and Phosphatidylserine are engulfment “eat-me “signals for phagocytes but are prevented when the CD47 “don’t eat me” signal binds to its macrophage receptor SIRPa. How this is regulated on the target cell membrane is not well understood.
Thanks to a cell model adapted to STROM imaging and single-particle tracking, we interrogate how the distribution of these molecules on cell surface correlates to plasma membrane alteration, SIRPa binding, and cell engulfment by macrophages. Apoptosis induces CRT clustering into blebs and increase CD47 mobility. Modulation of integrin affinity impacts the CD47 mobility on the plasma membrane without effect on SIRP binding, whereas CD47/SIRPa interaction is suppressed by cholesterol destabilization. SIRPa no longer recognizes CD47 localized on apoptotic blebs. Thus, the decrease of lipid order of the plasma membrane, by inducing inaccessibility of CD47 signal, is a key in the modulation of the phagocytosis process, with promising implication to cancer immunotherapy.

QuanTI-FRET: a method for quantitative FRET analysis in widefield imaging

Presenter: Aurélie DUPONT

Laboratoire Interdisciplinaire de Physique, LIPhy UMR 5588, Saint Martin d'Hères, France

Förster Resonance Energy Transfer (FRET) allows for the visualization of nanometer-scale distances and distance changes. This sensitivity is regularly achieved in single-molecule experiments in vitro but is still challenging in biological materials. Despite many efforts, quantitative FRET in living samples is either restricted to specific instruments or limited by the complexity of the required analysis. With the recent development and expanding utilization of FRET-based biosensors, it becomes essential to allow biologists to produce quantitative results that can directly be compared.

Here, we present a new calibration and analysis method allowing for quantitative FRET imaging in living cells with a simple fluorescence microscope. Aside from the spectral crosstalk corrections, two additional correction factors were defined from photophysical equations, describing the relative differences in excitation and detection efficiencies. The calibration is achieved in a single step, which renders the Quantitative Three-Image FRET (QuanTI-FRET) method extremely robust. The only requirement is a sample of known stoichiometry donor:acceptor, which is naturally the case for intramolecular FRET constructs. We show that QuanTI-FRET gives absolute FRET values, independent of the instrument or the expression level. Through the calculation of the stoichiometry, we assess the quality of the data thus making QuanTI-FRET usable confidently by non-specialists.

Super-resolved live-cell imaging using Random Illumination Microscopy

Presenter: Claire ESTIBAL

Centre de Biologie Intégrative, UMR 5547, Toulouse, France

Current super-resolution microscopy (SRM) methods suffer from an intrinsic complexity that may curtail their routine use in cell biology.
Based on speckled illumination and statistical image reconstruction, easy to implement and user-friendly, Random Illumination Microscopy (RIM) is unaffected by optical aberrations on the excitation side, linear to brightness, and compatible with multicolor live cell imaging over extended periods of time.
RIM's inherent simplicity and extended biological applicability, particularly for imaging at increased depths, could help democratize SRM in cell biology laboratories.

A fiber-based endomicroscope designed for full Mueller endoscopic polarimetric imaging

Presenter: Marc FABERT

XLIM, UMR CNRS7252, Limoges, France

Nous présentons un endomicroscope à fibre optique conçu pour l'acquisition d'images polarimétriques de Mueller 4x4 de tissus biologiques in situ. La technique polarimétrique utilisée ici est basée sur une méthode différentielle à deux longueurs d'ondes (MDDLO) qui permet d'éliminer la contribution de la fibre optique sur le signal polarimétrique mesuré. Nous obtenons ainsi des images polarimétriques complètes de 62500 pixels à la vitesse de 0,5 image/s à travers une fibre de 2 m de longueur. Les images sont construites grâce à un balayage rapide de la région d'intérêt opéré par une microsonde cylindrique distale (longueur 30 mm, diamètre 3 mm) spécialement conçue pour permettre de la mise en oeuvre de la MDDLO, rendant compatible cette technique avec les applications de diagnostic *in vivo*.

Orthogonality Breaking polarimetric microscopy of mitotic chromosomes

Presenter: Julien FADE

Institut FOTON, UMR 6082, RENNES, France

We present an original polarization imaging technique, implementing microwave photonics approaches and called orthogonality breaking (OB) microscopy, which allows to provide informative polarization images from a single scan of the sample (cell). Comparison of images of several cell lines at different stages of the cell cycle obtained by OB polarization microscopy and fluorescence confocal images shows that an endogenous polarimetric contrast related to the birefringence (retardation) properties of the sample occurs on the chromosomes compacted during cell division. Additional results on tissues such as collagene or liver fibers will be also presented.

Coherent anti-Stokes Raman scattering dynamic speckle illumination microscopy

Presenter: Eric Michele FANTUZZI

Aix Marseille Univ, CNRS, Centrale Marseille, Institut Fresnel, UMR 7249, Marseille, France

Dynamic speckle illumination microscopy consists of shining the sample with several different CARS speckle patterns and compute the variance to get the resulting image. We investigate here the ability of CARS dynamic speckle illumination to perform z-sectioning in wide field CARS imaging. Dynamic speckle illumination microscopy consists of shining the sample with several different CARS speckle patterns and compute the variance to get the resulting image. We investigate here the z-sectioning of a sample with this technique and present preliminary results demonstrating the enhanced contrast compared to the wide-field CARS imaging system.

Fluorescent polymer-aptamer probe for the selective labeling and dSTORM super-resolution imaging of cell surface nucleolin

Presenter: Arnaud FAVIER

Laboratoire d'Ingénierie des Matériaux Polymères (IMP), Villeurbanne, France

Nucleolin is a protein involved in numerous diseases like cardiovascular and neurological diseases, inflammation, bacterial and viral infection, in addition to its implication in many cancers [1]. An efficient labeling of nucleolin in cellulo is thus very important to better understand its role and functions.
Fluorescently labeled polymer chains represent a very attractive new class of probes that are smaller than fluorescent nanoparticles and brighter than single organic dyes. In the past few years, we have developed a modular platform, combining i) poly(N-acryloylmorpholine-stat-N-acryloxysuccinimide) copolymer chains synthesized by RAFT controlled radical polymerization at the azeotropical composition [2], and ii) multiple organic chromophores covalently bound along the polymer chain. The resulting water-soluble and biocompatible fluorescent polymer probes exhibit an enhanced brightness and an improved resistance to photobleaching compared to organic dyes [3]. Two types of chain-end functionalized probes were then developed to label proteins: Amine-reactive probes for the synthesis of highly bright conjugates with native proteins [4], and ligand-polymer probes for the site-specific labeling of recombinant proteins [5].

In the present study, the objective was to synthesize probes for the selective detection of endogenous nucleolin at the cell surface using advanced fluorescence microscopy techniques, especially dSTORM super-resolution imaging. We thus investigated the synthesis of fluorescent polymer chains presenting multiple AF647 fluorophores and at one chain-end a nucleotidic aptamer (AS1411) able to recognize nucleolin via a unique shape-specific interaction. Aptamer oriented conjugation to the fluorescent polymer chain was successfully achieved by strain-promoted alkyne-azide cycloaddition (SPAAC) bio-orthogonal click chemistry. Various experimental conditions were studied and the final products were characterized by UV-Vis spectroscopy, NMR and gel electrophoresis. The ability of the fluorescent polymer-aptamer probes to selectively label nucleolin was then confirmed both in vitro and in cellulo. Thanks their small size (<10nm) and high brightness, these probes could thus represent an advantageous alternative to classical immunofluorescence. Various evaluations showed that the polymer-aptamer probes indeed enabled an efficient detection of endogenous nucleolin by fluorescence microscopy. In particular, cell surface nucleolin could be observed by dSTORM super-resolution imaging with a high localization precision.

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[2] A. Favier et al., Polymer, 45, 7821 (2004)
[3] C. Cepraga et al., Polym. Chem., 4, 61 (2013)
[4] D. Duret et al. Polym. Chem., 9, 1857 (2018)
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Numerical auto-focus in Digital Holographic Microscopy

Presenter: Corinne FOURNIER

Laboratoire Hubert Curien, UMR 5516, Saint-Etienne, France

Healthcare can benefit from the development of new, simple and effective cell imaging devices to perform medical diagnosis faster and at lower cost. Since many living cells are transparent to visible light, staining is often used to improve their contrast, but it is impossible for in vivo observation because it degrades the sample. Furthermore the staining step is time consuming. Phase imaging techniques are also employed, but quantitative information on the phase of the object is difficult to obtain.

However, this information, which makes possible cells to be discriminated (type, state of life, etc.), can be important for their classification, leading to efficient medical diagnosis. Holographic microscopy techniques (including for example digital holography or diffractive tomography), are advanced techniques that can provide such quantitative information. They are based on the principle of interference between the object beam (light beam passing through the object) and a known reference beam. The resulting holograms are digitally processed to reconstruct phase objects in different planes. In-line holography is experimentally simple and can lead to low-cost devices in which the sample translation stage can be less accurate and therefore less expensive. Actually, focusing can be performed numerically, provided that the sample is already positioned in space.

We present here a methodology allowing this accurate positioning (<50nm). It consists in inserting known objects (beads for example) into the sample and in reconstructing them (i.e., estimating their 3D spatial coordinates and their sizes) using hologram reconstruction algorithms based on inverse parametric approaches. The microscope slide surface can then be accurately 3D reconstructed (position and tilts estimates). This method is compared to the state of the art.

Optical Coherence Tomography : charaterization and application to collagen-based cultural heritage artefacts

Presenter: Giulia GALANTE

Laboratoire d'Optique et Biosciences (LOB), Palaiseau, France

LC-OCT microscopy (Line-field Confocal Optical Coherence Tomography) combines OCT microscopy (Optical Coherence Tomography) with confocal microscopy. This technique provides 3D imaging with micrometer resolution in all three directions. This microscope designed to work in contact, immersion or no contact mode, is a non-destructive and non-invasive technique for the study of cultural heritage artefacts.
Three types of cultural heritage artefacts are studied during this project:

- Fluid collections: study of the impact of the fixation and preservation fluids on the structure of the skin of specimen preserved in jars.
- Gilt Leather: study of the upper varnish layer (thickness and composition)
- Wooden musical instruments: study of the various varnish layers (thickness and composition)

First, the characterization of the system (resolution in the three directions, field of view, imaging depth) is performed. Then, various culture heritage samples are studied to demonstrate the potential of the LC-OCT. Finally, image processing is developed to automatically detect the various interfaces observed in the OCT image and to calculate in each point of the 3D volume the layer thicknesses.

Automatic segmentation of organoids' nuclei spatial distribution in 3D with StarDist

Presenter: Xareni GALINDO

Institut Interdisciplinaire de Neurosciences (IINS), Team Sibarita, Bordeaux, France

3D cell cultures have emerged as the new gold standard in fundamental cell biology because of their higher physiological relevance compared with 2D cultures. These 3D aggregates, named multicellular spheroids or organoids, are by definition aimed at recapitulating the functions and the cellular architecture of organs in a miniature version. In parallel, high-content imaging 1 has unfold as a powerful technique to statistically determine physiological and morphological patterns by acquiring thousands of samples from several different conditions. In this context, we have developed the soSPIM 2, a single-objective light-sheet-based microscopy technique whose unique architecture allows non-invasive high-content imaging of organoids 3. It has however shifted the bottleneck from microscopy acquisition to computational analysis: quantifying in robust manner thousands of live organoids over time requires the development of new segmentation algorithms robust to experimental parameters such as uneven illumination or high-density packing of nuclei.

In recent years, usage of deep learning has followed an exponential rise in biology (730 publications in 2020) and revolutionized the way biological data are analyzed 4. Among the most impressive recent works, StarDist 5 is a convolutional neural network designed for segmenting star-convex objects such as cell nuclei, in 2 and 3 dimensions. Nevertheless, directly using StarDist for 3D segmentation is hardly achievable since high-quality pre-trained networks are only available for the 2D version. Such an observation is related to the lack of currently available annotated data of 3D nuclei.

