Mifobio 2023: Workshops list

A001-Segmentation and classification under Napari

Proposer/Coanimator

Pierre Weiss
Clément Cazorla

Abstract

The goal of this tutorial is to present a deep learning based methodology to derive quantitative analyses from biological images. We will be particularly interested in the classification of cells or nuclei from 2D or 3D fluorescence images. We will explore the use of two modern tools :
- CellPose 2 and its human in the loop feature. https://www.cellpose.org/
- Svetlana, a new classification plugin developed by the organizers https://www.napari-hub.org/plugins/napari-svetlana

The participants can bring their own dataset to test the methodology on their particular sample of interest.

The tutorial will be given by Clément Cazorla (PhD) and Pierre Weiss (CNRS researcher), both located in Toulouse.

Educational goal

At the end of this workshop the participants will :
- Get more familiar with Napari and some of its plugins.
- Learn how to use the more advanced features of CellPose, including its “human in the loop” feature, allowing to improve the segmentation results.
- Use the Svetlana plugin, to
1) annotate the segmentation results efficiently.
2) learn how to choose a neural network architecture for classification
3) train the neural network with the annotations from step 1). In particular, we will explore advanced training options such as learning rates, batch size, data augmentation.
4) use the resulting network to classify a collection of segmented images
5) explore interpretation modules such as CAM to explain how the neural network takes its decisions

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A003-Automated acquisition of organoids in high-throughput assays for cancer research

Proposer/Coanimator

Nicolas Bodier
Al hassan Cassé

Abstract

Organoid, an in vitro 3D culture, has extremely high similarity with its source organ or tissue, which creates a model in vitro that simulates the in vivo environment. Organoids have been extensively studied in cell biology, precision medicine, drug toxicity…which have been proven to have high research value. Periodic observation of organoids in microscopic images is essential for organoid research but it is difficult and time-consuming to perform manual screens for organoids. It’s in this way, to help research, we would like to have the opportunity to present our workshop at Mifobio

First, to show a potential application in cancer research using organoid technology to facilitate drug testing. Indeed, if organoids still fail to model immune system accurately, we can demonstrate the use of co-cultures between organoids and lymphocytes after different immune-treatments and the read-out associated:
1) Lymphocytes recruitment (migration of lymphocytes inside the tumor)
2) Lymphocytes engagement (analyze of granzyme or perforin marker)
3) Positive response (Decrease of organoids size or proliferation status by KI67 marker)
To do this, we'll show the implementation of the workflow to prepare the samples (description of the material & method) and in the second part to detect, acquire and analyze automatically organoids in high-throughput assays. Indeed, the automated acquisition and detection should be realized thanks to a Spinning Disk microscope from Olympus using the associated CellSens imaging software.
For the images analysis, we 'll perform a spatial 3D analysis at cellular and subcellular level using different machine learning methods from Imaris software.

Educational goal

The main aim of this workshop is to explain how to set up a workflow to felicitate organoids cancer assays by reducing time consuming using through automation detection, acquisition and analysis of organoids.
At the end of the workshop:
1) Participants 'll be aware about the possibility to set up a similar workflow for organoids research including theorical knowledge of samples preparation and read-out of cancer research.
2)They 'll be able to practice on Spinning disk using the CellSens software to develop an automatic protocol of acquisition.
-Quick presentation of the software interface
-Setting parameters to detected marker and perfom 3D acquisition
-Configure automatic detection and acquisition
3) We 'll present the solutions on organoids images implemented using Imaris software to quantify:
- analyze size of organoids and KI67 proliferation marker
- number of lymphocytes migrated inside the organoids
- number of lymphocytes expressing a marker of engagement (Granzyme or perforin)
And we'll explain how to launch the batch analysis module from Imaris

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A004-Unmixing of spectrally identical fluorescent proteins by lifetime imaging

Proposer

Joachim Goedhart

Abstract

Fluorescence lifetime unmixing enables the independent imaging of two or three fluorophores that have identical emission spectra. The separation is based on differences in the excited state lifetime. This imaging method is of interest as it increases the options for multiplex imaging. (It aligns with my talk on the engineering of fluorescent proteins).

We have engineered fluorescent proteins by screening for lifetime variants (https://doi.org/10.1038/nmeth.1415). As a result, we have identified and characterized fluorescent proteins with different fluorescence lifetimes and have demonstrated that these can be used for lifetime unmixing (https://doi.org/10.1529/biophysj.107.125229). In this workshop, we will discuss the requirements for lifetime unmixing and the types of fluorescent probes that are available. A sample with two different Cyan Fluorescent

Proteins (CFPs) that are targeted to two different cellular locations will be used (https://doi.org/10.1111/jmi.12168). The conditions for imaging and analysis will be discussed and the participants will have the opportunity to do the acquisition and tweak imaging conditions. The workshop has a focus on the data acquisition, but aims to explain and show the data analysis&visualization (using the software of the microscopy setup) as well. The outcome of the experiment is a set of two images with the distribution of the two cyan fluorescent proteins.

Educational goal

The workshop will demonstrate the basics of unmixing on cyan fluorescent proteins, but this methods can be generally applied to fluorophores with different lifetimes.
At the end of this workshop the participants will be able to acquire and analyze data to perform the unmixing of (any) fluorophores based on lifetime.
The sample will depend on a publicly available plasmid (https://www.addgene.org/60491/), so the participants can reproduce the sample preparation in their own lab.
The participants will have sufficient knowledge to set up unmixing in their own lab and on their own samples (if they have access to a lifetime imaging setup).

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A007-Label-free correlative microscopy of 3D samples: from light sheet to confocal microscopy

Proposer/Coanimator

Laetitia Pieruccioni
Corinne Barreau

Abstract

Many imaging modalities are now available. These techniques are generally complementary and can be used to improve the speed of acquisition, the image resolution or the signal-to-noise ratio. Correlative imaging offers the advantage of being able to combine several microscopy techniques on the same sample and to extract the specificities of each, thus freeing oneself from these compromises. In the workshop we propose, we have developed a pipeline to large and complex sample, unlabeled samples with light sheet microscopy to get a 3D view of the entire sample, then image the same sample with confocal microscopy and re-match the two images.

Educational goal

At the end of the workshop, participants will realise that no one microscope is better than another, but that it is the combination of two (or more) types of microscopies that allows them to obtain more accurate results for their biological questions.
Participants will learn the basic principles of confocal and light sheet microscopes through a brief presentation of the equipment and by handling samples under the different microscopes.
The presentation of correlative microscopy on optically different microscopes will make them aware that sample preparation prior to imaging is all the more important.
The participants will also learn about correlative microscopy and the registration of two types of acquisitions for image processing and analysis.

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A008-Automated reconstruction of massive amount of super-resolution single particle trajectories

Proposer

David Holcman

Abstract

Single particle trajectories have revealed the dynamics of key channels and receptors at synapses, as they play a key role in shaping learning and memory.
Statistical analysis coupled with stochastic modeling revealed the organization of subcellular compartments at a nanoscale resolution. We recently developed a model, analysis and the associated ImageJ software. This approach can be be applied to study diffusion and active motion. The biophysics properties can be extracted, leading often to high resolution heterogeneous mapping for the local protein distribution. In this workshop, I will present models and analysis of super-resolution trajectories as well as ImageJ plugin software. We will test it on neuronal membrane and also in sub-cellular networks such as the endoplasmic reticulum. We will present how the estimators are build and various fast algorithms to reconstruct potential wells and their geometrical organization.

Reference:
Parutto P, Heck J, Lu M, Kaminski C, Avezov E, Heine M, Holcman , High-throughput super-resolution single-particle trajectory analysis reconstructs organelle dynamics and membrane reorganization.
D. Cell Rep Methods. 2022 Aug 22;2(8):100277. doi: 10.1016/j.crmeth.2022.100277

Educational goal

At the end of this workshop the participants will be able to
1-install and manupultate the software
2-Try it on several data sets
3-Understand the biophysical concept
4-Apply it to their own data.

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A010-Lifetime STED microscopy to resolve the organization of the Golgi apparatus at super-resolution in plant samples

Proposer/Coanimator

Christel Poujol
Magali Grison

Abstract

Stimulated emission depletion (STED) microscopy is a powerful super-resolution microscopy technique that has achieved significant results in breaking the resolution limit of conventional confocal microscopes. In recent years, STED technology has continued to evolve, in particular with the aim of reducing the intensity of depletion lasers.
In this workshop, we will use a STED microscope that has been combined with a fluorescence lifetime equipment, a combo known as lifetime STED microscopy. A phasor approach is used to improve the photon screening based on their lifetime and so to improve the resolution with less amount of depletion light.
We applied this lifetime STED technique to plant tissue (Arabidopsis thaliana root tip) with the aim of imaging the organization of the Golgi apparatus at super-resolution and more precisely challenge the hypothetical presence of an Endoplasmic Reticulum (ER)-Golgi Intermediate Compartment (ERGIC) in plant cells. Stunningly,

lifetime STED microscopy revealed the presence of ERGIC by clearly differentiating a thin tubulo-vesicular structure from the background noise. Moreover, we successfully performed Lifetime STED in two-colors (for Alexa 594 and ATTO647N) and visualized that this tubulo-vesicular network is mostly independent from either the medial-cisternae of the Golgi apparatus or the ER-exit sites (ERES), although some associations are clearly visible with these structures. Together, the lifetime STED approach allowed us to unravel a structure that remained previously unidentified in plants and thus represents a major step forward the understanding of Golgi organization in plant cells.

Educational goal

At the end of this workshop the participants will be able to...

- Understand the operating principles of STED microscopy
- Understand the constraints related to STED microscopy
- Understand the operating principles of lifetime STED microscopy
- Discover the advantages of the lifetime STED technique (ratio noise/signal)
- Be familiar with the preparation of samples for two-colors STED imaging

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A011-Microscopy (meta)data management

Proposer/Coanimator

Guillaume Gay
Julio Mateos langerak

Abstract

The tremendous rise in size and complexity of bio-imaging data is confronting the research community with the need to manage their data and metadata in a more structured way. This exposes researchers with a specific set of challenges involving, not only the use of new software tools, but also in the way they interact with data, their format, location and the way they are accessed. These challenges have been acknowledged by research institutions and funding bodies with the push towards actionable Data Management Plans and the generalization of open science and open data policies . Comprehensive data management strategies need to be put in place in your lab.
Among the goals of these policies is to improve the “readability” of data and prevent its loss, support reproducibility and combat fraud. As stated by Jason Swedlow et al. as early as 2003: “It is possible to interpret images only if we know the context in which they were acquired”
The goal of this workshop is to equip you with the necessary knowledge and tools to set up good practices in your day to day work and, according to the FAIR (Findable Accessible Interoperable Reusable) principles, make your data easy to find, reuse, share and publish.

