Journées de Rencontre Imabio 2026

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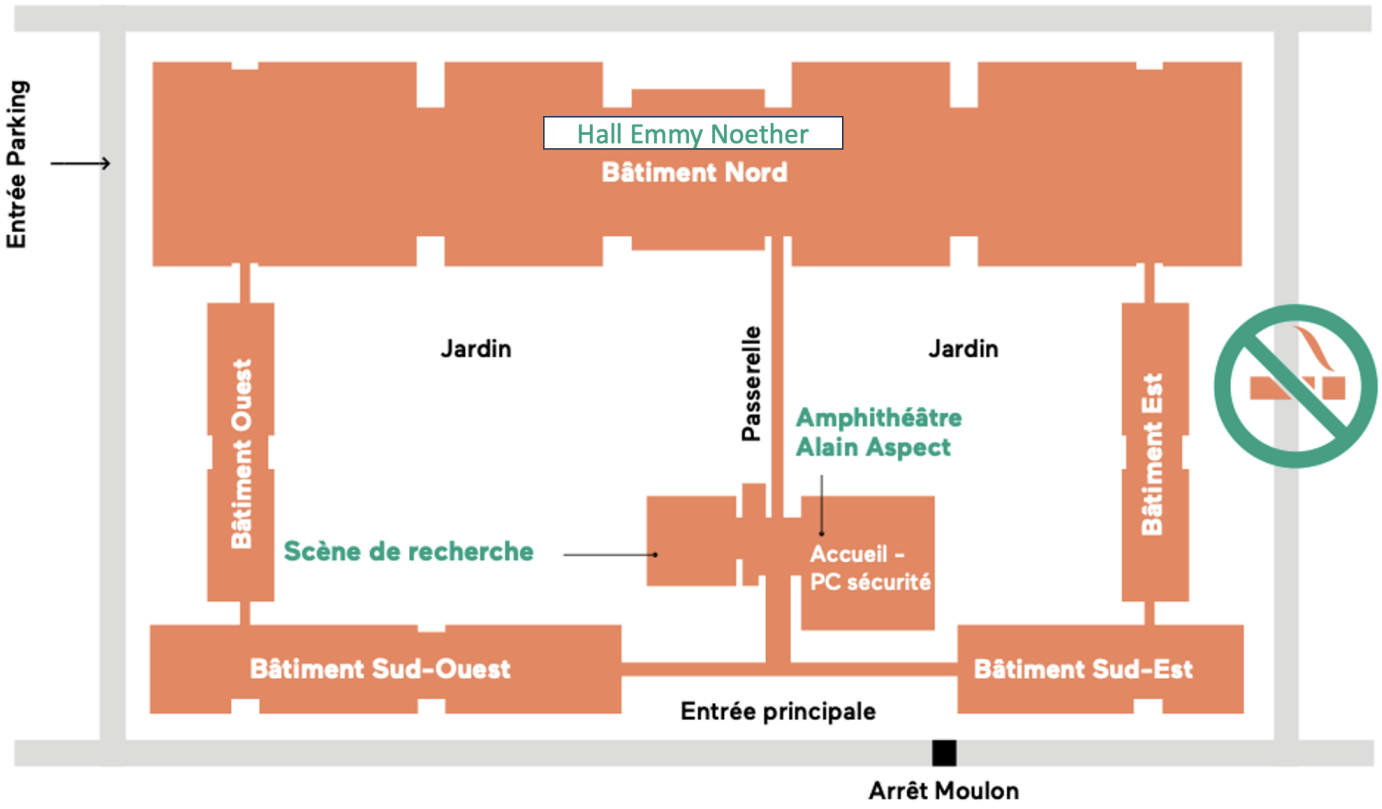

Supported by

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Invited speakers are highlighted in blue. The lectures will take place in the Alain Aspect Auditorium, the breaks in Emmy Noether hall.

Conference Program – Tuesday, June 9th

Time	Event / Presentation
12:00 - 01:30 PM	**Registration & Lunch Boxes** *Hall Emmy Noether*
01:30 - 02:00 PM	**Presentation of ImaBio Research Group** *Opening remarks and network objectives*
	Session 1: Imaging deeper, imaging faster: pushing spatio-temporal limits
02:00 – 02:45 PM	**Vincent Villette.** Monitoring neuronal spiking and subthreshold activities in cerebral and cerebellar microcircuits using optical methods in head-fixed behaving mice.
02:45 – 03:30 PM	**Amaury Badon.** Structural and functional imaging of multicellular assemblies using optical coherence tomography and light field microscopy.
03:30 – 03:50 PM	**Frédéric Pain**. Wide field flow mapping at the microcirculation level using speckle imaging : of Mice and Men.
03:50 – 04:10 PM	**Abigail Illand**. Multi light-sheet RESOLFT for fast multicolor live-cell imaging.
04:10 – 04:25 PM	*Industrial Talk.* **Simon Labouesse - RIMEO**. LiveDRIM: Letting Go of the Wheel in Super-Resolution.
04:25 – 04:50 PM	**Coffee Break & Poster Session**
04:50 – 05:10 PM	**Solène Prudhomme**. Serial third-harmonic microscopy for label-free brain-wide mapping of myelin.
05:10 – 05:30 PM	**Oscar Saavedra-Villanueva**. Harmonic nanoparticles and multiphoton microscopes enable high flow rate cell detection.
05:30 – 05:50 PM	**Joséphine Morizet**. A novel two-photon 3D random-access AOD-based microscope with temporal focusing for improved optical sectioning.
05:50 – 06:05 PM	*Industrial Talk* **Feriel Terras - Phasics**. Quantitative Phase Imaging made easy with SID4 QLSI.
06:10 – 07:10 PM	**Keynote Session:** *Sophie Brasselet* Next challenges in Single Molecule Orientation and Localization Microscopy (SMOLM).

Conference Program – Wednesday, June 10th

Session 2: From molecules to behaviour: multiscale and in vivo imaging of biological systems

09:00 – 09:45 AM	**Kate Grieve.** Full Field OCT from patient to laboratory - live imaging in retina.
09:45 – 10:05 AM	**Antoine Le Gall**. From Live Imaging to Behavioral Mapping: Quantifying Emergent Motility in Predatory Bacteria.
10:05 – 10:25 AM	**Vaky Abdelsayed**. Polarization-Resolved Second Harmonic Generation Microscopy Used to Assess Spatio-Temporal Dynamics of Cervix Remodeling during Murine Gestation.
10:25 – 10:55 AM	**Coffee Break & Poster Session**
10:55 – 11:40 AM	**Patrick Lemaire.** Imaging Inter-Individual Morphological and Molecular Variations to Unravel Ascidian Developmental Logic.
11:40 –12:00 PM	**Pierre Bost**. Universal characterization of tissue architecture in spatial omics data using pair correlation functions.
12:00 – 12:20 PM	**Diala Wehbe**. Optical Characterization of Endogenous Fluorescence in Mouse Prefrontal Cortex for Improved Signal Unmixing in Neuroimaging.
12:20 – 12:40 PM	**Matheus Arana**. Label-free 3D Imaging of Cardiac Organoids by Multimodal Nonlinear Microscopy.
12:40 – 14:30 PM	**Lunch Break & Poster Session**

Session 3: Quantitative imaging and single-object tracking in complex environments

02:30 – 03:15 PM	**Guillaume Baffou.** Multimodal cameras for single-shot imaging of intensity, phase, polarization and color.
03:15 – 03:35 PM	**Junwoo Park**. Multi-timescale scanning reveals molecular memory hidden in SMT data.
03:35 – 03:55 PM	**Alexandre Clausolles**. Agile two-photon microscope for fast 5D single-particle translation and rotation tracking.
03:55 – 04:15 PM	**Laurent Le**. Enhancing confocal microscopy assets with SPAD arrays and photon-resolved microscopy.
04:15 –04:30 PM	*Industrial Talk.* **Bruker**. Comment imager la biologie à toutes les échelles, de la vésicule extracellulaire au comportement?
04:30 – 05:00 PM	**Coffee Break & Poster Session**
05:00 – 05:45 PM	**Merlin Lange.** Building a Multimodal Atlas of Vertebrate Development.
05:45 – 06:30 PM	**Amir Nahas.** Noise correlation inspired elastography for in vivo stiffness mapping using optical coherent imaging systems.
06:30 – 06:50 PM	**Jan Pytel**. Near-infrared quantum-dot based adaptive optics for in-depth in vivo two-photon neuroimaging in the mouse cortex.
06:50 – 07:10 PM	**Laura Paggi**. Coupling two photon microscopy with sub-micron resolved infrared spectroscopy to investigate collagen alteration.
07:15 PM	**Gala Dinner** *Arts & Science* *In the presence of the artists: Léa Dedola, Ikse Maitre, and Marie Truffier*