In this project, we manually annotated 4657 3D nuclei spanning from 6 different organoids/spheroids acquired with the soSPIM system. This currently represents the only ground truth dataset of this size for 3D nuclei, and we plan to release it to the scientific community. We then used it to train StarDist in 3D and we developed an evaluation pipeline computing several quantitative metrics to assess its segmentation quality. Finally, we used our trained StarDist model to automatically segment hundreds of organoids in routine and in 3D, helping to quantify their nuclei spatial distribution.

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Whole Cell 3D Single Molecule Localization Microscopy using single-objective Selective Plane Illumination microscopy (soSPIM)

Presenter: Rémi GALLAND

Institut Interdisciplinaire de Neurosciences (IINS), Bordeaux, France

Light microcopy offers to the cell biologists the capability to monitor living samples in conditions similar to their native environment. Combined with fluorescent probes, it opened the unique opportunity to image proteins of interest with a very high specificity and in a minimally invasive manner within 3D living samples. Since about fifteen years, 3D fluorescence microscopy for biology is undergoing an in-depth revolution with the advent of Light-Sheet Fluorescence Microscopy (LSFM or SPIM) technics which enable to tackle many limitations of standard 3D imaging technics in term of photo-toxicity, speed and sensitivity. Common LSFM implementation use at least two objective lenses positioned perpendicularly one from the other to create a thin sheet of light and collect the fluorescence signal respectively. However, this multi-objective architecture comes with some drawbacks. It prevents the possibility to use bulky high numeral aperture objective and limits the achievable imaging throughput due to complex sample mounting.

To overcome those limitations, we developed an alternative architecture, called soSPIM (single-objective Selective Plane Illumination Microscope), which uses a single objective to create both the excitation light-sheet and collect the fluorescent signal. It relies on the integration of 45° mirrors directly into the imaging devices in place of the excitation objective along with a laser beam steering unit. I will discuss the multiscale imaging capability of the soSPIM technology which allows to image the nanoscale organization of proteins within single cells up to the development of living 3D organoids by varying the size and design of the imaging devices. Especially, I will present the recently engineered multiwell devices, named JeWells, that enable to turn the soSPIM system into a high-content screening platform of living 3D cellular cultures (spheroids/organoids) greatly improving the imaging throughput of 3D imaging technics.

Cataloger and impomero: painlessly annotate and import your microscopy images in omero

Presenter: Guillaume GAY

Turing Center for Living Systems, CENTURI, Marseille, France

The cataloger web app (https://github.com/centuri-engineering/cataloger) that helps user create "cards" describing their microscopy experiments. It is coupled with impomero https://github.com/centuri-engineering/impomero, a tool to monitor and automatically import and annotate data from the microscope.

The objective of these tool is to ease data annotation by the biologists while minimizing annotation terms multiplication and keeping controlled vocabularies.

Tubulin Polyglutamylation Differentially Regulates Microtubule-Associated Proteins

Presenter: Mariya GENOVA

Janke Lab, Institut Curie, UMR3348, Orsay, France

Tubulin post-translational modifications (PTMs) control and diversify the functions and mechanical properties of the microtubules as a cytoskeletal element involved in essential cellular processes, among which are cell migration and intracellular transport. One of these modifications is polyglutamylation, which is the reversible, enzymatically catalyzed addition and removal of single glutamate units in the form of a side peptide chain on a- and on tubulin carboxy-terminal tails. This modification is highly enriched in neurons, where it can act as a rheostat for mitochondrial transport along axonal microtubules. Recent studies from our team had demonstrated that abnormally increased levels of tubulin polyglutamylation can lead to neurodegeneration in transgenic mouse models lacking the major enzymes involved in the regulation of this modification in neuronal tissue (Magiera et al. 2018; Bodakuntla et al. 2021).

To determine the molecular mechanisms underlying the functions of polyglutamylation, we here use tubulin purified from brains of animals lacking defined PTMs to demonstrate in vitro that physiologically relevant polyglutamylation levels and patterns can regulate the binding affinity and functions of diverse microtubule-associated proteins (MAPs). Furthermore, by differentiating between glutamylation on a- and on b-tubulin, we show that MAP-microtubule interaction can be regulated by glutamylation of one of the subunits, or their affinity can be modulated by the strength of the electrostatic charge on the microtubule surface. Altogether our findings present evidence in support of the “tubulin code” hypothesis, which predicts that tubulin PTMs can be a key regulator of microtubule-MAP interactions.

Intracellular dynamics and viscosity microscopy with quantitative phase imaging and holography

Presenter: Clémence GENTNER

Institut de la Vision, Photonics department, UMR7210, Paris, France

Many processes in microfluidics and biology are driven or affected by local viscosity. Global viscosity measurements, particularly in cells, can be achieved using several methods like AFM, surface measurement of acoustic waves, biomembrane force probe, micropipette aspiration or Dynamic Light Scattering (DLS). Local viscosity measurements inside a cell are conducted via in-cell transducers either in active or passive motion. Contactless optical microscopy-based techniques have clear advantages, but non-fluorescent probes are still mostly within the upper part of the “micro” range.

The relatively large scattering cross-section of metallic or plasmonic nanoparticles offers a way to reduce probe size and increase spatial resolution using 3D superlocalization. Instead of relying on the statistics of lateral motion, which averages properties over the path of the particle, we chose to rely on rotation, which often occurs on shorter space and time scales. Moreover, the short exposure time of ultrafast cameras is not compatible with the detection of weak scatterers unless high power illumination is used, which is impossible in living media. Heterodyning is a powerful alternative: by using a frequency beating at frequency ΔF (up to several MHz), any variation occurring at ΔF in the sample can be imaged on a dark background after demodulation, even with a low frame rate, sensitive camera. Using heterodyne holography we imaged a) the Brownian rotation of gold nanorods over a broad frequency range (0-10 MHz) and b) the rotation of magnetic nanorods in an external field. After calibration, a single measurement or a frequency scan can give access to high resolution quantitative viscosity microscopy.

Switching mechanism of the fluorescent protein rsEGFP2 at cryogenic temperature

Presenter: Oleksandr GLUSHONKOV

Institut de Biologie Structurale, CNRS, Univ. Grenoble Alpes, CEA, Grenoble, France

Many super-resolution fluorescence microscopy experiments are carried out with chemically fixed samples, potentially generating artefacts. Flash cooling and working under cryogenic conditions would better preserve biological samples and would open the door to cryo-correlative studies with cryo-EM[1]. In the case of single-molecule localization microscopy applications (SMLM), the challenge is to find fluorescent probes capable to photoswitch below the glass transition temperature.

Switching of commonly used organic dyes cannot be induced by switching buffers in the absence of diffusion at low temperature. Fluorescent proteins, on the other hand, are more promising probes for cryo-nanoscopy as demonstrated in recent studies[2,3]. However, the number of suitable fluorescent proteins remains limited and their photoswitching, that typically requires conformational flexibility, is hampered at cryogenic temperature. In this work we are studying the low temperature switching capability of the rsEGFP2 proteins and its variants to understand the involved mechanisms and to optimize their switching efficiency. We observed that the reversibly switchable fluorescent protein rsEGFP2 undergoes a cryo-switching mechanism, which is different from the typical room temperature cis-trans isomerization of the chromophore.

The structural data suggest that the off-state is reached without major conformational changes, possibly via the triplet state or photoinduced protonation, which would explain why rsEGFP2 is able to switch despite restricted conformational freedom at cryo-temperature. Additionally, the observed blue-shifted (compare to the RT spectrum) absorption peak of the protonated protein serves as another indication of a different switching mechanism. Finally, the spectroscopic ensemble-level measurements show an improved off-to-on photoswitching efficiency of the rsEGPF2 protein after blue shifting respectively the activation laser, which could allow a better cryoSMLM data collection strategy.

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Compressive Raman Technology

Presenter: Clément GRAND

Institut Fresnel, UMR 7249, Marseille, France

Raman spectroscopy offers high chemical selectivity associated with micrometric optical resolution [1]. This method uses the vibrational spectrum of molecules which can be considered as a real "bar code" for the identifiers. In practice, the Raman scattering process is extremely weak and leads to weak signals associated with long integration times (ms) hardly compatible with imaging [2]. To overcome this difficulty, we present and validate the Raman Compressed technology using optimal binary spectral filters and a single-channel detector making it possible to estimate and detect known chemical species with a time per pixel which may be only a few tens of s.

Gradient-index lens widefield microscopy

Presenter: Quentin GRÉSIL

Laboratoire Photonique Numérique et Nanosciences, UMR CNRS 5298, Talence, France

Exploring the topology and rheology of the brain extracellular space (ECS) unfolds new approaches to understand neurodegenerative diseases. Single-particle tracking (SPT) of single-wall carbon nanotubes (SWCNTs) has been previously employed as near-infrared fluorescent probes to map diffusion and dimensionality of the brain ECS, with nanoscale precision, in slices.
This project aims to accommodate single-particle tracking of SWCNTs in-vivo. To transcend the insufficient photon depth of penetration, the use of Gradient-Index (GRIN) lenses enables to access deeper portions of the brain without critical abduction of live animal tissues. In this work, we manufacture a widefield microscope conjugated to a 0.7 NA, aberration-corrected GRIN lens to achieve SPT of SWCNTs.

Label free super resolution using blind structured illumination in stimulated Raman scattering

Presenter: Julien GUILBERT

Laboratoire Kastler Brossel (LKB), Paris, France

Super-resolution fluorescence imaging is the workhorse of biologists. However, fluorescence requires use of exogenous probes that may alter the sample's natural properties. Recently, a growing interest has emerged to exploit label-free Raman-based techniques exploiting the intrinsic non-linear vibrational response of molecules. Nevertheless, proposed super-resolution techniques in Raman processes often require high laser powers that could damage biological sample, or reduce the excitation volume resulting in lower signal levels. Moreover, as light goes deeper inside tissues, wavefront distortion arises inside a scattering medium generating a speckle pattern precluding most super resolution techniques to be used.

The proposed work is focused on enabling super resolution for Raman imaging deep inside biological tissues using low optical power levels. The technique we present here uses speckles as structured illumination patterns through a coherent Raman scattering process. After various speckle realizations, we reconstruct a super-resolved image without knowing the exact speckle illumination used to illuminate the sample. As a results, we demonstrate that the method allows to go beyond coherent Raman scattering raster scanning technique resolution, while maintaining sectioning ability and low power density levels.

Development of Tomographic Diffractive Microscopy

Presenter: Olivier HAEBERLE

Institut de Recherche en Informatique, Mathématiques, Automatique et Signal, IRIMAS EA7499, Mulhouse, France

Tomographic microscopy requires completely rethinking the data acquisition chain in unlabeled optical microscopy, to obtain enriched images with increased resolution, by coupling optics (light-specimen interaction), instrumentation (capture and transport of this information), and computer science (processing of this information).
The latest advances in the field will be presented, among them the possibility of building an "all-in-one" microscope allowing phase imaging, bright field or dark field microscopy, oblique or Rheinberg illumination, Huffman modulation, phase contrast, DIC etc ..., by recalculating the images from tomographic data.

Gold Loaded Polymeric Nanoparticles for SWIR Imaging

Presenter: Lucie HAYE

Laboratoire de Bioimagerie et Pathologies (LBP), Illkirch-Graffenstaden, France

Synthesis and characterisation of luminescent nanoprobes loaded with different concentration of gold nanoclusters. Study of the spectroscopic properties of the obtained nanoparticles emitting in the SWIR region.

A clearing protocol for pigmented roots : make symbiotic micro-organisms visible in 3D

Presenter: Mathilde HÉRICHÉ

UMR Agroécologie pôle interactions plantes micro-organismes équipe mycorhize (prof. D. Wipf), UMR 1347 IPM, Dijon, France

Three-dimensional imaging of large samples is limited by refraction and absorption of light by tissue components (lipids, pigments, etc.). In order to limit these phenomena and allow the acquisition of images over the entire root thickness, it is necessary to make the sample transparent by depigmenting and homogenising the different refractive indices within the tissue, via chemical treatments. This technique, called clearing, allows a three-dimensional analysis of the fluorescently labelled structures of interest on thick samples. More than twenty different tissue clarification techniques are based on this principle. Most of them have been developed and optimised for the clarification of animal tissues; in particular soft tissues such as brain. Although known in plants for several decades, only a few recent methods are compatible with fluorescent labelling and their effectiveness rely on clearing chlorophyll organs (leaves and stems of Arabidopsis thaliana).