We will review the theoretical and practical aspects of the data life cycle, the FAIR principles, and how they apply to microscopy data. We will focus on the REMBi (Recommended Metadata for Biological Images) recommendations by the QUAREP-LiMi (Quality Assessment and Reproducibility for Instruments & Images in Light Microscopy) consortium. We’ll then introduce the Investigation Study Assay (ISA) framework as a data organization principle and end up with the more practical aspects of image data management, such as:
- relevant data management plan entries
- naming and file hierarchy (ISA framework)
- metadata management, ontology usage
- use of public archives

We encourage participants to bring along data sets so that we can discuss the implementation on their own data.

Educational goal

We'll start by reviewing key notions as the data life cycle, the FAIR principles, and how they translate to microscopy data, with a focus on the REMBi (Recommended Metadata for Biological Images) recommendations by the QUAREP-LiMi (Quality Assessment and Reproducibility for Instruments & Images in Light Microscopy) consortium.
We'll then introduce the Investigation Study Assay (ISA) framework as a data organization principle.
After this theoretical part, we will look into key aspects of microscopy images management:
- relevant data management plan entries
- naming and file hierarchy (ISA framework)
- metadata management, ontology usage
- use of public archives

For this practical part, we will work on one or two concrete examples brought by the workshop participants.

At the end of this workshop the participants will be able to setup a comprehensive and FAIR data life cycle for their microscopy data

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A012-Imaging transcription at the single cell level during zebrafish development

Proposer/Coanimator

Maelle Bellec
Jeremy Dufourt

Abstract

Live imaging represents a powerful technique for the understanding of dynamic processes in biology, such as embryonic development. Recent advances in microscopy highly improve signal-to-noise ratios as well as spatial and temporal resolutions. Furthermore, development of new fluorescence markers allows a better quantification of protein expression and transcriptional dynamics in vivo. This workshop aims at imaging transcription in live, within whole zebrafish embryos in order to extract transcriptional dynamics. To do that, we will use the MS2/MCP system which consists of MS2 loops that upon transcription will be recognized by a MS2 Coat Protein fused to GFP. We will first show how to prepare and mount a living sample adapted for confocal microscopy. We will then explain how to make imaging settings in order to get a good signal to noise ratio and high temporal resolution with this system. We will use the newly developed Lattice Light Sheet microscope (ZEISS LLS7) to be able to image at a high time frequency.

Educational goal

This workshop will provide an introduction to the techniques used for live imaging of transcription in zebrafish embryos. Participants will learn how to prepare living zebrafish embryos and monitor gene expression. In addition, participants will gain hands-on experience with high-resolution confocal imaging and analysis techniques, enabling them to visualize and quantify dynamic changes in gene expression. The workshop will also discuss how to interpret the resulting data and apply it to answer specific biological questions. Finally, participants will have the opportunity to discuss and share ideas with other researchers in the field. This workshop will provide an invaluable opportunity to gain experience and knowledge in the expanding field of live imaging of transcription in zebrafish embryos.

At the end of this workshop the participants will be able to prepare living zebrafish embryos, to image transcription with a high temporal resolution.

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A014-DEEP NAPARI

Proposer/Coanimator

David Rousseau
Herearii Metuarea

Abstract

NAPARI is a recent software which has been promoted for the bioimage analysis community by the Chan Zuckerberg Initiative. It enables fast prototype interface for python script. As a consequence NAPARI constitutes a good tool for deep learning developers eager to easily allow their non coding collaborators to press button ergonomic user interfaces. In this workshop you will learn your first step in NAPARI starting from its installation up to the development of your own first basic NAPARI plugin. We will teach you how to make this plugin accessible on the NAPARI platform. Finally we will empower you with deep learning NAPARI plugin adapted to segmentation, classification or object detection. A demo of human in the loop approach of AI will be demonstrated with this plugin.

Educational goal

Participants, coming with at least basic notions of programming in Python or gifted with fearless enthusiasm to develop their programming skills, will learn the basic principle of how NAPARI works. At the end of this workshop the participants will be able develop their own NAPARI plugin including for machine and deep learning.

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A015-Fluorescence-based techniques to study ER–mitochondria contact sites

Proposer/Coanimator

Yves Gouriou
Nicolas Bertocchini

Abstract

Mitochondria-ER contact sites (MERCS) are playing critical roles in various homeostatic functions, such as calcium/redox homeostasis, lipid transfer, mitophagy/autophagy and regulation of organelle dynamics. Hundreds of proteins have been shown to either have a structural or functional role in these contacts. ER-mitochondria tethering is a tightly regulated process which is modulated in conditions such as starvation, ER stress and mitochondrial dynamics modifications. Despite diverse proteins have been reported to participate in MERCS, their role in several cellular pathways and pathologies remain to be fully elucidated. Biochemical and imaging approaches have been used to address these questions. However, MERCS are dynamics and their abundance can vary rapidly, which make their characterization a real challenge. Currently, not only one experimental approach has to be used to fully characterize an organelle contact site, but a combination of different methods. Old methods such as, cell fractionation can be used to isolate ER-mitochondria interaction in combination with electron microscopy methods to assess morphological MERCS structure. Fluorescence-based techniques are also widely used, which allow a more dynamic assessment of MERCS, despite a lower resolution. In this workshop, we will present the combination of three fluorescent-based techniques: in situ proximity

ligation assay (PLA), a contact site sensor based on split-GFP (SPLICS) and a novel FRET-based proximity biosensor (MERLIN-FRET) to study MERCS in cells and tissues sections. We will analyze ER-mitochondria interactions in a model of type 2 diabetes. Indeed, disruption of MERCS is an early causal trigger of hepatic insulin resistance and steatosis observed in obesity and type 2 diabetes.

Educational goal

1) teach the participants the advantage/disadvantage of the experimental approaches to monitor contact sites
2) demonstrate the technical feasibility of fluorescent-based techniques to study MERCS in cells and tissues
3) present an example of experimental data analysis and quantification using SPLICS for mapping organelle contact sites

At the end of this workshop the participants will be able to compare the different approaches available for monitoring contact sites. They will be particularly aware of the advantages and limitations of the methods based on fluorescence. They will participate in the acquisition and quantification of organelle contact sites in a cell line and tissue samples.
During the workshop, “Slido” tool will be used to engage conversations with live polls, questions/answers, word clouds or surveys. This will allow to check in real-time the understanding of the participants and to collect feedback.

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A016-Parallel long-term live imaging of developing C. elegans larvae with microfluidics

Proposer/Coanimator

Wolfgang Keil
Eliot Schlang

Abstract

Long-term live imaging of developing multicellular organisms is mostly performed in embryos, which remain in a fixed position and do not grow substantially during image acquisition. Imaging post-embryonic stages in intact animals is incomparably more difficult because samples are moving, feeding, and growing while development takes place.
The objective of the workshop is to introduce participants to the model organism C. elegans and a simple yet powerful microfluidic system to image its post-embryonic development that we now routinely use in our lab (Berger et al. Development 2021). In this system, C. elegans larva are confined in small micro-channels. Channels are periodically constricted with pressure to limit movement and allow high-resolution volumetric imaging. The microfluidics device enables imaging up to 40 developing animals in parallel for periods of up to 2 days to capture cell-divisions and gene expression time courses at single cell resolution throughout the animal body.
In the workshop, we will setup a microfluidics experiment with a strain in which skin stem cells and their descendants are labelled with mCherry and a key stem-cell fate-determining transcription factor is CRISPR-tagged with split-GFP. We will observe and track stem cell divisions as well as the evolution of split-GFP intensity over time in the individual cell lineages. A data set obtained in our lab with an identical strain will be used to introduce participants to post-acquisition analysis, focusing on challenges specific to moving samples, low-intensity fluorescent reporters and large 5D imaging datasets.

Educational goal

Participants will be introduced to the roundworm C. elegans, a powerful and one of the most widely used animal model organism for development and in-vivo cell biology. At the end of the workshop, the participants will be

aware of the specific challenges that imaging a moving, feeding and growing biological organism at high spatiotemporal resolution brings and how microfluidics, automated image post-processing and cell-tracking can help overcome some of them. Apart from the imaging requirements, participants will be introduced to management and analysis of large imaging data sets files, resulting from the 5D acquisitions of several animals in parallel. Specifically, participants will be made aware of:
(1) Handling and loading live C. elegans into microfluidics devices
(2) Low-intensity endogenous fluorescent reporters, phototoxicity associated with time lapse imaging
(3) Temperature control of the setup
(4) Large volume image acquisition,
(5) Automated post-acquisition image registration
(6) Cell tracking, segmentation, and extraction of quantitative features such as single-cell gene expression time courses

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A018-3D Single Molecule Localization Microscopy on reconstituted systems: purified proteins and model membranes

Proposer/Coanimator

Feng-ching Tsai
Simli Dey

Abstract

This workshop aims to show the participants how to perform 3D Single Molecule Localization Microscopy on reconstituted systems: purified proteins and model membranes having shapes like those observed in endocytosis

Endocytosis is essential for numerous cellular functions; when failing, it causes many human diseases including cancers and neurodegenerative diseases. Mechanistically, endocytosis begins with deforming a flat donor membrane into a thin tubule or a vesicular bud; then membrane fission occurs to separate the nascent carrier from the donor membrane. The typical diameter of the endocytic neck is around 100 nm with various neck lengths. Thus, the endocytic necks have a saddle-like shape. Notably, several membrane curvature sensor BAR proteins have been implicated in endocytosis, for instance, by self-assembling at the neck to constrict the tubular neck and by regulating actin assembly at the neck to facilitate neck fission. In our project, we aim to reveal the molecular organization of BAR proteins at saddle-shaped membranes as in endocytic necks.

