# Projects

**Project 1: "Imaging prefrontal cortex neurons encoding social information"**
**Instructor: Francisco de los Santos (*Max Planck Institute for Metabolism Research, University of Cologne, Germany*)**

Students will perform calcium imaging of neurons in the prefrontal cortex (PFC) in mice exploring an intruder animal of the same (experiment B1) and of a different sex (experiment B2), to detect neurons responding to social stimuli.
On day 1, the surgical steps will be explained including injection of virus encoding the calcium sensor and implantation of the lens probe, followed by a hands-on demonstration of stereotaxic attachment of the baseplate to the animal. The latter procedure is necessary to create a permanent docking station for the microendocsope, and will allow us to perform the subsequent experiments of the course. On day 2, aspects to consider when planning behavioural experiments using 1-photon calcium imaging will be discussed, and the nVista recording system and AnyMaze tracking platform will be introduced. After habituation to the microendoscope and arena, neural activity will be imaged while mice explore an enclosure with a conspecific allowing for social interaction. Mice will be recorded on 2 subsequent days, followed by one day of scoring of the social interactions. During the last 4 days of the project, pre-processing and analyses will be performed. Caiman, the Matlab-based, pre-processing pipeline for 1p-imaging data, will be introduced and used on the recorded images (for quality control, motion correction and trace extraction). Then, behavioural data will be processed (using Matlab). Finally, socially modulated cells will be identified and compared across experiments, followed by presentation and discussion of the results.
Main Techniques:

*Microendoscopes*
*Stereotactic surgeries*
*Behaviour*
*Matlab analysis*

**Project 2: "Imaging hippocampal place cells in freely moving animals"**
**Instructor: Hanna Van Den Munkhof (*Max Planck Institute for Metabolism Research, University of Cologne, Germany*)**

Students will perform calcium imaging of hippocampal CA1 neurons across 3 days in mice exploring the same arena to detect place fields and their stability over time.
On day 1, the surgical steps will be explained including injection of virus encoding the calcium sensor and implantation of the lens probe, followed by a hands-on demonstration of stereotaxic attachment of the baseplate to the animal. The latter procedure is necessary to create a permanent docking station for the microendocsope, and will allow us to perform the subsequent experiments of the course. On day 2, aspects to consider when planning behavioural experiments using 1-photon calcium imaging will be discussed, and the nVista recording system and AnyMaze tracking platform will be introduced. After habituation to the microendoscope and arena, neural activity will be imaged while mice explore a free-

feeding enclosure containing a range of stimuli (food, water, conspecific and a novel object). Mice will be recorded for 4-6 trials (of 5min?) on 3 subsequent days. During the last 4 days of the project, pre-processing and analyses will be performed. Caiman, the Matlab-based, pre-processing pipeline for 1p-imaging data, will be introduced and used on the recorded images (for quality control, motion correction and trace extraction). Then, behavioural data will be processed (using manual scoring and Matlab) and recordings longitudinally registered. Finally, place fields will be detected and compared across days, followed by presentation and discussion of the results.

Main Techniques:

*Microendoscopes*
*Stereotactic surgeries*
*Behaviour*
*Matlab analysis*

**Project 3: "FRET imaging of NMDA receptor co-agonists dynamics"**
**Instructor: Petr Unichenko (*Institute of Cellular Neurosciences, University of Bonn Medical School, Germany)***

Activation of excitatory glutamate receptors of the NMDA subtype (NMDARs) requires binding of the ligand glutamate and of a co-agonist: glycine or D-serine. Interestingly, glycine is not only an NMDAR co-agonist but also a primary ligand for inhibitory glycine receptors (GlyRs), which are widely expressed throughout the mammalian brain. Therefore, measuring extracellular glycine dynamics is essential to gain a deeper insight into glycinergic modulation of neuronal activity. It also provides the basis for understanding pathology of brain disorders involving NMDARs and/or GlyRs such as schizophrenia, depression, anxiety or epilepsy.

