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7th International Symposium
FRONTIERS IN NEUROPHOTONICS

6th-9th October, 2025

Centre Broca Nouvelle-Aquitaine,
Bordeaux (France)

Speakers

Francisco Balzarotti, Austria
Abraham Beyenne, USA
Liangyi Chen, China
Rosa Cossart, France
Daniel Côté, Canada
Johann Danzl, Austria
Valentina Emiliani, France
Linlin Fan, USA
Ali Shaib, Germany
Angela Getz, Netherland
Ralf Jungmann, Germany
Luke Lavis, USA
Christophe Leterrier, France
Sabine Levi, France
Marina Mikhaylova, Germany
Tomoko Ohyama, Canada
Dmitri Rusakov, UK
Markus Sauer, Germany*
Kate Smith, USA
Ilaria Testa, Sweden*

And more!

Scientific Committee

Anna Brachet (Bordeaux)
Daniel Choquet (Bordeaux)
Laurent Cognet (Bordeaux)
Paul De Koninck (Laval)
Yves De Koninck (Laval)
Mathieu Ducros (Bordeaux)
Flavie Lavoie-Cardinal (Laval)
Marie-Eve Paquet (Laval)
Jean-Baptiste Sibarita (Bordeaux)

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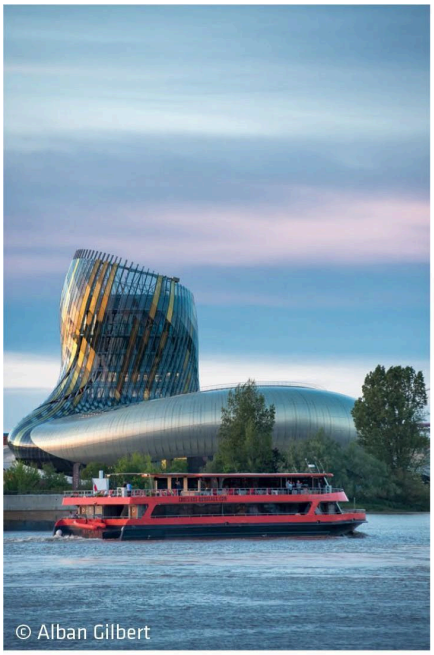
*keynote speakers

Welcome to Bordeaux !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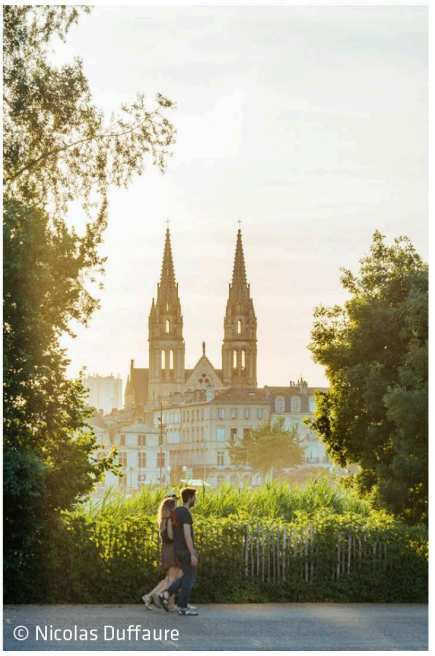
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Your accommodation
IBIS Bordeaux Centre Mériadeck***

The ibis Bordeaux Centre Mériadeck offers you an unforgettable immersion in Bordeaux. Its location is the perfect starting point for exploring the city and its unmissable Golden Triangle. Park your vehicle in our covered car park and start your adventure on foot, by tram or by bike. Make the most of it: we rent electric bikes! To finish off in style, enjoy the sunset and book a table at the Bistrot de Mériadeck.

To reach the Centre Broca Nouvelle-Aquitaine : Tram A: “Palais de Justice” ➡ “Saint-Augustin”, then ~10 min of walk (see map further).

35 Cours Maréchal Juin, 33000 Bordeaux

Social events

Welcome cocktail: on Monday.
From 7:00pm till 9:00pm.
For all the participants.
Location: Centre Broca Nouvelle-Aquitaine.

Wine and cheese: on Tuesday.
From 7:00pm till 8:00pm.
For all the participants.
Location: Centre Broca Nouvelle-Aquitaine.

Gala dinner: on Wednesday evening, from 7pm (see page 9).
Only for participants registered to the gala dinner.
Bus transportation is included.
Location: Château Carbonnieux.

Scientific program

Monday 6th October

- 14:30 – Registrations
- 16:15 – Welcome
- 16:30 - **Marina Mikhaylova**, Humboldt Universität zu Berlin, Germany
  Synaptic tethering of microtubule minus-ends by SHANK3 and CAMSAP2 shapes dendrites in parvalbumin neurons
- 17:00 - **Simon Haziza**, Stanford University, USA
  Imaging the membrane voltage activity of specific neuron-types across spatiotemporal scales in behaving animals
- 17:15 - **Mathieu Johnson**, Concordia University, Canada
  Mesoscale reconstruction of 3D microstructure and fibre orientation in whole brains with automated serial tilted polarization sensitive optical coherence tomography
- 17:30 - **Olivier Thoumine**, Institut Interdisciplinaire de Neurosciences (IINS), France
  Neurospheres from primary rodent brain cells to study the 3D organization and function of synapses at high resolution
- 17:45 - **Fabrice Harms**, Imagine Optic / mu-Imagine, France
  Adaptive Optics solutions for high-resolution fluorescence microscopy applied to Neuroscience

Keynote speaker

- 18:00 - **Markus Sauer**, Universität Würzburg, Germany
  Molecular resolution fluorescence imaging in cells

- 19:00 - Welcome cocktail

Tuesday 7th October

- 8:30 - Welcome Coffee
- 9:00 - **Ali Shaib**, University Medical Center Göttingen, Germany
  Democratizing nanoscale imaging at molecular resolution
- 9:30 - **Rosa Cossart,** Institut de Neurobiologie de la Méditerranée, France
  How stable are cortical representations during development? Insights from longitudinal neuronal activity imaging in vivo
- 10:00 - **Liangyi Chen**, Institute of Molecular Medicine, China
  COrtex-wide miniature Mesoscopic Technique (COMET) enables functional analysis of dorsal cortex networks at single-cell resolution in freely moving mice

- 10:30 - **Robert B. Quast,** Centre de Biologie Structurale (CBS), France
  The Conformational Landscape of Metabotropic Glutamate Receptors Resolved by Multicolor Single Molecule FRET
- 10:45 - **Renaud Ginet**, Argolight
  Argolight Solutions: Advancing quantitative fluorescence microscopy with reproducible QC
- 11:00 - Coffee Break
- 11:30 - **Valentina Emiliani**, Institut de la Vision, France
  All-optical circuits manipulation in head-restrained and freely moving mice
- 12:00 - **Ralf Jungmann**, Max Planck Institute of Biochemistry, Germany
  From DNA Nanotechnology to Biomedical Insight: Towards Single-Molecule Spatial Omics

- 12:30 - **Session Poster** / Lunch Break

- ***CANCELD!*** *14:30 -* ***Kate Smith****, University of Colorado – Anschutz Medical Campus, USA*
  Release your inhibitions: understanding the nanoarchitecture of GABAergic inhibitory synapses
- 15:00 - **Abraham Beyenne**, Janelia Research Campus, USA
  Mapping dopamine release with nanoscale precision: insights into neuromodulatory release and signaling
- 15:30 - **Baptiste Marthy**, Aix-Marseille Université, France
  Label-free quantification of organelle trafficking inside a single axon
- 15:45 - **Charles Ducrot**, Institut Interdisciplinaire de Neurosciences (IINS), France
  Investigating the nanoscale localization of synaptic proteins by dual photon-electron microscopy using engineered fluoro-nanogold probes
- 16:00 - **Hanna Manko**, Institut d'Optique Graduate School, France
  Microscope stabilization for drift-free single-particle tracking at depth in brain tissue
- 16:15 - **Luc Moog**, Coherent
  Innovations in Ultrafast Lasers for Nonlinear Microscopy
- 16:30 - Coffee Break
- 17:00 - **Johann Danzl**, Institute of Science and Technology, Austria
  Reconstructing brain tissue at synaptic resolution with light microscopy

Keynote speaker

- 17:30 - **Ilaria Testa**, Department of Applied Physics at the School of Engineering Science, Sweden
  Dynamics imaging of proteins at the nanoscale empowered by computation

- 19:00 - Wine & Cheese

Wednesday 8th October

- 8:30 - Welcome Coffee
- 9:00 - **Francisco Balzarotti**, Research Institute of Molecular Pathology, Austria
  Photon-Efficient Localization with MINFLUX Imaging and Tracking

- 9:30 **- Angela Getz**, Vrije Universiteit Amsterdam Center for Neurogenomics and Cognitive Research, Netherland
  A pipeline for single molecule imaging of endogenous synaptic proteins in brain tissue
- 10:00 **- Dmitri Rusakov**, UCL Queen Square Institute of Neurology, UK
  Nano-diffusion in the brain monitored with time-resolved fluorescence anisotropy imaging
- 10:30 **- Étienne Herzog**, Institut Interdisciplinaire de Neurosciences (IINS), France
  A tale of neurophotonics applied to synaptosomes: dopamine neuromodulation from varicosities to dopamine hub synapses
- 10:45 **– Thomas Ferhat**, NKT Photonics
  Lasers and fibers for neuroscience
- 11:00 - Coffee Break
- 11:30 **- LinLin Fan,** The Picower Institute for Learning and Memory, USA
  All-optical physiology reveals synaptic bases for learning and memory in behaving mammals
- 12:00 **- Ivo Calaresu**, Institut Interdisciplinaire de Neurosciences (IINS), France
  Extracellular matrix tunes the diffusion of pathological immunoglobulins
- 12:15 **- Mehdi Madi**, Abbelight
  Abbelight Nanoscopy Solution – Unlocking Spatial Proteomics at Single-Protein Resolution

- 12:30 - **Session Poster** / Lunch Break

- 14:30 **- Daniel Côté**, CERVO Brain Research Center, Canada
  Imaging myelin and molecules in the brain
- 15:00 **- Tomoko Ohyama**, McGill University, Canada
  A positive feedback loop between sensory and octopaminergic neurons underlies nociceptive plasticity in Drosophila larvae
- 15:30 **- Gonzalo Sanchez**, Dept. of Medical Cell Biology, Uppsala University, Suede
  GABA-induced Calcium Signaling in the Primary Cilium of Neurons
- 15:45 **- Chiara Galizia**, Institut Interdisciplinaire de Neurosciences (IINS), France
  Single particle tracking of GluA2 and NLG1 in organotypic hippocampal slices using lattice light-sheet microscopy
- 16:00 **- Théo Dudon**, Institut Interdisciplinaire de Neurosciences (IINS), France
  Mechano-dependent structural plasticity of the axon initial segment
- 16:15 **– Fériel Terras**, Phasics
  Label-free Quantitative Phase Imaging with QLSI: from intracellular dynamics to tissue organization
- 16:30 - Coffee Break
- 17:00 **- Somen Nandi**, Institut d'Optique, France
  Ultrashort Carbon Nanotubes with Luminescent Color Centers: Bright NIR-II Nanoemitters for Advanced Neurophotonics
- 17:30 **- Luke Lavis**, Janelia Research Campus, USA
  Building brighter fluorophores for advanced imaging and sensing

- **18:00 - Gala dinner, Château Carbonnieux**

Thursday 9th October

- 9:00 - Welcome Coffee
- 9:30 - **Christophe Leterrier**, NeuroCyto, France
  The axonal cytoskeleton down to the nanoscale
- 10:00 - **Rochelin Dalangin**, Centre de recherche CERVO, Canada
  Engineering a genetically encoded fluorescent sensor for D-serine
- 10:15 - **Jiesie Feng**, State Key Laboratory of Membrane Biology, Peking University School of Life Sciences, China
  A Red-Shifted NE Sensor for Multiplexed Imaging of Noradrenergic Dynamics In Vivo
- 10:30 - **Gerti Beliu**, Regensburg Center for Ultrafast Nanoscopy (RUN), Germany
  Labeling Strategies for Structurally Inaccessible Epitopes – Quantitative Mapping of Receptor Architectures in Neurons
- 10:45 - **Helge Schmidt**, Toptica
  Automated Femtosecond Fiber Delivery for Multiphoton Microscopy
- 11:00 - Coffee Break
- 11:30 - **Sabine Levi**, Laboratoire Plasticité du Cerveau, France
  Decoding GABAergic Synapse Structure and Dynamics
- 12:00 - **Florelle Domart**, Institut Interdisciplinaire de Neurosciences (IINS), France
  3D MINFLUX combined with DNA-PAINT reveals the orientation and arrangement of Bassoon at the active zone of hippocampal neurons
- 12:15 - **Jérémie Barral**, Institut de l'Audition – Institut Pasteur, France
  Fast 2-photon stimulation using holographic patterns
- 12:30 - Conclusion
- 13:00 - Lunch Box

More details

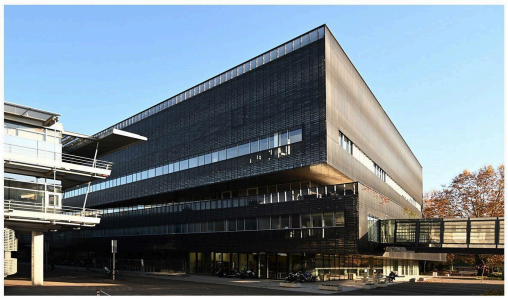
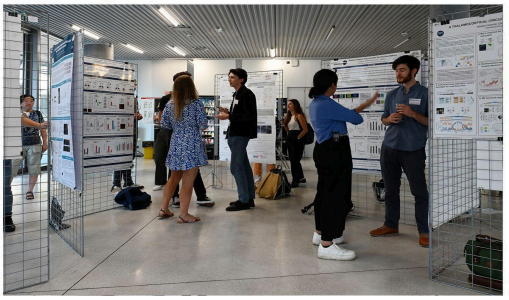
https://frontiersneurophotonics.org/2025-schedule/

Talk guidelines

Keynote: 45' + 15' Q&A
Invited speakers: 25' + 5' Q&A
Short talks: 12' + 3' Q&A

Conference venue

Broca Center (“Centre Broca Nouvelle-Aquitaine”)
Bordeaux Neurocampus
Campus Carreire - University of Bordeaux
Bordeaux, France

How to get there?

By taxi

Closest campus entrances:
40 Rue Albert Marquet

Location on Google map:
Search for “Centre Broca”
(Mind the name Broca, used for an auditorium on another Campus!)

https://goo.gl/maps/p4JE9mnJVMQ9hzz5A

By tram: Tram stop "Saint-Augustin"

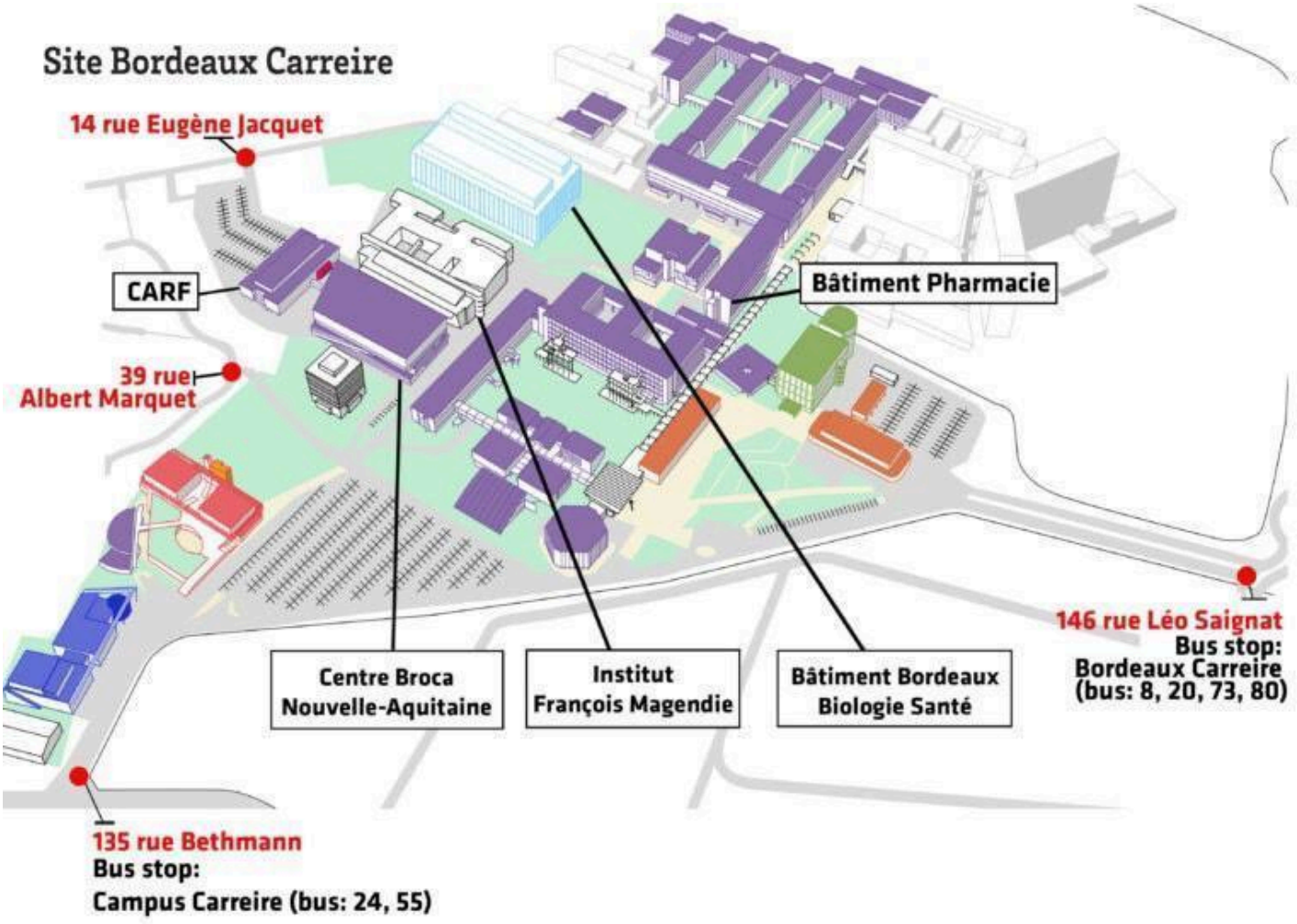
From the airport:
Take the tram A. Stop at Saint-Augustin.