We have reconstituted in vitro systems composed of purified BAR proteins and model lipid bilayers having endocytic-neck shapes. Given the nanometer-scale membrane necks, to reveal the molecular organization of BAR proteins at the neck, it is absolutely required to use 3D Single Molecule Localization Microscopy (SMLM). In this workshop, we will demonstrate the following work packages

(1) SMLM detection of membrane shapes using membrane reporter Cy5-PC and DiI
(2) SMLM detection of AX647 labelled BAR proteins at saddle-shaped membranes
(3) Single particle tracking of lipids on membranes using Atto647N-PE to reveal lipid dynamics

Educational goal

Given the expertise of our team in the reconstitution of model membrane systems, we want to share our recent experience of performing SMLM in the reconstitution systems. Although rich information about protein

organizations in cell membranes have been obtained in cellular systems, for many cases, it remains a challenge to interpret the obtained results due to the overwhelmingly complex cellular environment. Having a well-controlled biochemical environment, reconstitution systems thus provide an ideal platform for deciphering molecular mechanisms that are relevant in cell biology.

In this workshop, we will show the participants how to perform 3D SMLM on model membranes associated with purified proteins by realizing the three work packages. We will guide the participants step-by-step the preparation procedure of the reconstitution system, the choice of fluorescent probes for SMLM, and the corresponding data analysis. We will explain the participants the resulting images and their biological relevance.

To better involve the participants in our workshop, in WP 1, we will ask a volunteer to prepare model membranes using GUVs that we will prepare in advance. Then, we will ask the volunteer to perform a titration experiment by flowing in different amounts of fluoresce dye DiI to detect membrane structures using STORM. This will allow the participants to notice that it is important to have the right amount of fluorescent dyes when performing STORM.

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A020-Whole workflow for plant expansion microscopy: from sample preparation to image analysis

Proposer/Coanimator

Magali Grison
Mónica Fernández-monreal

Abstract

Expansion microscopy (ExM) is a relatively new super-resolution method based in the isotropic dilation of the biological sample in order to overcome the diffraction limit of conventional microscopy. Since its development in 2014, many laboratories have been implementing and adapting the technique to their needs.
Intercellular communication is critical for multicellularity, and evolution gave rise to distinct mechanisms to facilitate this process. Plants have evolved remarkable intercellular structures -the Plasmodesmata (PD) pores- which interconnect virtually all cells within the plant body, establishing direct continuity of both plasma membrane, endoplasmic reticulum and cytosol. Although, PD are critical for development, environmental adaptation, defense signaling, and spreading of viruses.
Due to their nanoscopic size, 40 nm width x 100 nm length, PD are quite easily visible in electron microscopy but not with conventional photonic microscopy. To overcome this limitation, we applied Expansion Microscopy in Arabidopsis thaliana root tip and obtain a resolution never achieve before.
In this workshop, we show that ExM can also be applied in plants, more particularly in Arabidopsis root tip, and we present a whole workflow including microscopy acquisition, expansion and distortion analysis and image treatment.

Educational goal

After a brief presentation of the biological project, we will show how to optimize the classical ProExM protocol from Ed Boyden's lab to plant samples. That includes digestion of the wall previous to immunolabeling, embedding in an acrylamide hydrogel, expanding the sample, and acquiring with a confocal microscope. The acquisition needs to be done before and after the expansion to calculate the expansion factor and the distortion of the sample by an open source software (FIJI and ICY).

We will show samples of different stages of the protocol (immunolabeled roots, mounted roots, pre-expansion vs post-expansion labeling, unexpanded and unmounted gels, mount of expanded gels in the chamber and find the expanded root in the microscope), and we will discuss a troubleshooting panel for the expansion in plants (failures, distorsion, etc).
At the end of this workshop, the participants will be able to follow a protocol to mount the Arabidopsis root tip in a coverslip for gel embedding, to expand the sample and to mount the gel in the acquisition support. Then we will present the acquisition configuration and the image treatment by open source softwares. We will finally show some examples of our experiments.

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A021-Simple and cost efficient Arduino-based drug delivery system for Intracellular dynamics analysis

Proposer/Coanimator

Elvire Guiot
Erwan Grandgirard

Abstract

Visualization and analysis of the intracellular signaling dynamics rely on fast and high sensitivity microscopy. Most of imaging modalities available in core facilities and research laboratories such as fast confocal, spinning disk and wide-field fluorescence microscopes are equipped with the required modules to record a specific signaling event in a live cell environment. However, drug delivery onto the sample usually requires additional commercial perfusion and injection system to be installed around the microscope setup.
We will present and describe a simple and cost-efficient Arduino-based drug delivery system that can be easily implemented on any microscopy setup. The system can precisely adjust and control real-time drug delivery using a remote connection and triggering by the acquisition software. It will be demonstrated by running a live cell imaging experiment and could be apply to analyze fast intracellular dynamics such as calcium intracellular release, or activation of a biosensor, etc.
A second part of the workshop will demonstrate how to optimize the microscopy set-up (wide-field, spinning disk or fast confocal) and the imaging protocol for recording the dynamics of an intracellular process upon drug application. As a proof of principle in the context of the workshop, we proposed to image in real time the labelling of different cellular compartments by applying specific fluorophores (Hoechst for the DNA, Mitotracker for the mitochondria).
To reach the final goal of the workshop that is to monitor a dynamic event at the signal cell level, analysis strategies will be introduced. Several powerful and user-friendly solutions for automatic detection of the objects of interest such as nucleus segmentation now exists based on classical intensity threshold method (ImageJ/Fiji), or deep learning model (Stardist/Cellpose). Then the final point of the workshop will be the measurement and plot of the nucleus intensity variation during the time-lapse.

Educational goal

The first part of the workshop will focus on the design of the Arduino system for drug delivery and on the principles of electronic triggering with an Arduino. The open source hardware Arduino is becoming more and more popular thanks to its inexpensiveness and ease to customize. The Arduino board includes a physical programmable circuit board, the microcontroller and an Integrated Development Environment (IDE) that runs on your computer. The IDE is used to write and upload computer code to the physical board. We will demonstrate how with a simple code, you can send signals to the digital and analog output, interface and remotely trigger various peripheral devices with your acquisition software.
In the second part, we propose here an application where an Arduino base device is used to remotely deliver a drug in a live cells sample at a precise time point of a time lapse acquisition sequence. The start of drug delivery

will be triggered by TTL state change command driven by the acquisition software to the Arduino board. The biological application could be for example an intracellular calcium imaging project or the activation of a biosensor by a signaling cascade. Since such project need specific cells lines that include suitable receptors and also access to specific drugs, different buffers …, we propose in the purpose of the MiFobio Worshop to demonstrate the efficiency of our setup with a textbook case ("cas d'école"): the imaging protocol will be apply to a live cell labeling experiment. Specific fluorophores will be delivered to target different organelles or subcellular compartments: 1) DAPI for nucleus staining, 2) MitoTrackerRed for Live Mitochondriae labeling and 3) a specific drug altering the mitochondria morphology. The imaging protocol will be set up with the goal to draw attention to parameters that need to be optimized: photobleaching consideration, resolution, temporal sampling to accurately record the studied process kinetics. To conclude the workshop, a post-acquisition processing and analysis steps can be introduced: 1) detection/segmentation of the cell nucleus 2) measurement of the intensity variation to follow the labelling kinetics of the nuclear DNA.
At the end of this workshop, the participants will know how to:
• Understand, modify and load a simple code on an Arduino board
• Interface the Arduino board with a microscopy system to remotely control a pump or perfusion system for a time-precise delivery of a drug
• Optimize a time-lapse acquisition protocol for fluorescence intensity quantification taking onto account the specificities, performances and limits of the microscopy hardware parts
• Have a reflexion 1) on the critical parameters to take care for a proper quantification 2) on the analysis strategy that can be applied for the monitoring of the dynamic event at the signal cell level.

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A022-OMERO data management tools, FAIR enough?

Proposer/Coanimator

Guillaume Maucort
Marc Mongy

Abstract

The production of microscopy data has been exponential over the last years and the community is facing new challenges in the way it manages such data and the associated metadata. A proper management is key to scientific reproducibility as the amount of data produced needs to be properly labelled and archived for accessibility and reuse.

The community has issued a set of rules to follow, known as the FAIR principles, to ensure Findability, Accessibility, Interoperability, and Reusability of the data that are starting to be enforced by scientific journals. Day-to-day users of machines producing image data need to learn the good practices associated with the FAIR data life cycle.

OME Remote Objects (OMERO, www.openmicroscopy.org) is an integrated solution to answer these issues and manage the data over its lifetime, from acquisition to publication.

During this workshop, we will demonstrate the simplicity and power of this solution through hand-on practical exercises going through the different stages of the data life cycle driven by OMERO and its dependencies. We will particularly explore OMERO's tools for data and metadata management, database management and exploration. We will show you how to use OMERO for papers figures generation, or data publication for external access.

Over the last years, more and more facilities have deployed their own instance of an OMERO server to help them deal with FAIRisation of their data. Recently OMERO servers have been deployed on local nodes for external

users to access. Eventually, they will be linked to local mesocenters and be accessible to everyone. Such new tools becoming available will only become relevant with users aware of their advantages and trained to the basic functions available, which is one of the main goals of this workshop.

This workshop does not require any prior knowledge. Attendees are encouraged to bring their own data.

Keywords: Data management, FAIR principles, OMERO, Image sharing.

Educational goal

The main point of this workshop is to promote good practices regarding data management, through the respect of FAIR principles and the use of OMERO as a microscopy image management platform.

We first present a theoretical introduction focused on the needs to have proper data management and the concepts of FAIR principles.

We will then present OMERO as a data management solution which can become the centerpiece of the data life cycle (production, storage, access for analysis, diffusion, archive).

After this, attendees will be guided through the usage of Omero:
- OMERO-web interface and functions
- Data management basics
- Metadata management
- Annotations and key-value pairs
- OMERO.iviewer interface and figure creation
- Import data with OMERO.insight (various file formats to display supported range)
- Example of analysis plugin

Attendees with own data will be encouraged to upload it on the OMERO server and work directly on it.

Through these manipulations, we wish to convince people of the interest of centralized data management tools, in particular OMERO, for accessibility from everywhere (via the web or directly through analysis tools such as ImageJ, Napari, QPath, ...) and will present the solution currently deployed with accessible nodes all over France.

During the whole workshop, we will emphasize the advantages of the FAIR principles allowing:
- Findable data: through filenames, annotations, tags, ...
- Accessible data: publication and diffusion available from the web client
- Interoperable data: use of a standard format OME-tiff for downloads
- Re-usable data: tracked metadata

At the end of the workshop, the attendees will be able to:
- Have a better understanding of the OMERO data management solution and it's links to the "FAIR" principles
- Navigate through the database, with import/export functions.
- Annotate and complete metadata on datasets.
- Create a figure.
- Better understand the need of a structured and persistent data management and sharing solution.