Together with the lab of Colin Jackson (ANU, Canberra) our lab has recently constructed a first optical FRET-based glycine sensor and investigated hippocampal glycine signalling (Zhang et al., 2018). In this project we will monitor extracellular levels of glycine during different modes of neuronal activity using this novel glycine sensor in hippocampal slices using two-photon excitation fluorescence microscopy. We will first establish the general protocol for immobilizing the sensor in extracellular space and the required tests to ensure its stability. We will then carry out experiments that explore which neuronal activity patterns drive extracellular glycine transients in the hippocampus. Analysis will be performed using fluorescence intensity and lifetime recordings (fluorescence lifetime imaging, FLIM).

Main Techniques:

*Transfection methods for slice cultures*
*Two-photon microscopy*
*FLIM*

**Project 4: "Assessing the mechanosensitive properties of the membrane periodic skeleton (MPS) in neurons"**
**Instructor: Filipe Nunes Vincente (*IINS, Université de Bordeaux, France*)**

Mechanotransduction events are genuine signals regulating cell migration, proliferation and differentiation in physiological and pathological processes such as immune response, development and metastasis. In neurons, mechanotransduction is involved in neurodevelopment, limb elongation and brain trauma. Neuronal growth is sensitive to the local mechanical environment and peripheral axons are often subjected to considerable strains, especially during limb flexion. Mechanical stretch by itself promotes axonal growth and neurotransmitter clustering. However, the molecular mechanisms involved in axonal mechanosensing are yet to be clarified. In this regard, recent studies using super-resolution microscopy (SRM) have unveiled the presence of a subcortical actin-spectrin lattice in axons, known as the membrane periodic skeleton (MPS) [1]. The MPS is composed by circumferential F-actin rings spaced by a period of ~190 nm, interconnected by spectrin tetramers. Although the biological functions of the MPS are unclear, recent evidence suggests a link between MPS dynamics and axonal physiology. Therefore, the MPS by itself might be a mechanosensitive structure, with the actin-spectrin scaffold acting as a shock buffer. For this project, we aim to characterize the molecular mechanisms underlying MPS mechanosensing.
In this regard, we have recently developed a unique cell stretching device compatible with SRM and single particle tracking (SPT). This method enables to study the nanoscale deformations and reorganizations of individual proteins inside mechano-sensitive structures. We will employ a combination of cell stretching and SRM or SPT on different biological systems (hippocampal neurons, Drosophila neurons, chicken spinal cord explants). There are two major classes of SRM: stochastic approaches based on Single Molecule Localization Microscopy (SMLM: PALM, STORM, PAINT) that use time and space decorrelation of single molecule emission [2,3] ; or targeted light-structuring techniques that control the emission states at precisely defined positions in the sample (STED, RESOLFT) [2,3]. We have extensively used several of these techniques in our team to characterize protein dynamics and nanoscale organization of mechanosensitive structures [4,5,6]. Using our device, we will image MPS components (e.g. βII-Spectrin, actin) with SRM (STED or PAINT) after live stretching followed by fixation of hippocampal neurons. This will allow to assess the nanoscale reorganization of MPS in response to external mechanical forces. In parallel, we will study the dynamics and acute mechanical responses of MPS components (e.g. βII-Spectrin, actin) by combining simultaneous SPT (sptPALM) and live stretching on Drosophila neurons or chicken spinal cord explants.
Main techniques:

*Super resolution microscopy (STED and PAINT)*
*Drosophila preparation*
*Chicken spinal cord explants*

**Project 5: "RESOLFT nanoscopy to study the fast reorganization of dendritic spines"**
**Instructor: Ani Jose (*IINS, Université de Bordeaux, France*)**