From the city center:
Take the tramway line A, direction “Mérignac”, “Le Haillan”, “Pin Galant” or “Aéroport”. Stop at Saint-Augustin (15 minutes from the city center).

From the tram station “Saint Augustin”

The Broca Center is at about 8 min walk from the tramway station.
Take the street under the chimney and **follow the pedestrian white path and the arrows “Centre Broca”.**
https://goo.gl/maps/p4JE9mnJVMQ9hzz5A

The campus



Gala dinner

Wednesday 8th October

From 6:00PM till 22:00pm

A bus will transport all registered participants to the gala dinner.
Château Carbonnieux is one of the oldest and largest wine estates in the Bordeaux region.
Its history extends over almost 8 centuries and its vineyard over a hundred hectares. A bus will take all participants to the gala dinner.
Belonging to the Perrin Family since 4 generations, the whole vineyard is located around the castle at the top of a superb gravel hill.
All the wines produced depend on the Pessac-Léognan AOP.
The vines cultivated in a sustainable and eco-responsible agriculture are devoted to exclusively producing red and dry white wines, both of which are qualified as **Grands Crus Classés de Graves**.

Oral presentations

Invited speakers

Francisco Balzarotti

Research Institute of Molecular Pathology, Austria

Photon-Efficient Localization with MINFLUX Imaging and Tracking

Co-Authors

- Alessandro Passera (Research Institute of Molecular Pathology)
- Alba Gomez-Segalas (Research Institute of Molecular Pathology)
- Paul Welzl (Research Institute of Molecular Pathology)
- Juan Maya (Research Institute of Molecular Pathology)

Super-resolution fluorescence microscopy has transformed optical imaging by overcoming the diffraction barrier, providing access to the nanometer scale in biological systems. Techniques such as STED and PALM/STORM achieve remarkable resolutions by controlling fluorophore emission, yet their performance is fundamentally constrained by the finite photon budget of fluorescent probes and the maximum emission rate of single molecules. These limitations manifest as a trade-off between spatial and temporal resolution: in imaging, the attainable resolution is capped by the total number of emitted photons, while in tracking, the finite emission rate dictates how finely molecular trajectories can be sampled. The MINFLUX concept—Maximally Informative Luminescence Excitation—addresses these limitations by making each detected photon more informative. Instead of relying on the shape of the emitted fluorescence pattern, MINFLUX localizes molecules through sequential probing with structured excitation beams containing an intensity zero.

By monitoring fluorescence as this zero is displaced, the molecular position is inferred with drastically improved photon efficiency. Consequently, MINFLUX imaging and tracking attain resolutions in the range of 1–3 nm in three dimensions and follow molecular dynamics with unprecedented temporal fidelity. The approach has been extended to multicolor 3D imaging, live-cell applications, and, more recently, to multi-emitter tracking, expanding its utility for studying molecular interactions and conformational changes in complex biological environments. In this presentation, I will review the principles of MINFLUX, from its photon-statistics rationale to its beam-structuring strategies and localization algorithms, and illustrate how these innovations reshape the limits of fluorescence microscopy. I will conclude by discussing the most recent capabilities of MINFLUX in imaging and tracking.

Back to program

Abraham Beyene

Howard Hughes Medical Institute, USA

Mapping dopamine release with nanoscale precision: insights into neuromodulatory release and signaling

In this talk, I will discuss the development and application of an optical dopamine biosensor developed from functionalized graphitic nanomaterials. The tool can be flexibly deployed to measure dopamine release across spatial scales. At the level of individual varicosities, we demonstrate that the sensor can help visualize dopamine release and its dynamics from a single release site with quantal sensitivity. At the scale of neuronal processes, the biosensor enables simultaneous measurement of dopamine release from hundreds of varicosities distributed across single or multi-neuronal axonal arbors in dissociated primary neurons. Furthermore, in intact tissue, the biosensor enables mapping of dopamine release in brain slices and in vivo, revealing hotspots corresponding to putative single release sites. A significant discovery from these measurements is the observation that dopamine release is spatially organized into distinct hotspots. These hotspots arise from varicosities that are found juxtaposed against dopamine receptor-expressing processes, and exhibit synapse-like organization and ultrastructure when examined using immunofluorescence and electron microscopy. These findings suggest that neuromodulatory release sites, such as those of dopamine, share organizational principles with classical synapses, enabling precise and efficient modulation of target structures.

Back to program

Liangyi Chen

Institute of Molecular Medicine, China

COrtex-wide miniature Mesoscopic Technique (COMET) enables functional analysis of dorsal cortex networks at single-cell resolution in freely moving mice

Co-Authors

- Changliang Guo (Peking University)
- Guohua Qiu (Peking University)
- Mian Xie (Peking University)
- Xiang Liu (Peking University)
- Haocheng Long (Peking University)

To understand how distributed neuronal networks integrate sensory input, process information, and generate behavior, large-scale recording of single-cell activity across multiple cortical regions in freely moving animals is essential. Although miniaturized microscopes with genetically encoded calcium indicators allow cortical imaging in unrestrained mice, achieving single-neuron resolution across nearly the entire dorsal cortex remains challenging. Here, we present COMET (Cortex-wide Observational Miniature Mesoscopic Technique), a lightweight (3.75 g) head-mounted mesoscope capable of wide-field imaging (up to 10 mm diameter, 78.54 mm^2) at 9.3 Hz with uniform resolution (7.7–10 µm). COMET enables simultaneous recording of up to 15,000 neurons in freely behaving mice within a 30-minute session without altering natural behavior. Using a computational pipeline for denoising, signal extraction, and neuronal activity analysis, we reveal that functional connectivity for memory formation emerges within three trials during auditory trace fear conditioning. Moreover, we demonstrate that nearly the entire dorsal cortex participates in social behaviors. COMET thus provides a transformative tool for investigating large-scale neural dynamics underlying complex behaviors, opening new avenues to study distributed cortical computations in naturalistic settings.

Back to program

Rosa Cossart

Institut de Neurobiologie de la Méditerranée, France

How stable are cortical representations during development? Insights from longitudinal neuronal activity imaging in vivo

Understanding cortical circuit development requires tracking neuronal activity across days in the growing brain. While in vivo calcium imaging now enables such longitudinal studies, automated tools for reliably tracking large populations of neurons across sessions remain limited. In this talk, I will present a novel cell-tracking method based on sequential image registration, validated on calcium imaging data from the barrel cortex of mouse pups over one postnatal week. Our approach enables robust long-term analysis of several hundreds of individual neurons, allowing quantification of neuronal dynamics and representational stability over time. Using this method, we identified a key developmental transition in neuronal activity statistics, marking the emergence of arousal state modulation. Beyond this key finding, our method provides an essential tool for tracking developmental trajectories of individual neurons, which could help identify potential deviations associated with neurodevelopmental disorders.

Back to program

Daniel Côté

CERVO Brain Research Center, Quebec, Canada

Imaging myelin and molecules in the brain

Coherent Anti-Stokes Raman Scattering (CARS) microscopy is an essential label-free technique for visualizing myelinated structures in the brain, capitalizing on the strong endogenous contrast provided by the high lipid content of myelin. CARS contrast is typically tuned to the lipid-rich structures of myelin sheaths. Myelin imaging with CARS intensity allows for the precise calculation of morphometric parameters, including the g-ratio (the ratio of the axon diameter to the fiber diameter). However, standard CARS imaging suffers from excitation polarization dependence due to the highly organized orientation of the lipid membranes within the myelin sheath, affecting image intensity and complicating accurate quantitative analysis. We have used the technique to identify differences in myelination between groups who died by suicide following early life adversity and control groups.
We have also used a spectroscopic form of coherent Raman specifically designed for Deep Brain Stimulation (DBS) surgery guidance. Proof-of-concept studies using a small form factor probe (250 μm outer diameter) integrated within a DBS electrode demonstrated high-resolution WM/GM delineation in intact nonhuman primate brain tissue, achieving low-resolution high wavenumber (HWN) spectra acquisitions in as little as 10 ms. Most recently, this spectroscopic approach was successfully deployed to acquire CARS spectra inside the brain of a fresh human cadaver during a simulated DBS procedure. Using a custom optical probe integrated into a commercial DBS lead, CARS measurements differentiated White Matter (WM) and Gray Matter (GM) along the insertion trajectory, showing an 82% correspondence with histological classification.

Back to program

Johann Danzl

Institute of Science and Technology Austria, Austria

Reconstructing brain tissue at synaptic resolution with light microscopy

Co-Authors

- Mojtaba R Tavakoli (Institute of Science and Technology Austria, Klosterneuburg, Austria)
- Julia Lyudchik (Institute of Science and Technology Austria, Klosterneuburg, Austria)
- Michał Januszewski (Google Research, Zürich, Switzerland)
- Vitali Vistunou (Institute of Science and Technology Austria, Klosterneuburg, Austria)
- Nathalie Agudelo Dueñas (Institute of Science and Technology Austria, Klosterneuburg, Austria)
- Jakob Vorlaufer (Institute of Science and Technology Austria, Klosterneuburg, Austria)
- Christoph Sommer (Institute of Science and Technology Austria, Klosterneuburg, Austria)
- Caroline Kreuzinger (Institute of Science and Technology Austria, Klosterneuburg, Austria)
- Bárbara Oliveira (Institute of Science and Technology Austria, Klosterneuburg, Austria)
- Alban Cenameri (Institute of Science and Technology Austria, Klosterneuburg, Austria)
- Gaia Novarino (Institute of Science and Technology Austria, Klosterneuburg, Austria)
- Viren Jain (Google Research, Mountain View, CA, USA)

Reconstructing the cellular architecture of brain tissue at the level of individual synaptic connections requires nanometer scale resolution, comprehensive structural contrast, and imaging across spatial scales. Volume electron microscopy has been the technological basis for reconstructing connectomes in diverse organisms, but obtaining molecular information is challenging. Light microscopy provides major opportunities for integrating molecular and functional information into neuronal circuit reconstructions, but has been limited to reconstructing sparse subsets of cells.

Here, I will discuss our recent development of light microscopy based connectomics (LICONN) [Tavakoli *et al*., Nature (2025)]. LICONN is based on a specifically engineered hydrogel expansion technology that provides the resolution, signal-to-noise-ratio and volumetric imaging capabilities required for tracing even the thinnest neuronal structures and densely reconstructing cellular tissue architecture at synaptic resolution using deep-learning methods. LICONN is directly compatible with labeling and multicolour imaging of specific molecules while imaging can proceed with standard off-the-shelf microscopes, facilitating adoption of the technology.

Back to program

Valentina Emiliani

Vision Institute, Sorbonne University, France

All-optical circuits manipulation in head-restrained and freely moving mice

The genetic targeting of neuronal cells with activity reporters, such as calcium or voltage indicators, has driven a paradigmatic shift in neuroscience, where photons have replaced electrons to monitor large-scale brain activity at cellular resolution. In parallel, optogenetics has demonstrated that targeting neurons with photosensitive microbial opsins enables the transduction of photons into excitatory or inhibitory electrical currents, allowing minimally invasive activation or silencing of neuronal signals. The combination of these approaches opens the way to all-optical interrogation of circuits, providing unique opportunities to study complex neuronal dynamics with minimal invasiveness. Achieving all-optical circuit manipulation at single-cell resolution in scattering tissue has, in turn, spurred the development of sophisticated wavefront-shaping strategies. When coupled with multiphoton excitation, these techniques enable in vivo control of neuronal circuits with high spatial and temporal precision across large brain volumes[1] In this presentation, we will highlight our most recent advances in pushing the frontiers of all-optical circuit manipulation. In particular, we will show how genetically encoded voltage indicators compatible with two-photon excitation can increase the precision of neuronal activity readout, with example experiments demonstrating in vivo multi-target holographic voltage imaging in the mouse brain and zebrafish larvae [2,3].We will also present a newly developed holographic two-photon endoscope that enables calcium imaging at up to 80 Hz, together with multi-target excitation, in both superficial and deep brain regions of freely moving mice.

1. V. Emiliani, E. Entcheva, R. Hedrich, P. Hegemann, K. R. Konrad, C. Lüscher, M. Mahn, Z. H. Pan, R. R. Sims, J. Vierock, and O. Yizhar, Nat. Rev. Methods Primer 2, 55 (2022).

2. R. R. Sims, I. Bendifallah, C. Grimm, A. S. M. Lafirdeen, S. Domínguez, C. Y. Chan, X. Lu, B. C. Forget, F. St-Pierre, E. Papagiakoumou, and V. Emiliani, Nat. Commun. 15, 5095 (2024).

3. C. Grimm, R. R. Sims, D. Tanese, A. S. Mohamed Lafirdeen, C. Y. Chan, G. Faini, E. Putti, F. Del Bene, E. Papagiakoumou, and V. Emiliani, (2024).

Back to program

Linlin Fan

The Picower Institute for Learning and Memory, USA

All-optical physiology reveals synaptic bases for learning and memory in behaving mammals

Co-Authors

- Doo Kyung Kim ((The Picower Institute for Learning and Memory, USA)
- Joshua Jennings (The Picower Institute for Learning and Memory, USA)
- He Tian (Department of Chemistry and Chemical Biology, Harvard University, USA)
- Peter Wang (The Picower Institute for Learning and Memory, USA)
- Charu Ramakrishnan (The Picower Institute for Learning and Memory, USA)
- Sawyer Randles (The Picower Institute for Learning and Memory, USA)
- Yanjun Sun (Department of Neurobiology, Stanford University, USA)
- Elina Thadhani (The Picower Institute for Learning and Memory, USA)
- Yoon Seok Kim (The Picower Institute for Learning and Memory, USA)
- Sean Quirin (The Picower Institute for Learning and Memory, USA)
- Lisa Giocomo (Department of Neurobiology, Stanford University, USA)
- Adam Cohen (Department of Chemistry and Chemical Biology, Department of Physics, Harvard University, USA)
- Karl Deisseroth (Department of Bioengineering, Department of Psychiatry and Behavioral Sciences, Stanford University, Howard Hughes Medical Institute, USA)

The ability of the brain to learn from and remember experiences lies at the heart of our existence and individuality. Learning has been associated with modifications of synaptic and circuit properties, but the precise changes storing information in mammals have remained largely unclear. Addressing these questions requires the ability to measure and manipulate synaptic transmission and plasticity during behavior.
In this talk, I will present our progress in developing and applying all-optical physiology techniques to track synaptic plasticity in awake animals during association formation. By combining genetically targeted voltage imaging with targeted optogenetic activation and silencing of pre- and post-synaptic neurons, this approach offers the required accessibility, temporal resolution, and subthreshold precision, enabling optical probing of synaptic function between identified cells with millisecond and millivolt precision.
Using these cellular-resolution all-optical interrogation, we have identified the synaptic implementation of hippocampal behavioral timescale plasticity and depolarization-induced suppression of inhibition (DSI) in vivo. These results define a methodology to resolve synaptic plasticity during learning and memory in behaving mammals. More broadly, the all-optical tools will provide opportunities to establish direct and causal links between synaptic properties, circuit dynamics, and behavior with unparalleled spatial, temporal, and subthreshold resolution.