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A024-Obtenir la PSF d'un système de microscopie de fluorescence

Proposer/Coanimator

Emmanuel Soubies
Valentin Debarnot

Abstract

En microscopie de fluorescence à haute résolution 3D, les techniques numériques jouent un rôle crucial, que ce soit pour la déconvolution, la microscopie à illumination structurée (SIM) ou la microscopie par localisation de molécules (SMLM). Tous ces problèmes de reconstruction/restauration computationnelle nécessitent une très bonne connaissance de la réponse du système qui est principalement encodée dans la fonction d'étalement du point (i.e. point-spread function PSF). En effet, il est essentiel d'être en mesure d'obtenir une bonne estimation de la PSF afin d'assurer une reconstruction fidèle à l'échantillon observé. En pratique, il y a deux écoles: ceux qui font confiance à une PSF théorique calculée à partir des paramètres optiques, et ceux qui préfèrent une PSF expérimentale estimée à partir d'acquisitions de microbilles fluorescentes.

Dans ce contexte, cet atelier a pour objectif de présenter aux participants l'importance de la PSF pour les algorithmes de reconstruction, les différentes façons d'obtenir des PSF, et les difficultés liées à cette estimation. Les participants seront formés à l'utilisation d'outils open-source (sous-forme de plugins ImageJ) leur permettant de réaliser cette tâche.

Educational goal

At the end of this workshop the participants will be able to

- use open-source tools to estimate the PSF of a system from micro-beads images.
- generate PSF from theoretical models.

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A025-AFM and FluidFM to probe the hydrophobic properties of living cell surfaces

Proposer/Coanimator

Audrey Audrey beaussart
Cécile Formosa

Abstract

Hydrophobicity of microorganisms is an important surface property that allows them to adhere to a variety of abiotic surfaces but also to other cells. In that sense it can be a determining factor in the pathogenicity of microorganisms, or on their capacity to form biofilms on surfaces. However, measuring the hydrophobic properties of complex surfaces such as living cells using classic methods (water contact angle measurements, adhesion to hydrocarbons) is usually a challenge. In this practical, we will demonstrate how atomic force microscopy and its microfluidic version, FluidFM, can be used to measure and quantify these hydrophobic properties at the micro- and nano-meter scales in liquid. For that, the first steps will be to immobilize living microalgae (Parachlorella kessleri) and yeasts (Saccharomyces cerevisiae) on functionalized surfaces. Then to evaluate the global hydrophobicity of cells, a hydrophobic probe such as an air bubble formed at the aperture of a microfluidic AFM cantilever using FluidFM, can be used to probe the cell surface in force spectroscopy experiments. In this case, the bubble formed is micrometer-sized, and will allow to quantify the global hydrophobic properties of a given cell. But if the goal is for example to reveal nanoscale hydrophobic structures

on cells, a smaller hydrophobic probe is needed. In this case, a gold AFM tip can be functionalized using a CH3-terminated thiol to make it hydrophobic, and used also in force spectroscopy experiments. Because the edge of the tip that will be in contact with the cell surface is of a few nanometers, information at the nanometer-scale can be collected. This practical is open to any scientists interested in cell surface hydrophobicity, and competences in AFM or biophysics are not required.

Educational goal

AFM, over the years, has made its way through biology laboratories mainly because of its nanoscale resolution imaging capacities of cells in liquid, thus under physiological conditions. But AFM is also a force machine, able to sense forces at the piconewton level, and this way, it can provide various types of information on cell surface interacting behaviors using specific probes. FluidFM is a recent advance in the field of AFM, and allows to combine it with microfluidics, enlarging in a significant manner the field of possibilities for bio-applications. Thus the first educational goal of this practical will be to introduce the general principle of AFM. In particular, the theory behind force spectroscopy measurements will be explained to understand how a probe moves during the experiments and what is measured precisely. An important aspect here will also be to introduce FluidFM, which is a rather new tool still not used in many labs (two in France). Explanations on how it combines AFM with microfluidics, its mode of operation and the possibilities it offers will be discussed.
Then, as a second aspect, the immobilization strategies that will be used for P. kessleri and S. cerevisiae will be detailed. This is an important step as the immobilization method used needs to allow a firm immobilization of cells without denaturing them. Immobilization methods for various types of microorganisms can be discussed at that stage with the participants interested in other types of cells.
Then the production of the hydrophobic probes will be explained. First, FluidFM will be used to show how air bubbles can be formed at the aperture of microfluidic cantilevers and what the characteristics of these bubbles are. Then, gold AFM tips functionalization with thiols will be explained and detailed. This will represent a good starting point to discuss AFM tip functionalizations strategies. Finally, the experiments performed will allow collecting force curves out of which information on the hydrophobicity can be extracted. The analysis of these force curves is a key point and will be detailed also to help the participants see the potential of the demonstrated techniques to quantify with high precision the hydrophobic properties of cells surfaces.

At the end of this workshop the participants will be able to install an AFM tip in the set-up, to align the laser, to adjust the parameters required for force spectroscopy measurements, and to analyze a force-distance AFM curve in order to extract the main information.

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A027-Single-cell force spectroscopy to probe cell-cell interactions using AFM and AFM combined with microfluidics

Proposer/Coanimator

Cécile Formosa
Sofiane El-kirat-chatel

Abstract

Yeast cell-cell interaction is a fundamental step in the formation of biofilms. Many cell surface components can be involved in these interactions, such as specific adhesion proteins or cell surface polysaccharides. While these cell surface components are to some extent identified, little is known about the force and the nature of the interactions they form with their homologs or other molecules on opposite cell surfaces. Recent advances in atomic force microscopy techniques have enabled researchers to gain insight into the molecular interactions of microorganisms. In particular, the force spectroscopy-based technique called single-cell force spectroscopy (SCFS) has made it possible to understand the forces driving cell-cell interactions and biofilm formation. In this

technique, a single microorganism cell is immobilized at the edge of a tipless cantilever, and used as a probe pushed against another cell immobilized on a surface. This technique has recently been implemented using AFM combined with microfluidics, where microfluidic cantilevers are directly connected to a pressure controller. In this case, a single cell can be aspirated at the aperture of a microfluidic cantilever, used for force spectroscopy measurements, and released afterward, allowing to catch another cell and repeat the process. This practical proposes to demonstrate how these two techniques can be used to measure, quantify and characterize the interactions between cells of the model yeast Saccharomyces cerevisiae. It is open to all scientists interested in cell-cell interactions of any type, and does not require any competences in AFM or biophysics.

Educational goal

AFM, over the years, has made its way through biology laboratories mainly because of its nanoscale resolution imaging capacities of cells in liquid, thus under physiological conditions. But AFM is also a force machine, able to sense forces at the piconewton level, and this way, it can provide various types of information on cell surface interacting behaviors using specific probes. The combination of AFM with microfluidics is a recent advance in the field of AFM that significantly expands the scope of possibilities of this technology for bio-applications. The first educational goal of this practical will be to introduce the general principle of AFM. In particular, the theory behind force spectroscopy measurements will be explained to understand how a probe moves during the experiments and what is measured precisely. An important aspect here will also be to introduce how the microfluidic version of AFM works, what are its mode of operation and the possibilities it offers.
Then, the second aspect will be to explain the basics for using an AFM, which are installing a cantilever, aligning the laser to follow its movement, and moving it over the surface to attach a cell at the edge of it. At this stage, the participants will have the opportunity to practice these operations directly and this way get a real sense of the technical requirements for these types of experiments. After demonstrating how yeast cell-cell interactions (S. cerevisiae) can be probed using tipless cantilevers, a demonstration of how AFM combined with microfluidics can be used to aspirate and release cells will be performed. This second approach allows rapid and well controlled cell immobilization on AFM cantilevers and therefore gives access to higher statistical analysis than classical SCFS with tipless cantilevers. However, microfluidic cantilevers are not suitable for all cell yet (for instance for small bacteria), while tipless cantilevers are. These aspects will also be discussed at this stage.
Finally, the experiments performed will allow collecting force curves out of which information on the nature of the interactions (physico-chemical or specific interactions), on their strength, and on the effect of contact time duration can be extracted. The analysis of these force curves is a key point and will be detailed also to help the participants see the potential of the demonstrated techniques to understand the molecular mechanisms underlying cell-cell interactions and the process they are involved in.
At the end of this workshop the participants will be able to install an AFM tip in the set-up, to align the laser, to adjust the parameters required for force spectroscopy measurements, and to analyze a force-distance AFM curves in order to extract the main information.

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A028-Microfluidics and microscopy for plant biology

Proposer/Coanimator

Camille Raillon
Nelson Serre

Abstract

Plants and microalgae constantly trigger fast and complex responses to reconcile their internal state with their environment. Microfluidics allows precise control of the flow of media, while modern microscopy techniques allow to image samples at high spatio-temporal resolution. Combination of microfluidics and microscopy allows to observe and characterize the dynamic of plants and microalgae growth and development in control and challenging conditions (a/biotic stresses).
The objective of this workshop is to transmit tools to study plants and microalgae behaviors by coupling microscopy and microfluidics. During this workshop, we will demonstrate that microfluidics is a powerful tool to study biological samples at high spatio-temporal resolution. To do so, we propose 2 measurement benches: 1/

Microscopic observations of root behavior (Arabidopsis thaliana) and 2/ hydrodynamic trapping and observation for single cell (microalgae of 5 - 20 µm). Each measurement bench has a dedicated microfluidic chip: a manually closable microfluidic chip to study A. thaliana roots and a hermetically glass bonded microfluidic chip for microalgae. Control of flow will be established using a fully commercial microfluidic system or syringe pumps to demonstrate the versatility of microfluidic systems. The incredible dynamic behavior of plant roots will be demonstrated through real-time microscopic observation of roots growing first in control media then in response to salt stress induced by NaCl.
To study microalgae organelle behavior with and without stress (nutritional deficiency), we will take advantage of the autofluorescence of the chlorophyll to locate the chloroplast relative to other stained organelles such as lipid droplets and vacuoles and quantitatively measure their respective number and volume.
At the end of this workshop, the participants will be able to prime microfluidic chips, input samples, and observe samples in vivo using time-lapse and/or 3D live cell imaging.