However, data on root clearing are missing. For this reason, we developed a clearing protocol in a plant of agronomic interest with tannins: Vitis vinifera. After obtaining a good level of transparency, we carried out different fluorescent labelling and we demonstrated that this protocol is suitable with mycorrhized tannic root.

Structured illumination for Single Molecule Localization Microscopy

Presenter: Abigail ILLAND

Institut des Sciences Moléculaires d'Orsay (ISMO, Orsay, France

Single Molecule Localization Microscopy (SMLM) is widely used in bioimaging, both for structural imaging and tracking. The molecular localization usually relies on a centroid technique that strongly relies on the point spread function shape. Axial localization is usually obtained by introducing a PSF engineering method.

These conventional localization strategies lead to lateral/axial precision that varies along the depth of field. So the depth of observation with the optimal localization precision will be reduced. We propose an alternative strategy where the introduction of a modulated excitation can be used to retrieve the single molecule position. This new approach is based on the extraction of the phase of the modulated fluorescence signal to retrieve the position information, which makes it possible to extract the localization with a gain in precision compared to classical methods. And the use of modulated excitation will be possible at different focal depth with the same precision localization.

Single-shot quantitative aberration and scattering length measurements in mouse brain tissues using an extended-source Shack-Hartmann wavefront senso

Presenter: Sophia IMPERATO

Laboratoire de Physique et d'Etude des Matériaux (LPEM), Paris, France

Functional fluorescence imaging in mammalian brain tissues remains challenging due to scattering and optical aberration-induced loss in signal and resolution. Correction of aberrations using adaptive optics requires their measurement deep in scattering tissues.

Here, we show that an extended-source Shack-Hartmann wavefront sensor (ESSH) allows quantitative aberration measurements through fixed brain slices, up to four times the scattering length of the tissue. We demonstrate in particular that this wavefront measurement method based on image correlation is more robust to scattering compared to the current centroid-based approach. Taking advantage of its geometry, the ESSH wavefront sensor provides also a measurement of the tissue scattering length.

Extra kinetic dimensions for label discrimination

Presenter: Ludovic JULLIEN

PASTEUR, Département de chimie, Ecole normale supérieure, PSL University, Sorbonne Université, CNRS, Paris, France

Due to its sensitivity and versatility, fluorescence is widely used to detect specifically labeled biomolecules. However, fluorescence is currently limited by label discrimination, which suffers from the broad full width of the absorption/emission bands and the narrow lifetime distribution of the bright fluorophores.

We overcome this limitation by introducing extra kinetic dimensions through illuminations of reversibly photoswitchable fluorophores (RSFs) at different light intensities. In this expanded space, each RSF is characterized by a chromatic aberration free kinetic fingerprint of photochemical reactivity, which can be recovered with limited hardware, excellent photon budget, and minimal data processing. This fingerprint was used to identify and discriminate up to 20 among 22 spectrally similar reversibly photoswitchable fluorescent proteins (RSFPs) in less than 1s. This strategy opens promising perspectives for expanding the multiplexing capabilities of fluorescence imaging.

Biophysical exploration of _LFA-1 mediated adhesion in cytotoxic T lymphocytes

Presenter: Claire LACOUTURE

Institut Toulousain des Maladies Infectieuses et Inflammatoires, INSERM UMR1291 - CNRS UMR5051, Toulouse, France

LFA-1 is a key-player protein for T-cells adhesion and activation, from the beginning of the immunological synapse to the final killing of the target. As an integrin, LFA-1 has several possible conformations, each one presenting a different affinity with its ligand ICAM-1 : it can switch from low affinity to high affinity conformations, which is mediated by interactions with the cytoskeleton. Furthermore, this high affinity conformation is organized in a ring, that is composed of nanoclusters of a dozen of proteins. However, the composition of these nanoclusters and their accurate link with the T-cell activation remains unclear.

A study by STED microscopy allowed us to investigate in detail the composition of these nanoclusters. Especially, colocalisation observations and analyzis argue that each nanocluster is composed by high affinity and low affinity LFA-1 at the same time. Otherwise, by analyzing these clusters with machine-learning on STED pictures, an increase of their number is observed with the increase of the T cell stimulation. This suggests that higher level of T cell activation induces a better adhesion, which is followed by a more efficient killing of the target cells, that is measured by the automatized microscope Opera Phenix (PerkinElmer). In perspective of this work, all these data will be integrated in a quantitative model of the CD8 T cell immunological synapse, in order to better understand the role of adhesion via LFA-1 in the immune response of these lymphocytes.

Through the microscope: Novel approaches in host-pathogen interaction direct observation and inferred modelling in Zebrafish.

Presenter: Valério LAGHI

Institut Pasteur, Paris, France

How do viruses propagate from organ to organ – particularly to the brain, and how does the innate host response, or drugs, counter this?

Our project starts from biology and develops towards modelling and bioinformatics. We will exploit an established model of viral infection of zebrafish larvae, in which the distribution of infected cells can be imaged in the whole body over time. We aim to create a new method of analysis and reconstruction of viral spreading from tissue to tissue, and to understand the relative impact of the host interferon (IFN) response and antiviral drugs on the mechanism of viral dissemination, with a particular focus on brain invasion.

We use SINV strains encoding for fluorescent proteins co-expressed with late or early viral proteins. Target cells, infection course and the innate immune response, are monitored using transgenic reporter zebrafish, larvae made unable to respond to type I IFNs, and larvae treated with antiviral drugs. Firstly, we use high throughput methods to screen large number of samples infected with SINV to determine the general route followed by the virus and lay down a first mathematical model based on two compartments (CNS and Outside CNS).
Based on assessments of viral burdens and modelling of dynamics, we will derive the parameters of viral growth (burst size, half-life of infected cells, estimation of target cells). After having determined the temporal frames of infection, the involved tissues and the base model, we move towards high resolution confocal imaging to be able to identify in vivo the key cell types involved. 3D reconstruction will be used to focus on discovering the molecular mechanism used to invade the central nervous system, the cellular population involved, the kinetic rules associated to the viral biophysics in each compartment of the specified model.

Chlorophyll fluorescence as a tool to study plant's health status

Presenter: Aliénor LAHLOU

PASTEUR - SONY CSL Paris, UMR 8640, Paris, France

Photosynthesis harvests light energy to fuel a set of (photo)chemical reactions that end up consuming carbon dioxide and produce dioxygen and biofuels. Each of these are of high interest to science and industry in the context of climate change. Photosynthetic microorganisms such as microalgae can be farmed to perform photosynthesis. A precise knowledge of algae status and performance is required to adapt the farming conditions and use the best-performing strains. To this aim, we exploit auto-fluorescence of photosynthetic organisms as a reporter of photosynthesis. The objective is to perform early diagnostics on plants to selectively detect specific stresses early on, before they have visual - irreversible - effects.

To reach this goal, we have developed a dedicated microscope for algae diagnosis, which we currently use to explore the dispersion of the auto-fluorescence response of individual algae cells to illumination sequences at various light intensities.
Here we show how we collect the fluorescence signal of individual algae to study their performance at photosynthesis. We focus on the regulation mechanisms developed by algae under light stress and evaluate the distribution of the dynamics of response.

Comparison of different clearing and acquisition methods for 3D imaging of murine intestinal organoids

Presenter: Louison LALLEMANT

Repeat Expansions & Myotonic Dystrophy, UMRS974, Paris, France

An organoid is a three-dimensional multicellular structure that shows realistic micro-anatomy of an organ. This in vitro model mimics the in vivo environment, architecture and multi-lineage differentiation of the original organs and allows to answer many interesting biological questions. For these reasons, they are widely used in stem cell, regenerative medicine, toxicology, pharmacology, and host-microbe interactions research. In order to study organoids, microscopy is very useful: It is possible to make three-dimensional reconstruction of serial sections, but it is time consuming and error-prone.

Here we propose an alternative solution: Tissue clearing reduces the dispersion of light because it homogenizes the refractive index of the tissue, allowing sample observation throughout its thickness. We have compared different clearing techniques on mouse intestinal organoids using different acquisition methods.

Probing biomechanics with a microfluidic aspiration pipette: from lipid membranes bending modulus to spheroids rheology

Presenter: Sylvain LANDIECH

Laboratoire d'Analyse et d'Architecture des Systèmes (LAAS), Toulouse, France

Cell membranes are submitted to many deformations: exo/endocytosis, cell division, contact with a substrate, other cells. Even if actual biological plasma membranes are complex (presence of proteins, coupling with the cytoskeleton), the mechanical properties of lipid bilayers, their main constituent, are thus important quantities. Micropipette aspiration is the gold standard method to measure the bending rigidity and area expansion modulus of a membrane [1]. It consists in measuring the increase of area of a giant unilamellar vesicle (GUV) upon aspiration. It is quantitative, but is limited to single objects, does not permit to change easily the surrounding medium, and is long and tedious to realize. Microfluidic systems are emerging to manipulate the micron-sized objects of soft-matter, with the promises of high-throughput, control over media composition, automation. This technology is thus also perfectly suited to study larger living systems such as spheroids, which are aggregates of cells of the order of 100µm, used as a model of healthy or tumor tissue. The physical properties of tissues play a major role in many biological processes such as embryonic morphogenesis, wound healing, cancer growth or metastasis. Micropipette aspiration allows to measure the rheology of these aggregates that can be modeled by a viscoelastic fluid.

After many attempts to exploit standard microfabrication methods to miniaturize GUV aspiration [2], we recently developed a more efficient on-chip micropipette thanks to an original approach derived from [3]. This innovative technology permits the integration of any trap shape to block vesicles, cell or spheroids. In particular, we integrated cylindrical traps (constituting the pipette) that were very difficult to obtain with the previous microfluidic approaches. Up to 7 vesicles or 5 spheroids could be blocked in parallel. We measured on simple lipid compositions (Dioleoyl Phosphatidyl Choline) the bending and stretching moduli which were quite compatible with literature (15+-5 kBT and 250+-25 mN/m respectively). We are currently characterizing less-known, more complex systems: effect of cholesterol, influence of copolymer micelles nanoparticles. By analyzing the dynamics of the cell flow in the micropipette we can measure the elasticity, the viscosity and the surface tension of the spheroid. For spheroids composed of mouse pancreatic cancer cells, the values we obtained are in line with the literature.
In conclusion, our approach enables on-chip micropipette measurements, with a better throughput than the standard aspiration setup. Microfluidics is versatile, and we are currently working at variants enabled by design changes: rheology of spheroids under growth pressure, effect of shear on vesicles.

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An adaptive microscope for the imaging of biological surfaces

Presenter: Loïc LE GOFF

Aix Marseille Univ, CNRS, Centrale Marseille, Institut Fresnel, Turing Center for Living Systems, Marseille, France

Scanning fluorescence microscopes are now able to image large biological samples at high spatial and temporal resolution. This comes at the expense of an increased light dose which is detrimental to fluorophore stability and cell physiology. To highly reduce the light dose, we designed an adaptive scanning fluorescence microscope with a scanning scheme optimized for the unsupervised imaging of cell sheets, which underly the shape of many embryos and organs.
The surface of the tissue is first delineated from the acquisition of a very small subset (0.1%) of sample space, using a robust estimation strategy (figure a). Two alternative scanning strategies are then proposed to image the tissue with an improved photon budget, without loss in resolution (figure b). The first strategy consists in scanning only a thin shell around the estimated surface of interest, allowing high reduction of light dose when the tissue is curved. The second strategy applies when structures of interest lie at the cell periphery (e.g. adherens junctions). An iterative approach is then used to propagate scanning along cell contours. We demonstrate the benefit of our approach imaging live epithelia from Drosophila melanogaster. On the examples shown, both approaches yield more than a 20-fold reduction in light dose and up to more than 80-fold- compared to a full scan of the volume. These smartscanning strategies can be easily implemented on most scanning fluorescent imaging modality. The dramatic reduction in light exposure of the sample should allow prolonged imaging of the live processes under investigation.