Back to program

Angela Getz

Vrije Universiteit Amsterdam, Center for Neurogenomics and Cognitive Research, The Netherlands

A pipeline for single molecule imaging of endogenous synaptic proteins in brain tissue

Co-Authors

- Maxime Malivert (Functional Genomics Department, Center for Neurogenomics and Cognitive Research, Vrije Universiteit Amsterdam, The Netherlands), (Bordeaux Imaging Center, Université de Bordeaux, France)
- Magali Mondin (Bordeaux Imaging Center, Université de Bordeaux, France)
- Chiara Galizia (Interdisciplinary Institute for Neuroscience, Université de Bordeaux, France)
- Bram A.A.J.W. Willems (Functional Genomics Department, Center for Neurogenomics and Cognitive Research, Vrije Universiteit Amsterdam, The Netherlands)
- Dario Crippa (Functional Genomics Department, Center for Neurogenomics and Cognitive Research, Vrije Universiteit Amsterdam, The Netherlands)
- Blanca Martín Muñoz (Interdisciplinary Institute for Neuroscience, Université de Bordeaux, France)
- Cécile Lemoigne (Interdisciplinary Institute for Neuroscience, Université de Bordeaux, France)
- Matthieu Sainlos (Interdisciplinary Institute for Neuroscience, Université de Bordeaux, France)
- Daniel Choquet (Bordeaux Imaging Center, Université de Bordeaux, France), (Interdisciplinary Institute for Neuroscience CNRS UMR 5297, Université de Bordeaux, France)
- Mathieu Ducros (Bordeaux Imaging Center, Université de Bordeaux, France)

The number and dynamic nanoscale organization of synaptic AMPA receptors defines synaptic transmission efficacy and is regulated by a variety of activity-dependent plasticity mechanisms. Visualizing these receptor trafficking and clustering processes at high-resolution – at the level of endogenous receptors in intact synaptic networks – is a key remaining challenge to understand how organizational diversity defines synapse-type-specific function, how this is remodelled during *bona fide* synaptic plasticity, and how these processes are disrupted in synaptopathies. A major technical hurdle has been the development of effective molecular labeling strategies and imaging technologies. We have recently reported a new molecular toolkit for high-resolution imaging of endogenous GluA2-containing AMPA receptors in integrated slice models. This is based on genetic knock-in of the biotin acceptor peptide tag, molecular strategies for target-specific biotin ligase expression, and small high-affinity imaging probes based on avidin family biotin binding proteins. Here, we have further engineered and optimized avidin-based imaging probes to develop superresolution imaging of endogenous AMPAR nanoscale organization and live diffusion-trapping processes. To achieve high-speed nanometric scale localization precision for imaging inside brain slices, we implemented single molecule localization microscopy (SMLM) techniques, including DNA-PAINT and single-particle tracking, with lattice light-sheet microscopy (LLSM). We developed an active image optimization (AIO) method and implemented adaptive optics (AO) with LLSM. Controlled PSF shaping with the deformable mirror of our AO-LLSM allows 3D reconstructions that capture the entire dendritic spine profile in a single acquisition. These advanced imaging techniques with improved spatiotemporal resolution will allow more precise exploration of the molecular mechanisms underlying synaptic diversity and plasticity in integrated networks.

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Ralf Jungmann

Max Planck Institute of Biochemistry, Germany

From DNA Nanotechnology to Biomedical Insight: Towards Single-Molecule Spatial Omics

Super-resolution fluorescence microscopy is a powerful tool for biophysical and biological research. The transient binding of short fluorescently labeled oligonucleotides (DNA-PAINT) can be leveraged for easy-to-implement multiplexed super-resolution imaging that achieves molecular-scale resolution across large fields of view. This seminar will introduce recent technical advancements in DNA-PAINT including approaches that achieve sub-10-nm spatial resolution and spectrally unlimited multiplexing in whole cells followed by recent developments in novel protein labeling probes that have the potential to facilitate DNA-barcoded labeling of much of the proteome within intact cellular environments. Applications of these new approaches will be discussed in cell surface receptor imaging and neuroscience. Visualization and quantification of cell surface receptors at thus far elusive spatial resolutions and levels of multiplexing yield fundamental insights into the molecular architecture of surface receptor interactions thus enabling the future development of more refined “pattern”-based therapeutics. A key approach in implementing these methods has been to leverage standard off-the-shelf fluorescence microscopy hardware as a tool for spatial omics, thus democratizing the ability to visualize most biomolecules and probe their network-wide interactions in single cells, tissues, and beyond with single-molecule-based "Localizomics”.

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Luke Lavis

Janelia Research Campus, USA

Building brighter fluorophores for advanced imaging and sensing

Specific labeling of biomolecules with bright, photostable fluorophores is the keystone of fluorescence microscopy. An expanding method to label cellular components utilizes genetically encoded self-labeling tags, which enable the attachment of chemical fluorophores to specific proteins inside living cells. This strategy combines the genetic specificity of fluorescent proteins with the favorable photophysics of synthetic dyes. Over the past decade, our laboratory has pioneered novel organic chemistry methodologies for synthesizing small-molecule fluorophores. Through this work, we have elucidated structure–activity relationships, culminating in the development of dyes with enhanced quantum efficiency and photostability across the entire visible spectrum. These fluorophores seamlessly integrate with self-labeling tags, facilitating sophisticated microscopy techniques, such as single-molecule and super-resolution modalities. Our chemistry also enables further modifications to fine-tune spectral and chemical properties for advanced experiments in increasingly complex biological samples such as functional imaging in intact organisms.

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Christophe Leterrier

CNRS-Aix Marseille University, France

The axonal cytoskeleton down to the nanoscale

The intricate arborization and molecular identity of axons is maintained for decades, but must also continuously adapt to changes in the environment and modulate the activity of neurons. Axons fulfill these paradoxical demands thanks to a unique cytoskeletal organization that ensures the coordinated transport, anchoring and assembly of axonal components. In our lab, we use super-resolution microscopy to delineate and map the nanoscale architecture of cytoskeletal structures within the axon: the periodic actin/spectrin submembrane scaffold, presynaptic actin assemblies, clathrin-coated pits, microtubule bundles. We are exploring their molecular organization and functions by combining versatile labeling approaches, correlative live-cell / super-resolution / electron microscopy and quantitative analysis that allow for high-content, nanoscale interrogation of the axonal architecture.

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Sabine Lévi

Laboratoire Plasticité du Cerveau, France

Decoding GABAergic Synapse Structure and Dynamics

Our team investigate the cellular and molecular mechanisms underlying synapse formation, maturation, and pruning during development, as well as the regulatory processes driving the plasticity and activity-dependent remodeling of mature synapses. Our work primarily focuses on inhibitory GABAergic synapses within the hippocampus.

Our experimental strategy spans multiple scales, from single-molecule analyses to behavioral phenotyping. At the cellular level, we dissect molecular pathways in primary hippocampal neuron cultures through pharmacological and genetic perturbations, combined with high-resolution live-cell imaging. Advanced single-molecule approaches, including Quantum Dot-based Single Particle Tracking (QD-SPT) and super-resolution nanoscopy (STORM/PALM), enable the quantitative mapping of membrane dynamics and the spatial organization of synaptic nanodomains that orchestrate receptor distribution, signaling cascades, and synaptic efficacy.

To establish the in vivo relevance of these mechanisms, we employ pharmacological manipulations, stereotaxic delivery of AAV vectors encoding mutant proteins or shRNAs, and genetically engineered mouse models. Synaptic and neuroglial interactions are analyzed through high-resolution 3D imaging and computational pipelines (Metamorph, Imaris), while behavioral assays probe how nanoscale remodeling of synaptic nanodomains influences hippocampus-dependent processes such as memory, social behaviors, anxiety, and susceptibility to epileptogenic activity.

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Marina Mikhaylova

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Synaptic tethering of microtubule minus-ends by SHANK3 and CAMSAP2 shapes dendrites in parvalbumin neurons

The microtubule cytoskeleton plays an essential role in establishing and maintaining neuronal polarity. In neurons, microtubules are initially generated at the centrosome which gradually loses this role in development. Non-centrosomal microtubules are stabilised by the microtubule minus end-binding protein CAMSAP2. Nonetheless, how and where microtubule minus-ends are anchored in dendrites of mammalian neurons remains an open question. Here, I am going to speak about our recent work where we show that dendritic microtubules are directly attached to the postsynaptic density of excitatory synapses through binding of CAMSAP2 to the scaffolding protein SHANK3. This process is particularly relevant for parvalbumin-positive GABAergic neurons in which excitatory synapses are located directly on the dendritic shaft. This association is furthermore strongly enhanced in the Autism Spectrum Disorder (ASD)-associated SHANK3L68P mutation which leads to an increase of synaptic CAMSAP2 levels. Increased CAMSAP2 presence at the synapse results in greater microtubule association with excitatory shaft synapses, altered microtubule dynamics, and a simplified dendritic tree in parvalbumin neurons, potentially contributing to ASD pathology.

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Tomoko Ohyama

McGill University, Canada

A positive feedback loop between sensory and octopaminergic neurons underlies nociceptive plasticity in Drosophila larvae

Co-Authors

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- Sophia Q. Zhao (McGill University)

Adaptive modulation of nociceptive behavior based on prior experience is essential for effectively responding to environmental threats. In Drosophila larvae, nociceptive escape behaviors are typically robust and stereotyped; however, emerging evidence suggests that these responses can be modulated by experience and internal state. Here, we demonstrate that repeated optogenetic activation of nociceptive sensory neurons enhances both the likelihood and intensity of nocifensive rolling, reflecting a form of behavioral sensitization. Using multiphoton imaging combined with optogenetic stimulation, we found that this heightened responsiveness is accompanied by a sustained increase in activity within nociceptive sensory neurons, indicating that plasticity arises, at least in part, within the sensory compartment.

We identified the neuromodulator octopamine—functionally analogous to norepinephrine in vertebrates—as a critical regulator of this sensitization. Signaling through the octopamine receptor OAMB in nociceptive neurons is required to maintain elevated nociceptive gain, and feedback from a specific class of octopaminergic neurons, the ventral unpaired median (VUM) neurons, amplifies sensory neuron output. Furthermore, using a GPCR activation-based octopamine sensor (GRAB-OA), we observed increased octopamine signalling in nociceptive neurons following repeated stimulation.

Together, these findings reveal an experience-dependent positive feedback loop in the nociceptive system, in which neuromodulatory circuits dynamically tune behavioral output based on prior experience.

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Dmitri Rusakov

UCL Queen Square Institute of Neurology, UK

Nano-diffusion in the brain monitored with time-resolved fluorescence anisotropy imaging

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The majority of electrical and molecular interactions in the brain, both inside and outside cells, are mediated by the rapid diffusion of small molecules such as ions and neurotransmitters. Because these interactions often take place within nanoscopic spaces, their timing is largely governed by the quasi-instantaneous diffusivity of such molecules, a property that can be altered in pathological states. For example, the spatiotemporal activation profile of neuronal receptors is shaped by the interstitial mobility of their ligands, both within and beyond the synaptic cleft. Likewise, spike propagation and neuronal firing depend on fast macromolecular movements, meaning that local medium viscosity could influence the gating of spike-driving sodium channels.

To understand of these processes, we have developed time-resolved fluorescence anisotropy imaging (TR-FAIM), a technique that enables direct monitoring of nanoscale diffusivity of small molecules inside and outside brain cells in living brain slices. More recently, we have adapted TR-FAIM for in vivo measurements in the intact mouse brain, applying it under a sensory stimulation paradigm. This talk will present and discuss our ongoing progress in mapping and understanding nano-diffusion dynamics in the brain.

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Markus Sauer

Universität Würzburg, Germany

Molecular resolution fluorescence imaging in cells

Over the past decade, super-resolution fluorescence imaging by single-molecule localization has evolved as a powerful method for subdiffraction-resolution fluorescence
imaging of cells and structural investigations of subcellular structures. However, although refined single-molecule localization microscopy (SMLM) methods can now provide a spatial resolution in the one-digit nanometer range on isolated molecules, that is, well below the diffraction limit of light microscopy, translation of such high spatial resolutions to sub-10 nm imaging in cells or tissues remains challenging. This is mainly caused by the insufficient labeling density and linkage error achieved using standard labeling methods. Furthermore, even if high density labeling can be realized fluorophore communication via different energy pathways can prevent reliable molecular resolution fluorescence imaging in cells. In my contribution I will introduce and discuss different methods to bypass these limitations. One is based on physical expansion of the cellular structure by linking a protein of interest into a dense, cross-linked network of a swellable polyelectrolyte hydrogel. By combining ~8-fold Expansion Microscopy (ExM) with direct stochastic optical reconstruction microscopy (dSTORM) on post-expansion immunolabeled samples we resolve the 8-nm periodicity of a,ß-heterodimers in microtubules and the polyhedral lattice in clathrin-coated pits with nanometer resolution in intact cells. Furthermore, I will demonstrate that 2-color Ex-dSTORM reveals the molecular organization of endogeneous RIM scaffolding proteins and Munc13-1, an essential synaptic vesicle priming protein, in ring-like structures with diameters of 20-30 nm at the presynapse in hippocampal neurons.
Furthermore, I will discuss an alternative approach that uses genetic code expansion (GCE) and click labeling of unnatural amino acids to introduce fluorophores site-specifically into multimeric proteins with minimal linkage error. Using resonance energy transfer between fluorophores separated by less than 10 nm, information about the distance of the fluorophores in cells separated by only a few nanometers can be unraveled using fluorescence photoswitching characteristics. Using time-resolved fluorescence detection in combination with this so-called photoswitching fingerprint analysis interfluorophore distances of only a few nanometers can be reliably resolved, even in living cells. Finally, I will demonstrate that the use of these tools in combination with fixed and live-cell lattice-light-sheet microscopy can be used advantageously to decode the molecular interplay of endogenous CD20 on tumor cells with therapeutic antibodies.

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Ali Shaib

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Democratizing nanoscale imaging at molecular resolution

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The structural visualization of individual proteins has traditionally relied on high-end techniques such as cryo-electron microscopy, which remain inaccessible to most laboratories due to cost, complexity, and technical barriers. One-step Nanoscale Expansion (ONE) microscopy offers a practical alternative, enabling near-molecular resolution imaging using standard fluorescence microscopes. By physically expanding biological specimens approximately 1000-fold in volume and labeling them directly with conventional fluorophores, ONE microscopy reconstructs protein morphologies through fluorescence fluctuation analysis. The method achieves resolutions of around 1 nanometer or better, allowing direct visualization of membrane-associated protein conformations as well as the shapes of soluble purified proteins across a variety of biological environments. ONE microscopy also proves valuable in clinical contexts. In cerebrospinal fluid from individuals with Parkinson's disease, it resolves the ultrastructure of protein aggregates relevant to pathology. More recently, the technique revealed direct contacts between small cell lung cancer cells and nodose ganglion neurons, suggesting functional hijacking of the glutamatergic pathway and pointing to a possible mechanism of neurovisceral interaction in cancer. By using standard instrumentation and accessible reagents, ONE microscopy actively moves forward the effort to democratize high-resolution structural imaging. It challenges the exclusivity of existing structural methods and opens molecular-scale analysis to a broader scientific community, aligning technological advancement with accessibility and equity in research.

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Kate Smith (CANCELD!)

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Release your inhibitions: understanding the nanoarchitecture of GABAergic inhibitory synapses

GABAergic inhibitory synaptic transmission is crucial for regulating circuit function and plasticity, essential processes for learning and cognition. In the central nervous system, synaptic inhibition is mediated by GABAA receptors (GABAARs), hetero-pentameric ligand-gated ion channels that are activated by GABA and clustered at the inhibitory post-synaptic domain iPSD. The number of GABAARs clustered at the iPSD is a major driver of synaptic strength and this clustering changes during plasticity and pathology to increase or reduce synaptic efficacy. Here we combine multiple super-resolution microscopy approaches to investigate novel nanoscale subsynaptic mechanisms that contribute to the diversity and plasticity of inhibitory synaptic structure and function.

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Ilaria Testa

Department of Applied Physics at the School of Engineering Science, Sweden

Dynamics imaging of proteins at the nanoscale empowered by computation

Capturing the dynamic interplay of proteins and organelles within synapses requires microscopy methods with high spatial and temporal resolution. STED and RESOLFT optical nanoscopy demonstrated great potential for live cell recording by using patterned light and reversible photo-switching. Despite the latest improvement in both switching probes and optical schemes, the length and speed of the time-lapse is still limited by photobleaching and sampling. Here, we present two new approaches based on deep learning to restore low-SNR or under-sampled images, which pushes the experimental boundaries of RESOLFT nanoscopy achieving up to 5 times longer imaging with 10 times lower dose of light per frame, or a 4-fold increase in imaging speed. The increased speed and length of time lapses enable to follow vesicles budding in and out from intact mitochondria, as well the actin meshwork formation and dissociations opening up new possibility for dynamic data acquisition at the sub-organelle level.

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Oral presentations

Short talks

Jérémie Barral

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Fast 2-photon stimulation using holographic patterns

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Two decades after its introduction, optogenetics – a biological technique to control the activity of neurons or other cell types with light – remains a cutting edge and promising tool to study biological processes. Its increasing usage in research varies widely from causally exploring biological mechanisms and neural computations, to neurostimulation and sensory restauration. To stimulate neurons in the brain, a variety of approaches have been developed to generate precise spatiotemporal light patterns. Yet certain constrains still exists in the current optical techniques to activate a neuronal population with both cellular resolution and millisecond precision. Here, we describe an experimental setup allowing to stimulate a few tens of neurons in a plane at sub-millisecond rates using 2-photon activation. A liquid crystal on silicon spatial light modulator (LCoS-SLM) was used to generate spatial patterns in 2 dimensions. The image of the patterns was formed on the plane of a digital micromirror device (DMD) that was used as a fast temporal modulator of each region of interest. Using fluorescent microscopy and patch-clamp recording of neurons in culture expressing the light-gated ion channels, we characterized the temporal and spatial resolution of the microscope. We described the advantages of combining the LCoS-SLM with the DMD to maximize the temporal precision, modulate the illumination amplitude, and reduce background activation. Finally, we showed that this approach can be extended to patterns in 3 dimensions. We concluded that the methodology is well suited to address important questions about the role of temporal information in neuronal coding.

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Gerti Beliu

Regensburg Center for Ultrafast Nanoscopy (RUN), Germany

Labeling Strategies for Structurally Inaccessible Epitopes - Quantitative Mapping of Receptor Architectures in Neurons

High-resolution optical visualization of neuronal proteins requires strategies that allow for precise labeling of defined sites under native conditions. Yet many targets, including receptor subunits and auxiliary proteins, harbor extracellular epitopes that are structurally inaccessible, conformationally regulated, or incompatible with standard tagging. As a result, conventional labeling often fails to resolve features critical for receptor organization and function.