Educational goal

Participants will first learn the basics of microfluidics, what it is, how to implement it and the pros and cons of this method when used in combination with fluorescence microscopy to study terrestrial plants and microalgae. Putting emphasis on the use of microfluidic chips, we will give a simple overview on methods to fabricate microfluidic chips. Also, we will transmit our know-how concerning the proper manipulation of microfluidic chips. Both hermetically glass sealed or manually closable microfluidic chips will be described and manipulated by the participants.

Regarding microfluidics for plant roots (model: Arabidopsis thaliana seedlings), participants will have hands-on experience to transfer seedlings into an open chip, manually seal the chip and conduct in vivo and real-time observations of a treatment switch. Here, we will use NaCl which produces a strong cellular osmotic stress with a visible biological reaction under the microscope. We will follow the root behavior before and after treatment as well as the arrival of a fluorescent tracer in the treatment medium. We will also discuss other kinds of treatments and biochemical stains/marker lines with examples. Caveats to avoid and tips to make experiments more successful will be described as well as possible methods to conduct post experimental image analysis (quantifications of root elongation, fluorescence…).

Concerning microfluidics for microalgae (model: Coelastrella and Chlamydomonas microalgae), participants will learn about organelle behavior under stress, with and without nutritional deficiency. Microalgae lipid droplets will be stained with Nile Red and vacuoles with CellTracker Blue. We will also discuss current methods used for image post-processing, including a recent Fiji macro we developed to count organelles and measure their volume relative to the cell volume.

At the end of this workshop, the participants will have general knowledge of what microfluidics is, the possible system to implement and more technically they will be able to prime microfluidic chips, input biological samples and do observations for time-lapse and/or 3D live cell imaging.

This workshop is open to biologists, physicists and chemists. There are no prerequisites to attend the workshop.

References:
1. Kim, H. S., Weiss, T. L., Thapa, H. R., Devarenne, T. P. & Han, A. A microfluidic photobioreactor array demonstrating high-throughput screening for microalgal oil production. Lab Chip 14, 1415–12 (2014).
2. Serre NBC., Kralik D., Ping Y., Slouka Z., Shabala S. & Fendrych M, AFB1 controls rapid auxin signalling through membrane depolarization in Arabidopsis thaliana root. Nature Plants 7-9, 1229-1238 (2021).
3. Yanagisawa, N., Kozgunova, E., Grossmann, G., Geitmann, A. & Higashiyama, T. Microfluidics-Based Bioassays and Imaging of Plant Cells. Plant Cell Physiol 62, 1239–1250 (2021).

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

Proposer/Coanimator

Delphine Muriaux
Swain Jitendriya

Abstract

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)

Educational goal

At the end of this workshop the participants will be able to:
-know better about STED technology
-perform STED 2D and 3D cell imaging
-visualized virus producing cells and viruses with STED imaging
- viral cluster analysis and quantification
-prepare cytoskeleton (actin, tubulin) live staining and imaging using live STED probes
-image live cell cytoskeleton image acquisitions
-multicolor STED imaging at high resolution on fixed cells (below 70nm in xyz)

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

Proposer/Coanimator

Sébastien Lyonnais
Delphine Muriaux

Abstract

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

Educational goal

Atomic Force Microscopy is experiencing its revolution in the field of biology with the development of integrated Bio-AFM that provide faster and robust force-spectroscopy modes adapted to live-cells analysis in buffer. In addition, the integration of AFM scanners on top of an inverted microscope allows a simultaneous localization of cellular domains and proteins to correlate near-field AFM-FS maps with wide-field imaging. The expected evolution of Bio-AFMs will require extensive integration with the most advanced optical techniques (super-resolution) and will therefore require strong interplay between "classic" microscopy and AFM specialists. The main goal of this workshop will be to provide an overview of the state of the art of Bio-AFM and AFM-FS for the community to anticipate these future developments. There will be three objectives for this workshop: (1) To allow the participants to know the latest AFM technologies for the study of live cells (2) to understand the formation of an image in AFM according to the different imaging modes most often used in AFM, (3) to learn the basics of force spectroscopy for the mechanical analysis of cells. These 3 points will be exemplified on model 2D-cell culture expressing structural viral proteins (HIV-1 Gag) or plasma membrane/cytoskeleton modifying protein of the I-BAR family. Cells expressing these proteins will present membrane protrusions and modifications that should locally induce modification of cell stiffness and membrane elasticity. These two examples will allow the audience to understand the interests and limitations of AFM in liquid on live sample, and the exciting prospects for the future development of AFM by combining it with super-resolution microscopies. On the biological side, the audience will explore the basis of virus assembly at the cell plasma membrane and the possible changes in cell mechanics upon changes in cell membrane shapes due to either viral buds or cell protrusions induction. 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.

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A034- 3D Deconvolution

Proposer/Coanimator

Ferréol Soulez
Daniel Sage

Abstract

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.

Educational goal

At the end of this workshop the participants will:
- understand the main concepts of deconvolution
- know the conditions (for the sampling in particular) required to use deconvolution
- know how to use the DeconvolutionLab and EpiDEMIC plugins
- identify characteristic artifacts caused by deconvolution algorithms

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

Proposer/Coanimator

Dorian Champelovier
Arnim Jenett

Abstract

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.

Educational goal

At the end of this workshop the participants will be able to prepare their own samples, run 'Jobs' and HCI acquisitions on the NIKON A1R microscope and will be able to run data management and automatic bioimage analysis on the basis of the fsdb.

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

Proposer/Coanimator

Jérémie Teillon
Charly Rousseau

Abstract

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.

Educational goal

The attendees will learn to:

> Prepare and acquire the samples:

- Use tissue optical clearing to make the brain transparent
- Immuno-label the neurons of interest on an entire brain
- Acquire images with the light sheet microscope

> Use image analysis software to:

- Detect cells of interest
- Map the cells to brain regions
- Compare several experimental conditions.

We will explain principles of tissue clearing and whole organ labelling strategies through a Powerpoint presentation opened to discussion and questions. We will give practical tips and tricks.
Image acquisition on the light sheet microscope will be shown on an actual light sheet microscope. We will discuss the proper choice of acquisition parameters with the attendees, and can test them in real time with them.
Image analysis will be done on the ClearMap2 software in real time.

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A038-Event-based Single-Molecule Localization Microscopy for fast and dense high resolution imaging

Proposer/Coanimator

Clément Cabriel
Ignacio Izeddin

Abstract

Single-molecule localization microscopy (SMLM) is suitable for high resolution imaging in fixed samples. While it is live compatible, a number of applications remain challenging due to the tradeoff between temporal and spatial samplings, particularly when the system studied displays heterogenous protein densities or dynamic processes at different temporal scales.

We aim to image biological samples at high speed thanks to a new approach to SMLM using an event-based sensor in place of scientific cameras (sCMOS/EMCCD). This will be particularly relevant to answer biological questions requiring time-resolved high resolution imaging of transient structures in living cells. Event-based sensors are affordable commercially-available matrices of independent, asynchronous pixels sensitive to intensity variations. Their response time is very fast and a given frame rate needs not be chosen before the experiment. This can be used to image processes at various dynamic scales, or to detect blinking molecules among overlapping fluorophores that stay in a bright state.

After describing the working principle of such event-based sensor and the experimental setup used for SMLM, we explain how data acquisition and processing are achieved in practise. We then image fixed biological samples in the dSTORM regime (alpha-tubulin AF647), first at normal Point Spread Function (PSF) density, and then at high density where the PSFs overlap significantly, causing camera-based methods to fail. In such a situation, the event-based sensing allows detecting only the moment when a molecule turns on or off, making it distinguishable from other molecules emitting in its diffraction-limited vicinity. We explain how to compare the performances with camera-based approaches to highlight a vast increase of image resolution and fidelity compared to frame-based SMLM.

We finally discuss possibilities of assessing multiscale diffusion in event-based Single Particle Tracking.

Educational goal

This workshop aims at introducing a new approach for single-molecule microscopy based on event-based sensors, and make it so to democratize its use in non-specialists labs. We will give an introduction of event-based sensors, their specificities compared to scientific cameras, their strengths and weaknesses. The participants will learn how they can be used in the context of fluorescence imaging, and more precisely for high-resolution imaging of biological samples using single-molecule localization (PALM/dSTORM). This will be illustrated with fixed COS-7 cells immunolabelled with Alexa Fluor 647 targetting the alpha-tubulin. They will learn about the experimental implementation of this technique and how a standard camera-based SMLM microscope can be easily converted into an event-based SMLM setup. The unusual nature of the data (compared to cameras) will be emphasized and we will describe precisely the different steps in the data processing, and what are the perspectives to improve them. Participants will be able to see experiments running and will have the opportunity to bring their own dSTORM/PALM/DNA-PAINT samples. Incidentally, we will propose methods to assess the spatial resolution of SMLM imaging and how they can be used in practise. Finally, we will highlight the added value of event-based SMLM for fast high resolution bioimaging in the high PSF density regime, in particular how it can be used to assess the temporal evolution of the protein organization over a timescales of a few minutes only. We also show how the technique can be easily adapted to Single Particle Tracking to follow dynamic processes at different time scales. We encourage participants to bring their own samples for SMLM or SPT experiments.

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A041-Photomanipulation of embryos of brown algae

Proposer/Coanimator

Stéphanie Dutertre
Bénédicte Charrier

Abstract

The embryos of kelp (large brown algae; here it will be the species Saccharina latissima) develop from a fertilised egg (diameter ~ 20µm) immersed in sea water. After fertilisation, the zygote is surrounded by a wall of polysaccharides, sort of sticky envelope that undoubtedly protects the embryo from attacks by bacteria or other aggressive agents. This envelope might also provide mechanical resistance to the embryo. The workshop will focus on photomanipulation methods to destroy this polysaccharide envelope, either in a very punctual way or on a large surface. We will use pulsed UV microscopy on embryos of 2 to 8 cells, growing in a microfluidic chip. The microfluidic chips is used here to force the embryos to lie in the X/Y focal plane, in order to keep the laser at similar power for all embryos and all embryo regions (the embryos grow vertically otherwise). We will then quantify the mechanical effect of “de-zyping” or “piercing” the envelope by measuring the change in cell volume (the envelope prevents the cell from swelling in response to its osmotic pressure).

