Super-resolution and advanced microscopy applied to neurosciences

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« The functional nano-architecture of axonal actin »

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 actin-based structures within the axon: the periodic actin/spectrin submembrane scaffold, intra-axonal hotspots and trails, and presynaptic actin assemblies. 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.

Mathieu Ducros
Bordeaux Imaging Center, France
« Lattice Light-Sheet Microscopy applied to neuroscience research »

Lattice light-sheet microscopy (LLSM) is a recent fluorescence microscopy technique based on selective plane illumination microscopy (SPIM) that presents several key advantages: intrinsic optical sectioning, very fast imaging, low photo-toxicity and sub-micrometric spatial resolution. The great benefits of LLSM for fast 3D cellular imaging are now well established in various fields of biology. At the Bordeaux Imaging Center (BIC) we built a LLSM setup in 2017 that is now used in routine, mainly for experiments in the field of neurosciences. We also added a photo-stimulation module to perform FRAP or uncaging experiments at spatially and temporally controlled regions of interest. With our LLSM we performed studies in acute and organotypic rodent brain slices. Sub-micrometric neuronal compartments such as spines could be imaged down to ~ 30 µm below the tissue surface with temporal resolutions up to 200 frames/s. We demonstrated the performances of LLSM in several published and ongoing studies: measurement of AMPA receptor diffusions at spines, vesicular transport in dendrites, spontaneous and stimulated local calcium activity in neurons and astrocytes, extracellular space and glutamate imaging.
This presentation will first cover the principles, advantages and limitations of LLSM, and then showcase the most advanced neuroscience studies that were performed at the BIC core facility.

Yannick Schwab
EMBL, Heidelberg, Germany
« Precise targeting for volume electron microscopy, a multimodal approach *»*

Volume electron microscopy methods (vEM) are powerful to study the complex ultrastructure of cells. Based on serial imaging of either resin sections (ssTEM, ssET and array tomography) or of sequentially ablated bock surface (SBEM and FIB-SEM), they uniquely capture cells and organelles shapes and interactions in three dimensions. Resulting datasets can lead to detailed morphological quantifications. High resolution vEM techniques are often performed on large samples, whether

multicellular organisms or tissues, which opens to studying cell-cell interactions within their micro-environment. In some cases, such analyses are performed on a subset of cells, selected for their particular phenotype or for their identity. Targeting the acquisition to these regions is thus interesting as it optimizes dramatically the acquisition time, the sampling throughput and the amount of data generated. Because direct targeting within the embedded specimen is almost impossible at the EM, multimodal correlative methods have been developed to establish with precision the position of the volume of interest.
This talk will describe the targeting methods that are developed at EMBL. They rely on 3D maps built from fluorescence microscopy or X-ray imaging, and on specific workflows to accurately and semi-automatically approach the regions of interest prior to EM imaging. Example applications will show how to image selected regions of interest in multiple specimens including models used to study different infection stages of the malaria parasite and marine protists sampled in the field.

Pierre Hener
INCI Institute, Strasbourg, France

« STED microscopy in Strasbourg: presentation of the confocal microscope Leica Stellaris 8 STED from the "in vitro" imaging platform *»*

Stimulated emission depletion (STED) is one of the super-resolution techniques that allows to overcome the diffraction limited resolution of optical microscopes. Using a donut shaped STED laser beam, fluorophores in the outer region of the diffraction limited spot generated by excitation laser are depleted. Finally, only the fluorophores in the non-overlapping region between excitation laser and STED laser are able to fluoresce. This leads to a size reduction of the effective fluorescent spot. Moreover, by combining the fluorescence lifetime information to the STED signal thanks to the TauSTED technology, the signal to background ratio and the image quality are greatly improved, even at low excitation and STED powers.
The "in vitro" imaging platform of the ITI Neurostra is located at the Institut of Integrative and Cellular Neurosciences (INCI) and is part of the RISEst network. We recently acquired a confocal microscope Leica Stellaris 8 STED. The STED module integrated in this system provides an easy way to image beyond the diffraction limit. The microscope is equipped with a white light laser allowing an excitation from 440 nm to 790 nm, 3 hybrid detectors and a 775 nm depletion line. Thus, the system is compatible with many "STEDable" fluorophores (such as AlexaFluor 594, Atto 647N, Star635P…) allowing up to 5 colors STED acquisitions. During the presentation, the " in vitro " imaging platform will be introduced and the system will be described together with several examples of ongoing projects.

Hosted by Manon BOIVIN, Marianne LEMEE, Pierre TILLIOLE