Conference Program – Thursday, June 11th

Session 4: Nano- and quantum-enabled sensing technologies for biology

09:00 – 09:45 AM	**Kirstine Berg-Sorensen.** NV-centers in diamond for biosensing and associated imaging.
09:45 – 10:05 AM	**Maxime Meghnagi**. In-depth, large–field-of-view 3D SMLM using nanochip-based ModLoc illumination.
10:05 – 10:25 AM	**Thomas Bugea**. High-throughput correlative magnetic tweezers-TIRF to investigate biomolecular reactions at the single-molecule level.
10:25 – 10:45 AM	**Coffee Break & Poster Session**

Session 5: Microscopy and AI - with GDR IASIS

10:45 – 11:15 AM	**Claire Lefort.** Boosting biomedical imaging with nonlinear optical microscopy: balancing AI and physics-based computational methods.
11:15 –11:35 AM	**Rajeev Manick**. Semi supervised GAN for smart microscopy, fast and data efficient cell cycle classification.
11:35 – 12:05 PM	**Sophie Achard.** Statistical network analysis, application to brain connectivity.
12:05 – 12:35 PM	**Thomas Walter.** Computer Vision for histopathology and spatial transcriptomics.
12:35 – 01:30 PM	**Lunch Break & Poster Session**
01:30 – 02:00 PM	**Corinne Fournier.** Physics based Deep Learning in Computational Imaging.
02:00 –02:30 PM	**Nicolas Faure.** Automatic Bacterial Classification in Microscopic Images of Positive Blood Culture Gram Smears.
02:30 – 03:30 PM	**Round Table: AI and Microscopy**. *Chaired by Anatole Chessel and Elsa Angelini*

Poster Presentations

Poster sessions will be held during all coffee and lunch breaks in Emmy Noether Hall.

Presenter	Poster Title
Frédéric Pain	Wide field flow mapping at the microcirculation level using speckle imaging : of Mice and Men
Emmanuelle Chaigneau	Analytical model enabling the correction of absorption and scattering in dual-color ratiometric multiphoton microscopy of brain tissue.
Xiaotong Yuan	Improving FLIM metabolic imaging speed with denoising filters
Vincent Maioli	Open-source tube lens design and applications to oblique plane microscopy
Antoine Hubert	Open-source compact design of two-photon light-sheet microscope and associated thermal effects characterization
Ghalia Kaouane	Nuclear eustress and mecanotransduction in hMSCs: Effects on tissue growth and cell differentiation in 4D in vitro model
Léo Larraz	New fluorescent biosensors to vizualise ion concentrations changes in plant cells
Claudia Köhler	Evaluation of Fast-Acting Borinic Acid Probes and Halo-Tag Conjugation for Spatially Resolved H2O2 Detection
Mariana Fernandez Guataqui	Study of the photostability of fluorescent proteins derived from StayGold
Carolina Chieffo	Photocrosslinking Coumarines for studies of peptide-membrane interactions (PHOCUS)
Baptiste Grimaud	Fluorescent nanodiamonds functionalized with neurotropic peptides for the measurement of intraneuronal transport
Ugo Arles	Label-free investigation of healthy human muscle using colocalized multimodal M-CARS hyperspectral analysis
Leonardo Pestana Legori	Hyperspectral Tomographic Diffractive Microscopy: Research and Applications
Tom Mariotte	Replication dynamics of Haloferax volcanii
Katia Belcram	Systematic 3D analysis of metaphase cells to investigate cell and spindle architectures in the root meristem
Salvatore Azzollini	VertX integrates dynamic full field optical coherence tomography (DFFOCT): a non invasive label free high resolution imaging technique for living tissues
Emelyne Le Lay	Etude du remodelage cellulaire durant le cycle infectieux du virus de la vaccine
Bérengère Dalmais	Monitoring the 3D-dynamics of the cytoskeleton during the plant cell cycle
Evangelos Gioukakis	Single molecule studies of viral capsid assembly
Erwan Le Floch	Endoscopy probe for early in vivo detection of gastrointestinal cancer by stimulated Raman histology
Roméo Suss	Visualising the self-assembly process of icosahedral viruses through innovative microscopy
Laurence Dubreil	Non linear microscopy of plant cuticle: A promising approach to characterize crystalline cellulose fiber organization in cell walls
Lucile Chanay	Multiphoton microscopy: a cutting-edge tool to study cultural heritage materials
Marie-Claire Schanne-Klein	Polarization-resolved Second Harmonic Generation Microscopy Identifies Collagen Disorganization as a predictive biomarker of Immune Checkpoint Inhibitor resistance in Mismatch Repair-deficient Endometrial Cancer
Gaël Latour	Cartographie de l'organisation des lamelles de collagène sur l'épaisseur totale du tissu cornéen par microscopie SHG

Amaury Autric	6D imaging of single molecules in polarization multi-focus microscopy
Benjamin Blain	Single Molecules Localizations Microscopy to study oxidative DNA damage repair mechanisms and transcription factors
Clément Cabriel	Resolution improvement and multicolor demixing with event-based SMLM
Xiaoke Kerry Chen	Capturing the diffusion dynamics of alpha-synuclein in neurons with event-based single molecule localization microscopy
Laura Paggi	Coupling two photon microscopy with sub-micron resolved infrared spectroscopy to investigate collagen alteration
Timothée Tremblais	Imagerie SHG et 2-PEF du papier dans le cadre patrimonial
Léa Gelo	Cell biomechanics using optical tweezers and fluorescence microscopy
Grégoire Lelu	Quantitative polarimetric wavefront imaging for single-molecule orientation and localization microscopy
Aymeric Leray	Quantitative analysis of fluorescence fluctuations spectroscopy by combining autocorrelation and time integrated cumulants
Jean-Jacques Greffet	Digital-Holography Nanoparticle Tracking Analysis (DH-NTA)
Gizem Nuran Yapici	Towards quantum microscopy of cell electrical and mechanical activity using Nitrogen-Vacancy centers in diamond nanopillars
Nasri Bassous	Quantum sensor based on NV centers in diamond nanopillars for imaging mechanical stresses in cells
Bruno Combettes	Seeing is just the beginning. Powerful AI tools to analyze label free 3D images. Example with collagen matrix.
Pierre Bourdoncle	OpenCID : gestion, analyse, traitement et partage de données d'imagerie volumineuses
Emmanuel Faure	MorphoNet 2.0: Interactive and AI-driven curation of large-scale 3D biological imaging datasets

Session 1: Imaging deeper, imaging faster: pushing spatio-temporal limits

Monitoring neuronal spiking and subthreshold activities in cerebral and cerebellar microcircuits using optical methods in head-fixed behaving mice.

V. Villette[1]

[1] *Institut de Biologie de l'École Normale Supérieure, Paris*

Abstract:
To understand how information is represented, processed, and propagated in the brain, technologies for recording transmembrane potential in vivo in neuronal populations with high fidelity will be essential. Direct cellular voltage imaging in vivo, was limited by the speed and sensitivity of both indicators and imaging modalities. We developed two multiphoton microscopy techniques (ULoVE and 3D-CASH) enabling the optical recordings of cortical population voltage signal at single cell and high temporal resolution (>2kHz) in awake behaving animal circumventing limitations of classical imaging approaches. Improved Genetically encoded voltage indicators enable to record up to 500 μm of depth during tens of minutes (up to 1 hour). Also, red shifted versions enable to multiplexed with calcium indicators offering a large palette of tools to study cell and network mechanisms. In addition to present the journey we made with those optical developments and GEVI improvements, I will also shortly present the context of our physiology researches within the cerebellum that seeded and strengthened our interest to achieve fast populational GEVIs multiphoton optical recordings.