These methods are based on protocols recently developed by the supervisors of the workshop
1. Boscq S, Dutertre S, Theodorou I, Charrier B. Targeted Laser Ablation in the Embryo of Saccharina latissima. J. Vis. Exp. (181), e63518, 2022 doi:10.3791/63518 (video:https://vimeo.com/showcase/9923480)
2. Clerc T., Boscq S., Attia R., Kaminski Schierle G.S., Charrier B., Näubli N.F. Cultivation and imaging of S. latissima embryo monolayered cell sheets inside microfluidic devices. Bioengineering 2022, 9(11), 718; https://doi.org/10.3390/bioengineering9110718

Educational goal

The concept is to be able to destroy a component of a cell that provides mechanical resistance to the tissue. We will use the special biological tissue that is the embryo of the brown alga Saccharina (looks like a rugby ball ~ 40µm long and ~15µm large). It displays a loose and sticky outer envelope all around the embryonic cells. In

brown algae, the chemical composition of cell wall differs of that of plant by 90%. Therefore, using algae will make a different experimental case.
The aim of the workshop is to estimate the stiffness of the envelope in different location at the surface of the embryo. The main spatial reference is the apico-basal axis. Atomic force microscopy would not be relevant to assess the mechanical stiffness of this envelope because 1. we aim to get information about stiffness in the X/Y axes of the envelope (tangential to the cell surface) and not in the Z-axis (perpendicular to the cell surface), what AFM usually does, 2. the envelope is very thick and sticky, making accurate AFM measurement difficult.
Therefore, we will assess stiffness of the outer envelope by measuring cell deformation in response to laser ablation. Brown algal cells, alike plant cells, are turgid. Therefore, releasing the mechanical resistance of the outer envelope should make cells swell immediately. The effect will be even stronger if the plasma membrane is pierced (cell will be emptied of its content). Only the stiffness of the remaining cell wall could maintain the initial cell dimensions (as measured before the ablation). We will then compare cell size before and after ablation. The more different the cell size before and after the ablation, the softer the envelope.
Laser ablation will target 4 main positions along the apico-basal axis of the embryo: 1) apical area (tip of the "rugby ball"), 2 and 3): two positions in the centered area, defined as the "flanks" and 4) basal area (bottom of the "rubgy ball"). Immunochemistry showed that a specific polysaccharide is present along the flanks. Therefore, we will compare the response of the 4 positions to laser ablation.
The envelope is made of cellulose, alginates and fucans, that are all polysaccharides. Cellulose can be stained in vivo with calcofluor. We will use both Brightfield (BF) and fluorescence microscopy to detect and localise the envelope thanks to its predictable location. Its viscous property makes it blurry in BF and its fluorescence when stained with calcofluor will further help us localise it.
Details about the equipment:
The embryo of Saccharina is about 30-50µm in length. The microfluidic chip should be observable on a confocal microscope with a 40X water objective. The algae should be observable in both BF and fluorescence.
For the photomanipulation experiment, the microscope should be equipped with a photomanipulation head with a pulsed UV laser allowing to define very precisely the irradiation area with the laser (in point or ROI mode). Cell size before and after the ablation of the outer envelope will be measured using the software tools or Fiji from photos taken during the experiment.

At the end of this workshop the participants will be able to:

1. manipulate a pulse UV laser confocal microscope.
2. Understand the constraints in this unusual biological background provided by brown algae: these organisms are made of unique cocktails of molecules and chemicals and are photo-sensitive.
3. assess the difference in stiffness of the 4 positions along the apico-basal axis of the algal embryo.

Trainees will be invited to suggest other experimental conditions to destroy the envelope and assess its stiffness (possible crossed-fertilisation with other MiFiBio workshops on site).

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A043-A practical review of several 3D-culture methods for the generation of hollow or solid organoids/spheroids with a unique cell-type, how environment matters

Proposer/Coanimator

Laetitia Andrique
Alessandro Furlan

Abstract

In this workshop we will illustrate how using several methods for generating organoids influences the final topology, histology and differentiation of cells.
As a substitute for hiPSCs that, for obvious safety reasons we cannot use in the context of MiFoBio, we will take advantage of a versatile alternative, i.e., an immortalised epithelial human cell line.
The attendees will implement different methods used to generate 3D aggregates of cells, in the cell culture room. The different methods differ in many ways, both in the generation process and the final cellular topology.
We will follow the growth of cell aggregates, in time and provide to the attendees the images of the structures that will illustrate how they are different in terms of initial state and growth.
The participants will as well carry out some methods to label and monitor cell growth by using fluorescent dyes.
Finally, we will show how to prepare and mount the different samples for microscopy depending on their topology, providing tips and tricks that would help the attendees to set-up their own experiments back in their labs.

Educational goal

At the end of this workshop the participants will be able to produce with several easy and basic protocols, 3D cell aggregates (spheroids, tumoroids, epithelium and cysts).
They will have an overview of these techniques with practical tips, and we will show them how to easily mount them for imaging.
For the one how already know some of these methods to generate organoids, they will be able to compare with other protocols and it will be an opportunity to share their expertise with the others participants.
For the beginners, this worshop will give them some keys to start in 3D culture.

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A044-Structured illumination microscopy with a chip based light source

Proposer/Coanimator

Andrea Bassi
Alessia Candeo

Abstract

The workshop will focus on the technique of Structured Illumination Microscopy (SIM), a widely used method for obtaining high and super-resolution images of biological specimens. The workshop will cover how to convert a standard widefield microscope into a SIM setup using a compact, integrated optical device that employs phase-shifted waveguides as a light source. Participants will gain hands-on experience with the SIM optical setup, alignment, and image acquisition process. Additionally, the workshop will provide training on the reconstruction of SIM images using a dedicated napari plugin, a python-based software for image visualization and analysis. The plugin is designed to be user-friendly, enabling participants with both programming and non-programming skills to utilize the software.

Educational goal

The educational goal of the workshop is to provide a hands-on understanding of techniques and the alignment procedures used in Structured Illumination Microscopy (SIM).
We will demonstrate the steps required to align two coherent laser beams and focus them onto the pupil of a microscope objective using a fluorescent liquid cuvette. This activity has the goal of training the participants interested in understanding the working principles of SIM and are participating to optical system development projects.

The participants will acquire images of biological slides and observe the resulting sinusoidal patterns (when visible), using custom python software. This activity has the goal to illustrate the steps required for resolution enhancement with SIM.
The acquired data will then be reconstructed using a napari plugin, specifically designed to enhance the resolution in one dimension. The resolution enhancement will be limited to one direction, using a one-dimensional sinusoidal pattern, to make the presentation simpler. The goal of the activity is to demonstrate the resolution improvement with SIM and understand the basic principles of the reconstruction algorithms.
At the end of this workshop the participants will be able to align a SIM microscope and reconstruct enhanced-resolution images.

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A045-Coordinate-based quantification of multidimensional and multicolor single-molecule localization microscopy data

Proposer/Coanimator

Florian Levet
Abdelghani Neuhaus

Abstract

Over the last decade, single-molecule localization microscopy (SMLM) has revolutionized cell biology, making it possible to monitor molecular organization and dynamics with spatial resolution of a few nanometers. By identifying the molecule coordinates instead of producing images, SMLM holds an important paradigm shift towards conventional fluorescence microscopy. Consequently, dedicated analyzing tools and methods have been developed to properly quantify SMLM data.

In this workshop we will present various analytical methods designed to quantify single-molecule localization microscopy (SMLM) data directly from the localization coordinates. In particular, we will review clustering, segmentation and colocalization methods, for both 2D and 3D SMLM data.

This workshop will focus on various state of the art methods that use localization coordinates for quantification. In particular, we will present:

- Clustering
  - K-Ripley function
- Segmentation
  - DBSCAN
  - SR-Tesseler
- Colocalization
  - CBC (Coordinate-Based Colocalization)
  - Clus-DoC
  - Coloc-Tesseler

The participants will apply those techniques on custom simulations, experimental data (microtubules in 2D and 3D, mitochondria, synaptic proteins, adhesion site) as well as datasets acquired during MiFoBio when possible. We will also discuss how experimental parameters, such as blinking, photoactivation, labelling efficiency, drift and chromatic aberration, can affect the quantifications and how they can be taken into account. All the exercises will be performed on Point Cloud Analyst (PoCA), a new intuitive software platform that can handle multidimensional SMLM data.

Educational goal

Both theoretical and practical aspects of the techniques will be presented. We will discuss the pros and the cons of the presented methods by summarizing their capabilities with respect to a few categories (ease of use, number of parameters, interpretation of the results, graphical feedback, etc.). We will highlight the necessity to understand that experimental parameters have an effect on quantifications, this effect having to be taken into account to properly interpret the results, Finally, the participants will learn to use PoCA, the legacy of SR-Tesseler and Coloc-Tesseler.

At the end of this workshop the participants will be able to choose the most appropriate methods to their problem, whether it be for structural or colocalization analysis, and use it in the framework of PoCA.

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A052-Two photon imaging of plant tissues with photo switchable Dronpa

Proposer/Coanimator

Marija Smokvarska
Jessica Pérez sancho

Abstract

In this workshop we will describe the advantages of using photo switchable tags in combination with two-photon microscopy using transgenic Arabidopsis plants stably expressing DRONPA.
Imaging of thick samples present the problem of light absorption and light scattering, making high-resolution deep imaging impossible for traditional, including confocal, fluorescence microscopy. Two-photon microscopy allows imaging in vivo tissues up to one millimeter depth because it uses near infra-red excitation, which minimizes light absorption and scattering. At the same time, the need of absorption of two photons simultaneously greatly reduce the out of focus excitation, and thus limits photo bleaching and reduce background. Two photon excitation is in accordance with most of the conventional fluorophores; we, however, want to introduce the photo switchable cytosolic molecule DRONPA, a coral-derived 28-kDa fluorescent protein that may be reversibly switched between a fluorescent on-state and a non-fluorescent off-state by irradiation with light. By combining DRONPA and the high resolution excitation of two-photon microscopy, we will activate DRONPA fluorescence in one single cell of the Arabidopsis root and follow it diffusion over time into neighboring cells.
We will describe the sample preparation and imaging protocol, then participants will practice preparing and mounting the samples themselves. We will compare the results obtained by two-photon vs confocal microscopes and discuss advantages and disadvantages. At the end of the workshop we will discuss other possible applications of this method to answer to other biological questions.