My research addresses this limitation through chemical biology–based strategies tailored to constrained proteins in live cells. By combining genetic code expansion with fluorogenic bioorthogonal labeling, we enable site-specific access to previously inaccessible epitopes in their native conformational context, without perturbing domain architecture or membrane topology [1–4]. To support quantitative structural readouts, we have developed protein-based nanorulers (Picorulers) that act as internally calibrated reference systems [5]. These allow for benchmarking of labeling efficiency and nanoscale distance (sub 10 nm) measurements directly in the imaging context [6].

Together, these strategies establish a modular framework for mapping structural accessibility and nanoscale organization of neuronal proteins in living systems.

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Ivo Calaresu

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Extracellular matrix tunes the diffusion of pathological immunoglobulins

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Effective neuronal communication and network function rely on the coordinated diffusion of molecules across varying distances within extracellular spaces (ECS). Our comprehension of spatial and temporal dynamics underlying these processes is critical for understanding physiological and pathological processes. Recent studies emphasize the extracellular matrix (ECM) as a complex and dynamic barrier that regulates the mobility of circulating molecules. We propose here a framework of advanced imaging techniques to address diffusion processes from the macro to the nanoscale in living brain slices. First, we exemplify brain ECS heterogeneity studying the fine-tuned rheology of the developing hippocampus. Transcriptomic screening of ECM protein genes led us to identify area-specific differences. By adenoviral transgene delivery in organotypic slices we modified ECM in order to abolish rheological disparities between hippocampal CA1 and CA3 subfields. Single-particle tracking (SPT) upon ECM manipulation confirmed the possibility to locally reduce macromolecular diffusion. Using multi electrode array, we performed extracellular field potential recordings on organotypic hippocampal slices exposed to pathological immunoglobulins. Through hindered diffusion, ECM manipulation successfully mitigated area-specific burden resulting from pathological macromolecules exposure.

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Rochelin Dalangin

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Engineering a genetically encoded fluorescent sensor for D-serine

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The last two decades have seen a growing interest in the role of D-amino acids within the nervous system. In particular, D-serine is now recognized as a key neuromodulator in its role as a more potent co-agonist than glycine for N-methyl-D-aspartate receptors (NMDARs), which are widely recognized as the key receptor responsible for synaptic plasticity. It has also been historically considered as a key molecule released by astrocytes in the tripartite model of the synapse. Accordingly, aberrations in D-serine signalling have been consistently associated with several pathological conditions, including schizophrenia, Alzheimer's disease, and epilepsy. However, despite our understanding of D-serine's role in the nervous system, the molecular mechanisms that govern its dynamics remain unclear, with recent works challenging its role as a gliotransmitter. Thus, a more thorough understanding of D-serine dynamics is necessary to properly understand its role in both healthy and disease states. To address these gaps in knowledge, tools with the requisite spatiotemporal resolution, such as genetically encoded fluorescent protein-based indicators, are necessary to monitor D-serine dynamics. To date, the only genetically encoded indicator for D-serine is a FRET-based indicator, called DserFS, based on a bacterial periplasmic binding protein (PBP) that was computationally redesigned to bind D-serine. PBPs are ideal scaffolds for sensor engineering because they are orthogonal to neurons, offer large changes in fluorescence in response to ligand binding, and can be targeted to arbitrary cellular compartments. However, DserFS shows a limited dynamic range relative to single fluorescent protein-based indicators and requires exogenous addition of purified protein for imaging in brain slices, significantly limiting its potential use in vivo. Here we present our work on engineering a genetically encoded single fluorescent protein-based indicator for D-serine using the redesigned PBP from DserFS. Indeed, our results indicate that our D-serine sensor is bright, shows large fluorescence changes in the presence of D-serine, and has good membrane localization. We anticipate that our new D-serine indicator will open new avenues for investigating D-serine dynamics within the nervous system.

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Florelle Domart

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3D MINFLUX combined with DNA-PAINT reveals the orientation and arrangement of Bassoon at the active zone of hippocampal neurons

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Neurotransmitter release and membrane retrieval occur locally inside presynaptic terminals. These processes require precise spatial and temporal coordination relying on an intricate molecular machinery constituting the presynaptic active zone. However, the exact nano structural organization of this machinery is not yet fully elucidated. Scaffolding proteins like Bassoon are suggested to extend as filamentous structures from the presynaptic plasma membrane into the synaptic terminal, and synaptic vesicles are positioned both within and outside the network formed by these filaments. This raises questions as to whether these scaffolding structures exhibit static or dynamic properties, necessitating a more detailed understanding of the nanoscale organization of the active zone machinery. To investigate the nanoscale organization of the active zone super-resolution techniques like STED and STORM microscopy have been used. However, these techniques have limitatons, especially in their 3D spatial resolution capacity. Compared to these techniques, 3D MINFLUX microscopy offers superior 3D precision of localization below 10 nm, allowing one to refine the organization of the active zone machinery. In this study we used 3D MINFLUX combined with DNA-PAINT to analyze the positioning and orientation of the scaffolding protein Bassoon in presynaptic terminals of glutamatergic synapses from hippocampal neurons, achieving a localization precision of 5 nm. This approach allowed us to spatially resolve the N-terminal and the C-terminal region of Bassoon and confirm and refine the notion that Bassoon exhibits a specific orientation at the active zone, with the C-terminal region directed toward the synaptic cleft and the N-terminal region positioned toward synaptic vesicles. Moreover, the location of individual Bassoon molecules was determined in 3D at 5 nm resolution, yielding an unprecedented image of the distribution of Bassoon at the active zone.

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Charles Ducrot

Institut Interdisciplinaire de Neurosciences (IINS), France

Investigating the nanoscale localization of synaptic proteins by dual photon-electron microscopy using engineered fluoro-nanogold probes

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To meet the constantly improving spatial resolution offered by advanced microscopy techniques, there is a need for small probes that label endogenous proteins of interest with high specificity, minimal distance to target, strong penetration in restricted cellular compartments, and compatible with both photonic and electron microscopy (EM). This is particularly true for studying synapses, tight neuronal connections bridged by adhesion complexes such as neurexins (NRXNs) and neuroligins (NLGNs).

In this context, a recently developed knock-in mouse model expressing N-terminally tagged neuroligin-1 (bAP-NLGN1) enabled the cell-specific biotinylation and selective isolation and imaging of endogenous NLGN1 using streptavidin conjugates, without overexpression (Ducrot et al., PNAS 2025). This approach revealed that NLGN1 is not limited to excitatory synapses, as previously believed, but also present at inhibitory synapses. Furthermore, super-resolution imaging and electron microscopy (EM) highlight that bAP-NLGN1 forms in the synaptic cleft a subset of nanodomains, which contain each a few NLGN1 dimers and whose number positively scales with the post-synapse size.

To allow for the orthogonal labeling of different synaptic proteins, for instance GFP-NRXN1β and bAP-NLGN1, we developed and characterized a small GFP nanobody (NB)-gold conjugate compatible with both fluorescence and EM, and capable of efficiently accessing confined synaptic spaces. Accessible lysine residues on the GFP NB were mutated such that only one gold nanoparticle of 1.4 nm was attached to the N-terminal primary amine through NHS reaction. Meanwhile, an α-helix tag was fused the GFP-NB C-terminus, allowing its subsequent recognition with a second nanobody anti ALFA-tag conjugated to an Alexa dye. Live cells expressing GFP-tagged proteins (NRXN1β or AMPA receptor subunits) were labelled with the GFP-NB-gold and the Alexa Fluor-conjugated anti-ALFA nanobody. After fixation, gold particles were silver-enhanced, and samples were imaged by epifluorescence and dSTORM before processing for EM. The NB-gold showed high specificity for GFP-tagged proteins, with excellent penetration in tight membrane environments, including synaptic contacts, where individual gold particles could be resolved and counted.

This novel monovalent probe represents a powerful and versatile tool for labelling the extensive repertoire of GFP-fusion proteins at the nanoscale level, bridging super-resolution and ultrastructural imaging.

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Théo Dudon

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Mechano-dependent structural plasticity of the axon initial segment

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Axons are subjected to mechanical forces not only during limb movement in the peripheral nervous system but also within the brain, where they interact with surrounding mobile structures like dendritic spines and glial cells, as well as components with varying stiffness, such as the extracellular matrix. In axons, spectrin-actin forms the periodic-membrane-skeleton (MPS), only revealed by super-resolution. The MPS's periodic architecture and composition exhibit features of a mechanosensitive structure. αβ-spectrin tetramers can unfold under force and the MPS is composed of adhesion, actin-binding and signaling proteins, some identified as mechano-sensor in non-neuronal cells. However, direct evidence of MPS mechanosensing is still lacking.

MPS composition differs from the distal axon to its initial segment (AIS), leading to the recruitment of different scaffolding and signaling proteins suggesting distinct mechanosensing. In this context, my thesis focuses on investigating the mechanical response of the MPS within the AIS, a domain essential for maintaining neuronal polarity and initiating action potentials.

Here, I will present our findings on the characteristics of the AIS in neurons cultured on substrates of varying stiffness. I will also describe the analysis workflow we developed to semi-automatically quantify AIS morphology, including its size and position along the axon. Overall, our study provides novel insights into how mechanical cues influence the organization of the AIS, highlighting its potential role as a neuronal mechanosensor.

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Jiesi Feng

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A Red-Shifted NE Sensor for Multiplexed Imaging of Noradrenergic Dynamics In Vivo

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Norepinephrine (NE) is a key neuromodulator involved in regulating arousal, stress response, mood, and cardiovascular function. Green fluorescent GRAB-NE sensors have enabled high-resolution visualization of NE dynamics in vivo. However, to dissect the complex interplay between NE and other signaling molecules, there is a growing need for spectrally distinct NE sensors that allow multiplexed imaging. To meet this demand, we developed rGRAB-NE2.0 (rNE2.0), a red-shifted, genetically encoded NE sensor based on the GPCR activation-based (GRAB) strategy. rNE2.0 exhibits a strong fluorescence response ($\Delta F/F_0$ ~600%), nanomolar sensitivity (~40 nM), fast sub-second kinetics, and minimal downstream signaling. Importantly, rNE2.0 shows high specificity for NE, with negligible cross-reactivity to structurally similar dopamine. In vivo, rNE2.0 reliably reports endogenous NE release in the lateral hypothalamus during tail suspension. To ensure compatibility with optogenetic tools and GFP-based indicators, we eliminated blue-light–induced photoswitching artifacts, resulting in a stable and versatile sensor suitable for multi-color imaging. Together, these advances make rNE2.0 a valuable tool for dissecting noradrenergic activities in both physiological and pathological contexts.

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Chiara Galizia

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Single particle tracking of GluA2 and NLG1 in hippocampal slices using lattice light-sheet microscopy

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The reversible diffusion-trapping of synaptic proteins, including receptors, plays a key role in synaptic plasticity and function. While numerous studies have investigated receptor dynamics, most of them have been conducted in dissociated neuronal cultures, which fail to fully recapitulate the complex physiological environment of the brain. To better understand how receptor trafficking contributes to physiological synaptic function, it is essential to study protein dynamics in brain tissue and correlate them with neuronal activity. Therefore, we aim to track these proteins using a small, minimally interfering tag in organotypic hippocampal brain slices.

Specifically, we employ an engineered monomeric streptavidin bound to sparsely biotinylated AP-tags in AP-GluA2 or AP-NLG1 knock-in mice. This minimally invasive tagging strategy enables accurate targeting and characterization of the distinct diffusion properties of the proteins under near-physiological conditions. To image their behavior in tissue, we use lattice light-sheet microscopy, which provides high-resolution, plane-specific illumination with minimal phototoxicity and photobleaching.

Furthermore, the introduction of astigmatism via a deformable mirror enables precise three-dimensional tracking of protein diffusion. We are currently validating this approach by testing various fluorescent probes and proteins under basal conditions in both 2D and 3D, to characterize their diffusion dynamics in brain slices and set the stage for coupling with electrophysiological recordings.

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Simon Haziza

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Imaging the membrane voltage activity of specific neuron-types across spatiotemporal scales in behaving animals

Co-Authors

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Recent methods for optical voltage imaging allow studies of neural spiking at a resolution of single action potentials. However, optical instruments for monitoring the aggregate, population-level voltage dynamics of identified neuron-types have lacked the sensitivity to track high-frequency ($\gtrsim$10 Hz) voltage waves and oscillations. This technology gap has impeded studies of how specific neuron-types shape the spatiotemporal dynamics of the brain's rich set of electrical oscillations. Here, we present two TEMPO (Transmembrane Electrical Measurements Performed Optically) technologies with unprecedented sensitivity for monitoring high-frequency transmembrane voltage activity up to ~100 Hz, from one or two cell-types in behaving animals (Haziza et al, Cell, in press). TEMPO has 3 key ingredients: (1) Co-expression of one (or two) fluorescent genetically encoded voltage indicators to track neural activity and a reference fluor to track optical artifacts (e.g., from hemodynamics and brain motion); (2) A dual-color fluorescence sensing or imaging apparatus; (3) Computational unmixing of optical artifacts from the fluorescence voltage traces. Notably, TEMPO differs from extracellular electric field potential recordings, which capture contributions of multiple unidentified neuron-types, are influenced by electrode shape, orientation, and composition, and include volume-conducted signals originating up to ~1 cm away from the recording electrode. We built fiber-optic and imaging systems for TEMPO studies of neural dynamics in freely moving and head-fixed behaving mammals, respectively. The fiber-optic apparatus, termed 'uSMAART' (ultra-Sensitive Measurement of Aggregate Activity in Restricted cell-Types), attains ~10-fold greater sensitivity than prior fiber photometry systems. The imaging system captures population-level neural voltage activity across a ~7-mm-wide field-of-view with a spatial resolution >10 times finer than the densest electrocorticography recordings. With these instruments, we resolved propagating voltage waves and oscillations up to high-gamma (~100 Hz) frequencies, as well as cross-frequency coupling between oscillations of distinct frequencies in the transmembrane potentials of individual neuron-types. With dual cell-type TEMPO, we characterized the joint dynamics of excitatory and inhibitory neurons during hippocampal ripples and visual cortical processing. Overall, TEMPO technologies will allow neuroscientists to examine how specific neuron-types interact to shape the brain's electric field dynamics and to probe the contributions of voltage waves and oscillations to animal behavior in both health and disease.

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Etienne Herzog

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A tale of neurophotonics applied to synaptosomes: dopamine neuromodulation from varicosities to dopamine hub synapses

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Dopamine, an essential brain neuromodulator involved in reward prediction and motor control, is released by dopaminergic (DA) neurons that represent less than 0.01% of brain neurons. Yet, DA neurons innervate most areas, particularly the striatum. To isolate fluorescent DA varicosities (synaptosomes) we developed fluorescence activated sorting for biological microparticles. In a thorough characterization of DA synaptosomes, we provided their proteome, identifying 57 proteins specifically enriched. We also revealed their adhesion to glutamatergic, GABAergic, or cholinergic synapses. We named these multipartite assemblies “dopamine hub synapses” (DHS). Upon association with the DA moiety, glutamatergic synapses exhibit a significant increase of pre- and postsynaptic markers. Thus, DHS may support previously un-anticipated local dopaminergic signaling, however the basic features of dopamine release are still largely unknown.

Hence, we further studied the ultrastructure of fluorescent DA and glutamatergic (GLU) synaptosomes with cryo-correlative light and electron microscopy coupled with tomography. We observed that DA synaptosomes display ~10 times fewer vesicles than GLU which are also bigger and less round. Vesicle organization at the single nanometer scale indicates that most GLU synaptosomes have tethered and primed vesicles, indicative of a readily releasable pool, while only 39 % of DA synaptosomes have tethered vesicles, which appear not to be primed. In addition, GLU synapses associated with DA terminals (in DHS) contain more primed vesicles than others. While DA varicosities do not form genuine synapses, their adhesion to cortico-striatal synapses may convey a local regulation of synaptic release properties.

Through this example related to DA neuromodulation, my presentation will illustrate how several modalities of neurophotonics applied to synaptosomes convey a wealth of detailed mechanistic information on neurotransmission.

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Mathieu Johnson

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Mesoscale reconstruction of 3D microstructure and fibre orientation in whole brains with automated serial tilted polarization sensitive optical coherence tomography

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A comprehensive mapping of the structure of white matter connectivity in the brain – its connectional architecture – is crucial to our ability to understand how brain function gives rise to behaviour in health and disease. This understanding is critically important, as connectivity (axons/fibres connecting neurons/regions) and microstructure (e.g., degree of axonal myelination, density) are two main determinants of optimal brain function. Previous work has assessed white matter connectional architecture through invasive tract-tracing in non-human animals and ex-vivo dissection in humans or through non-invasive diffusion-weighted magnetic resonance imaging and probabilistic tractography. The former approaches provide accurate and highly detailed connectivity mappings of functionally specific axons (e.g., limited to a specific receptor) with extreme spatial specificity (i.e., at the microscale), and the latter groups together hundreds of thousands of axons to provide probabilistic connectivity for the entire brain with poor reproducibility and spatial specificity (at the macroscale). These approaches fall short of capturing the intricacies of neural circuitry at the multi-cellular to regional scales – the mesoscale – which is critical for understanding the mechanistic links between connectivity, communication, and brain function in health and disease.

Motivated by the fundamental lack of insight into white matter architecture at the mesoscale, we developed an automated serial histology tilted polarization-sensitive optical coherence tomography (as-psOCT) platform to enable rapid quantification of the structural organization supporting circuit-level brain function. Our platform pairs a large automated xyz stage, tilting arm, and vibratome with an off-the-shelf psOCT system (ThorLabs Telestro) for serial histology – extracting both estimates of tissue microstructure (OCT intensity) and in-plane fibre orientation (birefringence-dependent polarization). Using the rodent cerebellum as a model system of regional connectivity that is constrained by a restricted set of inputs/outputs (and therefore simplified in comparison to cerebral circuits), we have characterized white- and grey-matter microstructure in 3D (up to 10x10 micrometer in-plane resolution) and are building the algorithms, tools, and pipelines to combine multiple orientation maps acquired at different tilt angles to reconstruct 3D maps of fibre orientation for psOCT anatomical tractography.