Structural and functional imaging of multicellular assemblies using optical coherence tomography and light field microscopy

A. Badon[1]

[1] *Laboratoire Photonique, Numérique et Neurosciences, Bordeaux*

Abstract:

Wide field flow mapping at the microcirculation level using speckle imaging : of Mice and Men

F. Pain[1]

[1] *Laboratoire Charles Fabry, Institut d'Optique*

Abstract:
Dynamic laser speckle contrast imaging (LSCI) has been developped in the last decades for wide field (up to 15 x 15 cm^2) imaging of blood flow at the level of the microcirculation (arterioles, capillaries and veinules). The technique relies on the camera recordings of time varying speckle patterns arising from photon backscattered by tissues illuminated with a coherent source. For a given camera exposure time, the recorded image is blurred accordingly to the rate of the spatial changes of the speckle patterns. These are related to the movement of the scatterers witin the tissue and ultimately to the local blood flow. Multiple exposure laser speckle(MESI) imaging has been developped as an improved variant of the techique, allowing to seperate the contribution of static and mobile scatterers and experimental noises. I will show and discuss how we have implemented and validated this approach in regards of the specific constraints of small animal and human imaging. The performances and pitfalls of the different instrumental choices and data analysis algorithms were assessed on simulations and custom phantom models of microcirculation. The resulting imagers have been applied first, for imaging cerberal blood flow in mice models of obesity during sensory stimulation and, second, for the imaging of human limbs at rest and during dynamical physiological challenges.

Multi light-sheet RESOLFT for fast multicolor live-cell imaging

A. Illand[1]

[1] *ISMO, Université Paris Saclay*

Abstract:
Light-sheet microscopy enables fast and gentle volumetric imaging of live samples. In particular, high NA oblique plane microscopes are suitable for intracellular cell imaging. However, diffraction imposes a fundamental trade-off between light-sheet thickness and propagation length. Reversibly switchable fluorescent proteins (RSFPs), commonly used for RESOLFT (REversible Saturable OpticaL Fluorescence Transitions) microscopy, can overcome this limitation by confining fluorescence emission to planes thinner than the excitation light sheet, and thus, improving the spatial resolution beyond the diffraction limit.

Here, we implemented a system in which a highly modulated three-dimensional illumination pattern switched RSFPs into the OFF state above and below the light sheets. This process imprints thin planes of ON-state fluorophores throughout the sample, which are then rapidly read out by light-sheet excitation. The parallelization of the OFF switching speeds up the recording and lowers the demand on the number of proteins ON-OFF cycles, which minimizes out of focus switching fatigue. Using this strategy, entire volumes of up to 100x80x15 μm^3 are acquired in less than one second with an isotropic resolution of 250 nm.

Furthermore, in sub-cellular light-sheet imaging only a fraction of the camera sensor is typically used. By introducing an active optical element in front of the detector, we exploit the available sensor area to simultaneously capture additional information without compromising acquisition speed. This offers the possibility to get multi-color information with minimal constraints.

LiveDRIM: Letting Go of the Wheel in Super-Resolution

S. Labouesse[1]

[1] *RIMEO*

Abstract:

Understanding biological function requires imaging fine structures, mechanical organization, and fast, rare dynamics in living systems. Random Illumination Microscopy (RIM) enables robust super-resolution imaging under live conditions without complex illumination control, making it compatible with a wide range of biological samples including bacteria, cells, tissues, and organoids.

LiveDRIM is a new dynamic super-resolution microscopy module. It delivers 90 nm resolution in live samples with high contrast, low phototoxicity, and imaging up to 300 μm deep. With frame rates up to 12.5 Hz, it enables the study of fast dynamics and rare events, with applications in structural biology, cellular biomechanics, and dynamic studies.

Serial third-harmonic microscopy for label-free brain-wide mapping of myelin

S. Prudhomme[1], **H. Blanc**[1], **C. Le Yannou**[2], **G. Le Dréau**[2], **J. Livet**[2], **B. Stankoff**[3], **A. Desmazières**[3], **C. Stringari**[1], **P. Mahou**[1], **E. Beaurepaire**[1]

[1] *Laboratoire d'Optique et Biosciences, CNRS, Inserm, Ecole polytechnique, Institut Polytechnique de Paris, 91120 Palaiseau, France* [2] *Vision Institute, Sorbonne University, INSERM, CNRS, Paris, France* [2] *Paris Brain Institute, Sorbonne University, Pitié Salpêtrière Hospital, INSERM, CNRS, Paris, France*

Abstract:
Understanding the role of myelin in brain development and neurological function requires large-scale mapping of myelin in neural tissue with subcellular precision. Third harmonic generation (THG) microscopy offers label-free myelin imaging potential [1,2], yet its suitability for brain-wide analysis and its specificity for myelinated axons require systematic validation. We present an update of our serial color multiphoton approach (ChroMS) [3], which can detect THG and two-photon fluorescence (2PEF) signals in fixed mouse brains at arbitrary depths. Leveraging wavelength mixing for multicontrast nonlinear imaging [4], ChroMS achieves channel registration with sub-micrometer accuracy across large-scale images, enabling precise comparisons of THG and 2PEF signals. Our work aims to analyze the specificity of THG for myelinated axons across different brain regions through systematic comparison with proteolipid protein (PLP) markers in transgenic mouse lines. This analysis will quantify the reliability of THG as a label-free myelin indicator, and enable to derive metrics of myelin distribution at different scales based on label-free THG signals, in turn paving the way to the analysis of myelin distribution in development and disease models.

[1] Farrar et al, Biophys J 2011, doi:10.1016/j.bpj.2011.01.031

[2] Morizet et al, Optica 2025, doi:10.1364/OPTICA.562091

[3] Abdeladim et al, Nat Comm 2019, doi:10.1038/s41467-019-09552-9

[4] Blanc et al, ACS Photonics 2023, doi:10.1021/acsphotonics.3c01104

Harmonic nanoparticles and multiphoton microscopes enable high flow rate cell detection

O. Saavedra-Villanueva[1]

[1] *PAnTher, UMR703, INRAE, ONIRIS*

Abstract:
Preclinical validation of the safety of cell therapy-based strategies requires a detailed understanding of the biodistribution of the transplanted cells within the host organism. The detection of cells in the circulatory system and

the identification of any possible aggregation state provide critical insights. However, this task demands deep penetration, fast imaging, high selectivity, subcellular resolution, and high throughput. We have demonstrated the detection of human stem cells in whole blood at high flow rates. This was achieved using a multiphoton-based inflow detection approach in a microfluidic system [1]. The approach is based on nonlinear optical microscopy with a diffractive scan perpendicular to the direction, enabled by a rapid wavelength-swept laser [2]. In our new study, we assess and characterize the detection of live human stem cells in whole blood (unfiltered or diluted) under physiological flow rates using our previous methodology. We investigated various experimental conditions, such as laser power, cell concentration in blood, and flow rate. Assessment of cell viability as a function of laser power. Our results show there is no significant difference in cell viability even at high laser power. Stem cells were labeled with lithium niobate (LNO) harmonic nanoparticles (HNPs), which exhibit both second and third harmonic generation (SHG and THG, respectively). The combination of this setup and LNO-labeled cells enabled imaging at 4,000 frames per second with a pixel dwell time of 2.5 ns. Automated LNO-labeled cell detection and segmentation allowed the extraction of key parameters, such as single cell diameter, velocity, and recognition between single cells and aggregates. This approach has been trialed to detect stem cells in the bloodstream of transplanted rats shortly after their intracardiac injection. After tracking the transplanted cells in the blood, it is essential to determine where the cells are distributed within the recipient's body. Therefore, shortly after the transplantation, some rats were sacrificed, and their organs were collected for further investigations. We used a second innovative multiphoton microscope, based on a SWIR laser, to scan 1 mm-thick tissue sections. Thanks to the HNPs, which played a key role in clearly distinguishing the cells from endogenous SHG and THG signals in tissues, we have shown here our ability to find injected stem cells deep in the tissue.