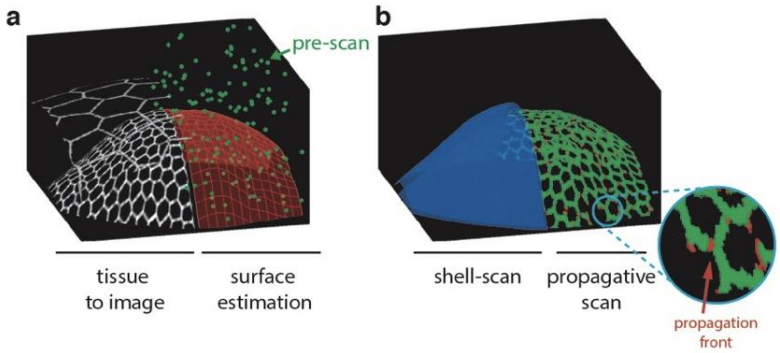

Figure 1. Principle of smart-scan: (a, left): drawing of epithelial tissue to image. (a, right): a highly fractional prescan of the volume (0.1%) allows to mathematically estimate the surface of interest. For imaging, we can then either scan a thin shell around the surface (b,left), or algorithmically propage imaging along cell contours (b, right).

Abouakil, F., Meng, H., Burcklen, MA. et al. An adaptive microscope for the imaging of biological surfaces. Light Sci Appl 10, 210 (2021). https://doi.org/10.1038/s41377-021-00649-9

Near infrared II imaging technology for biomedical applications

Presenter: Xavier LE GUEVEL

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In vivo optical imaging is nowadays widely used for cancer diagnosis and image-guided therapy with various devices that have already entered into the clinic. However, current fluorescence imaging is worth improving in spatial and temporal resolution in deep tissues. A novel approach relies on collecting optical signal from the second near-infrared window between 900 to 1700 nm called Shortwave InfraRed (SWIR) or NIR-II. The use of this wavelength range considerably reduce light scattering and absorption by blood and tissues in vivo improving sensitivity and spatial resolution in depth. We showed how the use of NIR-II imaging technique in mice models enable to monitor the vascularisation with high resolution thanks to the development of new bright NIR-II emitting contrast agents and the use of post-imaging processing based on Monte Carlo restoration and deep learning.

Resonances at fundamental and harmonic frequencies for selective imaging of sine-wave illuminated reversibly photoactivatable labels

Presenter: Thomas LE SAUX

PASTEUR, Département de chimie, École normale supérieure, PSL University, Sorbonne Université, CNRS, Paris, France

Dynamic contrast exploits kinetics to discriminate a label against a spectrally interfering background. Imposing light oscillations to a reversibly photoactivatable label induces oscillations of the photochemical rate constants. Even for rate laws linearly depending on the concentrations, the product of a photochemical rate constant and a concentration leads to harmonic generation in any observable linearly depending on the concentrations.

We introduce HIGHLIGHT (pHase-sensItive imaGing of reversibly pHotoactivatable Labels after modulatIon of activatinG ligHT), which consists in submitting reversibly photoactivatable fluorescent labels to sine-wave illumination of large amplitude. We show that Fourier amplitudes of concentration and fluorescence oscillations at harmonic frequencies exhibit resonances in the space of the control parameters of illumination. Choosing the frequency and the mean light intensity singularizes a label associated with a given kinetics. We evidence that several non-redundant resonant observables can be simultaneously retrieved from a single experiment by using phase-sensitive detection. Moreover, we demonstrate that labels out of targeted locations can be easily discarded in an inhomogeneous spatial profile of illumination. This simple and general strategy is used for selective and intrinsically confocal imaging of four spectrally similar reversibly photoswitchable fluorescent proteins that had not been discriminated so far. The protocol opens roads for simplified optical setups at reduced cost and easier maintenance.

Improved single molecule localization from dual-objective microscopes with ZOLA-3D

Presenter: Benoit LELANDAIS

Imagerie et Modélisation (IMOD), Institut Pasteur, Paris, France

In single molecule super-resolution microscopy, the resolution is limited by the number of photons that is collected from individual fluorophores. Using dual opposing objectives to collect photons from both sides of the sample enables collection of twice more photons, hence allowing an improvement of the localization precision. Introduction of astigmatism by means of cylindrical lenses permits to reconstruct 3D images, but sample thickness leads to spherical aberrations. We recently developed ZOLA-3D, a software that enables 3D SMLM image reconstruction using a realistic modelling of engineered PSFs, and which accounts for spherical aberrations. Here, we describe an extension of ZOLA-3D for the 3D localization of molecules imaged by a dual-objective microscope equipped with two cameras.

The localization method is optimal as it combines the images from both cameras while using realistic PSF models. Cramér Rao bounds confirm that both the theoretical localization precision and the axial range are improved using dual objectives. Simulated data show that our software achieves optimal precision on single emitters, and that the use of two cameras allows to improve localization precision for high emitter density by reducing detection ambiguities. The software also performs registration of the two images and drift correction. Reconstructions of tubulin filaments in 3D show that the dual objective system improves resolution not only because more photons are collected, but also because detection ambiguities are reduced.

SMLM with light sheet illumination for deep cell imaging

Presenter: Mickaël LELEK

Imagerie et Modélisation (IMOD), Institut Pasteur, Paris, France

Single molecule localization microscopy methods like PALM and STORM overcome the optical diffraction limit by a factor of 10. However, thee methods are limited to imaging a single focal plane in the sample because they generally use TIRF or HILO illumination. In TIRF, only the first hundred nanometers above the coverslip are imaged while in widefield deeper focal plane imaging is permitted but the out of focus planes are photobleached preventing their imaging in high resolution. In Single Plane Illumination (SPIM) a single focal plane is illuminated by a thin light sheet, thereby leaving the out of focus planes free of photobleaching. Scanning the light sheet then enables volumetric imaging of the cell with a confocal resolution.

Taking advantage of both methods, and following previous work, we aimed to perform SMLM using a thin light sheet illumination in order to achieve volumetric high resolution microscopy of the entire cells. We present the development of a SPIM system for SMLM, whch allows to perfrom high resolution microscopy imaging deeper in the cells. The microscope is based on a commercial body using 2 high NA and high magnification objective lenses oriented at 120° from each other.

Combination of ultrafast two-photon microscopy with tissue clearing for studying physiopathology

Presenter: Lhorane LOBJOIS

Institut Toulousain des Maladies Infectieuses et Inflammatoires, INSERM UMR1291 - CNRS UMR5051, Toulouse, France

Imaging into deep tissue can be reached by two main modalities: light-sheet or two-photon microscopy. To finely decipher the 3D architecture of tissues at the cellular level, the two-photon approach seems to be the best choice due to its resolution. An ultra-fast multi-photon setup was used to compensate for the slowness usually associated with this laser scanning technique. Indeed, the combination of GaAsp sensitive detectors with a resonant scanner, an ultra-fast z-piezo, and a large field of view (FOV 1mm*1mm) result in a high-throughput two-photon microscope (up to 60fps). Moreover, in any case, light scattering and absorption limit the imaging deepness into thick samples. Clearing methods are often used to circumvent this issue, as they homogenise the refractive index. However, only a few of them allows to perform immunolabelling and/or keep the endogenous fluorescence inside the tissue.

This study focuses on two different clearing methods: the recently published Fast Light-Microscopic analysis of Antibody-Stained wHole organ (FLASH) method was used for clearing immuno-labelled human placenta; and the RapiClear (SunJinLab) commercial solution was used for clearing immuno-labelled mouse colon. This poster aims to illustrate the possibilities of high-throughput two-photon imaging combined with sample clearing for studying physiopathology.

CEMIPAI: a unique BLS3 Microscopy Facility to analyze infectious samples at the nanometer scale

Presenter: Sébastien LYONNAIS

Centre d'étude des maladies infectieuses et Pharmacologie anti-infectieuse (CEMIPAI), Montpellier, France

La nouvelle génération de microscopes à force atomique (AFM), à haute vitesse et couplés à la fluorescence, adaptés spécifiquement pour la biologie (Bio-AFMs) permet de disposer de boites à outils remarquablement puissantes pour l'étude morphologique et nano-mécanique des virus, des bactéries et des cellules infectées à l'échelle nanométrique, et sur du matériel vivant. L'acquisition en chaque point des forces d'interaction entre la pointe et l'échantillon (spectroscopie de force) associe désormais une image topographique avec les propriétés biophysiques et mécaniques (déformabilité/élasticité, adhésion, etc.) en milieu liquide. Cette imagerie multimode peut être réalisée en fonction du temps pour donner accès à la dynamique des objets avec des niveaux de détails et de rapidité qui s'améliorent avec chaque nouvelle génération d'instrument, pour combiner imagerie de fluorescence et imagerie AFM.

Nous avons installé un tel combiné bio-AFM dans le laboratoire de biosécurité de niveau 3 de la plateforme régionale CEMIPAI afin d'explorer les potentialités de cette nouvelle imagerie pour la virologie en particulier. Nous présenterons ici plusieurs exemples d'imagerie qualitative et quantitative par AFM couplée à l'imagerie optique de virus (SARS-CoV-2, VIH, HTLV, Arbovirus, grippe), bactéries et cellules infectées ; montrant les perspectives uniques de ce type de technologie pour apporter des informations nouvelles (des mesures biophysiques jusqu'alors inaccessibles) sur le cycle de vie des agents pathogènes de classe 3.

High throughput screening and analysis of osteogenic cell differentiation on biomimetic coating plates using HCS fluorescent microscopy

Presenter: Paul MACHILLOT

CEA/IRIG/DS/BRM, ERL5000, Grenoble, France

Here we study cells behavior on other surfaces than those traditionally used in cell biology (glass, polystyrene), especially to use biomimetic coatings of controlled stiffness and carrying bioactive molecules (BMP2 growth factor; involved in osteogenic differentiation). Made of natural biopolymers, these coated films are deposited using an robust automated process with a liquid handling robot at the bottom of 96-well cell culture glass plates. Advantageously, these biomimetic films are optically transparent, which enable studies using optical microscopies.
Using an HCS microscope (IN Cell Analyzer 2500 – Molecular Devices) we develop a high throughput automated acquisitions of fluorescent imaging of these biomimetic-cultured cells. Coupled with a high troughput analysis of these images using ImageJ macros, we are able to study cell behavior (adhesion/spreading) and osteogenic cell differentiation such as nuclear localization of transcription factors.

Multimodal label-free imaging approach to monitor spinal cord injury in mice

Presenter: Clara MANESCO

Laboratoire Charles Coulomb (L2C), University of Montpellier, CNRS, Montpellier, France.

Aim: With the objective to investigate the mechanisms underlying absence of spontaneous axonal regeneration following spinal cord injury (SCI) we employ a multimodal label-free imaging approach to monitor glial scar in a mice SCI model.

Method: To determine the relevant structural signature, and the nanobiomechanical behavior of healthy and injured spinal cord tissue we combine the non-linear, multiphoton microscopy (MPM) technique with force measurements via atomic force microscopy (AFM). The glial scar at different post lesion time-points is investigated with these two techniques to monitor structural and elasticity (Young modulus) changes of the tissue.

Results: 2-photon excited fluorescence (2PEF) and second harmonic generation (SHG) signals of excised mice SC injured tissues were recorded in MPM at 72h, 1week and 6 weeks post-lesion. The MPM images revealed the apparition of a strong SHG signal at 1week post injury, due to the formation of fibrillar collagen fibers (collagen type I) by the injury site in the glial scar. At 6 weeks post-injury, the SHG signal is more intense and a higher number of fibers are detected in average. We further assessed the preferential orientation of the collagen bundles performing polarization dependent measurements of the SHG signal. The AFM based force spectroscopy measurements have been performed at the same post-lesion time-points to map the elastic properties of the healthy (grey and white matters) and injured (lesion) parts in the spinal cord tissue. The results suggested an increase of the lesion area stiffness over time that could be correlated with the apparition of fibrillar collagen observed in MPM, indicating the presence of a fibrotic process seven days after injury, that develops in time. As tissue stiffness is a regulator of neuronal growth, such kind of measurements might help to understand why adult mammalian axons do not regenerate after an injury. Our next step is to investigate the effect of a treatment (pharmacological transient depletion of microglia/macrophage in mice that underwent SCI) on the structure and mechanical properties of the lesion site at 1 week and 6 weeks post injury.