This comprehensive strategy aims to overcome the limitations of existing approaches to reveal fundamental insights into the principles of organization of connectional architecture at the mesoscale in the cerebellum and beyond – with the aim of developing a framework for mechanistic understanding of brain circuits for linking with brain function and behaviour in health and disease.

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Hanna Manko

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Microscope stabilization for drift-free single-particle tracking at depth in brain tissue

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Super-resolution microscopy techniques, enable study of the structural organization and dynamics of neuronal cells and extracellular spaces within the brain at the nanoscale level. However, most high-resolution methods require relatively long acquisition times. As a result, stabilizing sample drift is crucial for obtaining high-quality structural or accurate single-molecule tracking data. While various approaches exist to correct lateral sample drift, maintaining precise focus remains a significant challenge.

In this work, we demonstrate that applying a cross-correlation-based method to label-free, homogenized differential phase contrast images (hDPC) enables precise, real-time 3D microscope stabilization. This approach is compatible with both trans- and back-illumination modalities, making it suitable for a wide range of sample types. We validate its effectiveness in single-particle tracking experiments performed in both fixed and live brain tissue.

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Baptiste Marthy

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Label-free quantification of organelle trafficking inside a single axon

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The study of organelle trafficking traditionally relies on fluorescent protein tagging methods. These tags enable the visualization of organelle lifecycles, refining models of cellular trafficking. However, fluorescence-based techniques face significant limitations. First, fluorescent proteins are susceptible to photobleaching and phototoxicity, which reduces the observation window for dynamic, long-lasting cellular events. Second, only labeled compartments are made visible, challenging the construction of a comprehensive map of cellular processes when most components cannot be imaged simultaneously.

In this work, we show that quantitative phase imaging (QPI) allows to acquire label-free images of organelle trafficking within a single axon in-vitro. Neural network are reconstructed using microfluidics where single axon can be isolated. QPI quantifies the dry mass of biological structures over extended period of times, without perturbation. We demonstrate that QPI can measure distinct cargoes behaviors (such as dry mass evolution, speed and pauses) while providing contextual information about the axon's morphology, its branches, and its connections with other neurons. We are currently correlating QPI with fluorescence imaging, advancing towards computational in-silico labeling.

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Somen Nandi

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Ultrashort Carbon Nanotubes with Luminescent Color Centers: Bright NIR-II Nanoemitters for Advanced Neurophotonics

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The integration of high brightness and nanoscale dimensions in short-wave infrared (SWIR) emitters is pivotal for advancements in bioimaging, photonics, and quantum science. However, the development of such nano-emitters has remained a significant challenge. In this study, we present ultrashort carbon nanotubes, with lengths substantially below 100 nm, functionalized with luminescent color centers (uCCNTs), as exceptionally bright emitters in the near-infrared second bological window (NIR-II) of the SWIR spectrum [1].
Our investigation reveals that the remarkable brightness of these uCCNTs arises from the mitigation of quenching defects typically present in dispersed carbon nanotubes. Through a combination of ensemble and single-nanotube photoluminescence measurements, we observe nonlinear emission behaviors and determine photoluminescence quantum yields reaching up to 20% at the single-nanotube level. Notably, the NIR-II brightness of these ultrashort emitters surpasses that of conventional visible emitters, including quantum dots [1].
Upon rendering the uCCNTs biocompatible, we demonstrate their applicability in advanced neurophotonics techniques, such as point-spread function engineering and high-resolution, three-dimensional single-particle tracking within thick brain tissue. These findings position functionalized uCCNTs as a promising platform for deep-tissue, high-resolution imaging in the NIR-II window, offering new avenues for exploration in neurophotonics.

References:
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GABA-induced Calcium Signaling in the Primary Cilium of Neurons

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The primary cilium is a solitary organelle present in almost every neuronal cell type that extends into the extracellular space for detecting a variety of signals. Several GPCRs have been identified as ciliary receptors, though the transduction underpinnings and its impact on the cell's function remain mostly unknown.
Here we show in 3-weeks-old primary cultures that the cilia of neurons is an active organelle displaying spontaneous calcium dynamics, furthermore the compartment is not affected by the neuronal calcium activity taking place in the cytoplasm thus suggesting that ciliary GPCRs drive the organelle's own signaling. In addition, we also show that the metabotropic GABA receptor subtype 1 (GABA-B1) localizes to primary cilium of neurons, but not other non-neuronal cell types, across different regions of the mouse brain with the strongest enrichment in striatal neuronal cilia.
Finally, activation of GABA-B1 receptors with low dose of the agonist baclofen (100nM) initiates calcium signaling that is restricted to the cilium. We have recently shown that a similar GABA-B1 ciliary pathway is operative in insulin producing cells of the pancreas where the receptor appears to engage non canonical downstream partners to trigger the calcium transients, notably without forming an hetero-dimer with the cognate GABA-B2 receptor.
We suggest that this novel GABAergic ciliary pathway with excitatory features provides neurons with a parallel input adjoining the known actions of GABA as the major inhibitory neurotransmitter in the brain.

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Olivier Thoumine

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Neurospheres from primary rodent brain cells to study the 3D organization and function of synapses at high resolution

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To improve our understanding of synapse assembly, there is a need for robust, easy-to-use, and physiologically relevant in-vitro models allowing for the controllable formation of neuronal contacts in a reasonable time, whose structure and function can be investigated using advanced microscopy. To address this challenge, we engineered 3D cultures from rodent primary nerve cells, that we call “neurospheres”. When plated into ultra-low attachment wells, dissociated rat hippocampal cells spontaneously assemble in compact spheroids of highly reproducible dimensions (100-300 µm), that can be precisely controlled by adjusting the number of seeded cells. Neurospheres contain a mix of neurons and astrocytes and grow over time in culture, through the combination of glial cell proliferation and neurite extension. Neurospheres can be immunostained in fluid phase, and sparsely electroporated for the multi-colour visualization of recombinant synaptic proteins. Within the finite volume of neurospheres, neurons extend an elaborate network of axons and dendrites, forming numerous excitatory and inhibitory synapses identified at the structural level by immunofluorescence and electron microscopy. Live calcium imaging of neurospheres reveals periodic oscillations whose amplitude negatively scales with frequency, and that are driven by glutamatergic synaptic activity. We imaged synapses in 3D at high resolution in these samples using expansion microscopy as well as DNA-PAINT under single objective-based light sheet illumination, SoSPIM (Galland et al. Nat Meth 2015). We showcase the potential of neurospheres to investigate synaptogenesis by focusing on the neurexin-neuroligin adhesion complex, i.e. visualizing trans-synaptic contacts between neurexin-1β and neuroligin-1 and modulating synapse formation by varying the neuroligin-1 expression level in neurons. Finally, we formed neurospheres from a tagged knock-in mouse strain (Ducrot et al., PNAS 2025), to visualize endogenous neuroligin-1 in the synaptic cleft by electron microscopy. Overall, neurospheres represent a standardized and cost-effective culture system compatible with existing labelling toolkits to study synapse structure and function at high resolution in 3D. When combined to the high content capability of soSPIM and dedicated image analysis pipeline, this culture model could be extended to drug screening applications.

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Robert B. Quast

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The Conformational Landscape of Metabotropic Glutamate Receptors Resolved by Multicolor Single Molecule FRET

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Neuroreceptors are activated through a complex interplay of conformational changes. Single molecule FRET (smFRET) allows to monitor these structural rearrangements with sufficient time resolution to identify transient states and quantify dynamic equilibria but requires labeling with donor and acceptor dyes at appropriate positions. In contrast to classical labeling strategies relying on proteinogenic functionalities or large protein tags, reactive non-canonical amino acids (ncAA) are small (1-2 kDa) and allow labeling at desired position using genetic code expansion and biorthogonal click chemistries in a minimally invasive manner.

To decipher the conformational landscape of the human metabotropic glutamate receptors 2 (mGluR2), we developed several conformational 2-color FRET sensors through incorporation of a single[1] and two distinct, reactive ncAAs combined with bioorthogonal conjugation of donor and acceptor fluorophores. We then studied ligand induced conformational rearrangements of single receptors in a carefully optimized detergent mixture[2] by smFRET using confocal, pulsed interleaved excitation and multiparameter fluorescence detection. We find that a synergy with positive allosteric modulators, acting over a long-range functional link, is required to fully activate these multidomain neuroreceptors. Finally, through the establishment of an orthogonal triple labeling strategy we design 3-color smFRET sensors, by combining double ncAA and SNAP-tag labeling, and provide evidence for a previously unknown, pre-active, intermediate state in equilibrium with the active state upon ligand activation that we could not resolve by classical 2-color smFRET[3]. We conclude an activation model where orthosteric and allosteric ligands act on different conformational equilibria between coexisting states to fine-tune mGluR2 activation. Our work highlights the power of minimally invasive, ncAA-based, bioorthogonal labeling to dissect domain-specific conformational rearrangements of neuroreceptors using smFRET.

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[3] Bonhomme L, Bilgen E, Clerté C, Pin JP, Rondard P, Margeat E, Lamb DC, Quast RB. JACS 2025, https://doi.org/10.1021/jacs.4c18364

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Oral presentations

Sponsors communications

Adaptive Optics solutions for high-resolution fluorescence microscopy applied to Neuroscience

Represented by

- Fabrice Harms – Imagine Optic / mu-Imagine – Orsay, France

We report on the benefits of Adaptive Optics (AO) for high- and super-resolution fluorescence microscopy, as a means to increase image signal, resolution and contrast by correcting sample induced aberrations in depth. We present main AO methods and required components, as well as their implementation in several microscopy setups applied to neuroimaging. Specifically, we present AO implementations in optical sectioning microscopes such as two-photon, Light-Sheet and Lattice Light-Sheet used for SMLM. Using these setups, we show significant image improvement of brain structures such as e.g. neurons, axons, dendrites, in samples including animal models (living mouse, Zebra Fish, drosophila) and brain tissue, at large depths down to several hundreds of microns. The gain in signal and resolution brought by AO provides several advantages : 1. Improvement of quantification based on e.g. cell segmentation, or of the sensitivity of functional recordings, 2. Minimization of phototoxicity, and 3. Deeper imaging.

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Abbelight

Abbelight Nanoscopy Solution - Unlocking Spatial Proteomics at Single-Protein Resolution

Represented by

- Mehdi Madi, Abbelight, Cachan, France

Single-Molecule Localization Microscopy (SMLM) offers unprecedented access to the nanoscale organization of proteins with molecular precision. However, its broader adoption in life sciences has been hampered by constraints in field of view, multiplexing capability, and workflow complexity.

The Abbelight **SAFe platform** addresses these challenges through an integrated suite of innovations: patented **ASTER illumination** for large and uniform fields of view, **Spectral Demixing** for simultaneous multicolor imaging, and **Ultimate 3D nanoscopy** for isotropic resolution across all dimensions. Together with automated **Exchange DNA-PAINT** and optimized **reagent kits**, the platform enables high-throughput, multi-target imaging while preserving single-protein resolution.

For neuroscience applications, this workflow spans from **live-cell TIRF imaging** to **super-resolution SMLM** and **single-particle tracking** (SPT), providing powerful insights into the dynamic and nanoscale organization of synapses, the architecture of the membrane periodic skeleton (MPS), and other key neuronal structures.

Coupled with **NEO software** for automated acquisition and analysis, the SAFe platform delivers a robust, scalable, and user-friendly solution for spatial proteomics. By bridging advanced hardware and software, together with ready to use kits, Abbelight empowers neuroscientists to accelerate discovery and deepen understanding of brain organization at the molecular scale.

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Innovations in Ultrafast Lasers for Nonlinear Microscopy

Represented by

- Luc Moog, Coherent Corporation

Multiphoton microscopy continues to find new applications across diverse fields such as neuroscience, cancer studies, and immunology. There is also a pursuit to leverage the benefits of multiphoton imaging for clinical and diagnostic applications, driven by both research advancements and commercial developments. Alongside this diverse range of applications, there is a growing array of probes, laser beam manipulation techniques, and optical delivery schemes that are aimed to expand the functionality of multiphoton microscopy. In turn, these advancements drive continuous progress in femtosecond laser sources, which are essential for driving nonlinear excitation processes in these imaging techniques. In this presentation we will review novel developments in femtosecond lasers and their interplay with emerging imaging techniques. Among these, we will look at the establishment of compact, fixed wavelength laser sources and how they help to drive targeted imaging applications in neuroscience and imaging of freely behaving animals. In addition, these sources can benefit nonlinear imaging applications in clinical endoscopy or in surgical room environments. The advancements in tunable femtosecond sources will also be reviewed, where the expanded functionality makes them a tool of choice to power up advanced multi-modal imaging, or multi-user setups. We will also describe how an increasing refinement of the optical integration helps to streamline the laser usage in the laboratory and (pre-) clinical environments.

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Hamamatsu Photonics France

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PHOTON IS OUR BUSINESS

Lasers and fibers for neuroscience

Represented by

- Thomas Ferhat, NKT Photonics

In this presentation, we will demonstrate through user cases how photonic crystal fibers, supercontinuum and ultrafast laser can enable research in neurosciences.

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Argolight

Argolight Solutions: Advancing quantitative fluorescence microscopy with reproducible QC

Represented by

- Renaud Ginet, Argolight

Fluorescence microscopy has become a cornerstone of modern bio-imaging, evolving from a purely qualitative visualization technique into a powerful quantitative tool. This shift places increasing demands on data reliability, reproducibility, and comparability across instruments, laboratories, and time. However, fluorescence microscopes are inherently prone to performance drifts: light sources lose intensity, optical alignments shift, detectors change sensitivity, and environmental factors introduce additional variability. These biases, if left unmonitored, can significantly compromise quantitative measurements and the scientific conclusions that rely on them.

Argolight addresses this challenge with innovative quality control (QC) solutions, built around proprietary Argoglass® technology. This unique fluorescent material embeds stable and durable reference patterns into reusable slides and plates, guaranteed for life. When combined with Argolight's Daybook software, these tools enable the measurement of more than 200 QC metrics across 13 tests, including lateral resolution, optical sectioning strength, field uniformity, co-registration accuracy, ... Unlike consumable samples such as fluorescent beads, Argolight slides require no preparation or special storage, ensuring both ease of use and long-term reproducibility.

These solutions empower scientists to validate experimental conditions, facility managers to monitor fleets of microscopes, and manufacturers to provide reliable quality assurance. Argolight tools are already trusted by leading research institutes such as Pasteur, Curie, Oxford, and Harvard, as well as major instrument manufacturers including Zeiss, Evident, or Andor.

By providing standardized, accessible, and reproducible QC methods, Argolight contributes to raising the global standards of fluorescence microscopy, ensuring confidence in results that matter for both science and healthcare.

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Label-free Quantitative Phase Imaging with QLSI: from intracellular dynamics to tissue organization

Represented by

- Feriel Terras
- Cassandra Borgane
- Benoit Wattellier

Optical microscopy research predominantly relies on fluorescence imaging, which provides molecular specificity and controlled depth of field. However, labeling procedures can introduce biases and suffer from photobleaching. Quantitative Phase Imaging (QPI) offers a powerful label-free alternative, enabling non-invasive access to structural and functional information with high sensitivity and temporal resolution. Based on wavefront sensing, and in particular Quadriwave Lateral Shearing Interferometry (QLSI), the Phasics QPI system can be implemented as a plug-and-play “phase camera” on standard microscopes.

This device enables multiscale investigations. At the single-cell level, QPI allows quantitative monitoring of cellular dry mass, a robust indicator of metabolism. Thermal QPI extends this capability by mapping temperature fields in real time, allowing the study of subcellular heat-shock dynamics triggered by plasmonic nanoheaters. Beyond single cells, QPI enables tracking of vesicle trafficking and organelle motion without perturbations associated with fluorescent probes. At the tissue level, combining QPI with retardance imaging highlights anisotropic structures such as collagen fibers, providing insights into fibrosis and extracellular matrix remodeling. These features make QPI particularly relevant for neurophotonics, where capturing subtle morphological and metabolic variations is essential to understand neuronal activity, cell–cell interactions, and pathological processes.

Altogether, QPI using wavefront sensing constitutes a versatile methodological platform, complementing fluorescence techniques and opening new perspectives for label-free, quantitative neuroimaging. In this presentation, we will briefly present the basis of technology. We will show a panorama of all possible applications, by means of various scientific studies made possible thanks to PHASICS SID4 cameras.

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Toptica

Automated Femtosecond Fiber Delivery for Multiphoton Microscopy

Represented by

- Helge Schmidt, Toptica

Replacing the free-space path between a Watt-level femtosecond laser and a two-photon microscope is a game-changer for microscopy. Delivering femtosecond pulses from the laser to the microscope via an optical fiber enables new microscope designs. Miniaturized microscopes head-mounted on freely moving mice were used to study neuronal activity with minimal influence on animal's natural behavior. Other implementations are endoscopic or hand-held devices equipped with nonlinear imaging capabilities. Thirdly, maneuverable microscopes which can rotate in almost every angle around the sample provide highest flexibility. Polarization-maintaining hollow-core fibers paired with femtosecond fiber lasers are commercially available nowadays. We present a hands-off automated fiber coupling based on TOPTICA's proprietary constant optical output level (COOL) technology. It eliminates manual alignment at installation, optimizes fiber coupling via GUI, and monitors fiber coupling efficiency internally.

