A029-3D STED microscopy for nanoscopic imaging of virus-host cell interactions.

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Super resolution fluorescence microscopy has become essential for cell biology studies. The recent advence of superresolution microscopies such as Stimulated Emission Depletion (STED microscopy, invented by Stefan Hell, Nobel Prize for Chemistry 2014) has revolutionized observation of biological samples, enabling lateral resolution of a few tens of nanometers on fixed or immobile samples, at first, then more recently on living cells. One advantage of STED compared to other superresolution optical methods is that a superresolution image is obtained rapidly with no need for data processing following acquisition. STED therefore represents a perfect tools to obtained on the nanometer scale and now in 3D (70nm in the 3 directions) and in multi-color. Here, we will apply this multicolor 3D nanotechnology to very small biological objects like fluorescent virus-like-particles and on non infectious labelled virus expressing host cells with labelled actin or tubulin cytoskeleton nano-filaments in fixed and living host cells.

The workshop will have 3 parts:

Part 1: presentation of the microscopie STED principle (J.Swain, IRIM , CNRS Montpellier) and of the virological system for STED imaging (D.Muriaux, IRIM CNRS Montpellier)

Part 2: image acquisition of fixed virus and infected cells labelled for 2D and 3D imaging + explanation for sample preparation (J.Swain/D.Muriaux) and virus size analysis on the microscope computer (comparing Confocal versus STED resolution in xyz) and using imagJ (show on a laptop during the workshop)

Part 3: Preparation of live cells expressing fluorescent virus-like particles (labelled with eGFP) with a live staining for F-actin or Tubulin and movie acquisitions with live STED 2 colors (D.Muriaux/J.Swain)

A032-Force spectroscopy on virus-like particle producing cells using atomic force microscopy

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Physical forces and mechanical properties have critical roles in cellular function, physiology and disease. Over the past decade, atomic force microscopy (AFM) techniques have enabled substantial advances in our understanding of the tight relationship between force, mechanics and function in living cells and contributed to the growth of mechanobiology. The objective of this workshop will be to provide a comprehensive overview of the use of AFM-based force spectroscopy (AFM-FS) to study the elastic properties and dynamics of living cells, expressing exogenous membrane or cytoskeleton modifying proteins. We'll first introduce the importance of force and mechanics in cell biology and the general principles of AFM-FS methods. We'll describe procedures for sample and AFM probe preparations, the various AFM-FS modalities currently available and their respective advantages and limitations, the integration of AFM imaging with optical microscopy. We also provide details and recommendations for best usage practices, and discuss data analysis, statistics and reproducibility. We exemplify typical AFM-FS experiments in focusing on live cells over-expressing either the HIV-1 structural P55Gag polyprotein, which assemble and bud at the cell plasma membrane to produce viral particles, or the membrane curving I-BAR protein IRSp53, which promotes membrane protrusions by interacting with actin cytoskeleton and help HIV-1 budding. Fluorescent viral and cellular proteins localized at the cell membrane will be used to correlate AFM force mapping with protein localization with the cell. At the end of this workshop, the participants will be able to understand the basic functioning of a modern bio-AFM and the grand challenges in the area for the next decade

A034- 3D Deconvolution

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The purpose of the deconvolution is to digitally compensate for the blur introduced by the microscope. In 3D microscopy, deconvolution improves images on several points:

- by increasing the resolution (along the axial direction in particular),
- by reducing noise (especially at low flux),
- by improving the contrast.

This makes deconvolution a valuable tool for improving post-processing such as segmentation.

This workshop proposes to demystify the deconvolution methods and offers a demonstration of open source deconvolution software.

It will be in 4 parts:

- a brief theoretical description,
- the important points for a successful deconvolution,
- comparison of classical methods with the DeconvolutionLab2 ImageJ plugin on simulated and real epifluorescence and confocal data.
- in case the PSF is not known we will guide users in the use of EpiDEMIC, an Icy blind deconvolution plugin for epifluorescence.

A036-High content 3D imaging of small specimens and automatic analysis in the context of screening applications

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In the context of (eco)-toxicology or developmental studies 3D imaging shows increased precision in detection and localization of the induced anatomic changes. The recent advances in tissue clearing facilitate performing studies like these on rather big/opaque specimens as zebrafish larvae or organoids.
Confocal laser scanning microscopy (CLSM) is a well established technique in the field of biological research. Moreover, resonant CLSM offers the perfect compromise between speed, resolution and depth of light penetration for this range of samples. By combining this technique with automation scripts it is possible to acquire volumetric images of a large number of specimens. As the analysis of the induced phenotypes must be statistically sound a high number of images is needed as input for the bioimage analysis.
In this workshop we present a high content screening (HCS) application based on a commercial confocal high content imaging system driven by automation scripts and an automatic data management and analysis pipeline of our own design (file system based database; fsdb). We will also present our tissue clearing protocol and the sample mounting procedure for HCS acquisition.

A037-Whole brain single cell resolution study of the mouse brain using iDISCO, light sheet microscopy and the ClearMap2 software.

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Whole-organ optical-imaging techniques have paved the way for anatomical studies of the entire central nervous system with single cell resolution. Coupled with tissue clearing and immunolabeling, immediate early gene expression or viral tracings, these techniques have opened the door to a new world of experiments in various biological fields: cancer research, developmental biology or neurosciences.
While these techniques provide structural and functional information of entire organs or even entire organisms far more complete than that of conventional 2D histology, and various microscopes have become readily available, the tissue preparation and subsequent data analysis does not rest on commercial kits and software but still relies on expertise and software under active development.
During this workshop, we propose to demystify this kind of experiments by showcasing 2 state of the art techniques in the field:

- whole brain tissue clearing and labeling using iDISCO
- whole brain data analysis using the ClearMap2 open-source software.

We will apply the iDISCO method to map brain activity by using a cellular marker of neuronal activity: c-Fos. The 3D scans will then be processed through the ClearMap2 pipeline to align the sample brain to the Allen Brain Atlas and automatically detect the labeled cells. We will finally compare the cell counts between regions and across experimental conditions. The software offers a simple graphical user interface with the aim to empower the end users to analyse their own datasets.
Our workshop is intended for anyone interested in studying cells of interest in entire mouse brains, from complete beginners to participants already familiar with clearing techniques but lacking experience pertaining to the quantification. Although we chose to present c-Fos, the techniques presented are applicable to other scientific questions such as the analysis of microglia activation, amyloids plaques, blood vessels or neuronanal connectivity.