Educational goal

At the end of this workshop the participants will be able to understand the principle 2-photon excitation microscopy, its advanteges over other optical microscopes for thick samples; plant sample preparation and genetic material needed, image acquisition with microscopy settings fine- tuning and image analysis. Participants will understand the photoswitchable properties of certain fluorophores to choose the best for their own needs.

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A054-Virtual reality for multidimensional data visualization and analysis

Proposer

Thomas Blanc

Abstract

Advances in the imaging field have revealed the complex 3D organization of biological samples at the molecular scale. Gaining an intuitive understanding of images of acquired structures requires a means to visualize and interact with the data in a natural way. Clearly, viewing complex multidimensional data on a 2D screen presents many limitations in this regard.

In recent years, Virtual Reality (VR) technology has emerged in many applications. The stereoscopic visualization of 3D data sets in combination with motion tracking offers a totally immersive experience. Moreover, with a VR headset and associated controllers, the user can visualize and interact with the data in a natural way.

This workshop is directed to biologists and physicists who deal with complex multidimensional data sets. The purpose of this workshop is to demonstrate how VR can change our perception of acquired imaging data and facilitate its interpretation and analysis.

In sessions open to all, participants are invited to test two VR software developed in our labs (Institut Curie and Institut Pasteur): (i) DIVA-viewer for pixel-based multidimensional images such generated in optical and electron microscopy, and (ii) Genuage for multidimensional point cloud data such as those generated in single-molecule localization experiments.

Participants are encouraged to bring their own datasets (image stacks or point cloud localization data) to try with the software.

Educational goal

Current imaging techniques are constantly evolving, revealing an ever-growing set of information on complex biological processes. While the dimensionality of the acquired datasets is growing, the means of visualizing and interacting with such complex datasets are still lacking. In this workshop, we aim to show that virtual reality (VR) offers a natural perception of complex data with an intuitive understanding of the underlying structure and dynamic as well as facilitate image and data analysis.

The complexity of the data currently generated by optical microscopy is steadily increasing. It is possible now to acquire not only the 3D evolution of living structures but also to image in different colors, and at resolutions that go beyond the diffraction limit (i.e. super-resolution methods). In super-resolution microscopy, data is generated as a set of point clouds coordinates for each imaged molecule. Other information can also be inferred such as local density, organization, molecular orientation, dynamical behavior, diffusion, etc. As visualizing these datasets is commonly done by performing a low dimensional embedding of this multidimensional data to project the information on a 2D screen, interesting features or properties can be lost in translation. Moreover, interacting with high dimensional data on a 2D screen is another point of limitation for many studies and simple manipulations such as selecting a region of interest become laborious and overly time-consuming. VR offers the means for a natural visualization of 3D data sets [1]. With DIVA-viewer [2] and Genuage [3] platforms, it will be possible to explore two different types of multidimensional data. DIVA-viewer is dedicated to interacting with pixel-based images stacks and Genuage for analyzing multidimensional point cloud data such as those generated in single-molecule experiments.

Two software will be presented:

DIVA-viewer: a VR software to visualize, interact and analyze multidimensional images stacks such as the ones acquired with advanced optical (e.g. confocal, light-sheet) and electron microscopes.

Genuage: a tool for visualizing and interacting with multidimensional point cloud data. Such data is generated for instance in single-molecule localization microscopy and can be overlaid with complementary information on molecular orientation, color, trajectories, and diffusivity.

For both software, the VR experience not only serves the purpose of visualization but also enables quantitative analysis of the data with a facilitated interaction with the 3D representation of the data. Examples include selection of specific areas and tracing by allowing the user to interact with the data while various algorithms are performing the necessary treatment.

References:

[1] M. El Beheiry, S. Doutreligne, C. Caporal, C. Ostertag, M. Dahan and J.-B. Masson, "Virtual Reality: Beyond Visualization," Journal of Molecular Biology 431, 1215–1321 (2019).

[2] El Beheiry M, Godard C, Caporal C, Marcon V, Ostertag C, Sliti O, Doutreligne S, Fournier S, Hajj B, Dahan M, Masson JB. DIVA: Natural Navigation Inside 3D Images Using Virtual Reality. J Mol Biol. 2020 Jul 24;432(16):4745-4749.

[3] Blanc, T., El Beheiry, M., Caporal, C. et al. Genuage: visualize and analyze multidimensional single-molecule point cloud data in virtual reality. Nat Methods 17, 1100–1102 (2020).

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A055-Endommagement ciblé de l'ADN par irradiation laser sur cellules vivantes à l'aide d'un microscope confocal

Proposer/Coanimator

Sandra Piquet
Audrey Chansard

Abstract

L'irradiation laser est une technique utilisée pour étudier la réparation de l'ADN dans son contexte chromatinien in vivo.
L'une des méthodes les plus répandues consiste à irradier le noyau en utilisant le laser 405 nm d'un microscope confocal sur des cellules pré-sensibilisées avec un agent intercalant de l'ADN (BrdU/ Hoechst …) afin d'induire des cassures double brin. Cette approche permet de mettre en évidence la dynamique de recrutement de divers facteurs de réparation préalablement fusionnés avec une protéine fluorescente dans des cellules de mammifères vivantes.

Nous présenterons ici en détails cette technique basée sur la micro-irradiation utilisant le laser d'un microscope confocal, permettant de visualiser instantanément la réponse aux dommages à l'ADN. Nous discuterons des avantages et des limites de cette méthode pour l'induction de dommages à l'ADN et présenterons brièvement une autre solution commerciale permettant d'induire des dommages de façon plus précise dans des compartiments subnucléaires spécifiques.

Educational goal

A l'issue de l'atelier, les participants seront capables d'endommager l'ADN à l'aide d'un microscope confocal. Ils auront également un aperçu de l'amélioration de la méthode par l'utilisation un module de pilotage laser.

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A056-Beginner's guide to the fabrication and use of microfluidic organ-on-chip systems

Proposer

Mathieu Hautefeuille

Abstract

Nowadays, microphysiological systems (MPS) recapitulate native microenvironments in vitro with high fidelity, to imitate physiological or pathological architectures and processes at multiple scales in space and time. Compatible with traditional biological analyses and microscopy (real-time or end-point measurements), they enable the precise study of tissue-like constructs and facilitate mechanistic studies. It has become possible to recreate the organization and functions of complete organs inside microfluidic systems called organ-on-chips (OoC), where the crucial role on tissue homeostasis of factors like multicellularity, mechanics, morphogens and flow-mediated cell-cell exchange has been demonstrated. A particular emphasis has been put on the importance of the interactions between epithelial cells and endothelial cells in all OoCs. For any model to be developed, it is critical to correctly design and fabricate MPS and OoCs in agreement with application-dependent specifications, for biology research and in particular, where microscopy tools are used. In spite of their advantages, developing these techniques may seem overwhelming for non experts and limits their broad adoption by biologists.
In this workshop, we will present how to readily fabricate one of the most important and widely used device: a 2-channel microfluidic chip where an epithelial and an endothelial channel are separated by a porous membrane.
We will provide a clear methodology for beginners to offer a solid background to start working in their own lab. Micromolds of the 2-channel chip will be used to replicate structures in PDMS and assembled into a functional microfluidic chip where channel-to-channel diffusion tests will be performed using fluorescent dyes with wide-field microscopy. Several critical tips/tricks will be provided at each stage (fabrication, interconection, flow) to help reproduce the device successfully and test it under their own microscope environment (widefield or confocal).

Educational goal

The aim of this workshop is to provide a general overview of what MPS and OoC technology are bringing to biomedical research labs both in applied and fundamental science. Our clear objective is to prove that the use of this technology has now become more affordable and that students, researchers and teachers should not refrain themselves from using it because it seems expensive and limited to experts. The rapid-prototyping strategies currently employed worldwide are now enabling the use of this technology in a lot of lab environments, even in the classroom, with a bit of creativity and enough confidence (that we expect to build rapidly in the workshop). In summary, we will provide a clear methodology for beginners to offer a solid background to start working in their own lab, with the help of a strong support of case studies and examples from the literature.

We will show that the factors typically limiting its adoption are technical and theoretical and that simple tools already available can help them gain confidence in designing their own systems and constructing their own devices.
After a short introduction about the technology behind the fabrication of MPS and OoC, we will explain how microfluidic chips must be thought of, with a particular focus on the parameters that are important when culturing cells, that are not necessarily described in the literature.

We will explain how to start designing the architecture, choose the adapted materials and select the biological elements to be used in the chips.
We will detail very precisely all the steps to obtain a viable microfluidic chip in the lab for cell culturing with a practical part on mold-replica and chip sealing for functional channel formation.
We will discuss and share good practice on how to implement the correct physicochemical conditions inside the model to support the desired phenotype inside the device depending on the cell types and the goals.

The practical section will be an ideal include tips/tricks from our own experience, as well as do's and don'ts collected from other users prior to the workshop and current participants

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A059-Expansion microscopy on mouse brain tissue slices: gain of resolution allowing deciphering of synapse substructure

Proposer/Coanimator

Pierre Hener
Yves Lutz

Abstract

Expansion microscopy (ExM) is a recently developed optical superresolution approach based on the original idea that physical separation of light point sources will overcome the limits of the conventional-diffracted microscope. Indeed, an expansion factor of 4 will give for example a lateral resolution of around 70nm compared to the 270 nm resolution of the non-expanded sample.

The tissue samples (cerebellum) will originate from mice injected in the spinal cord with an AAVvirus that express tdTomato. The targeted neurons project then to the cerebellum, allowing detection of specific synapses (mossy fibers) in that brain region. Our goal will be to observe the fine structure of the synapses and its internal organization. We will demonstrate the gain of resolution obtained using ExM.

No special pre-requisite is needed to attend this workshop. In order to facilitate the starting of such a project we will go through all the possible difficulties a beginner could encounter. In this workshop we will present a step by step detailed protocol based on the proExM (protein retention ExM) method published by Asano et al.

The specimen (here a tissue slice) in which proteins have been covalently anchored with acryloyl-X, SE (AcX), is embedded in an acrylamide/bis-acrylamide/sodium acrylate gel (hydrogel). Then, a homogenization /disruption step is performed by enzymatic digestion or by a high temperature treatment in presence of detergent. Finally, using the swellable property of sodium acrylate, the gel is expanded with water undergoing an isotropic three-dimensional expansion by 4-4.5 times in that case.

To go further we will also discuss about recent developments allowing even a better resolution with expansion factors of 10 or more.

Educational goal

At the end of the workshop the participants will have a complete overview of an ExM experiment.