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Posters

Nasim Akhtar

P1

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2P, 3P multimodal microscopy applied to neurophotonics

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Multiphoton microscopy, including two-photon (2P) and three-photon (3P) fluorescence techniques, is used in various fields, especially in neuroscience, to visualize in vivo and ex vivo tissue morphology and physiology at a cellular level with high resolution, less photodamage, and minimal invasiveness. In particular, the 1700nm excitation window, balancing tissue scattering and absorption, profides an absolute optimum for deep-tissue optical imaging. Yet, the combination of the 1700nm window excitation wavelength and 3P microscopy using red fluorophore (such as mCherry, tdTomato, etc.) is still an emerging technique for enabling biological investigations. Additionally, the fluorescence emission wavelength also plays an important for optimizing the imaging depth. In this context, the NIR emitting fluorophores for 2P excitation at 1700 nm remains underexplored due to a scarcity of suitable fluorophores and detectors for near-infrared emission.

Here, we investigate the use of single-walled carbon nanotubes (SWCNTs) as potential fluorescent probe for 2P at 1700 nm, leveraging their exceptional optical properties. Although 2P excitation of SWNTs have been demonstrated, their application for 2P imaging. Our research aims to bridge this gap by conducting 2P microscopy on individual SWCNTs. After conducting spectroscopic analysis on various individual SWCNTs, we selected the optimal fluorophore for two-photon excitation within the accessible range of the 1700 nm laser.

Furthermore, we performed Monte-Carlos simulations of the 2P and 3P fluorescence collection of the photon from the tissue. Finally, we validate this comparison experimentally in the brains. This research introduced the fluorophore for 2P and compared quantitatively experimental 2P and 3P microscopy in brain slices using the same excitation wavelength for deep imaging. Additionally, we measured the 2P absorption cross-sections of individual different SWCNTs samples using 2P spectroscopy to further optimize their use in bioimaging.

Frédéric Beaupré

P2

Université Laval, Canada

Detection of Synaptic Anomalies in STED Microscopy Using Deep Learning

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Synaptic dysfunction is a key hallmark of a plethora of neurodegenerative diseases. On a population level, synaptic dysfunction is heterogeneous and tends to worsen with disease progression. In this project, we frame the problem of detecting nanometric changes in synaptic protein organization as a deep-learning-based anomaly detection task. The detection and characterization of rare synaptic features is difficult, in part because synapses are less than 1µm² in size, they exhibit a wide range of shapes and internal structures, and their molecular organization is extremely regulated. Given the lack of precise knowledge of the anomalous events or structures of interest, our algorithm is centered around two objectives: (1) learn an expressive and general representation of the nominal data and (2) detect deviations from this learned representation which may reveal novel or rare synaptic structures. To tackle the first challenge, we designed STED-FM, a large-scale, self-supervised foundation model for robust and generalizable representation learning in super-resolution microscopy (Bilodeau, Beaupré et al., bioRxiv, 2025). It leverages a Vision Transformer architecture trained at scale with Masked Autoencoder (MAE) pre-training on a new dataset of nearly one million STimulated Emission Depletion (STED) microscopy images. STED-FM learned expressive latent representations without extensive annotations, leading to robust performance increase across diverse downstream microscopy image analysis tasks. To detect deviations from the learned distribution of nominal data (objective 2), we paired STED-FM with a diffusion model decoder, which is trained to reconstruct images of synaptic proteins with a conditioning mechanism leveraging STED-FM's representations. At inference, we can use our STED-FM/diffusion model pair to detect poorly reconstructed proteins as anomalous structures of interest. We evaluated our anomaly detection algorithm on structures of increasing complexity, from synthetic anomalies, to known rare synaptic morphologies such as perforated synaptic proteins.
The proposed methods could lead to discoveries of rare synaptic events that would be overlooked by classical detection and classification approaches of known features.

Maia Brunstein

P3

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Fast 2-photon stimulation using holographic patterns

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A fundamental question in neuroscience is to understand how patterns of neuronal activity can represent useful sensory or motor information to drive animal behavior. To build a causal link between brain activity and behavior, it is necessary to control the neuronal dynamics and observe how animal comportment is affected [1]. Addressing this challenge requires tools to manipulate a population of neurons in a very controlled manner, both spatially and temporally. This can now be achieved by optogenetics, a genetic manipulation that drives the expression of a light-modulated actuator in a defined cell type in order to modify cellular properties during illumination. A variety of approaches have been developed to generate precise spatiotemporal light patterns to stimulate the neurons on the brain. Yet certain constrains still exists in the current optical techniques to activate a neuronal population with both cellular resolution and millisecond precision. Here, we describe an experimental setup allowing to stimulate a few tens of neurons in a plane at sub-millisecond rates using 2-photon activation. We used a LCoS-SLM to generate a single spatial pattern in 2 or 3 dimensions containing multiple regions of interest and projected this pattern onto a DMD that was employed as a fast temporal shutter for each region of interest. In this way, the total laser power was efficiently dedicated to activating only potential targets without wasting laser power on unused DMD pixels. This approach had been applied to imaging application with the development of an encoded multisite 2P microscope [2] or to multi-site photolysis of caged neurotransmitters [3]. The main advantages of our optical developments are (i) to push the temporal resolution to the limitations of the opsin time constant, (ii) to reduce the complexity of the phase pattern calculation since only 1 phase pattern needs to be built, (iii) to enable real-time control of neuronal activity.
Using fluorescent microscopy and patch-clamp recording of neurons in culture expressing the light-gated ion channels, we characterized the temporal and spatial resolution of the microscope. We described the advantages of combining the LCoS-SLM with the DMD to maximize the temporal precision, modulate the illumination amplitude, and reduce background activation. We further demonstrate that this approach can be extended to patterns in 3 dimensions (see figure). This methodology is well suited to address important questions about the role of temporal information in neuronal coding.

Julia Chabbert

P4

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Molecular mechanisms of the dendritic Membrane-Associated Periodic Skeleton (MPS) activity-dependent remodeling

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The neuronal cytoskeleton is essential for maintaining structural integrity, supporting intracellular trafficking and enabling synaptic function. Super-resolution microscopy has revealed a highly ordered periodic lattice just beneath the membrane of neurites, known as the membrane-associated periodic skeleton (MPS). This structure consists of filamentous actin (F-actin) rings interconnected by spectrin tetramers. While the MPS is well characterized in axons, its study in dendrites is more challenging. First, dendritic F-actin exists under several nano-organizations (e.g. synaptic pool, rings, patches). Second, the dendritic MPS incorporates a broader range of spectrin isoforms than axons, where only β2-spectrin is typically present. Third, recent observations using STimulated Emission Depletion (STED) nanoscopy revealed that dendritic F-actin rings reorganize into longitudinal fibers during neuronal activity, a phenomenon not observed in axons. Thus, several questions remain unexplored: How do other proteins of the periodic lattice react to neuronal activity? What are the molecular mechanisms triggering this remodeling? What is the function of this dendritic remodeling?

We first hypothesized that β-Ca2+/calmodulin-dependent kinase II (βCaMKII), a major decoder of Ca2+ signals in neurons and F-actin stabilizer, is involved in activity-dependent F-actin rings remodeling. However, knockdown experiments combined with chemical stimulations of cultured hippocampal neurons showed no significant effect on actin reorganization, suggesting that other signaling pathways might be involved.

Adducin is part of the MPS and acts as an actin-capping protein stabilizing the spectrin-actin junctions in an activity-dependent manner. Using STED nanoscopy and autocorrelation analysis, we observed a disassembly of the periodic pattern of adducin in dendrites after glutamate/glycine or KCl stimulation. Given that protein kinase C (PKC) is known to phosphorylate adducin leading to destabilization of actin-spectrin junction, we are currently exploring its involvement as a potential regulator of dendritic MPS plasticity.

Together, this work highlights the compartment-specific regulation of the MPS and aims to shed light on molecular mechanisms governing its activity-dependent reorganization, which may contribute to membrane trafficking, dendritic signaling and synaptic plasticity.

Tatiana Charbonnier

P5

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Semi-acute degradation of spectrins using intrabodies coupled with bioPROTAC

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Spectrins are cytoskeletal proteins ubiquitously expressed in Metazoans where they play key roles in regulating cell shape and organisation. They form heterotetramers consisting of two α-spectrin subunits and two β-spectrins subunits, bound head to head. In neurons, only α2-spectrin and β2- to β4-spectrins paralogues have been described. While α2-spectrin is present in the whole neuron, β-spectrins are differentially expressed across neuronal compartments: β2- and β3-spectrins are found in the somatodendritic region, β4-spectrin is enriched at the Axon Initial Segment (AIS) and β2- spectrin is expressed along the axon. Several pathogenic variants affecting these cytoskeletal components have been implicated in various central nervous system disorders (collectively known as spectrinopathies), including developmental delay and autistic features. As part of my PhD project, I aim to develop innovative tools to study spectrin organization and function in physiologically relevant environments such as brain slices. In collaboration with Matthieu Sainlos, we have generated a panel of specific intrabodies targeting endogenous spectrins. These were selected from in-house libraries based on the 10th fibronectin type III domain (Fn3) scaffold and are designed to bind distinct domains of α- and β-spectrins, as previously demonstrated for synaptic MAGUK proteins. To enable functional modulation, we fused these intrabodies to a proteolysis-targeting chimera system (bioPROTAC), harnessing the proteasome to degrade the target proteins and thereby semi-acutely reduce spectrin levels in cells.
Here, I will discuss our current findings on the selective degradation of spectrins mediated by different ubiquitin ligases in COS cells and primary neurons, highlighting the potential of this approach for dissecting spectrin function in neural architecture and pathology.

Tiffany Cloâtre

P6

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Matching protein localization to synapse morphology in primary neurons by correlative super-resolution light microscopy

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Super resolution imaging provides unprecedented visualization of sub-cellular structures but the two main techniques used, Single Molecule Localization Microscopy (SMLM) and STimulated Emission Depletion (STED), are not easily reconciled. By building on a previous study (Inavalli et al., Nature Methods 2019), we developed a protocol to super-impose the nanoscale protein distribution reconstructed with SMLM to the sub-cellular morphology obtained in STED, by tracking cells on etched coverslips and registering images from two different microscopes with 30-nm accuracy. We applied this strategy to map synaptic proteins in primary rat hippocampal neurons.

Specifically, neurons were electroporated with DNA constructs coding for synaptic proteins before being plated, then cultured for 2 weeks to allow synapse development. We first imaged in PALM the endogenous scaffolding protein of excitatory synapses, PSD-95, using a recently characterized intrabody (Xph20) fused to the convertible protein mEos3.2. Then, we imaged GFP-actin that accumulates in dendritic spines using STED microscopy, after amplifying the GFP signal by immunolabelling with antibodies conjugated to Atto-647N.

We found that individual dendritic spines identified in STED contained from 0 to 4 discrete PSD-95 nanodomains detected in PALM. Those nanodomains had a relatively constant surface area, independently of their actual number in the spine, but the number of nanodomains was positively correlated with the projected area of the dendritic spines. This specific architecture of synapses in punctate nano-domains or -modules seems to be crucial in their response to plasticity mechanisms.

This protocol can easily be applied to study other synaptic targets including adhesion or cytoskeletal proteins that have been successfully tagged with mEos3.2 for PALM, for example synaptopodin which concentrates at the neck of dendritic spines. In cases where mEos3.2 fusion proteins are not available or functional, which is the case of the adhesion protein neuroligin-1, the PALM procedure can readily be replaced by dSTORM performed on neurons stained with appropriate primary and dye-conjugated antibodies. The long-term goal of the project is to establish an atlas of protein nanoscale localization within the profile of dendritic spines.

Célia Delhaye

P7

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Clustering and membrane dynamics of KCC2 differ at the surface of interneurons compared to principal cells

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KCC2, a membrane transporter that allows the efflux of chloride ions from the neurons, plays a crucial role in establishing the chloride gradient and regulating the polarity of GABAergic signaling. Its activity is set by its membrane clustering that depends on its “diffusion-capture”. Reduced KCC2 expression and elevated intracellular chloride levels are associated with various neurological disorders, including epilepsy and schizophrenia. Interneurons orchestrate the activity of pyramidal cells. An alteration of GABAergic signaling in Parvalbumin cells is sufficient to induce interictal-like activity. Here, we explored the cellular and molecular mechanisms underlying the regulation of KCC2 in interneurons versus principal cells of the hippocampus, and how they are modified in pathological conditions. Our results in primary rat hippocampal cultures reveal an increased membrane expression and clustering of KCC2 in interneurons compared to principal cells. We performed Quantum-Dot based single particle tracking to study the membrane dynamics of KCC2 on the surface of these two cell populations. Although KCC2 moves more rapidly across the membrane of interneurons, the proportion of transporters trapped at the GABAergic synapse is doubled on the surface of these cells. This could explain the increased clustering we observed at the membrane of interneurons. These differences in KCC2 expression, organization and membrane dynamics in interneurons versus principal cells could reveal different regulatory mechanisms for KCC2 at the membrane of these two cell populations. These differences could contribute to distinct vulnerabilities of these neurons to pathological conditions.

Domitille des Robert

P8

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Characterization of neuronal cultures growing on hydrogels with different stiffnesses

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Post-synaptic receptor trafficking plays an important role in synaptic transmission and plasticity. Receptor internalization occurs at endocytic zones (EZs) marked by clathrin. Clathrin is recruited to a membrane zone which invaginates and forms a vesicle which then moves into the cell. In cultured neurons, EZs are positioned near post-synaptic densities (PSDs) marking excitatory synapses in dendritic spines, as well as uncharacterized sites of the dendritic shaft (Blanpied et al., 2002; Lu et al., 2007). EZs are dynamic, with lifetimes of just a few minutes, in immature cultured neurons (6 days in vitro) but are static in more mature neurons, even though endocytic vesicles forms at these sites (Rosendale et al. 2017). In organotypic brain slices, the proportion of spines that contains EZs is slightly lower than in neurons growing in culture. Importantly, EZ dynamics were markedly changed, from very static in culture to highly dynamic in organotypic slices (median lifetime of around 60 s). We hypothesized that the reason for this difference lies in the different mechanics of cell adhesion to the substrate. Indeed, the brain is a very soft organ (Young modulus ~0,17 kPa) and it was shown in non-neuronal (HeLa) cells that EZ dynamics is also strongly affected by substrate rigidity (Baschieri et al., 2018).

To test our hypothesis, we developed methods to culture neurons on polyacrylamide gels of controlled stiffness and we characterized these cultures. Our results show that rigidity does not affect the dendritic morphology of neurons, the density and morphology of dendritic spines nor the density of pre and post-synaptic markers. We will then explore the molecular determinants and dynamics of EZ in this model.

Adèle Drouet

P9

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High-affinity detection of biotinylated endogenous neuroligin-1 at excitatory and inhibitory synapses using a tagged knock-in mouse

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Neuroligins (NLGNs) are important cell adhesion molecules mediating trans-synaptic contacts between neurons. However, the high-yield biochemical isolation and visualization of endogenous NLGNs is hampered by the lack of efficient antibodies. Thus, to reveal their subcellular distribution, binding partners, and synaptic function, NLGNs were extensively manipulated using knock-down, knock-out, or overexpression approaches, leading to controversial results. As an alternative to the manipulation of NLGN expression level, we describe here the generation of a knock-in (KI) mouse strain in which native NLGN1 was N-terminally tagged with a small biotin acceptor peptide (bAP) that can be enzymatically biotinylated by the exogenous delivery of biotin ligase. After showing that KI mice exhibit normal behavior as well as similar synaptic number, ultrastructure, transmission properties, and protein expression levels when compared to wild type counterparts, we exploited the fact that biotinylated bAP-NLGN1 can be selectively isolated or visualized using high-affinity streptavidin conjugates. Using immunoblotting and immunofluorescence, we show that bAP-NLGN1 binds PSD-95 and gephyrin and populates both excitatory and inhibitory synapses, challenging the historical view that NLGN1 is exclusively localized at excitatory synapses. Using superresolution optical and electron microscopy, we further highlight that bAP-NLGN1 forms in the synaptic cleft a subset of nanodomains, which contain each a few NLGN1 dimers and whose number positively scales with the postsynapse size. Overall, our study not only provides an extensively characterized KI mouse model which will be available to the scientific community but also an unprecedented view of the nanoscale organization of endogenous NLGN1.

Mathieu Ducros

P10

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Super resolution imaging inside brain slices with lattice light sheet microscopy and adaptive optics

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Super-resolution (SR) microscopy at depth within biological samples remains a challenging but critical endeavor, as it enables investigations at the biomolecular scale in intact and integrated tissues. In single-molecule localization microscopy (SMLM), imaging is typically performed near the surface (0–1 µm) of the sample, most often using total internal reflection fluorescence (TIRF) illumination. Achieving high-precision single-molecule localization deeper within tissue requires maintaining (1) high contrast between single molecules and background, and (2) a near-diffraction-limited point spread function (PSF).