[1] S. Karpf, et al. Harmonic Imaging of Stem Cells in Whole Blood at GHz Pixel Rate. Small 2024, 20, 2401472. https://doi.org/10.1002/smll.202401472

[2] Karpf et al. Spectro-temporal encoded multiphoton microscopy and fluorescence lifetime imaging at kilohertz frame-rates. Nat Commun 11, 2062 (2020). https://doi.org/10.1038/s41467-020-15618-w

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A novel two-photon 3D random-access AOD-based microscope with temporal focusing for improved optical sectioning

J. Morizet[1]

[1]*Institut de Biologie de l'École Normale Supérieure, Paris*

Abstract:
The two-photon 3D Custom Access Serial Holography (3D-CASH) microscope, based on the control of Acousto-Optic Deflectors (AODs), has enabled volumetric neuronal activity recordings in the kHz range in head-fixed behaving mice. Although the use of AOD-based holographic beam shaping to enlarge excitation volume efficiently improves fluorescent signals and mitigates in vivo motion-induced recording artefacts, it also introduces interference patterns ("hot spots") away from the excitation pattern, that may compromise the specificity of the detected neuronal signals in dense neuronal population labelling. In this study, we aim to enhance optical sectioning in the 3D-CASH microscope by implementing temporal focusing (TF). We demonstrated both numerically and experimentally the feasibility of combining TF with AODs despite inherent spatio-temporal pulse distortions generated by AODs. Using numerical simulations based on a ray-tracing method (Kostenbauder matrix theory), we showed that defocus and group delay dispersion can be successfully corrected at the center of the field-of-view by adding an Acousto-Optic Modulator (AOM) before the AOD in the TF configuration. Based on the development of a modular 3D-CASH set-up with TF, we experimentally confirmed that adding the AOM is critical to recover near transform-limited pulses at the focal plane. Finally, we demonstrated that hot spots induced by holography could be efficiently suppressed using TF, thus improving optical sectioning. By removing the background contribution to the total collected fluorescence, introducing TF to the 3D-CASH set-up should considerably enhance the specificity

and the signal-to-background ratio of activity recordings, paving the way for activity recordings in larger neuronal networks.

Keynote Session

Next challenges in Single Molecule Orientation and Localization Microscopy (SMOLM)

S. Brasselet[1]

[1]*Institut Fresnel, Aix Marseille Univ, CNRS, Centrale Med, Marseille*

Abstract:
Molecular organization in cells and tissues plays a crucial role in many biological processes. At the smallest molecular scale in such organizations, the orientation of proteins drives key events such as signalling interactions in immunology or force generation in mechanobiology. Today, such information is most commonly obtained using electron microscopy, which is incompatible with live imaging. Imaging the orientation of proteins down to the single molecule level is possible using fluorescence polarization microscopy, a method based on incorporating polarization information into single molecule localization microscopy (SMLM). We will describe how single Molecule Orientation and Localization Microscopy (SMOLM) exploits the orientation-sensitive coupling between optical excitation fields and molecular transition dipole moments, and show how controlling three-dimensional polarization states in high numerical aperture (NA) microscopy can yield important insights into the 3D orientation of single isolated molecules, including their orientational fluctuations [1,2]. We will illustrate the capability of SMOLM based on polarization split and/or pupil plane manipulation to image the organization of actin filaments in the cell cytoskeleton, which is generally challenging to access in super resolution imaging. We will also discuss the requirements for proteins' labelling strategies in polarization microscopy, including for live imaging [3]. Finally, we will address a few next challenges in SMOLM: addressing the fast rotational dynamics of single fluorescent labels, quantifying the orientation and chirality of chiral fluorescent labels where magnetic transitions play a role in addition to their electric transition dipoles, and transferring SMOLM approaches into dark field imaging in order to image complex 3D-polarized optical fields.

[[1] S. Brasselet and M.A. Alonso, Polarization microscopy: from ensemble structural imaging to 527 single-molecule 3D orientation and localization microscopy. Optica 10 (11), 1486-1510 (2023)

[2] S. Brasselet and M. Lew, Single-molecule orientation and localization microscopy. Nature Photonics 19, 925–937 (2025). https://doi.org/10.1038/s41566-025-01724-y

[3] C. Silva Martins et al. Genetically encoded reporters of actin filament organization in living cells and tissues. Cell 188 (9), 2540 - 2559.e27 (2025) https://doi.org/10.1016/j.cell.2025.03.003

Session 2: From molecules to behaviour: multiscale and in vivo imaging of biological systems

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Full Field OCT from patient to laboratory - live imaging in retina

K. Grieve[1]

[1] *Vision Institute / Quinze Vingts National Ophthalmology Hospital*

Abstract:

From Live Imaging to Behavioral Mapping: Quantifying Emergent Motility in Predatory Bacteria

A. Le Gall[1]

[1] *Centre de Biologie Structurale, Montpellier*

Abstract:
Advances in live-cell imaging now enable the observation of biological systems across multiple spatial and temporal scales, but extracting quantitative and interpretable information from such data remains a major challenge. In particular, linking molecular-scale dynamics to cellular and collective behaviors requires integrated approaches that combine imaging, tracking, and quantitative analysis.

Here, we present a multiscale imaging and analysis pipeline to study the motility of the predatory bacterium Myxococcus xanthus. Using complementary microscopy approaches, including large field-of-view time-lapse imaging (BactoTracker) and high-resolution fluorescence imaging (TIRF/HILO), we capture cell dynamics from subcellular protein localization to population-level organization over extended durations. Automated tracking provides dense trajectory datasets, from which we extract a comprehensive set of motility descriptors characterizing instantaneous cell behavior.

We then use dimensionality reduction techniques to construct a behavioral space that reveals distinct motility signatures associated with different genetic backgrounds and environmental conditions. This analysis shows that the two motility systems of M. xanthus, gliding and twitching, produce distinct and quantifiable behavioral patterns, and that their coordinated activity gives rise to emergent behaviors at both the single-cell and collective levels.

Together, these results demonstrate how combining advanced imaging modalities with quantitative trajectory analysis enables the reconstruction of functional behavioral states from live imaging data. This framework is broadly applicable to other systems where complex dynamics emerge across scales.

Polarization-Resolved Second Harmonic Generation Microscopy Used to Assess Spatio-Temporal Dynamics of Cervix Remodeling during Murine Gestation

V. Abdelsayed[1], **J. Pei**[2], **A. Ajmal**[2], **D. Giammattei**[2], **P. Mahou**[1], **G. Latour**[1,3], **J. Ramella-Roman**[2], **M.C. Schanne-Klein**[1],

[1] *Laboratoire d'Optique et Biosciences, CNRS - Inserm - Ecole Polytechnique, Institut Polytechnique de Paris, Palaiseau, France* [2] *Department of Biomedical Engineering, Florida International University, Miami, USA* [3] *Université Paris-Saclay, Gif-sur-Yvette, France*