Super-resolution microscopy techniques to investigate enzymes organization in bacteria

Presenter: Hanna MANKO

University of Strasbourg, CNRS, UMR 7021, Strasbourg, France

Bacteria are able to survive in various environment conditions by activating sophisticated machineries that allow to produce molecules with remarkable structural and functional diversity. These include non-ribosomal peptides (NRPs), a family of natural products with a broad spectrum of biological activities and pharmacological properties from which key antibacterial, antifungal,- antiviral, immunosuppressant, and anti-cancer drugs have been retrieved. The compounds are synthesized by non-ribosomal peptide synthetase (NRPS). Nevertheless, little is known about the organization and regulation of NRPS within native producer cells despite their uniqueness and importance. The understanding of NRPS spatial organization and their regulation at the single cell level is an absolute need for optimized NRP production in cellular or synthetic systems.

The biosynthesis pathway of pyoverdine, one of the small molecules called siderophores, was chosen as a model for study. This pathway, mediated by NRPS, can be activated by Pseudomonas aeruginosa in case of iron-deficient environment. In order to retrieve the information about organization and dynamics of the enzymes of interest we relied upon super-resolution microscopy techniques, including single molecule tracking of PAmCherry fusion enzymes and Single Molecule Localization Microscopy.

Future work includes two-color DNA-PAINT and two-color tracking experiments. To enhance the performance of the spectrally-resolved microscopy a U-net-kind Neural Network was utilized.

Fluorescence prediction in phase microscopy using complex deep learning

Presenter: Guillaume MAUCORT

Laboratoire Photonique Numérique et Nanosciences, UMR CNRS 5298, Talence, France

Ce poster présente une technique de prédiction de localisation de fluorescence par deep learning appliquée à de la microscopie de phase ainsi que les premiers résultats prometteurs sur des réseaux de mitochondries.
L'outil employé est une variation du réseau U-net modifié pour ajouter les éléments caractéristiques à notre problématique, notamment une gestion de données sous forme de nombres complexes afin de travailler directement sur le champ électromagnétique (intensité et phase).

Nous présentons également une métrique d'apprentissage et de caractérisation du modèle adaptée à ces conditions particulières.

Quantitative in-vitro optical monitoring of ROS production through nanoparticle imaging.

Presenter: Maxime MAUVIEL

Laboratoire d'Optique et Biosciences (LOB), Ecole Polytechnique, Palaiseau, France

The quantitative deciphering of signaling pathways is critical to understand and control physio-pathological processes. In complex diseases, such as some inflammation-related conditions, it is often necessary to identify in situ the loci, the timing and the quantity of the production of the involved molecules resulting of their complex interplay with the environment. This is particularly true for Rapidly Progressive GlomeurloNephritis (RPGN) causing terminal kidney failure due to a loss of the glomerulus filtration capabilities, caused by an uncontrolled de-differentiation and migration of specialized cells, such as parietal epithelial cells (PECs). Among numerous local cues, HB-EGF, PDGF and CD9 have been identified as key regulators of RPGN progression. As EGF and PDGF pathways are known to involve intracellular ROS production and since ROS are a key second messenger in signaling, it is assumed that ROS is a central regulator for RPGN.

However, the lack of efficient quantitative assays, both for cell response in a multicellular environment and for ROS detection, has so far hindered the understanding of the molecular mechanisms leading to this pathological phenotype at the organ scale. We are thus developing a three-layer biomimetic in vitro device (or “Glomerulus-on-Chip”) technology combined with single lanthanide-based luminescent nanoparticle imaging to quantitatively measure intracellular ROS concentration in systems of controlled complexity in order to identify critical mechanisms that could be the basis for in vivo methods of RPGN treatment or management. These photoluminescent lanthanide based nanoparticles (YVO4:Eu) are used as nanosensors to probe the cell oxidative response while our versatile microfluidic-based framework allows to quantitatively assay cell responses such as single or collective migration processes under various controlled stimuli.
Based on this multi-scale approach, we plan to (i) map the spatio-temporal pattern of ROS production in PECs submitted to controlled stimuli, notably during artificially induced migration, (ii) observe the effects of pharmacological treatments at the tissue, cell and molecular scales in different genetic backgrounds, to (iii) ultimately identify critical pathways controlling the pathological transition.
This work could pave the way to the development of a versatile framework for the screening of putative drugs before any clinical trial, and could thus be a major tool for rational treatment design for RPGN management.

Keywords: Nanoparticles, ROS, Organ-on-Chip, Glomerulus, RGPN, disease modelling.

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Exploring tissue morphodynamics using the photoconvertible Kaede protein in amphioxus embryos

Presenter: Lydvina MEISTER

Observatoire Océanologique de Banyuls sur Mer, BIOM UMR 7232, Banyuls sur Mer, France

Photoconvertible proteins are useful tools widely used in cellular biology to tag a protein or the membrane to study cell dynamics, organelles and proteins. Over the past decade, photoconvertible proteins have also been used for developmental biology applications to track the fate of a cell or subpopulation of cells in the embryo. The Kaede protein from stony coral Trachyphyllia geoffroyi undergoes irreversible photoconversion from green to red fluorescence when illuminated with UV light. The definitive switchable property of this protein allows studying cell fate and cell migration during embryonic development. Undertaking a cell tracing approach using photoconvertible proteins can be challenging when using unconventional animal models. In this protocol, we describe the use of Kaede to track specific cells during embryogenesis of the cephalochordate Branchiostoma lanceolatum. This protocol can be adapted to other unconventional models, especially marine animals.

Contactless mechanical characterization of microtissues using optogenetics

Presenter: Adrien MÉRY

Laboratoire Interdisciplinaire de Physique (LIPhy), Saint Martin d'hères, France

Mechanical signals have been shown to regulate a wide variety of biological processes, from cell proliferation and differentiation to tissue homeostasis. Yet, little is known about the way mechanical signals are stored and transmitted in tissues.
We combined 3D model tissues with recent advances in optogenetics. Micro fabricated tissue gauges simultaneously constrain the tissues and report their traction force. The use of photosensitive-ArhGEF11 fibroblasts allows for a precise spatio-temporal control of cell forces from the inside, as ArhGEF11 is a major regulator of cellular contractility.
Upon light stimulation, the tissue tension increased over several minutes before being regulated back to its baseline level. By varying the mechanical stiffness of the microcantilevers, we demonstrated that the baseline tension as well as the photo-induced contractility depended on the spring constant of the boundaries. Optogenetic control allows for stimulation of only parts of the tissue.

We thus observed that the photoactivation of the contractility of a group of cells propagate through the microtissue and impact the whole tissue tension. Moreover, we showed that the magnitude of the tension increase is proportional to the number of cells photoactivated and increase with the duration of culture of the tissues. Preliminary results seem to indicate this contraction increase is linked to an increased organization of tissues structure.

Direct Visualization of Horizontal Gene Transfer by Transformation in Live Pneumococcal Cells Using Microfluidics

Presenter: Isabelle MORTIER

Laboratoire de Microbiologie et de Génétique Moléculaires (LMGM - CBI - UMR5100), Toulouse, France

Natural genetic transformation is a programmed mechanism of horizontal gene transfer in bacteria. It requires the development of competence, a specialized physiological state during which proteins involved in DNA uptake and chromosomal integration are produced. In Streptococcus pneumoniae, competence is transient. It is controlled by a secreted peptide pheromone, the competence-stimulating peptide (CSP) that triggers the sequential transcription of two sets of genes termed early and late competence genes, respectively.

Here, we used a microfluidic system with fluorescence microscopy to monitor pneumococcal competence development and transformation, in live cells at the single cell level. We present the conditions to grow this microaerophilic bacterium under continuous flow, with a similar doubling time as in batch liquid culture. We show that perfusion of CSP in the microfluidic chamber results in the same reduction of the growth rate of individual cells as observed in competent pneumococcal cultures. We also describe newly designed fluorescent reporters to distinguish the expression of competence genes with temporally distinct expression profiles.
Finally, we exploit the microfluidic technology to inject both CSP and transforming DNA in the microfluidic channels and perform near real time-tracking of transformation in live cells. We show that this approach is well suited to investigating the onset of pneumococcal competence together with the appearance and the fate of transformants in individual cells.

Magnetic muscular multicellular aggregates: focusing on fluid-like properties of tissue models and driving macroscopic organization

Presenter: Irène NAGLE

Matière et Systèmes Complexes (MSC), UMR 7057, Paris, France

Creating in vitro models of skeletal muscle reproducing its multi-scale aligned structure and its function would offer a polyvalent platform for mechanobiology but also for the development of new drugs or gene therapies to treat muscular traumatisms or diseases. To address this challenge, the use of magnetic fields and forces, via the incorporation of biocompatible superparamagnetic nanoparticles (γ-Fe2O3) in the cells is promising. This magnetic labelling enables both the manipulation of cells at distance to create purely cellular aggregates of controlled shapes and the application of forces to measure their mechanical properties and to drive their differentiation into functional muscular cells. Using mouse muscle precursor cells C2C12, we obtained multicellular aggregates without support matrix of unprecedented size (1 mm). The aggregate deformations with a magnetic field enable to measure its macroscopic mechanical properties (surface tension, Young modulus).
We looked at the interplay between the individual cell properties (cell-cell adhesions, actin structure and tension) and the mechanical properties at the tissue scale revealing the importance of desmin disorganization in macroscopic rigidity and surface tension. By studying desmin-mutated muscle precursor cells (point mutations involved in desminopathies), we enhanced the fundamental role of the intermediate filament network architecture in this 3D tissue model.
A magnetic stretcher is now developed to stimulate mechanically for several days multicellular aggregates of muscle precursor cells trapped between two mobile magnets and induce their differentiation into fused aligned muscular cells. This magnetic stretcher represents a new tool to study cell deformation under stretching and muscle cell differentiation.

MiSiC, a deep learning-based method for cell segmentation in dense bacterial communities

Presenter: Swapnesh PANIGRAHI

Laboratoire de Chimie Bactérienne (LCB), UMR 7283, Marseille, France

Studies of bacterial communities, biofilms and microbiomes have an impact on health and ecology. Multimodal imaging of interacting bacterial communities requires new tools that can analyze very large datasets with dense information content. Here, we present MiSiC, a general deep-learning-based 2D segmentation method that automatically segments single bacteria in complex images of interacting bacterial communities with very little parameter adjustment, independent of the microscopy settings and imaging modality. The simple implementation of MiSiC and the relatively low need in computing power make its use broadly accessible to fields interested in bacterial interactions and cell biology.

Tracking memory formation in the honeybee visual circuit

Presenter: Marco PAOLI

Centre de Biologie Intégrative (CBI), Toulouse, France

Honeybees are able of associating an olfactory or visual stimulus with a sugar reward, providing an ideal model for the study of sensory coding and memory formation. While coding principles of odour perception have been extensively studied allowing us to acquire some insight on the nature of olfactory memory trace, our knowledge of the neural correlates of visual learning remains very limited. By coupling protocols of visual stimulation and learning with in vivo calcium imaging analysis with multiphoton microscopy, we provide a model for the investigation of how individual stimuli are coded along the different neutrophils of the visual system, and how (and where) memory traces are stored.

Nipah virus W protein inhibits and translocalize NF-κB p65 by harnessing 14-3-3-mediated repression

Presenter: Rodolphe PELISSIER

Centre International de Recherche en Infectiologie (CIRI), U1111, Lyon, France

The Nipah Virus (NiV) is an emerging virus that belong to the Henipavirus family. This zoonotic virus has for host the fruit bat and is responsible for yearly lethal epidemic episodes in Southern Asia with no approved vaccine or treatment. NiV is known to induce neurological/ severe respiratory symptoms as well as a total deregulation of the immune response of the infected host. The NF-κB pathway plays a crucial role in antiviral response as a main transcriptional regulator of the inflammation. We report that Nipah virus (NiV) inhibits the activation of the canonical NF-κB signaling via its nonstructural W protein and more precisely, via its specific C-terminal Domain (CTD). The nuclear localization of W protein and the interaction of W-Ser449 with 14-3-3 cellular protein results in the accumulation of 14-3-3 in the nucleus and the inhibition of NF-κB p65 phosphorylation and its translocalisation from the nucleus to the cytoplasm. This interaction between endogenous 14-3-3 and the NiV-W protein has also been observed in vivo in a model of non-human primate suggesting that NiV-We play a role in the down-regulation of the inflammatory response observed during the infection course.