The rapid and sustained structural remodeling of dendritic spines are known to be correlated with circuit plasticity during memory and learning. This structural plasticity is facilitated by fast molecular reorganizations of scaffolding proteins within the spines. Actin cytoskeleton is perhaps the most important scaffolding structure enabling spine remodeling, as they form the structural basis for dendritic spines. Another major scaffolding protein is the membrane-associated PSD-95, which is involved in anchoring synaptic proteins, and is believed to be one of the most important regulators of synaptic strength. Even though these structures have been studied for many years, the molecular mechanisms leading to spine remodeling are still subjected to debate. Various actin binding proteins (ABP) like the WAVE complex, Formin (FMNL2), Arp2/3 etc. are also known to be key players in regulating spine plasticity. But these studies relied on single particle tracking, which does not allow fast acquisition of super-resolved time-lapses, thereby lacking a global picture of reorganization of macromolecular structures occurring at short timescales (1-10s).
Optical microscopy, particularly fluorescence microscopy, is an important and widely used tool in life sciences due to its unique ability to observe cellular processes in living specimens with target-specific image contrast. However, the wave nature of light limits the ultimate resolution achievable with an optical microscope. Light originating from a point object does not converge to a single point at the image plane but spreads out in space to a finite-sized volume, thereby losing the high frequency information from the sample. But over the past two decades, various Imaging techniques have emerged which circumvents the diffraction limit, primarily by utilizing on-off transitions in fluorescent molecules[6]. Collectively called nanoscopy (or super-resolution microscopy), these imaging techniques revolutionized bio-imaging by delivering structural information previously hidden due to diffraction of light. Out of these techniques, RESOLFT (Reversible Saturable OpticaL Fluorescent Transitions) is particularly interesting for live neuron imaging, as it uses minimal light doses to circumvent the diffraction limit. RESOLFT nanoscopy have already demonstrated its ability to visualize the remodeling of dendritic spines and the rapid molecular reorganization within them. In this project, we will use the RESOLFT nanoscope that we have developed in our lab, to study the nanoscale organization of PSD 95 and ABPs within the spines and investigate how their reorganization leads to spine remodeling.
Main techniques:
*Super resolution microscopy: RESOLFT nanoscopy*

**Project 6: "Single synapse imaging of glutamate release and pre-synaptic $Ca^{2+}$ for study of pre-synaptic receptor function and short-term synaptic plasticity"**
**Instructor: Tom Jensen (*University College London, United Kingdom*)**

Control of the pre-synaptic neurotransmitter release machinery by $Ca^{2+}$ dependent signalling is crucial for information processing in the central nervous system. Yet, reliable and direct study of release dynamics and measurement of intrasynaptic signalling at small central synapses has only recently been achieved with multiphoton imaging and novel genetically encoded sensors. This project aims to provide the student with the technical training to apply intensity-based imaging of glutamate and/or $Ca^{2+}$ at individual synapses, and to learn the theoretical framework to analyse and interpret the results. During the

course the students will employ these methods in a pilot experiment to study how modulation of cannabinoid sensitive receptors during postnatal development influences the structure and function of hippocampal synapses.

Main Techniques:

*Transfection methods for use in hippocampal slice cultures*
*Patch-clamp electrophysiology*
*multiphoton Glutamate/Calcium imaging*
*Imaging and electrophysiology data analysis*

**Project 7: "Quantitative intracellular calcium measurements using fluorescent lifetime imaging microscopy"**
**Instructor: Kaiyu Zhen (*University College London, United Kingdom*)**

The aim of this project is to introduce basic theoretical background, up-to-date techniques, calibration procedures, common troubleshooting of FLIM systems, hands-on demonstration of calcium measurement using FLIM and analysis of FLIM data. At the end, students should have sufficient understanding of FLIM techniques, be able to perform simple FLIM calcium imaging on a ready-made FLIM microscope and obtain useful results by apply simple analysis. After the workshop, the students should be able to adapt the FLIM technique to their own projects where possible on an existing FLIM system.