Abstract:
Collagen plays an important role in cervix mechanics. The remodeling of collagen fibers during gestation softens the

cervix and facilitates fetus delivery [1]. It is crucial to understand this process as an accelerated cervical remodeling can lead to Pre-Term Birth (PTB), one of the leading causes of infant mortality worldwide. The techniques used so far were either low in resolution and lacking specificity to collagen or qualitatively conducted on small arbitrary regions of the cervix or a few time points of gestation [2]. In this study, we consider a large dataset from 18 mice at 7 different gestation days and we acquire several transverse sections from different depths in each murine cervix using polarization-resolved Second Harmonic Generation (p-SHG), which is very efficient for imaging collagen with a high specificity and sub-micrometer resolution [2, 3]. We conducted mosaicking in a fully automated way to acquire a series of p-SHG images (tiles) with a motorized stage to extend the field of view and image the whole cervical section (2 x 2 mm^2). Then, the intensity and orientation maps of the collagen fibers are obtained for each tile and stitched together to reconstruct maps of the whole section. We developed metrics to quantitatively assess collagen content, porosity and orientational order at different locations of the cervix, laterally over each section and axially across different cervical depths, and their temporal evolution throughout gestation. On the same samples, we also used Mueller Matrix (MM) imaging, a technique that is less resolved and specific to collagen but more clinically deployable, to quantify the orientational order and correlate it to our p-SHG results. Our p-SHG data showed that the lower cervix is richer in collagen that the upper cervix, confirming previous histological studies with a technique more specific to collagen [4]. We found that this collagen level increases during gestation, especially at this lower part. The density and size of pores also showed spatial and temporal differences. Notably, we found a higher porosity laterally at the part close to the cervical canal and a general depth-dependent increase of porosity throughout gestation, that agrees with the cervical softening we expect. The p-SHG orientation maps showed a high organization over all depths at the beginning (days 0-6) and a high disorder at the end of gestation (days 15-18). A progressive remodeling was observed before this later regime (days 12-14) with an increase of disorder from the upper to the lower cervix. The increase of disorder throughout gestation between these three regimes was also assessed with MM with a high correlation to p-SHG results. To conclude, thanks to our automated pipeline combining p-SHG mosaicking and collagen metrics analysis, the collagen structural changes that occur during murine gestation and the spatial heterogeneity of this remodeling. Furthermore, we have shown that some of these changes can be detected with MM, which is faster and cheaper than p-SHG, holding promise for PTB detection through a potential abnormal acceleration of this process.

[1] M. Muñoz-de-Toro, J. Varayoud et al, Nat. Cell Biol., 20(2), 75-84 (2003).

[2] J. C. Ramella-Roman, M. Mahendroo et al, ACS Photonics, 11(9), 3536-3544 (2024).

[3] V. Abdelsayed, J. Pei et al (paper in revision).

[4] A. Chatterjee, R. Saghian et al, Sci. Rep, 11(1), 4903 (2021).

Imaging Inter-Individual Morphological and Molecular Variations to Unravel Ascidian Developmental Logic

P. Lemaire[1]

[1] *Centre de Recherche en Biologie cellulaire de Montpellier*

Abstract:

Universal characterization of tissue architecture in spatial omics data using pair correlation functions

P. Bost[1]

[1]*Institut Curie*

Abstract:
Multiplexed imaging technologies now enable simultaneous measurement of hundreds to thousands of molecular features in intact tissues, offering unprecedented insight into cellular heterogeneity and spatial organization. However, existing quantitative frameworks for analysing spatial patterns, including distance-based metrics, neighbourhood analyses and graph-based models, either focus on local interactions or trade interpretability for predictive performance. Here we present PCF-SiM (Pair Correlation Function Sigmoid Modelling), a scalable and interpretable framework that parameterizes the pair correlation function to quantify spatial organization in multiplexed imaging data. PCF-SiM compresses complex spatial patterns into a small set of biologically meaningful parameters, enabling robust comparisons across cell types, samples and conditions. Applied to public spatial transcriptomics datasets, PCF-SiM outperfoms current methods and identifies previously unknown condition-dependent tissue remodeling in a mouse model of colitis. We further introduce a co-scaling strategy to detect cell types participating in shared spatial structures. Using newly generated clinical datasets from Hashimoto's thyroiditis and uveal melanoma liver metastases, PCF-SiM uncovers hierarchical organization of immune infiltrates and coordinated spatial interactions between lymphatic endothelial cells and tumour-infiltrating lymphocytes. Together, PCF-SiM thus provides a principled and interpretable framework for quantifying multiscale spatial organization in biological tissues.

Optical Characterization of Endogenous Fluorescence in Mouse Prefrontal Cortex for Improved Signal Unmixing in Neuroimaging

D. Wehbe[1], **A. Faure**[1], **C. Rimbault**[1], and **D. Abi Haidar**[1]

[1] *IJCLab*

Abstract:
Understanding the pathophysiology of psychiatric disorders such as schizophrenia requires reliable tools to probe neurotransmitter dynamics in vivo. Fluorescence microendoscopy, based on genetically encoded biosensors, has emerged as a powerful approach for monitoring neural activity during behavior. However, a major limitation lies in the difficulty of interpreting fluorescence signals, which result from a complex combination of exogenous biosensor emission and endogenous autofluorescence linked to metabolic activity. In this work, we investigate the endogenous fluorescence signature of the mouse prefrontal cortex in ex vivo tissues. We combine structural, spectral, and fluorescence lifetime imaging to provide a comprehensive characterization under both fixed and fresh conditions. A parametric study under two-photon excitation identifies the key optical parameters governing endogenous fluorescence. We further assess the temporal stability of these signatures following tissue excision using complementary imaging modalities. Our results define a critical time window over which endogenous optical properties remain representative and exploitable. Beyond methodological advances, this work establishes a framework for integrating endogenous and exogenous signals, enabling more robust signal unmixing and improved discrimination between metabolic and neurodynamic processes.

Label-free 3D Imaging of Cardiac Organoids by Multimodal Nonlinear Microscopy

M. Araña[1], **S. Sart**[2], **M. Pucéat**[3], **W. Supatto**[1]

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Abstract:
Cardiac organoids are powerful models for studying heart development, disease mechanisms, and drug responses. However, characterizing their three-dimensional architecture in a non-invasive manner remains challenging, as conventional fluorescence confocal microscopy requires exogenous labeling and is limited in imaging depth, potentially introducing artifacts that compromise physiological relevance. Here, we present a label-free multimodal nonlinear microscopy approach combining Second Harmonic Generation (SHG) and Third Harmonic Generation (THG) to image cardiac organoids in 3D beyond the depth limits of conventional confocal microscopy. SHG provides specific contrast for fibrillar collagen and myosin, enabling the assessment of fiber organization and maturation, while THG highlights lipid-rich interfaces and structural boundaries, offering complementary morphological information without any staining or genetic modification. Using this approach, we investigate the structural organization of collagen and myosin networks across different organoid maturation stages and in response to distinct culture protocols. Preliminary results reveal heterogeneous fiber architectures that evolve with maturation, as well as structural remodeling. By leveraging the intrinsic nonlinear optical properties of cardiac tissue, our approach enables deep, volumetric, and minimally perturbative imaging of organoids, opening new avenues for longitudinal studies of cardiac maturation and disease modeling in a fully label-free manner.

Session 3: Quantitative imaging and single-object tracking in complex environments

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Multimodal cameras for single-shot imaging of intensity, phase, polarization and color

G. Baffou[1]

[1]*Institut Fresnel, Marseille*

Abstract:
Fluorescence microscopy is widely regarded as the gold standard in bioimaging, owing to its high specificity and sensitivity. However, it suffers from several drawbacks, including photobleaching, phototoxicity, and invasiveness. As a result, there is a growing interest in label-free and non-invasive imaging approaches. Among these approaches, some rely on detecting the richness of the transmitted light, including its polarization, spectral content, wavefront, and phase. However, these techniques are often complex and difficult to implement on standard microscopes used in biology. Here, we show that all these observables can be retrieved using simple camera-based systems, modified by integrating optical elements in close proximity to the image sensor. We first present cross-grating wavefront microscopy (CGM) for wavefront and phase imaging of biological samples such as neurons and bacteria, among other models. This microscopy technique is based on the implementation of a 2-dimensional quadriwave (QLSI) grating in front of the camera sensor. We then demonstrate how we extended CGM toward polarization- and color-resolved imaging, preserving the compactness and simplicity of the device, for applications in single-shot phase-fluorescence microscopy and in imaging the complex vectorial field of light, highlighting specific organelles in living systems.