In this work, we combine lattice light-sheet microscopy (LLSM) with adaptive optics (AO) to achieve SR microscopy in brain slices at depths of up to ~50 µm. Our setup integrates a deformable mirror (DM) into the detection path of a LLSM system. LLSM enables high-contrast single molecule imaging in brain slices by producing a very thin (~0.5 µm) planar illumination that provides clean optical sectioning. A semi-automatic, image-based algorithm periodically refocuses the light sheet and optimizes the DM shape to correct for sample-induced optical aberrations. Thus our LLSM-AO microscope maintains diffraction limited resolution through an extended depth. We employ the DNA-PAINT SMLM technique to image neuronal synapse morphology, AMPA receptor clusters, and Beta IV spectrin geometry. Additionally, the DM allows us to introduce controlled astigmatism for 3D SMLM localization. Single-molecule localization precision at the surface reaches 15 nm. At depths greater than 20 µm, localization precision and detection density tend to decrease due to light sheet defocus and sample based optical aberrations. However, this drop can be significantly compensated thanks to our image-based optimization approach, thus maintaining a localization precision less than 20 nm up to 50 µm deep.

Overall, our LLSM-AO instrument and aberration correction method, combined with a labelling and SMLM toolkit such as DNA PAINT, provides a robust technique to image at the molecular scale within intact neuronal tissue.

Audrey Dufau

P11

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Relationship of ventral-striatum astrocytes with vglut1-dopamine hub synapses

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Dopamine is a major neuromodulator implicated in reward prediction and voluntary motor command among others. Psychostimulants challenge the dopaminergic system and induce synaptic plasticity in the ventral striatum, and of course become a public health issue when causing addiction. VGLUT1 glutamatergic synapses inervating the ventral striatum from projections arising from the cortex, hippocampus and basolateral amygdala can be regulated by dopaminergic projections.

Our lab demonstrated the existence of dopamine hub synapses in the striatum. These hubs are tripartite structures such as a glutamatergic synapse associated with dopaminergic varicosities. The features of VGLUT1 dopamine hub synapses in the ventral striatum is still poorly characterized. Astrocytes are glial cells which play a major role in neuronal modulation and plasticity . In a recent study, our collaborators describe the involvement of astrocytes in the plasticity induced by exposure to psychostimulants. Therefore, their association to the dopamine hub synapses is to be investigated.

To assess the association frequency of these hubs, we extract synaptosomes from the ventral striatum of VGLUT1 venus mice, perform Fluorescence-Activated Synaptosomes Sorting (FASS) and collect them on coverslips. We then perform Immunocytochemistry to monitor the degre of association of dopaminergic, glutamatergic and astrocyte processes in multipartite synapse structures.

This work may lead to a better comprehension of astrocytes role in relation to dopamine signaling in the ventral striatum.

Jiesi Feng

P12

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A Red-Shifted NE Sensor for Multiplexed Imaging of Noradrenergic Dynamics In Vivo

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Norepinephrine (NE) is a key neuromodulator involved in regulating arousal, stress response, mood, and cardiovascular function. Green fluorescent GRAB-NE sensors have enabled high-resolution visualization of NE dynamics in vivo. However, to dissect the complex interplay between NE and other signaling molecules, there is a growing need for spectrally distinct NE sensors that allow multiplexed imaging. To meet this demand, we developed rGRAB-NE2.0 (rNE2.0), a red-shifted, genetically encoded NE sensor based on the GPCR activation-based (GRAB) strategy. rNE2.0 exhibits a strong fluorescence response ($\Delta F/F_0$ ~600%), nanomolar sensitivity (~40 nM), fast sub-second kinetics, and minimal downstream signaling. Importantly, rNE2.0 shows high specificity for NE, with negligible cross-reactivity to structurally similar dopamine. In vivo, rNE2.0 reliably reports endogenous NE release in the lateral hypothalamus during tail suspension. To ensure compatibility with optogenetic tools and GFP-based indicators, we eliminated blue-light–induced photoswitching artifacts, resulting in a stable and versatile sensor suitable for multi-color imaging. Together, these advances make rNE2.0 a valuable tool for dissecting noradrenergic activities in both physiological and pathological contexts.

Gioele Giovanni Figus

P13

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Super-Resolution Morphological Analysis of Neuronal Structures Using Photon Resolved Image Scanning Microscopy

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Investigating neuronal morphology and its alterations is essential to shed light on the processes underlying both physiological and pathological large-scale phenomena. However, exploring in great details how neurons interact, organize, and modify their complex structure requires imaging technologies that overcome the resolution of conventional optical microscopy. Such super-resolution techniques surpass the diffraction limit barrier, enabling visualization of structures below 200 nm and thereby opening new avenues for morphological analysis. Among emerging methods, Image Scanning Microscopy (ISM) represents a promising solution to the long-standing trade-off between spatial resolution and Signal-to-Noise Ratio (SNR): by integrating Single Photon Avalanche Diode (SPAD) array detectors, where each element functions as an independent pinhole, ISM achieves a spatial resolution of ~120–150 nm without compromising fluorescence signal. In contrast, achieving a comparable resolution with conventional confocal microscopy would require a closed-pinhole configuration, which drastically reduces the number of collected photons and therefore limits image quality, especially in low-light or thick-sample conditions. In this project we apply PRISM (Photon-Resolved ISM), developed by Genoa Instruments, to primary neurons cultured in vitro, allowing photon-by-photon data acquisition and reconstruction by preserving the exact position and arrival time of each photon. This approach maximizes the information obtained from each sample and, due to the high temporal resolution of our system, lays a solid foundation for integration with functional imaging modalities such as Fluorescence Lifetime Imaging Microscopy (FLIM), with the aim of accurately correlating neuronal structures with molecular states and metabolic activity. In this work, we propose a multimodal framework that combines electrophysiological acquisitions, super-resolved morphological analysis and lifetime imaging, offering new insights into how nanoscale features contribute to neural computation and disease mechanisms. Our preliminary images demonstrate clear visualization of thin neuronal processes, enabling fine 3D morphological assessments. In the long term we envision applying this methodology to more complex samples, including 3D structures such as spheroids and brain organoids, with the goal of moving from animal-based to human-derived cultures.

Attila Ignácz

P14

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Syngap1 loss of function leads to altered intracellular calcium signalling and AMPA receptor stabilization

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The Syngap1 gene encodes the 'Synaptic Ras/Rap GTPase-activating' (SynGAP1) protein, one of the most abundant proteins of the postsynaptic density (PSD). Pathogenic mutations in Syngap1 often result in haplodeficiency (Syngap1+/-) and intellectual disability in humans.
SynGAP forms a complex with PSD95, a major postsynaptic scaffold protein, to accumulate at the PSD in basal conditions and repress synaptic activity, through AMPA receptor (AMPAR) trafficking and stabilization in the postsynaptic membrane. AMPARs are synaptic glutamate receptors responsible for most of the fast excitatory neurotransmission in the brain. AMPARs are in complex with auxiliary proteins, among which TARPs (transmembrane AMPA receptor regulatory proteins) are the main representants that enable AMPAR binding to PSD95 and synaptic stabilization. SynGAP1 acts via competing with TARPs on binding to PSD95 which contributes to SynGAP1 lowering synaptic strength, as well as Ras inhibition, which prevents AMPAR insertion to the plasma membrane.
In the present work we used Syngap1+/- mouse line, to investigate how haplodeficiency affects calcium activity on the network level as well as in individual postsynaptic dendritic spines. To this end, we expressed GCaMP in organotypic hippocampal slice cultures and measured spontaneous calcium activity using a Lattice Light Sheet microscope, developed in the Bordeaux Imaging Center. We observed an increase in both length and frequency of calcium events in Syngap1+/- cells compared to wild type, as well as a more synchronous activity.
Additionally, we performed uPAINT (Universal Point Accumulation Imaging in Nanoscale Topography) experiments using Fab antibody fragments against GluA1 and GluA2, on dissociated hippocampal cell cultures. The Fab was directly coupled to ATTO643, permitting single molecule tracking of AMPARs in the cell membrane. We used a Cas9-based knockdown of Syngap1 to induce loss of function in individual cells. Our results indicate an increase in stable surface AMPARs in the cells of Syngap1 loss of function.

Our findings are in line with the literature, where an excessive synaptic maturation rate is reported in Syngap1+/- mouse lines and provide further mechanistic explanation through increased AMPAR immobilization.

Marc Lebordais

P15

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Adapting nanoscale synaptic imaging protocols to zebrafish larvae

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As brain development progresses, synapses undergo fine-tuned regulation balancing stability with plasticity. Thus, out-of-balance synaptic maturation processes could result in brain disorders or diseases called synaptopathies. Several hypotheses have been proposed to underlie those conditions, such as alterations in synaptic formation, coupling, or the excitatory/inhibitory ratio. Moreover, nano-column organization between pre- and post-synaptic compartments has recently been observed in neural culture, and proposed to be necessary for efficient synaptic transmission. Therefore, characterizing nanoscale organization in developing neural circuits is a crucial step towards identifying aberrant patterns in synaptic development.

To date, neuronal cultures or brain slices from murine models are widely used to investigate nanoscale synaptic organization in physiological and pathological conditions. However, observations of such processes in the intact brain are limited. For this reason, the zebrafish (ZF) is a strategic model to study neurodevelopmental mechanisms. Indeed, its ex-utero development and transparency at the larval stage enable exceptional access to the whole-brain organization with microscopy. Although behavioral and brain-wide functional studies are increasingly established in this model, synaptic nanoscale microscopy protocols are still lacking. To that end, we have developed a workflow of optimized procedures relying on STimulated Emission Depletion (STED) microscopy combined with immunolabeling of pre- and post-synaptic proteins.

We first developed a montage for whole-mount ZF larvae, using a 3D-printed silicon mold, to be compatible with an inverted microscope. Then, we optimized an immunofluorescence protocol for STED microscopy of ZF synaptic proteins, focusing mainly on post-synaptic PSD-95 and Gephyrin, as excitatory and inhibitory scaffolding proteins, respectively, combined with the pre-synaptic protein Bassoon.

Our preliminary results reveal a strong improvement in STED imaging resolution compared to diffraction-limited microscopy, revealing diverse synaptic nano-organizations across multiple brain regions. Using a pySODA analysis package, previously developed in the lab (Wiesner et al., 2020), we will quantify the evolution of synaptic features, including coupling properties, at key developmental time points. Thus, by adapting super-resolution microscopy protocols for larval ZF, this work paves the way to uncover synaptic organization and maturation during early stage development.

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P16

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Adapting nanoscale synaptic imaging protocols to zebrafish larvae

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As brain development progresses, synapses undergo fine-tuned regulation balancing stability with plasticity. Thus, out-of-balance synaptic maturation processes could result in brain disorders or diseases called synaptopathies. Several hypotheses have been proposed to underlie those conditions, such as alterations in synaptic formation, coupling, or the excitatory/inhibitory ratio. Moreover, nano-column organization between pre- and post-synaptic compartments has recently been observed in neural culture, and proposed to be necessary for efficient synaptic transmission. Therefore, characterizing nanoscale organization in developing neural circuits is a crucial step towards identifying aberrant patterns in synaptic development.

To date, neuronal cultures or brain slices from murine models are widely used to investigate nanoscale synaptic organization in physiological and pathological conditions. However, observations of such processes in the intact brain are limited. For this reason, the zebrafish (ZF) is a strategic model to study neurodevelopmental mechanisms. Indeed, its ex-utero development and transparency at the larval stage enable exceptional access to the whole-brain organization with microscopy. Although behavioral and brain-wide functional studies are increasingly established in this model, synaptic nanoscale microscopy protocols are still lacking. To that end, we have developed a workflow of optimized procedures relying on STimulated Emission Depletion (STED) microscopy combined with immunolabeling of pre- and post-synaptic proteins.

We first developed a montage for whole-mount ZF larvae, using a 3D-printed silicon mold, to be compatible with an inverted microscope. Then, we optimized an immunofluorescence protocol for STED microscopy of ZF synaptic proteins, focusing mainly on post-synaptic PSD-95 and Gephyrin, as excitatory and inhibitory scaffolding proteins, respectively, combined with the pre-synaptic protein Bassoon.

Our preliminary results reveal a strong improvement in STED imaging resolution compared to diffraction-limited microscopy, revealing diverse synaptic nano-organizations across multiple brain regions. Using a pySODA analysis package, previously developed in the lab (Wiesner et al., 2020), we will quantify the evolution of synaptic features, including coupling properties, at key developmental time points. Thus, by adapting super-resolution microscopy protocols for larval ZF, this work paves the way to uncover synaptic organization and maturation during early stage development.

Anna Lovisotto

P17

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Alzheimer’s Tau Seeds-Induced Pathology Enhances Hippocampal Extracellular Diffusion

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The extracellular space (ECS) forms a complex and dynamic network that occupies approximately 20% of the brain's volume. It is filled with a cerebrospinal fluid-like solution enriched with extracellular matrix (ECM) molecules, which play a crucial role in regulating molecular diffusion. ECS properties are increasingly recognized as key modulators of protein mobility and, potentially, the propagation of pathological agents in neurodegenerative diseases. Despite the central role of tau protein aggregation in Alzheimer's disease (AD), the contribution of ECS and ECM dynamics to tau propagation remains largely unexplored.
In this study, we used wild-type mice injected with tau seeds purified from the brains of AD patients to model sporadic tauopathy. To assess extracellular diffusion, we employed quantum dot single-particle tracking (QD-SPT) in live hippocampal tissue, allowing us to probe nanoscale diffusion dynamics in situ. Our results revealed a significant increase in extracellular diffusion in tau-injected mice, particularly in specific hippocampal subregions. Diffusion profiles varied markedly between cell-dense and cell-sparse regions, reflecting local microstructural constraints.
Furthermore, we observed pathological alterations in ECM organization. Astrocytes exhibited abnormal internalisation of proteoglycans like aggrecan, and qPCR analyses revealed dysregulation of matrix-modifying enzymes, including upregulation of Galns. These findings suggest that tau-induced pathology alters ECM composition and turnover, contributing to enhanced diffusivity in the ECS. Interestingly, the highest diffusion rates were detected around autofluorescent cellular debris, possibly reflecting disrupted tissue architecture and impaired clearance mechanisms.
Together, our findings demonstrate that tau pathology induces structural and rheological remodeling of the hippocampal ECS, facilitating increased molecular diffusion. These changes may create a permissive environment for the extracellular spread of pathological tau species, highlighting ECS/ECM dynamics as a novel contributor to tauopathy progression and a potential therapeutic target in Alzheimer’s disease.

Our preliminary results reveal a strong improvement in STED imaging resolution compared to diffraction-limited microscopy, revealing diverse synaptic nano-organizations across multiple brain regions. Using a pySODA analysis package, previously developed in the lab (Wiesner et al., 2020), we will quantify the evolution of synaptic features, including coupling properties, at key developmental time points. Thus, by adapting super-resolution microscopy protocols for larval ZF, this work paves the way to uncover synaptic organization and maturation during early stage development.

Rohit Mangalwedhekar

P18

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3D Single-Particle Tracking using Short-Wave Infrared probes to study diffusion in Complex Soft Matter Systems as Models for neurobiological tissues

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Exploration of the brain extracellular space (ECS) using diffusing nanoparticles has gained interest in recent years. These regions are microenvironments for signalling molecules, neurotransmitters and toxic metabolites, and exhibit heterogeneities and spatial variations with changes in health, and development. Thus, understanding the physical aspects of diffusion in complex biological tissues is crucial to explain the nanoscale topology and local molecular mobility in the context of health and disease. In this regard, soft matter systems act as excellent biophysical models to investigate diffusion properties in a standardised, reproducible manner while also offering tunability and controlled complexity that is seldom achievable in biological tissues.

Here we present a soft matter system composed of packed emulsion droplets as a biophysical model to mimic ECS in tissues that can be studied with SWIR Single Particle Tracking (SPT). These bio-inspired emulsions used to study macroscopic tissue responses to mechanical forces and cell adhesion form a network of interstitial voids that provides a model system for various tissues, including brain ECS (Figure 1).

The exploration of these model samples can be achieved with the use of SPT strategies in the shortwave infrared (SWIR) range (950-1300 nm), particularly using color-center single-walled carbon nanotubes (CCNTs) of various lengths. Their exceptionally bright emission lies in the transparency window of biological tissues, enabling SPT at depths of hundreds of micrometres.

Using widefield optical sectioning, and a double helix point spread function for 3D SPT, we obtain correlative images of the droplet system with individual diffusing nanoparticles with a localization accuracy of less than 20 nm at SWIR wavelengths in the 3 dimensions. Using ultra-short CCNTs of ~50 nm length (with nanometer diameter), fluorescing at ~1100 nm as nanoprobes, we demonstrate 3D SPT at depths greater than 100 µm. Additionally, we show that the choice of CCNT surfactant allows for tuning their diffusion at the droplet surface or in interstitial volumes. Furthermore, we investigate the impact of the nanotube length on their exploration and diffusion. Finally, we also propose an opal model to study diffusion in rigid structures relative to droplets.

Amine Mehidi

P19

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Intrabody-based Tools to Resolve Endogenous Talin Domain Organization in Living Cells

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Talin is a multimodular protein composed of a globular head and a rod region containing 13 mechanosensitive domains. It connects the extracellular matrix to the actin cytoskeleton through integrin-based adhesions. Known as a key component of integrin adhesion structures (IAS), talin is now recognized as essential for the transmission and processing of mechanical forces across these structures. Its function depends on force-induced conformational changes that regulate interactions with multiple adhesion and signaling partners. Despite significant advances in understanding talin mechanobiology, current tools do not enable the study of the conformational states of endogenous talin at the domain level in living cells.

To overcome this limitation, we developed a panel of intrabodies, protein probes selected by directed evolution, which specifically recognize distinct talin domains. Used in fluorescence microscopy of live mouse embryonic fibroblasts (MEFs), these probes enable precise labeling of the targeted domains. Combined with advanced super-resolution microscopy techniques and proximity labeling approaches, these intrabodies enable dynamic and spatiotemporal studies of the interactions and organization of endogenous talin domains under physiological conditions. This innovative approach holds great promise for investigating mechanotransduction in complex systems, particularly in neuronal contexts, where adhesion dynamics are critical.