Our data suggest that NiV W increases the influx of 14-3-3 in the nucleus and enhances its negative feedback on NF-κB p65 pathway. Leading to the suppression of the host inflammatory response and giving the molecular insight in the pathogenesis of this highly lethal emerging zoonotic infection.

Pourquoi et comment chronométrer un ribosome en cours de traduction ?

Presenter: Karen PERRONET

Lumière Matière et Interfaces (LuMIn), Gif sur Yvette, France

Le ribosome est la machine moléculaire qui orchestre la synthèse des protéines dans tous les organismes vivants. Ce processus, par essence asynchrone, est difficile à étudier par des mesures d'ensemble. Nous expliquerons comment nous pouvons mesurer la vitesse de traduction de ribosomes eucaryotes uniques. Sur différents exemples, nous montrerons l'intérêt de ce type de mesure pour comprendre l'impact de la cinétique sur la traduction.

Relationship between ER Ca2+ depletion and UPR induction: experimental and modeling approach

Presenter: Ilaria PONTISSO

Calcium signaling and microbial infections / INSERM, UMRS 1282, Gif sur Yvette, France

The Endoplasmic Reticulum (ER) is the primary site of folding and quality control of one third of cellular proteins and is the major intracellular Ca2+ store. Depletion of the luminal [Ca2+] disrupts the correct folding environment leading to an alteration of ER homeostasis and accumulation of misfolded proteins inside the lumen. In order to restore ER proteostasis and normal cellular functions, cells have developed an adaptive mechanism consisting in 3 specific signalling pathways. This response is commonly referred to as the Unfolded Protein Response (UPR) and leads to an increase of the protein folding capacity of the ER and to homeostasis restoration. Although long-term and strong UPR activation is much studied, the consequences of more physiological and small amplitude luminal Ca2+ depletions on the early activation of UPR has been largely unexplored.

In my project I intend to study how moderate Ca2+ depletion impacts on the activation of the signalling pathways of the UPR. My approach will be centred around the development of a data-driven computational model that will allow to decipher, formalize, and quantify this intricate signalling pathway. Ca2+ imaging experiments by use of Genetically Encoded Ca2+ indicators targeting the ER will be combined with Immunoblots, qPCR and imaging data to reveal the links between Ca2+ and UPR activation. Given that luminal Ca2+ depletion and alteration of correct ER proteostasis are involved in a variety of pathologies such as diabetes, neurodegenerative diseases or cancer, a better understanding of the reciprocal crosstalk between Ca2+ and UPR will provide insight into the mechanisms of progression of these diseases.

Polarimetry techniques in microscopy: from single quantum-rods orientation imaging (QROM) to reveal topographic features in the deca-nanometer range to complete polarimetry imaging to probe chirality

Presenter: Guillaume RAFFY

Institut des Sciences Moléculaires, UMR5255, TALENCE, France

Soft materials are, in a variety of cases, constituted of nano-structures of self-organized small organic molecules and a solvent (organic solvent, oil, water), and can be found in many industrial and technological applications, as well as in bio-materials. It is of major importance to control the molecular packing and the objects' nano-structures, being at the origin of their properties.
However, their development requires nowadays imaging techniques that are more evolved and provide deca-nanometer resolution in solvated materials, and potentially dynamically. In this presentation, we will discuss fluorescence polarimetric approaches used to characterize organic nano-fibers which present several constraints that inhibit the application of current super-resolution reconstruction imaging based on single molecule detection: the nanostructures are 100% made of fluorescent chromophores (thus impossible to switch off enough to see isolated single molecule blinking); and the soft materials are based on organic solvents and not water (most of commercial dyes are not appropriate). We used anisotropic core-shell CdSe/CdS quantum-rods (QRs) (~ 5-6 nm wide, ~ 50-60 nm long) to probe the nanostructured surface of the fluorescent nanofibers (NFs) constituting an organogel of DDOA (2,3-didecyloxyanthracene). The QR's were modified with an appropriate molecular outer shell so that they were dispersible into the same solvent than the organogel, allowing the preparation of hybrid organogels containing NFs and QRs. TEM microscopy of desolvated sampes reveals that QRs align along the long axis of the NFs. QROM imaging (Quantum Rods Orientation Microscopy) of solvated gels exploits the linearly polarized emission of the QRs to establish an orientation map of each individual QR interacting with NFs.
This indirectly reveals the orientation of the underlying nanofibers' structure probed at the deca-nanometer scale [1]. The poster opens on complete emission polarimetry imaging (i.e. Stokes-vector mapping). More difficult to implement and calibrate, this technique could bring however great added value since it allows, through the measurement of the last Stokes parameter, the probing of the local chirality.

Scattering correcting wavefront shaping for three-photon microscop

Presenter: Bernhard RAUER

Laboratoire Kastler Brossel (LKB), Paris, France

Investigating the complex structure and functioning of biological systems through light microscopy represents a foundational pillar of the life sciences. However, the very complexity of the samples cellular structure leads to one of the main limitations of optical microscopy: light scattering confines it to the outer most tissue layers. In the past decades, multi-photon microscopy successfully extended the accessible depth ranges and on a different front, advances in optical wavefront shaping showed that scattering can be compensated for, even in regimes where light entirely lost its initial directionality.

Combining these two approaches, we establish scattering correction through wavefront shaping for three-photon fluorescence imaging and thereby extend the accessible depth ranges even further. Applying these techniques to an established neuronal imaging platform in the future we hope to open new frontiers for in-vivo brain imaging.

Single-molecule super-resolution imaging in reversibly cryo-arrested cells

Presenter: Louise RÉGNIER

Physico Chimie Curie - Institut Curie (PCC), UMR 168, Paris, France

Fluorescence optical imaging is an indispensable tool to study living cells and organisms at a microscopic scale. However, the spatial resolution of light microscopy is limited to a few hundreds of manometers because of the wave-like nature of light. Single molecule localization microscopy circumvents the diffraction limit by imaging a sparse distribution of single molecules at a time. The center of each single emitter is localized with a precision way below the diffraction limit of the optical microscope. A super-resolution image is the accumulation of all the centers of single-molecules recorded after repeating steps of bleaching and activation of different single-molecule subsets. However, a lethal chemical fixation is usually required prior to imaging in order to stop any molecular motion in the cell and to allow precise localization over typically several tens of minutes.

In order to observe the evolution of molecular organization in living cells, reversible cryo-arrest of cell was proposed as a mean to block molecular motion and acquire single-molecule localization images of the same cell at different time steps. Here we show a new implementation of the method with new, user friendly, design optimized for biological applications. Our experimental set-up includes a dedicated aluminum mount that enables homogeneous temperature control on the sample and efficient replacement of water by DMSO during the cooling process. The design allows direct on-stage cooling of cells and imaging with high NA objective. In addition, as the photophysics of fluorescent molecules can be affected at low temperatures. Here, several quantification tools are implemented to characterize the behavior of molecules and their emission properties at low temperature, such as SNR measurements and blinking behavior. Detections that belong to a same molecule are identified in order to correct for multiple detections and improve the localization precision.
Our approach offers a user-friendly mean to observe cellular organization at the molecular scale and to follow its evolution at different time steps.

Fluorescence detection and identification of antibiotic resistance biomarkers in bacteria using droplet microfluidics

Presenter: Justine RITI

Laboratoire d'études et de recherches en immunoanalyse (LERI), Gif-sur-Yvette, France

Antibiotic resistance (ABR) is a big threat to global healthcare today and there is an increasing need for efficient tools for the rapid detection of ABR biomarkers and antibiotic susceptibility testing in bacteria. We are currently developing microfluidic-based strategies to improve the sensitivity and multiplexing capabilities of current ABR detection methods. Particularly, digital microfluidics allows creating millions of microdroplets for the compartmentalization of single cells, offering several advantages over conventional, population-based methods in microbiology (resolution, quantification, automation, rapidity). Our aim is to compartmentalize single cells in nanoliter droplets and monitor in real-time bacteria growth profiles in the presence of antibiotics. We have implemented an automated fluorescence-based imaging (using resazurin/resorufin as fluorescence viability markers) and analysis technique for the identification of spherical droplets on chip and the quantification of the average intensity inside each droplet (around 2000 events over 10-hours time lapses). We plotted intensity distribution profiles for each cell culture condition (with and without antibiotics) to evaluate the encapsulation ratio (following a Poisson distribution for single cell events), quantify the initial bacterial concentration and growth rate. Using this method, we determined the minimal inhibitory concentration for a variety of tested drugs and bacterial strains on chip. Future work will involve the development of high throughput imaging methods on chip, and the implementation droplet based immunoassays for the simultaneous detection of ABR biomarkers.

Three-dimensional super-resolved imaging using Random Illumination Microscopy (3D-RIM)

Presenter: Benoit ROGEZ

Institut Fresnel, UMR 7249, Marseille, France

One of the current objectives in cell biology is to image large tissues with a resolution good enough to detect protein-protein interaction. To do so, many super-resolution microscopy techniques, enabling resolution down to a few tens of nanometers, have been developed during the last 20 years. If these techniques are well suited for fixed and/or thin samples, they present caveat which limits their use for the study of thick and/or living tissues.
First, techniques such as saturated fluorescence (STED), or photoactivated localization microscopies (STORM, PALM) usually require a high laser intensity to achieve sub-100 nm resolution, which can be toxic for living cells. Moreover, temporal dynamics is limited by the time required to scan the sample or reconstruct the image.
On the other hand, structured illumination microscopy (SIM) achieves similar resolution which a much lower laser intensity, and therefore reduced phototoxicity. But it requires a very good knowledge of the spatial distribution of the excitation intensity. It is therefore quite sensitive to aberration such as the diffusion by the sample itself, which limits its use on thick samples.

Recently, it has been shown that using series of random illumination (RIM), called speckle, it is possible to reconstruct an super-resolved image with a resolution of 120 nm transversaly and 300 nm axially, similar to what is doable with SIM microscopy. The main advantage of RIM over SIM is that it only requires a good knowledge of the spatial coherence of the speckle illumination, which is independent of aberration in the excitation path. This means that it is theoretically possible to keep a constant, sub-100 nm resolution, whatever the axial position in a thick sample.

Until now, this kind of 3D imaging using RIM illumination has been performed plane by plane, each plane being processed separately. Here, we propose an new approach where the entire volume is processed at once, with the aim to increase acquisition speed, and axial resolution.

Groupe de travail PPP groupe pour la conception et l’assistance dans la conception de prototypes scientifiques

Presenter: Brice RONSIN

Centre de Biologie Intégrative (CBI), UMR5547, Toulouse, France

Notre groupe réalise ce qui n'existe pas sur le marché et qui peut permettre de finaliser ou de réaliser vos expériences scientifiques en microscopie et/ou en biologie. Nous intervenons de la conception du système, de sa réalisation jusqu’à sa validation. Notre expertise nous permet de développer des logiciels d’analyse, de contrôle commande ou encore le développement de pilotes, et au niveau matériel par la réalisation de prototypes électroniques et mécaniques.

Ultrabright all-organic nanoparticles for single particle tracking in the brain extra-cellular matrix

Presenter: Morgane ROSENDALE

Institut des Sciences Moléculaires, UMR5255, Talence, France

To date, quantum-dots are the most widely used nanoparticles for bioimaging thanks to their unprecedented brightness and photostability. However, their inorganic core is inherently water insoluble, requiring them to be coated by solubilizing agents such as polyethylene-glycol (PEG). Moreover, most contain heavy metals, raising environmental concerns and limiting their clinical potential.