Multi-timescale scanning reveals molecular memory hidden in SMT data

J. Park[1]

[1] *Laboratory of Computational, Quantitative and Synthetic Biology (CQSB), Paris*

Abstract:
In this talk, we will present a theoretical approach to investigate molecular memory (i.e., temporal correlation) and bridge the theory to real data in SMT. Single-molecule tracking (SMT) in living cells routinely reduces anomalous dynamics to a single exponent (i.e., anomalous diffusion exponent or Hurst exponent) extracted from a mean square displacement (MSD) fit. This collapses physically distinct regimes — fractional Brownian motion (fBm) and confinement — into indistinguishable sub-diffusive values, obscuring the underlying mechanism. We introduce a multi-time-scale Hurst estimator $\hat{H}(\Delta)$ and its confidence interval. We model displacement-ratio statistics with a Cauchy likelihood that exploits every offset trajectory at a given lag rather than a single offset, giving an outlier-robust, bin-free estimator that uses every trajectory segment. The estimator incorporates exact fBm motion-blur and localisation-noise kernels with a per-detection Cramér–Rao bound on localisation precision. Scanning $\hat{H}$ across lags Δ turns a single number into a diagnostic curve whose shape identifies the regime: flat for true fBm, monotonically decaying for pure confinement. Applied to SMT data from H2B in human cells and Rad51 in yeast, the method resolves regimes that a single α/H cannot. Multi-Δ scanning exposes motion-regime information that a single exponent systematically hides, and we argue it can replace single-exponent fitting as the default readout for in vivo SMT.

Agile two-photon microscope for fast 5D single-particle translation and rotation tracking

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[1] *Laboratoire Lumière, Matière et Interfaces, ENS Paris-Saclay*

Abstract:
We present a two-photon microscopy setup to measure intraneuronal transport parameters in a 3D sample in a super localisation regime thanks to the non-linear optical response from nanoparticles (second harmonic generation (SHG)). We take advantage of a Digital Micromirror Device (DMD) to perform digital holography and change the focus position of the excitation laser. We create a pattern of excitation in the vicinity of the nanoparticle, which allows us to super-localise the particle in real time (millisecond regime), with a localization precision of less than 5 nm by maximum likelihood approach. The DMD is fast enough to track the nanoparticle during its motion. We also use the holograms to correct the wavefront and obtain thus a diffraction-limited spot at the laser focus. The tracking method has been tested on nanoparticles ($BaTiO_3$ nanospheres, $\sim$ 100 nm diameter) internalized in living cells displaying directional trajectories and typical go and stop phases.

We aim at completing the intraneuronal transport parameters, inferred from the $x(t), y(t), z(t)$ positions, with the measurement of the rotational movement of vesicles. This additional parameter is useful to understand how the molecular motors are driven along the microtubules. The nanoparticles' SHG signal depends on their crystalline axis and polarization of the excitation laser. By rotating the incident polarization and detecting along two orthogonal polarizations, we are able to track the translation motion as well as the rotation of the nanocrystal. Our first measurements display standard deviations around 1° for azimuthal and polar angles θ and φ.

Enhancing confocal microscopy assets with SPAD arrays and photon-resolved microscopy

L. Le[1]

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Abstract:
Fluorescence laser scanning microscopy (LSM) stands as a cornerstone in the life sciences, enabling the study of complex biological processes through a unique balance of spatial and temporal resolution, imaging depth, and live-cell compatibility, while remaining compatible with advanced approaches such as spectroscopy and non-linear excitation. Yet, conventional LSM detectors are intrinsically inefficient: traditional bucket detectors integrate fluorescence over time and space, discarding the rich spatial and temporal information potentially carried by individual photons.

To overcome this limitation, we have developed asynchronous read-out SPAD array detectors (1), enabling a new paradigm: photon-resolved microscopy. In this framework, every detected photon is tagged with multiple spatiotemporal signatures, unlocking information inaccessible to traditional detectors. In this presentation, I will highlight several applications of photon-resolved microscopy, with a particular focus on image scanning microscopy (ISM). I will present reconstruction approaches ranging from basic pixel reassignment to advanced deconvolution, showing how the spatial information captured by SPAD arrays improves resolution (2) and optical sectioning (3) while reducing illumination dose. I will then discuss the integration of ISM with fluorescence lifetime imaging (FLIM), demonstrating how photon-resolved data enable super-resolved structural and functional imaging, and how lifetime information can be exploited to achieve multi-target super-resolution ISM, greatly expanding multiplexed imaging capabilities. Finally, I will briefly outline how photon-resolved microscopy synergizes with other super-resolution modalities such as STED and MINFLUX, and how SPAD arrays extend its benefits beyond imaging to fluorescence fluctuation spectroscopy (4) and single-particle tracking (5).

1. Buttafava, M. et al. SPAD-based asynchronous-readout array detectors for image-scanning microscopy. Optica 7, 755 (2020).

2. Castello, M. et al. A robust and versatile platform for image scanning microscopy enabling super-resolution FLIM. Nat. Methods 16, 175–178 (2019).

3. Zunino, A. et al. Structured detection for simultaneous super-resolution and optical sectioning in laser scanning microscopy. Nat. Phot. 19, 888–897 (2025).

4. Slenders, E. et al. Confocal-based fluorescence fluctuation spectroscopy with a SPAD array detector. Light Sci. Appl. 10 (2021).

5. Bucci, A. et al. 4d single-particle tracking with asynchronous read-out single-photon avalanche diode array detector. Nat Comm. 15 (2024)

Building a Multimodal Atlas of Vertebrate Development

M. Lange[1]

[1] *Institut de la Vision, Paris*

Abstract:
Elucidating the developmental processes of organisms requires a comprehensive understanding of cell lineages across spatial, temporal, and molecular dimensions. Advances in imaging and omics technologies allow us to generate technically challenging multidimensional datasets that capture developmental dynamics at unprecedented resolution and across scales (from molecule to organism). In this talk, I will describe how we integrate light-sheet

microscopy with scRNAseq, yielding a time-resolved multimodal atlas of zebrafish development named Zebrahub (zebrahub.org). I will showcase its application in elucidating the differentiation of complex late pluripotent progenitors. Finally, I will show our recent effort leveraging Multiomic assays and tracking data to perform in silico experiments and gain mechanistic insights into development and differentiation.

Noise correlation inspired elastography for in vivo stiffness mapping using optical coherent imaging systems

A. Nahas[1]

[1] *Université de Strasbourg*

Abstract:

Near-infrared quantum-dot based adaptive optics for in-depth in vivo two-photon neuroimaging in the mouse cortex

J. Pytel[1,2], **A. Guillaume-Manca**[1,2], **B. Jeffries**[4], **C. Roux-Byl**[4], **T. Pons**[4], **P. Treimanyl**[3], **F. Harms**[3], **L. Bourdieu**[1], and **A. Fragola**[2]

[1] *Institut de Biologie de l'École Normale Supérieure, Paris*

[2] *Institut des Sciences Moléculaires d'Orsay, Université Paris Saclay*

[3] *Imagine Optic SA*

[4] *Laboratoire de Physique et Etude de Matériaux, ESPCI*

Abstract:

The study of fast biological phenomena at the cellular level has become possible even in depth in biological tissues in several animal models, thanks to efficient 3D microscopy techniques. In particular, two-photon microscopy enables the organization and activity of genetically-identified neural networks to be imaged in mice kept head-fixed. This type of recording can be easily performed in the upper layers (1-3) of the mouse cortex, but in the deeper layers (4-6), observation of subcellular structures such as dendritic spines and detection of neuronal activity becomes difficult due to scattering and optical aberrations. To overcome this difficulty, in particular regarding aberrations, Adaptive Optics (AO) has been implemented on several multiphoton microscopy setups. When based on direct wavefront sensing, rather than sensorless operation that is slow and limited in dynamic range, it provides a reliable live correction of the aberrations [1-3], improving the collected signal and the spatial resolution, thus allowing for imaging at greater depths. Nevertheless, the accuracy of aberration measurement remains challenging at large depths due to the scattering properties of the tissue and thus the low signal to background ratio on the wavefront analyser camera. Semiconductor nanocrystals (quantum dots) are bright, stable and tunable fluorescent nanoparticles. We have shown that they can be injected into the blood vessels to provide an improved source of ballistic fluorescence signal for the wavefront sensor as compared to reported dyes. We will demonstrate the biocompatibility and superior blood circulation time of CuInSe2/ZnS quantum dots emitting at 700nm, compared to a dye-dextran conjugate. These qualities make these quantum dots ideal guide stars for long-term imaging. Using this approach, we demonstrate that the wavefront can be efficiently and accurately measured deep inside the mouse brain. This enables aberrations to be corrected quickly (typ. 1-4s) and enhancing the contrast of images of fine structures up to 600 μm deep in the visual cortex of genetically labelled, anaesthetised mice. These promising

experiments pave the way to efficient aberrations correction up to 1mm, e.g. from the use of SWIR-emitting QDs, allowing the study of the whole mouse cortex in vivo.