Camille Mergaux

P20

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Exploring the interplay between the extrasynaptic surfaceome and extracellular matrix in hippocampal networks

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Neurotransmitter receptors are the main actors of neuronal communication. In synapses, they are organized into dense and structured clusters, allowing a fast and efficient binding of signaling molecules. Disruption of this organization impairs synaptic functions, such as long-term synaptic plasticity. These receptors are also present in the extrasynaptic membrane, where they are activated by ambient neurotransmitters. Their activation generates tonic and persistent currents particularly important for the modulation of neuronal excitability and dendritic integration. However, how neurotransmitter receptors, and more generally membrane proteins, are organized within this extrasynaptic compartment remains poorly understood. Brain cells are enwrapped in a dense mesh of proteins and polysaccharides, namely the extracellular matrix (ECM). This matrix is a well-known regulator of receptor signaling and ion channel transmission. Given ECM's regulatory roles, we test the hypothesis that ECM controls surface proteins (surfaceome) organization and related functions. Combination of single molecule localization and tracking microscopies (dSTORM, sptPALM) are mainly used to unveil extrasynaptic surfaceome organization in hippocampal neurons. Genetic tools are developped to modify ECM content in neuronal cultures. Preliminary results reveal that extrasynaptic surface proteins are organized within nanoscale complexes whose organization is changed when ECM content is modulated. While molecular mechanisms underlying this interplay and its functional consequences remain to be explored, these findings highlight the ECM’s role in neuronal surfaceome organization.

Aysha Shazmina

P21

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Three-photon holographic all-optical interrogation of neuronal circuits in depth

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This project aims to advance three-photon excitation (3PE) strategies for all-optical investigation of neuronal circuits in deep brain regions. Leveraging the intrinsic advantages of 3PE, such as reduced scattering due to longer excitation wavelengths and superior optical sectioning from the cubic intensity dependence, we aim to perform both imaging and precise at depth. We developed a custom-designed microscope for parallel 3P holographic excitation, and optimized protocols for precise neuronal activation. We report, for the first time, successful 3PE optogenetic activation of both excitatory and inhibitory opsins using soma-targeted illumination via computer-generated holography (CGH) combined with temporal focusing (TF). Furthermore, we demonstrate in vivo all-optical experiments in the mouse visual cortex at depths reaching up to 800 µm. These results highlight the potential of 3PE microscopy as a powerful tool for functional interrogation of deep-layer neuronal circuits with high spatiotemporal precision.

Antoine Reynaud

P22

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Multimodal imaging of the dynamics of endocytic zones in hippocampal neurons in dissociated and organotypic cultures

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Synapses within neuronal networks are highly dynamic structures, subject both to intracellular messages and to extracellular signals, leading to the phenomenon of long-term synaptic plasticity. Receptors present at the post-synaptic membrane, particularly in the region known as the post-synaptic density, are continually recycled by endocytosis and exocytosis, modulating the overall sensitivity of the post-synapse to ligands released into the synaptic cleft. Recycling of these post-synaptic receptors plays a major role in synaptic plasticity and is achieved in the case of internalisation mainly by clathrin coat structures (CCSs). The dynamics and activity CCSs can be modulated by both intracellular and extracellular signals. We have shown, with live cell spinning disk confocal microscopy of neurons expressing clathrin light chain fused to Halotag and the ligand labelled with JF645, that CCSs are very static in mature cultured hippocampal neurons. However, we show with lattice light sheet microscopy that in neurons in organotypic hippocampal slices, CCSs are much more dynamic, with a median life time of less than 2 minutes. We now explore the mechanisms of this difference. First, we vary the mechanical environment of neurons by growing neuronal cultures on substrates of controlled rigidity. Second, we modify the interactions of neurons with the extracellular matrix, in particular integrins, and neighbouring cells such as astrocytes with pharmacological blockers and knock-down/knock-out strategies. To do so, we have built a spectral image scanning microscope enabling live multicolour imaging of various types of culture preparation.

Manuel Rojo

P23

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When a problem becomes a tool: continuous high density labeling of cellular membranes with fluorescent secondary antibodies in conventional and in super-resolution microscopy

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In conventional wide-field or confocal microscopy, the detection of membrane proteins with specific antibodies apparently achieves homogeneous and continuous labeling of membranes and/or organelles. In super-resolution microscopy, however, the detection of separate membrane proteins or protein complexes hampers continuous labeling of cellular and organellar membranes. In this work, we show that secondary antibodies coupled to hydrophobic dyes (goat IgG coupled to atto 647N or atto 550) efficiently label cellular membranes, notably mitochondria, endoplasmic reticulum and plasma membranes. Membrane labeling requires solubilization of fixed cells with non-ionic detergents (Triton X-100) and is abolished upon fixation/permeabilization with solvents (cold methanol); implying that membrane labeling relies on binding of fluorescent IgG to detergent-resistant and solvent-extractable lipids. We further show that the signal of IgG-atto 647N and IgG-atto 550 can be amplified or modified with antibodies against goat IgG coupled to other fluorescent dyes. Finally, we establish that these commercially available reagents allow bright, continuous and high density labeling of cellular membranes with different super-resolution microscopy approaches: stimulated-emission-depletion (STED), direct stochastic optical reconstruction microscopy (dSTORM) and expansion microscopy (ExM).

Limeng Ruan

P24

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Polarization sensitive Single Nanoparticle Tracking in the near-infrared in the Brain Extracellular Space

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The extracellular space (ECS) is a system of interconnected regions limited by neuronal membranes and containing the interstitial fluid and the extracellular matrix (ECM), which plays a fundamental role in the diffusion of chemical signals and intercellular communication. Understanding its characteristics is crucial for advancing our knowledge of the physiology and pathology of the brain. The development of single nanoparticle tracking has provided a method to obtain the local diffusion behavior of molecules in live brain tissues. In particular, single-walled carbon nanotubes (SWCNTs) are highly efficient emitters in the near-infrared (NIR) domain, which are used as a probe for imaging and characterizing the brain ECS. While the study of SWCNT translational diffusion in ECS has been well established, their rotational diffusion characteristics remain underexplored. This would provide useful information about the local viscosity properties of the ECS as well as the local ECM organization.

To this aim, we developed a radially and azimuthally polarized (raPol) microscope working in the NIR inspired by single-molecule orientation-localization microscopy (SMOLM). Our system is based on the optical property that the fluorescence polarization emitted by SWCNTs is aligned with their backbone. The 3D orientation is represented by the azimuthal and polar angles. By engineering the dipole-spread function, we can obtain two different polarization images, which exhibit different shapes and intensities depending on the 3D orientation of the SWCNT. This allows us to get both 2D localization and 3D orientational information. Our analysis is based on vectorial phase retrieval and deep learning algorithms to achieve high-precision measurements. Our work provides a new approach for studying the rotational diffusion of SWCNTs and holds promising potential for applications in nanoscale brain imaging and material characterization.

Dimitrios Samouil

P25

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Modulation of NMDA receptor signalling by postsynaptic metabotropic glutamate receptors

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At excitatory synapses, the effects of glutamate are mediated by the cooperative actions of a large variety of glutamate receptor subtypes. How, at individual synapses, different glutamate receptors cooperate to shape synaptic transmission, and plasticity remains however poorly understood. Here, we specifically aim to resolve how different postsynaptic metabotropic glutamate receptor (mGluR) subtypes cooperate to modulate the function of the ionotropic NMDA-type glutamate receptor (NMDAR). To do so, we utilize CRISPR/Cas9-mediated genome editing tools to tag and localize endogenous mGluRs at and around excitatory synapses. To determine how different mGluR subtypes modulate NMDAR function we record miniature spontaneous calcium transients (mSCaTs) at individual synapses. We found that in hippocampal neurons, both endogenous mGluR5 and mGluR2 are expressed at most, but not all, excitatory synapses. Further, using super-resolution STED microscopy, we found that both receptor types are enriched at perisynaptic sites, seemingly excluded from the postsynaptic density. Single-molecule tracking experiments revealed, however, that mGluR2 is significantly more mobile than mGluR5. Pharmacological activation of mGluR2 or mGluR5 affected NMDAR-mediated calcium transients. Together, these results indicate that both group I and II metabotropic glutamate receptors are differentially expressed at excitatory synapses to exert control over NMDA receptor signalling. Untangling the regulatory mechanisms that allow glutamate receptor crosstalk at synapses will provide a firm understanding of how glutamate receptors cooperatively shape synaptic transmission and plasticity.

Silvia Sposini

P26

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Investigation of neuromodulator receptor endocytosis at single vesicle resolution in their physiological context

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Neurons express a variety of G protein coupled receptors (GPCRs), which profoundly condition neuronal physiology by activating intracellular signalling cascades in response to extracellular signals, including neuromodulators such as noradrenaline and dopamine. One mechanism GPCR use to tune their activity is membrane trafficking. Upon ligand binding, GPCRs are endocytosed through distinct mechanisms via interaction with different proteins (e.g clathrin, endophilin). Moreover, endocytosis is affected by membrane composition. With the aim of studying endocytosis at single event level in living neurons, where neuromodulator receptors are endogenously expressed, we adapted a high spatial-temporal resolution method combining pH sensitive probes and Total Internal Reflection fluorescence microscopy, called the pulsed pH (ppH) assay. With this, we monitored endocytosis of β-adrenergic receptors (βARs) in primary hippocampal neurons. We observed that agonist stimulation (10 µM isoproterenol) induces β2AR, but not β1AR, membrane clusters and increases only β2AR internalization rates. Moreover, whilst β1AR does not prefer either clathrin or endophillin2A, β2AR preferentially internalizes through clathrin. With the same approach, we investigated the effect of poly unsaturated fatty acids (PUFAs), lipids enriched in the brain, on the endocytosis of the Dopamine receptor 2 (D2R). We show that, in cells enriched in either ω3- or ω6-PUFAs, agonist-induced (10 µM quinpirole or dopamine) D2R endocytosis, but not that of other membrane receptors, is blunted compared to control cells. Specifically, PUFA enrichment affects recruitment of β-arrestin2 at D2R endocytic sites, sparing receptor clustering at the membrane. Together we have uncovered novel mechanistic details of GPCR endocytosis in their physiological context relevant to neuromodulation.

Daniele Stajano

P27

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TRPV1 Channels as Modulators of Synaptic Fate During Neurodevelopment: Insights from Super-Resolution Imaging

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During human neurodevelopment, physiological, activity-dependent synaptic loss reduces synapse density by approximately 50%, establishing the coherent microarchitecture and connectivity of the mature nervous system. This synaptic elimination is initiated by Long-Term Depression (LTD), a form of synaptic plasticity known for downregulating synaptic transmission. Sustained LTD and the induction of synaptic pruning require dephosphorylation of GSK3β at Ser9, which promotes PSD95 phosphorylation and subsequent autophagy.

Our group has shown that calcium channels act as integrators of extracellular signals following LTD, functioning as both sensors and effectors in pathways related to neuronal activity, neuromodulation, and inflammation, ultimately determining synaptic fate—whether maintenance or elimination.

Recent data from our lab identify the Transient Receptor Potential Vanilloid 1 (TRPV1) channel as a novel modulator of synaptic fate during both activity-dependent and inflammation-induced LTD. TRPV1 appears to influence pruning via regulation of GSK3β activity.

To gain structural insights into TRPV1 function at synapses, we focus on its nanoscale distribution during key neurodevelopmental windows of synaptic loss. Although TRPV1 surface levels are known to be reduced in a Shank3-related model of Autism Spectrum Disorder—a condition marked by altered synaptic density—its spatial organization at the synapse remains unclear.

Here, we employ direct Stochastic Optical Reconstruction Microscopy (dSTORM) with spectral demixing to visualize endogenous TRPV1, tagged through CRISPR-based strategies, in primary mouse hippocampal neurons. Preliminary findings reveal a developmental accumulation of TRPV1 at dendritic spines, particularly during the critical window for physiological synapse elimination.

These results suggest a potential regulatory role for TRPV1 in shaping synaptic architecture during neurodevelopment.

Vasika Venugopal

P28

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Bridging Synaptic Molecular Architecture to Function: a Multimodal Approach to Study Synapse Heterogeneity

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Connectomics studies have recently revealed the staggering complexity of neural circuits in the mammalian brain, arising from the highly organized diversity. Synapses play a pivotal role here, constantly remodelled by activity-dependent mechanisms to support adaptive behaviours such as learning and memory. They are also the locus of numerous psychiatric and neurodegenerative diseases, including autism spectrum disorders (ASDs) and Alzheimer's disease, where synaptic failure and maladaptation may precede symptom onset. Classical approaches typically consider synapses at the population level, i.e. the average synapse. However, synapses exhibit significant structural and functional heterogeneity. This includes variations in size, receptor composition, and plasticity. Super-resolution techniques have further revealed sub-synaptic diversity through nano-domains, highlighting the need for a deeper understanding of individual synapses. Depending on these parameters, individual synapses may exhibit different plastic properties and respond differently to activity-dependent, genetic or pharmacological perturbations, whether physiological, pathological, or therapeutic. Thus, investigation into synaptic heterogeneity begs to be addressed.

To fully comprehend rules that shape synapse heterogeneity, experimental approaches integrating multiple modalities at single-synapse resolution are necessary. Previous methods, while allowing monitoring of single synapses, show strong limitations, lacking either spatial, functional, structural or molecular correlates. Thus, we developed a strategy combining patch-clamp recordings with single- cell transfection, high-resolution live imaging, and expansion microscopy to enable: 1/ genetic manipulation and imaging of pre- and postsynaptic elements at individual synaptic sites embedded

in the local connectome; 2/ mapping and monitoring at structural, molecular, and functional levels to investigate their contribution to neuronal output; 3/ studying the impact of synaptic plasticity or disease-related mutations in a longitudinal manner. Here, we present an example, overexpression of Neuroligin-1, a key transsynaptic adhesion molecule implicated in ASDs, to understand synapse heterogeneity through perturbation.

Léa Villetelle

P29

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A Truncating Mutation in the GluN2B C-Terminal Domain Reveals Non-Ionotropic Roles of NMDARs in Neurodevelopment

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NMDARs are ionotropic glutamate receptors essential for synaptic plasticity and neurodevelopment. Among their subunits, GluN2B plays a critical role during early brain development. Recent sequencing efforts have identified rare de novo GRIN2B variants in individuals with autism spectrum disorder (ASD), intellectual disability (ID), epilepsy, and developmental delay. Interestingly, several of these mutations occur in the intracellular C-terminal domain (CTD) of GluN2B and induce no change in the intrinsic properties of the receptor, suggesting a role beyond ionotropic function. Here, we investigated the Y1004* mutation, which introduces a premature stop codon in the CTD, and is associated with ASD and ID. Despite its truncation, GluN2B-Y1004* traffics normally to the plasma membrane in cortical neurons, as shown by live-cell immunolabeling and confocal imaging. However, high-resolution analysis revealed a pronounced reduction in synaptic localization compared to wild-type (WT) GluN2B, indicating a deficit in synaptic anchoring. To assess receptor dynamics, we performed single-particle tracking using PALM and quantum-dot labeling (SPT-PALM and SPT-QD). These techniques showed that receptors containing Y1004* exhibited significantly higher lateral mobility, reduced confinement within synaptic domains, and shorter synaptic dwell times. These findings highlight the CTD's essential role in stabilizing NMDARs at synapses. Beyond affecting localization and mobility, the CTD truncation also altered the receptor's structural conformation. Using FLIM-FRET, we measured energy transfer between GluN1 subunits co-expressed with either WT or Y1004* GluN2B. In the WT condition, a stable FRET signal indicated a consistent GluN1-GluN1 proximity within the receptor. In contrast, co-expression with Y1004* led to a significant decrease in FRET efficiency, suggesting intramolecular rearrangement and increased conformational flexibility. These results demonstrate that the CTD is critical not only for synaptic anchoring but also for maintaining proper receptor architecture. A single truncated GluN2B subunit is sufficient to disturb the organization of the receptor complex, even in the presence of non-mutated GluN1. Our findings challenge the assumption that truncating mutations act solely via trafficking deficits and highlight the CTD as a key regulator of both NMDAR localization and structural integrity. These insights may help explain the pathogenic mechanisms underlying CTD-truncating GRIN2B mutations in neurodevelopmental disorders.

Xuesi Zhou

P30

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Deciphering the mechano-sensitive properties of the membrane periodic skeleton (MPS) in neurons

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Mechanosensing is crucial for the regulation of neuronal functions, such as neuronal development and synaptic transmission, as well as pathological events like traumatic brain injury. In neurons, spectrin/actin forms a unique structure known as the periodic membrane skeleton (MPS), a novel cellular organization discovered by super-resolution. The periodic architecture and molecular composition provide essential properties for it to function as a mechanosensing structure. However, direct evidence of MPS response to force is still lacking. To capture the mechanical response of the MPS at the nanoscale, we used a homemade cell stretching device compatible with super-resolution. We show that MPS exhibited a loading-rate-dependent response to mechanical forces. Slow deformations led to reversible spectrin extension and unfolding, while rapid loading induced irreversible spectrin-actin reorganization. The actin-capping protein adducin displayed reversible mechano-dependent dissociations from the MPS. Thus, the MPS is a mechanosensitive scaffold that actively and reversibly reorganizes in response to mechanical stimuli.a potential regulatory role for TRPV1 in shaping synaptic architecture during neurodevelopment.

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