To circumvent these limitations, our lab develops Fluorescent Organic Nanoparticles (FONs) as a promising, all organic, spontaneously watersoluble alternative. Prepared by self-aggregation of rationally designed hydrophobic dyes in water, it is possible to tune the properties of FONs by molecular engineering of their constitutive dyes. We have previously reported on green and near-infrared emitting FONs that could enter and be tracked in living cells, making them good candidates for drug delivery systems. However, stealth emitters can be of interest for tracking of cell-surface receptors or exploring the extracellular space.

In this work, we describe spontaneously stealth, size-tunable, ultrabright, red emitting FONs made from a novel quadrupolar dye. We report on the characteristics and properties of these FONs and show that they have no unspecific interactions with living cells. Thanks to their brightness and stability, we also have achieved single particle tracking in rat organotypic brain slices tens of micrometers deep in tissue. From these combined properties, we conclude that these novel FONs are promising candidates for the next generation of tools for bioimaging.

Optogenetic activation of contractility reveals that mechanical stresses transmit most efficiently perpendicularly to axis of mechanical polarization in cell doublets

Presenter: Artur RUPPEL

Laboratoire interdisciplinaire de la physique (LiPhy), Saint-Martin-d'Hères, France

Forces are known to be implicated in the regulation of processes, e.g. differentiation, morphogenesis or tissue homeostasis. Many of the molecular mechanisms for force generation and sensation in cells are well described, but little is known how these force signals propagate through a tissue. The development of optogenetic tools allows to generate force signals in cells with high spatiotemporal resolution, allowing to study the dynamics of force transmission between cells. Here we propose a minimalistic tissue model consisting of two MDCK cells to adress these questions. We standardize their morphology and systematically vary morphological parameters with the use of micropatterns.

Analyzing the spatiotemporal structure of heterochronic miRNA transcription using microfluidics live-imaging of nascent miRNA dynamics

Presenter: Shubham SAHU

Laboratoire Physico-Chimie Curie, UMR168, Paris, France

The expression of C. elegans heterochronic miRNAs during larval development, among them lin-4 and let-7, has previously been shown to be highly pulsatile, with expression peaking once per larval stage, phase-locked with the molting cycle (Hendriks et al., 2014; Kim et al., 2013, Perales et. al.,2014; Wynsberghe et. al.,2014). While the molecular mechanisms driving this pulsatile expression are unknown, our labs have previously identified two regulators of pulse amplitude. LIN-42/Period is also shown to dampen transcriptional output of miRNAs by negatively regulating the overall duration of transcription within each larval stage (Perales et. al.,2014; Wynsberghe et. al.,2014). Antagonistically, blmp-1 and elt-3 null mutants completely suppress lin-42(lf) phenotypes and BLMP-1 acts as pioneer factor to enhance miRNAs transcription output (Stec et al., 2021). All of the previous approaches to measure the transcriptional dynamics of miRNAs in-vivo have relied on fluorescent transcriptional reporters, limiting temporal and spatial resolution. Here, we present our ongoing efforts to overcome this limitation by monitoring miRNA transcription using MS2/MCP-GFP based RNA-localization.

By combining high-resolution long-term imaging with microfluidics (Keil et al., 2017) with extensive image registration, segmentation, tracking and image analysis, we reveal intriguing wave-like spatiotemporal transcriptional patterns of lin-4 in hypodermal cells as well as vulval precursor cells within larval stages. We also characterize the relationship between lineage descendancy, cell cycle and transcriptional timing among hypodermal and vulval precursor cells. Finally, we measure how these spatiotemporal features of transcription are altered in lin-42 and blmp-1 mutants. Our results establish a new approach to measuring live miRNA-dynamics in the C. elegans larva and provide quantitative insights into the complex spatiotemporal regulation of miRNA transcription underlying temporal cell-fate patterning in the C. elegans epidermis.

Synthesis and characterization of dual-color photoconvertible dyes for bioimaging

Presenter: Lazare SALADIN

Laboratoire de bioimagerie et pathologies (LBP), UMR 7021, Strasbourg, France

Les sondes fluorescentes photoconvertibles sont des outils puissants en imagerie car ils permettent le suivi de biomolecules ainsi qu'une utilisation en Microscopie à super-résolution. Pour l'instant peu d'exemple de petites molécules ont été décrites, ce sont surtout les paGFP qui domine ce domaine.
Différentes sondes photoconvertibles ont été synthétisées et caractérisées. Ces sondes peuvent passer de rouge au vert ou du vert au bleu sous irradiation LASER en cuvette ainsi qu'en cellule. Ces sondes cibles les mitochondries, la membrane plasmique ainsi que les gouttelettes lipidiques.µ

Inverse blebs in blastocoel formation

Presenter: Markus SCHLIFFKA

Institut Curie - Unité Génétique et biologie du développement, U934/UMR3215, Paris, France

Mammalian preimplantation development culminates in the formation of the blastocyst. The blastocyst contains the first mammalian lumen, which forms between cell-cell contacts by hydraulic fracturing. Pressurized fluid accumulates into a multitude of microlumens, which eventually empty their content into a single lumen in a coarsening process. However, the cellular events involved in the formation of microlumens and blastocoel positioning remain poorly characterized. As microlumens form, we observe previously unreported short-lived membrane protrusions growing inside the cells at cell-cell contacts. On the short time scale, we use spinning disk confocal microscopy to image the protrusion dynamics. We find a sequential recruitment of actin and myosin at the onset of protrusion retraction. Due to the similarity in shape and molecular dynamics to membrane blebs, we call these protrusions inverse blebs, since they grow inward. Using light-sheet microscopy, we find an increase in bleb number concomitant with microlumen appearance and a subsequent decrease during microlumen coarsening.

Therefore, inverse blebs appear at the right time and place to play a role in the coarsening and positioning of the blastocoel. Interfering with fluid pumping and adhesion molecule levels indicates that both extracellular hydrostatic pressure and tight confinement of cell-cell adhesion are necessary to trigger inverse blebs. We will further investigate what determines the sites of inverse bleb formation by interfering with actomyosin contractility, cell pressure and membrane curvature. We hope this will reveal the role of inverse blebs during the formation of the first mammalian lumen.

Forces shaping chromatin in the nucleus

Presenter: Vittore SCOLARI

Nuclear Dynamics, Institut Curie, UMR3664, Paris, France

The scale of 10^5-10^6 base pairs, human chromosomes are structured in topologically associated domains (TADs). These are regions of the chromosome within which contacts are more frequent that with adjacent regions, as measured by 'chromosome conformation capture' experiments such as Hi-C. TADs have been shown to result from the action of cohesin, a molecular motor. Cohesin binds to chromatin, reel it in, and extrude it as a loop. This process is called “loop extrusion”. In this realm, theoretical modelling is able to answer the following questions:

How much energy should the cell spend to maintain these structures? with how many concurrent motors? how fast? and how frequently?

The current approach to simulate loop extrusion uses explicit molecular dynamics simulations. This approach, while very flexible, limits our possibility (i) to explore the parameter space in an efficient manner and (ii) to dissect the observed effects under the lenses of a coherent analytical theory. I will present our original approach to simulate loop extrusion, that exploits an analytical solution of the probability distribution over a conformational space for the polymer model, and the action of extruders simulated in 1D. I will show how this approach allows highlighting the hallmarks of the out-of-equilibrium processes on chromatin conformation observed in experiments. Finally, this approach permits the definition of the Gibbs entropy of chromosome conformation. I will show how the application of this concept to simplified toy-models increases our analytical understanding of the loop extrusion process.

Real-time 3-D nanoparticle tracking in neurons using holographic excitation

Presenter: Florian SEMMER

Laboratoire lumière matière et interfaces (LuMIn), Gif sur Yvette, France

Neurodegenerative diseases such as Alzheimer's disease present abnormalities in intraneuronal transport, suggesting the relevance of measuring this key biological process. In 2017, a sensitive method to measure changes in intraneuronal endosomal transport has been reported in 2D cultures of neurons using fluorescent nanodiamonds (fNDs) [1]. The high brightness, photostability and absence of cytotoxicity allow fNDs to be tracked with 50 nm spatial and 50 ms time resolutions.
This nanoparticle tracking based approach applies also to multiphoton imaging, opening the possibility of transport measurement in vivo. We use nanocrystals possessing a large nonlinear second order optical response. First results indicate that the intraneuronal transport measurement can be inferred from nonlinear microscopy data, opening applications to thicker samples owing to the low background of multiphoton imaging. In order to get a high spatio-temporal resolution (around 10 nanometers at 1 ms), we are developing a two-photon microscope, based on a digital holography method [2]. A Digital Micromirror Device (DMD) is used as a spatial light modulator, allowing a fast 3D motion of the excitation volume. We aim at reaching a time resolution below the millisecond and super-localization regime in the tens nanometer range using orbital tracking.

[1] S. Haziza, et al. Nat. Nanotechnol. 12 (2017), 322.
[2] Geng, Q., Gu, C., Cheng, J. & Chen, S. Digital micromirror device-based two-photon microscopy for three-dimensional and random-access imaging. Optica 4, 674 (2017)

Super-resolution imaging of mitochondrial morphology reveals a close link between mitochondrial dynamics and oxidative phosphorylation in yeast

Presenter: Manish Kumar SINGH

Laboratoire de Biologie Moléculaire et Cellulaire des Eucaryotes, UMR8226, Paris, France

Mitochondria are tubular organelles delimited by two lipid bilayers: the outer membrane (OM) and the inner membrane (IM), which itself surrounds the mitochondrial matrix. The tubular morphology of Mitochondria is maintained by highly dynamic processes of fusion and fission of mitochondrial membranes as well as movements of mitochondrial tubules along cytoskeletal tracks.

Here, we report that the mitochondrial network of the budding yeast Saccharomyces cerevisiae undergoes extensive and previously unappreciated rearrangements during the metabolic transition from fermentation to respiration. Using super-resolution imaging with structured illumination microscopy (SIM) and photo-activated localization microscopy (PALM), we demonstrate that the mass, thickness, and the apparent number of mitochondria increase during respiration. More specifically, we show that thin mitochondrial tubules observed upon fermentation undergo regular, extensive, and stable Dynamin-mediated constrictions as cells switch to respiratory growth. We are now investigating whether these constrictions of mitochondrial tubules are caused by respiration or whether they have a more profound function in the efficiency of oxidative phosphorylation in yeast.

Identifying mechanisms by which cells sense multicellular density

Presenter: François SIPIETER

Institut Jacques Monod, UMR7592, Paris, France

Sensing cell density within a cohesive tissue is a fundamental property thought to underlie the regulation of tissue homeostasis in multicellular organisms. Yet, the mechanisms by which cells sense multicellular density remain largely unknown.
In an epithelium, the extent of cell adhesion to the extracellular matrix directly depends on cell density: the higher the density, the smaller the matrix area available for cell adhesion. Here, we tested whether cell-matrix adhesion functions as a sensor of local epithelial cell density. We hypothesized that changes in cell density are perceived within adhesion complexes through changes in molecular tensions, which subsequently confer density-dependence to downstream mechanosensitive pathways.

By using a multiplexed FRET biosensor strategy and dominant mutants and pharmacological inhibitors, we showed that cell density regulates focal adhesion growth and Vinculin tension, subsequently instructing Extracellular-signal Regulated Kinase (ERK) activity. Conversely, the Focal Adhesion Kinase (FAK) is also sensitive to cell density but only exert a permissive control on ERK activity.
We are now examining the molecular mechanisms by which molecular tensions in Vinculin instruct the activity of ERK.

Tomographic Diffractive Microscopy: Optimizing Sample Illumination

Presenter: Asemare Mengistie TADDESE

Institut de Recherche en Informatique, Mathématiques, Automatique et Signal (IRIMAS), Mulhouse, France

Tomographic diffractive microscopy, or Digital holographic tomography has a sequential nature of data acquisition. First, sets of diffracted fields from the sample at various illumination angles are recorded, and then recombined to numerically compute the 3D complex refractive index distribution of the object. In general, using limited number of illuminations is fast, however, the reconstructed image quality is poor mainly due to unrecorded object frequency components. Conversely, for high-resolution imaging, collecting enough diffracted fields could fill the Fourier space and improve the image quality but with slow output for fast imaging.