[1] K. Wang et al., Nat. Commun., vol. 6, p. 7276 (2015)

[2] R. Liu et al., Nat. Methods, vol. 16, pp. 615–618 (2019)

[3] S. Imperato et al., Optics Express 30(9), pp. 15250-15265 (2022)

Coupling two photon microscopy with sub-micron resolved infrared spectroscopy to investigate collagen alteration

L. Paggi[1], **J. Mathurin**[2], **A. Deniset-Besseau**[2], **M. Thoury**[3], **M.C. Schanne-Klein**[1], **L. Robinet**[4], **G. Latour**[1,5]

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Abstract:
Type I collagen is the most abundant protein in mammals, as the main component of skin, bones, tendons or cornea. Its multiscale hierarchal structure is fundamentally linked with its specific mechanical, chemical and optical properties. In skin, triple-helix biopolymers assemble into fibrillar bundles stabilized by intermolecular hydrogen bonds and covalent crosslinks. These fibrils, in turn, form the building blocks of micrometer-thick, entangled fibers. The resulting dense, three-dimensional collagen matrix endows tissues with stability and elasticity, explaining both its biological function and the widespread use of processed animal skin (e.g., parchment). Exposure to heat and humidity can disrupt these molecular assemblies, progressively degrading fiber-scale organization and ultimately, irreversibly, leading to gelatin formation through complete protein denaturation. Nonlinear two-photon microscopy (2PM) has emerged as a powerful label-free imaging tool for visualizing collagen organization and quantifying its degradation stages. While second harmonic generation (SHG) signals serve as endogenous markers of intact fibrils (arising from aligned, non-centrosymmetric peptide bonds), altered collagen exhibits a progressive increase in fluorescence following heat and humidity exposure [1]. Correlating 2PM contrasts with nanometer-resolved infrared spectroscopy has linked structural differences between native and denatured collagen to specific chemical signatures. Thermally altered isolated collagen fibrils and pure gelatin display similar behaviors: broadening of the amide I band and the emergence of a new carbonyl stretching mode near 1730 cm^{-1} suggesting molecular esterification [2]. Extending this multimodal, multiscale analysis to fibers extracted from bulk tissues subjected to thermal and humidity treatments could yield deeper insights into the dynamics underlying the diverse physicochemical signals observed.

[1] M. Schmeltz et al., "Noninvasive quantitative assessment of collagen degradation in parchments by polarization-resolved SHG microscopy," Sci. Adv., vol. 7, no. 29, p. eabg1090, Jul. 2021, doi: 10.1126/sciadv.abg1090.

[2] G. Latour et al., "Correlative nonlinear optical microscopy and infrared nanoscopy reveals collagen degradation in altered parchments," Sci. Rep., vol. 6, no. 26344, 2016, doi: 10.1038/srep26344.

Session 4: Nano- and quantum-enabled sensing technologies for biology

NV-centers in diamond for biosensing and associated imaging

K. Berg-Sørensen[1]

[1] *Technical University of Denmark*

Abstract:
The nitrogen-vacancy (NV) centers in diamond possess unique magneto-optic properties with fluorescence characteristics that allow scientists to initialize and discern different electron spin states at room temperature. As diamond is biocompatible, NV-centers in diamond has demonstrated itself as a novel and versatile material for biosensing and associated imaging. One result of the optical spin-state control is the ability to discern and thus detect magnetic field strength by optical means. This property may be applied, e.g., for microscale imaging of magnetic fields and magnetic signals when working with diamond crystals with a top layer of NV-centers. In addition, the population in the different electron spin states of the NV-centers depends on spin interactions with the surroundings, a property suggested applied for detection of free radicals inside cells by analysis of the signal from nanodiamonds rich in NV-centers, often referred to as fluorescent nanodiamonds (FNDs). Literature also suggests that the fluorescence spectra from such FNDs shows variation with the pH of the surrounding liquid, implying that FNDs may be used to determine variation in pH at the scale of the size of the FND. In a liquid or a cellular environment, an extended measurement sequence is often necessary. Therefore, to improve stability and avoid nanodiamonds diffusing away, we suggest optically trapping the FNDs. Analysis of the motion of the optically trapped intracellular FNDs further may give insights into the intracellular viscoelasticity. The presentation will introduce the different sensing modalities described above, discuss the advantages and disadvantages of the two approaches - diamond crystal with NV-centers vs FNDs - for bio-sensing, as well as present examples of actual studies from the labs of the speaker and her close collaborators.

In-depth, large–field-of-view 3D SMLM using nanochip-based ModLoc illumination

M. Meghnani[1]

[1] *ISMO, Université Paris-Saclay*

Abstract:
Extending imaging depth while preserving a large field of view (FOV) remains a major challenge in single-molecule localization microscopy (SMLM), particularly for volumetric imaging in biological applications. In ModLoc [1, 2], a time-modulated illumination scheme encodes the axial position of single emitters in the phase of their fluorescence signal, enabling sub-7-nm axial localization precision over an extended depth range. This approach has already proven effective for imaging complex biological samples such as tissues and spheroids with a 50 μm $\times$ 50 μm FOV. By miniaturizing the excitation module and increasing the accessible imaging volume, we extend this approach to single-shot SMLM imaging over a 1.8 $\times$ 100 $\times$ 100 μm^3 volume.

Here, we introduce a compact and mechanically stable implementation of ModLoc based on an engraved 3 cm $\times$ 3 cm nanochip [3], which directly generates the two excitation beams and the controlled phase shift required

to translate the interference pattern within the sample. Conjugating the beams to the back focal plane of the objective in a compact geometry improves stability and facilitates implementation.

To recover the axial position of each molecule, 4 phase-shifted intensity measurements must be acquired. To preserve localization events from short-lived emitters, we implemented a high-speed demodulation strategy based on galvanometric mirrors that records the 4 modulation states simultaneously on 4 distinct sub-arrays of a single camera frame. However, extending the FOV from 50 μm $\times$ 50 μm to 100 μm $\times$ 100 μm requires larger scan angles, which introduces optical aberrations and distorts the point spread function across the field. Therefore, we performed a comprehensive optical simulation of the full detection path and redesigned the set-up to 1) prevent cropping in both image and pupil planes, 2) control magnification throughout the system, and 3) minimize and compensate aberrations using off-the-shelves optics despite the large beam angles and the spatial extent of the optical signal. We will present a compact detection module that enables accurate phase retrieval across the full FOV.

Combined with the large axial capture range of a ?60 silicon objective, this implementation provides large-volume single-shot imaging. We will present the system characterization and a first application to cytoskeleton imaging in cells.

[1]. P. Jouchet, C. Cabriel, N. Bourg, M. Bardou, C. Poüs, E. Fort and S. Lévêque-Fort, "Nanometric axial localization of single fluorescent molecules with modulated excitation", Nature Photonics, vol. 15, no. 4, pp. 297–304, 2021.

[2]. A. Illand, F. Matos, M. Lengauer, P. Jouchet, E. Fort and S. Lévêque-Fort, "Time modulated illumination for single molecule localization microscopy", CLEO/Europe–EQEC 2025, Optica Publishing Group, 2025

[3]. M. Calvarse, P. Paiè, A. Candeo, G. Calisesi, F. Ceccarelli, G. Valentini, R. Osellame, H. Gong, M. Neil, F. Bragheri, A. Bassi, "Integrated optical device for Structured Illumination Microscopy", Optics Express, vol. 30, no. 17, pp. 30246–30258, 2022.