Main Techniques:

*Two-photon microscopy*
*FLIM system*
*Phasor analysis of complex FLIM data*

**Project 8: "A viral strategy for targeting and manipulating principal neurons: probing the glutamatergic synapse function at multi-synapse imaging approach"**
**Instructor: Olga Kopach (*Queen Square Institute of Neurology, University College London, United Kingdom*)**

Release of glutamate – the primary excitatory neurotransmitter in the brain – at the synapses of principal hippocampal neurons controls synaptic activity essential for information processing, memory and learning formation. Recent advances in engineering the neurotransmitter-sensing fluorophores have enabled monitoring glutamate within neural circuits using multiphoton imaging techniques. This project aims to provide students with the necessary technical skills and theoretical framework to apply the optical glutamate sensor (iGluSnRF) for monitoring glutamate release evoked by electric stimulation of afferent fibres in acute brain slices and to trace release events at multiple synapses simultaneously. A possibility of monitoring astrocytic 'sensing' of glutamate release at the synapses could be achieved by combining iGluSnRF and $Ca^{2+}$ imaging in astrocytes using genetically encoded fluorophores or bulk-loaded dyes. We will apply these approaches for live-cell imaging within CA3-CA1 pathway in an acute hippocampal tissue. The project aims to probe the glutamate release probability between different synapses.

Students are invited to design own questions and test their hypothesis on tissue with genetically encoded fluorophores. This will require some advanced planning to get virally transfected tissue for the study before the course. Alternatively, students can learn the technique of viral transfection via the intracerebroventricular injections in neonates and

develop skills on multiplex imaging with burst stimulation of Schaffer collaterals using the hippocampal slices expressing iGluSnRF.

Main Techniques:

*Viral transfection*
*Two-photon microscopy*
*Combine glutamate and calcium sensor for simultaneous measurements*

**Project 9: "Probing of astroglial $Ca^{2+}$ dynamics in organised brain tissue through fluorescent intensity and lifetime measurements"**
**Instructor: Olga Tiurikova (*Institute of Neurology, University College London, United Kingdom*)**

Electrically passive astroglia use internal Ca2+ waves to shape synaptic transmission and modulate physiological functioning of neuronal networks in the brain. Yet, one of the main challenges of studying Ca2+ dynamics in organised brain tissue associated with the technological limitations of monitoring and probing astroglia on a sub-microscopic scale. This project aims to provide the student with theoretical framework and necessary technical skills to record Ca2+ dynamics (baseline and evoked activity upon exposure to exogenous compound) in astrocytic syncytium of acute brain slices. This would include acute slices preparation, intracerebroventricular injection of genetically encoded Ca2+ indicator to neonatal pups, two alternative approaches of Ca2+-sensitive dyes delivery to the glial syncytium and two imaging strategies.

Main techniques:

*Intracerebroventricular AAV injection to neonatal mice*
*Two-photon microscope*
*FLIM measurements of Ca2+ dynamics*
*FLIM analysis*

**Project 10: "Multicolor multiphoton imaging of single synaptic release sites *in vivo*"**
**Instructor: James Reynolds (*Queen Square Institute of Neurology, University College London, United Kingdom*)**

Somatosensation has been observed to recruit intracellular calcium oscillations in cortical astrocytes *in vivo*; however, they also display constitutive and stochastic calcium activity even in the apparent absence of sensory stimuli. As such, there are constraints in relating astrocytic activity to distinct experimental inputs. One approach we are developing in the Rusakov lab involves multiplexed imaging of two compartments of the tripartite synapse. This approach allows us to spectrally isolate astrocytic calcium oscillations in proximity to synaptic structures or in concurrence with an indicator of genuine neurotransmission (such as calcium elevations in the presynaptic bouton). As such, we can record perisynaptic astrocytic responses to genuine neurotransmission at single-synapses.

The aim of the project is to perform multiphoton microscopy *in vivo*, measuring both the distribution of labelled presynaptic boutons in the barrel cortex, and their activity during a simple sensory stimulus (such as a tactile whisker displacement). Students will then design and implement an imaging experiment that leverages these observed patterns of neurotransmission in order to better decode concomitant astrocyte calcium activity.