To solve this dilemma, optimal sample scanning scheme is introduced. With the aim of optimizing Fourier space filling in the low diffraction regime, we propose several classes of sample scanning patterns, and study their respective Optical Transfer functions (OTF) for transmission, reflection and 4Pi TDM. Simulation on a phantom, and experimental results showed that 3D uniform angular sweeping fills the Fourier space optimally, delivering better resolution images.

Nanoscale dynamics of cell wall assembly during the cell cycle of Streptococcus pneumoniae

Presenter: Jennyfer TROUVÉ

Institut de Biologie Structurale (IBS), Grenoble, France

The bacteria cell wall is a three-dimensional sugar and peptide network that surrounds the cell. It confers a cell shape adapted to the ecological niche of the bacterium and protects the cell against mechanical stress exerted by the internal turgor pressure. Even though this process is essential for proliferation and survival of the bacteria, we still only poorly understand how the cell wall is assembled in space and time. Fluorescence microscopy is a method of choice to investigate cell wall assembly but a major drawback is the fact that in ovoid bacteria the cell wall is assembled within an annular region whose dimensions approximate the resolution limit of conventional fluorescence microscopy (~ 250 nm).

To gain access to nanoscale details of cell wall assembly, we set up a metabolic labeling approach using click chemistry and imaged cell wall synthesis by super-resolution fluorescence microscopy in the ovoid bacterium Streptococcus pneumoniae. Our nanoscale-resolution data (~ 30 nm) reveal unprecedented spatio-temporal features of cell wall assembly and fate along the cell cycle. We further used these data to simulate the morphogenesis of the ovoid cell in silico. Altogether, experimental and modeling analyses revealed how cell wall assembly and remodeling correlate with cell morphogenesis.

Mechanisms of IRSp53 driven filopodia formation

Presenter: Feng-Ching TSAI

Physico-Chimie Curie Lab (PCC), Institut Curie, UMR168, Paris, France

Filopodia are actin-rich membrane protrusions essential for cell morphogenesis, motility, and cancer invasion. It remains elusive how cells control filopodia initiation on the plasma membrane. To address this fundamental question in cell biology, we performed experiments in cellulo, in vitro and in silico. We showed that IRSp53, a membrane curvature sensor critical for filopodia generation, self-assembles into clusters on PIP2-containing membranes in the absence of signalling regulators as those found in cells. Our simulation work revealed that PIP2 is key for IRSp53 clustering. We further showed that IRSp53 is required for the recruitment of actin polymerases such as VASP to assemble actin filaments locally, leading to the generation of membrane protrusions filled with fascin-mediated actin bundles. By following filopodia growth in live cells, we revealed that fascin enhances filopodia elongation rate and stability.

Overall, our novel in vitro reconstitution system was able to faithfully mimic the generation of actin-rich filopodia. Moreover, our results indicate that once IRSp53 is active and able to bind to membranes and recruit downstream partners, it readily induces protrusion formation. However, by pulling membrane tethers from live cells, we observed, for the first time, that IRSp53 can only be enriched and trigger actin assembly in tethers at highly dynamic membrane regions where it is membrane-bound and presumed active. Our work thus supports a cellular regulation mechanism of IRSp53 in its attributes of curvature sensation and partner recruitment to ensure a precise spatio-temporal control of filopodia initiation.

Hybrid Adaptive Optics SPIM for bio-samples encased in synthetic matrix

Presenter: Divyendu Kishore VALAPPIL

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The necessity of light-sheet microscopy for imaging in the field of biology is well established. We have developed a combination of the basic selective plane illumination microscopy, and SPIM using adaptive optics into a single hybrid microscope. This system is ideal for understanding and analyzing biological samples encased in a synthetic matrix. This hybrid SPIM being driven by a single entity allows easy switching between the basic SPIM and the SPIM with adaptive optics. The setup grants easy comparison between the basic SPIM and the SPIM with adaptive optics. The point spread function using both parts of the hybrid setup shows the effect of using adaptive optics and the corrections. The effect of imaging through the synthetic matrix and thick adipose tissues is seen using the setup. The hybrid microscope is also used to image adipose tissues and measure the optical properties.

Separation of spectrally overlapping fluorophores using intra-exposure excitation modulation

Presenter: Hana VALENTA

Laboratory for Nanobiology, Department of Chemistry, KU Leuven, Belgium

Multicolor fluorescence imaging is an excellent method for the simultaneous visualization of multiple structures, although it is limited by the available spectral window. More labels can be measured by distinguishing these on properties, such as their fluorescence dynamics, but usually these dynamics must be directly resolvable by the instrument.
We propose an approach to distinguish emitters over a much broader range of light-induced dynamics by combining fast modulation of the light source with the detection of the time-integrated fluorescence. We demonstrate our method by distinguishing four spectrally overlapping photochromic fluorophores within Escherichia coli bacteria, showing that we can accurately classify all four probes by acquiring just two to four fluorescence images. Our strategy expands the range of probes and processes that can be used for fluorescence multiplexing.

Imaging host-pathogen interactions: Recruitment of Guanylate-Binding Proteins to two intracellular bacteria

Presenter: Stanimira VALEVA

Centre International de Recherche en Infectiologie (CIRI), Lyon, France

Guanylate-Binding Proteins (GBP) are interferon-inducible GTPases with a key role in innate immunity against intracellular pathogens (bacteria, viruses and protozoa). During infection with cytosol-dwelling Gram-negative bacteria, some GBPs are recruited to the lipopolysaccharide (LPS) in the bacterial outer membrane and promote the activation of inflammatory immune responses.
Few bacteria have adapted to thrive in the host cytosol, which is a nutritive but hostile space with an abundance of defence systems. Professional cytosol-dwelling bacteria have developed mechanisms allowing to either fight or hide from host immune responses.

We investigated recruitment of host GBPs to two such bacteria: Francisella novicida and Shigella flexneri. While S. flexneri carries a “typical” LPS, the LPS of F. novicida has an unusual structure, often associated with evasion of immune responses. Using quantitative image analysis, we identified key differences in the host and bacterial specificities that control GBP targeting of both species. Overall, GBPs targeted F. novicida with less affinity than S. flexneri and required additional factors to target F. novicida. Analysis of F. novicida mutants revealed that escape from GBP targeting is, at least in part, governed by the atypical LPS structure.

Effects of high UV-C doses on nucleoid organization and dynamics in the radiation resistant bacterium Deinococcus radiodurans

Presenter: Pierre VAUCLARE

Institut de Biologie Structurale, Groupe I2SR, Grenoble, France

Understanding the functional aspects of microorganisms in vivo, and more specifically that of bacterial chromosomes (nucleoids), still comes up against many technological obstacles in living imaging, which limits our investigations and our knowledge in this field.

However, thanks to the M4D cell imaging platform of the I2SR group (Integrated Stress Response Imaging – IBS Institute) which offers the combination of different methodological approaches chosen according to their performance criteria, it was possible to successfully highlight the remarkable organization and plasticity of the nucleoid of the most radioresistant bacterium: Deinococcus radiodurans. The choice of this fascinating, non-pathogenic, shell-like morphology bacterium is built on the wonderful plasticity of its condensed nucleoid (< 1µm3) which adopt multiple morphological changes during the cell cycle, a property which are managed by small DNA-associated architects-proteins. But also, D. radiodurans displays a particularly effective metabolism and DNA repair system which allows these bacteria to survive to tremendous doses of DNA-damaging radiation such as UV-C radiation (the most harmful UV radiation).

These two cellular specificities combined have led us to deepen our knowledge to understand the relation between the molecular dynamics of the nucleoid and the exceptional radioresistance properties of D. radiodurans to survive to lethal dose of UVC radiation. The technological challenge of this project has been depicted by various strategies and approaches using 3D conventional spinning-disk fluorescence imaging of live cells, flow cytometry, membrane and DNA dyes or engineered strains expressing labelled nucleoids-associated proteins.

The effector functions of Plasmacytoid Dendritic Cells are targeted toward virally infected cells

Presenter: Manon VENET

Centre International de Recherche en Infectiologie (CIRI), Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, École Normale Supérieure de Lyon, Univ Lyon, F-69007, Lyon, France

Plasmacytoid dendritic cells (pDCs) control the innate immunity against viral infections by rapid and massive production of type I and III interferons (IFN-I/III). These cytokines are notably produced upon the recognition of viral RNA by toll-like receptor and inhibits viral infection.

Our recent study uncovered that pDC activation requires physical cell contact with infected cells. This cell contact is established by molecular rearrangements at the contact site into a specialized platform. This structure favors the signal transfer to activate the pDC antiviral response, and we thus named the interferogenic synapse. Next, we further define the regulation of the effector functions of pDCs via a quantitative analysis of confocal microscopy imaging. We showed that the secretory machinery (Golgi) and its structural support, which involves the microtubule-organizing center (MTOC), both polarize in pDCs at the contact site with virally infected cells. This polarization drives the secretion of IFN-I targeted toward the infected cell, only when in direct contact. We further revealed that actin network is progressively depleted at the contact site, when MTOC and Golgi polarized, hereby likely enabling the docking and release of the secretory vesicles at contact with infected cells.

Altogether, our results demonstrated the establishment at the contact site of a secretory platform, which enables the subsequent the secretion by pDCs of antiviral cytokines targeted toward the infected cells. This regulation hereby confines the antiviral pathways at the infected site, thus limiting a systemic antiviral cytokine production, known to be deleterious to the host.

Objective assessment of corneal transparency in the clinical setting

Presenter: Maëlle VILBERT

Laboratoire d'Optique et Biosciences (LOB), Ecole Polytechnique, Palaiseau, France

PURPOSE. To develop an automated algorithm for clinical spectral-domain OCT images, capable of extracting quantitative transparency data for corneal tissue while correcting artifacts related to device characteristics and patient positioning. To establish a reference data set of transparency parameters for healthy corneas. To present a clinical application to quantitative post-graft follow-up.

METHODS. SD-OCT images (RTVue-100, Optovue) were recorded on n=85 normal corneas, w/ 2 images per eye. Inclusion criterion: subject registration for refractive surgery. Our automated pre-processing algorithm standardizes the raw images and computes a correction mask from each OCT image, derived from the Principal Component Analysis of the in-depth OCT signal. The mean stromal in-depth intensity profile is extracted and analysed using our previously developed method designed to quantify the photon mean-free path as an objective metrics of corneal transparency [Bocheux et al., 2019 https://doi.org/10.1371/journal.pone.0221707].

RESULTS. The mean coherent transmittance of young normal corneas (aged 31±13 years) lies between 46.8−55.8 % (CI95 of mean). There is no significant correlation between transparency and age nor between transparency and corneal thickness in this group.

CONCLUSION. Our data processing algorithm is capable of robust detection and correction of clinical SD-OCT image artifacts. It enables to determine in vivo numerical values for corneal transmission of coherent light using unmodified clinical diagnostic devices.

Linear and nonlinear microscopy in the short-wave infrared for in vivo deep mouse brain imaging

Presenter: Fei XIA

Laboratoire Kastler Brossel (LKB), Paris, France

Here, I will summarize the recent technical advances of using short-wave infrared (SWIR) for linear and nonlinear microscopy of in vivo deep mouse brains. The following three advances will be discussed:

1) a multicolor three-photon imaging scheme with a single-wavelength excitation and the combination of three-photon imaging with adaptive optics;

2) A multimodal optical coherence and three-photon imaging system for simultaneous label-free and fluorescence imaging of the mouse brain; 3) SWIR fluorescence and reflectance confocal microscopy for deep tissue imaging of the mouse brain at a depth comparable to that of two/three-photon microscopy, but at a much lower price.

ImaBio Young Scientists Network (IYSN)

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In 2018, the GDR ImaBio created an associated network of young researchers (IYSN for ImaBio Young Scientists Network). IYSN aims to bring together PhD students and post-docs from the bio-imaging community in France and connect them around key topics. Our goal is to offer specific opportunities to young researchers in terms of knowledge-transfer, training, as well as interacting with the industrial partners and other laboratories of ImaBio. The network welcomes all young researchers whose interests fall within the topics of GDR ImaBio (microscopy for biology from the nano- to macro-scale).

To increase the communication within the community, the IYSN organizes an interactive conference every year.

We are recruiting new members to the team! Come to see our poster and discuss with us!


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