High-throughput correlative magnetic tweezers-TIRF to investigate biomolecular reactions at the single-molecule level

T. Bugea[1]

[1] *Vrije Universiteit Amsterdam*

Abstract:
Magnetic tweezers (MT) represent a powerful and established single-molecule force and torque spectroscopy method, widely used to investigate protein-nucleic acid interactions. In MT, permanent magnets positioned above a flow chamber apply force and torque to nucleic acids tethered magnetic beads, allowing precise controlled stretching and twisting. The integration of high-resolution, large-chip CMOS cameras with fast GPU-based tracking algorithms enables the monitoring of hundreds of single nucleic acid molecules simultaneously and in real-time. Correlative techniques that combine mechanical manipulation with fluorescence imaging –such as MT or optical tweezers coupled to fluorescence microscopy – have provided remarkable insights into complex biomolecular mechanisms. Yet, these assays often suffer from limited throughput and statistics, making it difficult to capture the complete dynamic behavior of biomolecular reactions. Here, we present a major advancement in magnetic tweezers technology, enabling high-throughput correlative measurements at high spatiotemporal resolution. We developed a bespoke total internal reflection fluorescence (TIRF) microscope capable of simultaneously tracking thousands of fluorescently labeled biomolecules on a single sCMOS camera across multiple emission channels, all in real time. By integrating this high-throughput TIRF platform with magnetic tweezers, we achieve real-time, correlative MT-TIRF measurements on up to ∼400 individual nucleic acid molecules in parallel. We will demonstrate proof-of-principle experiments highlighting the capabilities of this system, including the characterization of

early events in bacterial transcription initiation. Additionally, we will present recent developments that extend our platform's performance toward near-kilohertz acquisition rates and subnanometer-scale spatial precision, paving the way for high resolution, high-throughput measurements at the single molecule level.

Session 5: Microscopy and AI

Boosting biomedical imaging with nonlinear optical microscopy: balancing AI and physics-based computational methods

C. Lefort[1], **L. Trautmann**[2], **S. Ferrat**[2], **U. Arles**[1], **S. Frachet**[3], **A. Danigo**[3], **M. Duchesne**[3], **J.-C. Pesquet**[2], and **E. Chouzenoux2**[2]

[1] *Institut de recherche XLIM, UMR CNRS 7252, Université de Limoges* [2] *Université Paris Saclay, Inria, CentraleSupélec, CVN* [3] *Department of Neurology, University Hospital of Limoges, Dupuytren Hospital*

Nonlinear optical microscopy, combined with advanced computational and signal-processing strategies, is transforming label-free biomedical imaging by providing access to biochemical information at subcellular resolution. While artificial intelligence is increasingly adopted in imaging pipelines, AI is not always the most relevant computational strategy. Through examples in muscle structure and pathology, we compare physics-based and AI-driven approaches, highlighting situations where deep learning becomes essential and others where simpler computational methods provide more robust and interpretable solutions. **Abstract:**

Semi supervised GAN for smart microscopy, fast and data efficient cell cycle classification

R. Manick[1]

[1] *Université de Rennes*

Abstract:
Modern optical microscopes are fully motorised, yet they are not truly smart unless they can adapt acquisition in real time to detected objects and biological events. This requires fast, robust, and transferable classification algorithms that can operate with limited annotated data.

In this talk I will present a semi-supervised generative adversarial network (SGAN) for data-efficient cell-cycle stage classification in microscopy. By combining labelled images, unlabelled microscopy data, and generated cellular images, the framework learns robust representations under low-resource conditions. On the five-class Mitocheck dataset, SGAN reached $93 \pm 2\%$ accuracy using only 80 labelled images per class and 600 unlabelled images. I will discuss how this compact model transfers across cell lines, labelling schemes, and microscopy settings, and how it can support real-time decision-making in automated smart microscopes.

TBA

S. Achard[1]

[1] *Laboratoire Jean Kuntzmann, CNRS, Université Grenoble Alpes*

Abstract:
The brain is the most complex organ of the human body. It is composed of around a hundred billion neurons that are interconnected with each others. It is nowadays possible to measure the activity of the brain while functioning using neuroimagery such as ElectroEncephalography (EEG), MagnetoEncephalograpy (MEG) or functional Magnetic Resonance Imaging (fMRI). Neuroimaging facilities are now available in most hospitals to make observations of the brain during task or resting-states. These has brought new perspectives for understanding evolution of pathologies such as neurodegenerative diseases of consciousness disorders, especially at the subject level. From a statistical point of view, neuroimaging provides many datasets with both spatial and temporal scales. For example, fMRI consists of a set of voxels (i.e. cubes) covering the whole volume of the brain for each time point. The side of one voxel is usually around 2 to 3 millimeters and one volume is acquired every 1 to 2 seconds. It is then needed to summarise these complex datasets to give a comprehensive model of the brain. Using these spatio-temporal datasets, a current goal is to infer brain networks, where one node of the network corresponds to a region of the brain and one edge corresponds to a connection or link between a pair of regions. The challenge is to define an accurate and interpretable measure of a connection between two regions. Indeed, neuroimaging provides a massive amount of data that can be difficult to process and analyse adequately to obtain reproducible results. In this talk, I will present methods for robust and statistically consistent estimation of networks using functional data analysis for multivariate datasets such as those observed in neuroimaging. We already obtained reliable results using wavelet correlations (FC) for both dead and anesthetized rats. The idea is to combine accurate correlations estimations for long memory time series and correction for multiple testing. We are currently working on using spatio-temporal characteristics of the datasets to exploit densities of correlations as a novel tool for quantile-based network construction.

Computer Vision for histopathology and spatial transcriptomics

T. Walter[1]

[1] *Institut Curie*

Abstract:
Whole slide images (WSI) are microscopy images of stained tissue sections, routinely used in cancer treatment centers for diagnosis, patient stratification, and treatment selection. WSIs are large and complex, often containing hundreds of thousands of individual cells, and their analysis requires specialized algorithmic approaches. Spatial Transcriptomics (ST) is a technique that maps gene expression to specific locations within a tissue, thus combining spatial information with transcriptomic data. ST enables the study of spatial organization of cellular processes and how they relate to tissue structure and function. However, ST presents several computational challenges, such as single-cell deconvolution, cross-modality prediction and image segmentation. In this lecture, I will present our recent developments addressing these challenges. I will discuss methods for predicting molecular subtypes of bladder cancer and genomic features such as homologous recombination deficiency (HRD) directly from WSIs. I will also describe our work on cell segmentation using image-based spatial transcriptomics data. Finally, I will

present a method for predicting single-cell gene expression from morphological phenotypes observed in H&E images, leveraging paired H&E and spatial transcriptomics datasets.

Physics-Informed Neural Networks (PINNs) in computational imaging

C. Fournier[1]

[1] *Laboratoire Hubert Curien, Université Jean Monnet, Saint-Etienne*

Abstract:
The combination of computing and imaging has led to the development of 'computational imaging'. This multidisciplinary field brings together image acquisition system design, computer science and image processing algorithms. Rather than relying on the conventional 'what you see is what you get' approach, computational imaging uses indirect measurements to reconstruct images that contain richer information (e.g. 3D, phase or polarisation) than conventional imaging systems. Advances in deep learning and neural networks have also had an impact on computational imaging. This presentation provides an overview of deep learning reconstruction methods based on physical forward models and presents an example of self-supervised reconstruction of holograms of Gram-stained microbiological samples.

Automatic Bacterial Classification in Microscopic Images of Positive Blood Culture Gram Smears

N. Faure[1]

[1] *bioMérieux SA, Grenoble*

Abstract:
Gram staining of positive blood cultures is one of the most routinely performed tests in clinical microbiology diagnostics. It plays a critical role in medical decision-making, as it guides the initial choice of antimicrobial therapy in life-threatening emergency situations. Despite its importance, this test is still largely performed manually, with little integration of artificial intelligence. This is due to several factors, including regulatory constraints in the context of in vitro diagnostics, technical challenges inherent to microscopic imaging, and variability in staining procedures that limits reproducibility. We will present the key challenges and specific characteristics of this task, and introduce a computer vision–based approach designed to address them. Our method paves the way toward large-scale automation and standardization of Gram stain analysis.