By the end of the project, students will be competent to perform such imaging experiments and will be able to tailor and generalise the approach to their own experimental needs.
Main techniques:

*In vivo multiphoton light microscopy*
*Intracranial injections (to introduce anterograde tracers and/or fluorescent proteins)*
*cranial window implantation*
*Image analysis (ImageJ, Matlab)*

**Project 11: "High spatial-temporal imaging of a heterogeneous population of synapses in neuronal cultures using the iGluSnFR probe "**
**Instructor: Phillipe Ribeiro Furtado De Mendonca ((*Institute of Neurology, University College London, United Kingdom*)**

Traditionally, electrophysiological assays have been preferably employed to unravel the dynamics of synaptic vesicle release and short-term plasticity. However, these approaches are based on indirect measurements from large population of synapses, which substantially impedes the understanding of the mechanisms that regulate transmitter release at the level of single presynaptic boutons.
This course aims to show how advances in live-imaging technique that enable overcoming these constraints when investigating synaptic activity. I will show how the recently developed fast fluorescent glutamate sensor (iGluSnFR) can be combined with patch-clamp electrophysiology to image the activity of dozens of presynaptic boutons simultaneously with high temporal resolution (in neuronal cultures). Most importantly, this course will highlight the importance of taking into consideration the natural synaptic heterogeneity present in cortical neurons.
Main Techniques**:**

*Primary cortical neuronal culture preparation*
*Whole-cell patch-clamp recordings*
*iGluSnFR image acquisition using high sensitivity camera*
*Electrophysiology and fluorescence data analysis using MATLAB*

**Project 12 : "Super-resolution shadow imaging in the mouse brain"**
**Instructor: Luc Mercier and Stephane Bancelin (*IINS, Université de Bordeaux, France*)**

The extracellular space (ECS) of the brain has an extremely complex spatial organization, which has long defied conventional light microscopy. Consequently, despite a marked interest in the physiological roles of brain ECS, its fine structure and dynamics remain largely inaccessible for experimenters. Leveraging recent advances in 3D-STED microscopy and fluorescent labeling, the Nägerl group has recently developed super-resolution shadow imaging (SUSHI) of brain ECS in living organotypic brain slices. SUSHI enables quantitative analysis of ECS structure and reveals dynamics on multiple scales in response to a variety of physiological stimuli. Because SUSHI produces sharp negative images of all cellular structures, it also enables unbiased imaging of unlabeled brain cells with respect to their anatomical context. Moreover, the extracellular labeling strategy greatly alleviates problems of photobleaching and phototoxicity associated with traditional imaging approaches. As a straightforward variant of STED microscopy, SUSHI provides unprecedented access to the structure and dynamics of live brain ECS and neuropil.

We will give a tutorial on the basic optical principles and instrumentation technology of STED microscopy, and a practical training on a custom-built two-color STED microscope providing 3D super-resolution.
We will then explore the potential of SUSHI imaging in brain slices to visualize fine contacts between brain cells (pre-post synapse, astrocytic processes, etc.) and perform time-lapse imaging of cell migration.
The student will finally implement and test various strategies to inject a fluorescent dye in the ECS of a living animal in order to perform SUSHI imaging in anesthetize mice.
Main techniques:

*Intracranial injections*
*STED microscopy*
*3D super resolution*
*SUSHI imaging*

**Project 13: "Dual color single particle tracking to study membrane receptors dynamics"**
**Instructor: Elena Avignone and Francois Maingret (*IINS, Université de Bordeaux, France)***

The spatiotemporal organization of neurotransmitter receptors in the postsynaptic membrane plays a fundamental role in synaptic transmission, and thus in storage and encoding information. Long considered as immobile at the plasma membrane, receptors are instead highly dynamic. Changes in their number, composition and distribution at the neuronal surface have been investigated in pathological and physiological conditions. However, receptors should be considered as part of a complex, as protein-protein interactions at the level of the plasma membrane may affect the surface dynamics. Nevertheless, due to technical limitations, up to day only few studies investigated more than one receptor at the same time.
The aim of this project is to investigate the functional cross-talk between two neuronal membrane receptors using single nanoparticle tracking methods. We will use quantum dot coupled to two different tags to track simultaneously two membrane receptors. You will choose the two receptors to track among a library of Flag- and GFP-expressing proteins available in the lab. The library includes receptors mediating fast glutamatergic and GABAergic transmission, metabotropic receptors (glutamate, GABA or catecholamine). We will particularly focus the study on how the dynamics (lateral mobility, distribution and synaptic retention) and the interaction of the two proteins of interest are affected by a specific treatment that you can propose (plasticity protocol, inflammatory context, toxicity etc.). The project will be discussed in advanced.
Main techniques:

*Dual color single particle tracking*

**Project 14 : Imaging tripartite synapses using super-resolution microscopy**
**Instructor : Janosch Heller (*FutureNeuro and Royal College of Surgeons in Ireland, Ireland*)**

Astrocytes play essential roles in the nervous system. The cells not only facilitate brain development, homeostasis, and metabolic support but they are also critical for the formation and regulation of synaptic circuits. Astrocytes have an extraordinary complex, sponge-like morphology that is nanoscopic at times. The cells occupy adjacent, non-overlapping tissue domains tiling the synaptic neuropil. Within the brain, excitatory

synapses are frequently approached or surrounded by fine astrocytic protrusions, often termed perisynaptic astrocytic processes (PAPs). The structural remodeling of synapses is thought to be a basis for learning and long-term memory formation. Also PAPs show use-dependent morphological plasticity that might work in concert with synaptic plasticity.
The main goal of this project is to decipher the plastic relationship between glial and synaptic proteins in the brain and how neuronal activity shapes astrocytic coverage of synapses.
In order to do that, we will visualize astrocytic proteins in the vicinity of synapses. We will use hippocampal neuronal cultures as well as acute hippocampal slices to study the nanoscale relationship between synaptic proteins and astrocytes using single molecule localization microscopy (SMLM). Additionally, we will explore the altered astroglial coverage of excitatory and inhibitory synapses in different conditions compatible with long-term synaptic potentiation or depression as well as in *in vitro* seizure models.
Altogether, this project will shed light on the plastic localisation of astrocytic proteins surrounding excitatory and inhibitory synapses in the brain and how neuronal activity shapes astrocytic coverage of synapses.
Main techniques:

*primary cell culture,*
*super-resolution microscopy*
*single molecule localization microscopy*

**Project 15: "Nanoscale organization and dynamics of synapsin condensates"**
**Instructors: Dragomir Milovanovic (*Charité University Clinic, Berlin, Germany*)**

Neurotransmission relies on the tight spatial and temporal regulation of synaptic vesicle (SV) cycle. Nerve terminals contain hundreds of SVs that form tight clusters. Despite being held together, vesicles are highly mobile within these clusters, so that they can be randomly recruited to the surface of the cell to release their content upon activation of the neuron. Several features of SV clusters suggest that they may be part of a distinct liquid phase in which one component of the phase are vesicles and the other a protein in the interweaving matrix. Recently, it was shown that synapsin 1, a highly abundant synaptic protein, forms a distinct liquid phase in an aqueous environment. Synapsin condensates are reaction centers able to sequester lipid vesicles. The phase of synapsin 1 rapidly disassembles upon phosphorylation by CaMKII, mimicking the dispersion of synapsin 1 that occurs at presynaptic boutons upon simulation. Thus, a minimal system of synapsin (with or without its binding partners) may sequester lipid vesicles, forming a distinct liquid phase. This mechanism helps to explain how hundreds of SVs are kept into distinct clusters while maintaining their high mobility.
The goal of this project is to quantitatively assess the organization and dynamics of synapsin condensates. The specific aims are:

a) Reconstitution of synapsin condensates using the purified synapsin 1 tagged with photoswitchable dye (i.e., mEOS3.2-Synapsin 1).
b) Recording and analyzing the diffusion properties of Synapsin 1 within the condensates and its exchange between the condensates.
c) Comparison of diffusion properties of synapsin 1 in reconstituted condensates, ectopically expressed in mammalian cell lines, and in primary neuronal culture.

d) Characterizing the capacity of synapsin-condensates to specifically recruit and confine the mobility of its interacting partners (e.g., Grb2).

Main techniques:

*confocal microscopy*
*single-molecule tracking*
*in vitro reconstitution of liquid phases*
*live-cell imaging*