Participants will leave the workshop knowing where to buy the good reagents, how to prepare and manipulate the gel, how to design incubation/gelation chambers, which disruption method is suitable for their own sample and how to perform image acquisition. Between all the steps, the signal will be monitored under the microscope.

Due to the real timeline of an ExM experiment (at least 2 days) we will come with different samples already pre-prepared, representing the different steps. Especially we will discuss about signal preservation and possible immuno-amplification depending on the disruption method used.

The Dapi DNA staining of the nuclei will allow us to evaluate the expansion factor by measuring nucleus diameter.

Moreover and finally we will mount the studied samples in 3D printed chambers that we designed for sample imaging (to be discussed).

A general protocol data sheet will also be provided to the participants.

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A061-Looking at protein structural dynamics using single molecule FRET

Proposer

Emmanuel Margeat

Abstract

In this workshop, we will learn how to perform quantitative single molecule FRET using a multicolor confocal microscope. We will use membrane proteins extracted from mammalian cells to monitor conformational changes associated with the binding of ligands or partner proteins. We will set up the acquisition on a dedicated, modular and commercial single molecule FRET microscope in confocal geometry. We will learn how to judge the quality of the data, perform data analysis in real time, and extract quantitative parameters such as FRET efficiency, that can lead to accurate distances between the fluorophores down to the Angstrom level. We will learn various tools to evaluate underlying structural dynamics of the labeled proteins including dPDA and H2MM analyses. As an application, we will perform smFRET on labeled G-protein coupled receptors (GPCR) solubilized in detergents, in the presence of various ligands, to understand their role on the conformational dynamics of the receptor.

Educational goal

At the end of the workshop, the participants will be able to perform a single molecule FRET or an FCS experiment on a sample solution, including : experimental design, adjusting the fluorescent molecules concentration depending on the experiment, data analysis using commercial or home-made software (Correlation spectroscopy, smFRET histogram, detecting and quantifying conformational changes using FRET, using different analysis)

They will be informed as well on how to site-specifically label their target protein, using unnatural aminoacids coupled with click-chemistry

A068-Microscopie à expansion sur différents échantillons infectieux (Autophagie norovirus / toxoplasme)

Proposer/Coanimator

Elisabeth Werkmeister
Sophie Salomé-desnoulez

Abstract

La microscopie par expansion est une technique de préparation d'échantillons basée sur l'ancrage des protéines de l'échantillon dans un hydrogel que l'on va venir dilater. Avec un microscope conventionnel, il est donc possible d'imager des structures très proches les unes des autres avec une résolution accrue puisqu'elles auront été spatialement séparées grâce à l'expansion. Cette technique a été publiée pour la première fois en 2015.

Durant ce TP, nous proposons d'imager plusieurs types de structures.

Un premier exemple consistera à observer des autophagosomes au sein d'une cellule infectée par un norovirus. L'autophagie est un processus biologique qui permet une dégradation de composants intracellulaires par un lysosome. Dans le cas d'une infection, les virus peuvent être entourés par des morceaux de membranes qui vont former un autophagosome. Cet autophagosome va ensuite fusionner avec un lysosome, et son contenu sera dégradé. Les autophagosomes expriment la protéine LC3 que nous aurons marquée au préalable (immunomarquage). L'objectif du TP sera d'observer ces structures sur des lamelles non expansées, puis sur des gels qui auront été expansés d'un facteur d'environ 4.

Le second exemple consistera à imager un parasite (Toxoplasma Gondii) dans lequel plusieurs protéines intracellulaires auront été immuno-marquées. De la même manière, nous comparerons les images obtenues avant et après expansion.

L'objectif étant de montrer l'intérêt de l'expansion sur différents types de structures.

Educational goal

Durant ce TP, nous souhaitons discuter de la méthode d'expansion que nous utilisons. Nous souhaitons comparer la microscopie avant/après expansion sur des structures très petites de type autophagosome, ou vésicules intracellulaires dans un parasite.
A la fin du TP, les participants auront des pistes pour réaliser une expérience de microscopie à expansion.

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A069-In Resin Fluorescence (IRF) Correlative Light and Electron Microscopy (CLEM) for plant tissues

Proposer/Coanimator

Clement Chambaud
Amandine Leroy

Abstract

Correlative Light and Electron Microscopy (CLEM) consists in imaging the very same cell or tissue area using first light followed by electron microscopy. CLEM approaches permit to identify the region of interest labelled by fluorescence and to access to the ultrastructural details of this region. Here, we will focus on one of them called In Resin Fluorescence (IRF) that we adapted for plant tissues. After sample preparation and sectioning, a tricky steps consist to catch enough fluorescent signal before ultrastructure observations. After introducing main principles of sample preparation for IRF, we propose to show tips and tricks allowing a correct light microscopy acquisitions with the help of Airyscan to get the most out of the fluorescence microscopy. We will finish by showing how to overlay light and electron acquisitions thanks to the free software ICY and the plugin EcCLEM.

Educational goal

At the end of this workshop the participant will be able to:
- Understand the crucial steps of sample preparation to maintain fluorescence together with a good ultrastructural preservation
- Tips and tricks for mounting CLEM sections between slide and coverslip for fluorescence acquisitions
- Parameters to catch enough fluorescent signals using confocal and AiryScan module
- Tips and tricks for unmounting CLEM samples without damage for ulterior TEM imaging
- Perform the overlay of fluorescence and TEM images to obtain CLEM images.

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A070-Atomic force microscopy automation, from microbes to mammalian cells

Proposer/Coanimator

Etienne Dague
Childérick Severac

Abstract

Atomic Force Microscopy (AFM) is an imaging technique able to address living cells. It is also a force machine providing critical information on the nanomechanical properties of living cells. However, the technology is limited because it is extremely time consuming. It is already a challenge to record data on 10 cells in a whole day. Due to this intrinsic limitation the technology is restricted to applications in the academic field. Even in this context, the small size of the data base investigated leads to sometimes inconsistent results in the literature.
It is therefore necessary to increase the throughput of the measurements and to propose solutions to realize force measurements on a larger number of cells in a reduced time. To achieve this goal we have developed a strategy for automating force measurements by AFM based on 2 points. I) directed immobilization of living cells according to well-defined cell arrays and ii) cell-to-cell movement and automatic AFM measurements thanks to an algorithm translated into a script executable on a commercial AFM.
Thanks to this system we are able to perform measurements on 500 to 1000 microbes (Saccharomyces cerevisiae) in 2 to 4 hours(1-3) or to record data on 50 mammalian cells (PC3-GFP) in 2 hours. This development

allows to access and address for the first time, by AFM, the problems of mechanical heterogeneity of cell populations.

(1)Proa-Coronado S., Severac C., Martinez-Rivas A., and Dague E. 2020 Beyond the paradigm of nanomechanical measurements on cells using AFM: an automated methodology to rapidly analyze thousands of cells. Nanoscale Horizons, 5, 131-138,
(2) Formosa C., Pillet F., Schiavone M., Duval R.E., Ressier L., and Dague E. 2015. Generation of living cell arrays for Atomic Force Microscopy studies Nature Protocols, 10, 1, 199-204
(3) Severac C., Proa-Coronado S., Formosa-Dague C., Martinez-Rivas A., and Dague E. 2021. Automation of bio-AFM measurements on hundreds of C. albicans cells JoVE 170.

Educational goal

The first step of the procedure is to prepare a matrix of cells. For microorganisms (Saccharomyces cerevisiae) we will provide patches in micro-structured Poly-Di-Methyl-Siloxane (PDMS) with cavities of the size of the microbe to be immobilized. The super- natant is deposited onto a PDMS stamp, and degassed for about 40 min. After 40 min the buffer is removed from the PDMS surface, and 200 ml of the cell solution are deposited and allowed to stand for 15 min at room temperature. The cells were then placed in the microstructures of the stamp by convective/capillary assembly. The PDMS stamp with cells was finally fixed on a Petri dish (FluoroDish FD35-100) and it was filled with 5 ml of acetate buffer solution to maintain the cells in liquid media.

We will then show how the automation script is called on the AFM, how it is parameterized to take into account the specifics of the cell array (cell spacing, number of cells in a row), and finally how it is executed. Participants will then be able to see the AFM tip move, and record data, automatically, from cell to cell.
In a second step, we will explain the method of directed immobilization of mammalian cells. We use fibronectin micropatterns obtained by microcontact printing. This process is described in an article which we will also make available to participants. Fibronectin is deposited on glass slides according to patterns determined by the structuring of a PDMS patch. Participants will be able to practice microcontact printing which is on the principle very simple. A drop of the anchor is deposited on the microstructured patch, dried, then the patch is stamped on the surface to be functionalized.
We will have previously prepared some cell chips and we will continue the workshop by showing the differences in the implementation of the automation script. Indeed, the displacements between the cells are necessarily larger and the displacement method is based on the optical microscope stage and not on the displacement of the piezo electric ceramic of the AFM. Once the sample of cells is in place and the script is set up, participants will again see the AFM tip moving and recording data, automatically, from cell to cell.
At the end of the workshop participants will have understood and experienced our strategy for automating AFM measurements at different scales. It is based on the combination of directed immobilization of cells and automation of the movement/measurement duo.

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A072-La microscopie par dSTORM pour cartographier les crêtes mitochondriales

Proposer/Coanimator

Claire Caron
Giulia Bertolin

Abstract

Le complexe V de la chaine de respiration mitochondriale est essentiel à la production d'ATP. ATP5A, sous-unité de ce complexe, est une protéine clé pour le maintien de la morphologie et de l'ultrastructure mitochondriale, et pour le fonctionnement de la chaîne respiratoire. Nous la retrouvons localisée dans les membranes des crêtes avec d'autres protéines comme MIC60 (site de contact mitochondriale et système d'organisation des crêtes, sous unité 60) et PHB2 (Prohibitin-2). Etudier leur distribution spatiale permettrait de nous donner des

informations sur les possibles interactions entre ces protéines, et d'obtenir une cartographie des crêtes mitochondriales avec une résolution spatiale jamais atteinte. La question posée est la suivante : Sommes-nous capables d'étudier cette distribution spatiale dans un sous compartiment restreint avec les outils de microscopie actuels ?
Pour répondre à cette problématique nous allons utiliser la microscopie de super résolution par dSTORM (Direct Stochastic Optical Reconstruction Microscopy) en modalité deux couleurs (2c-dSTORM). Grâce à cet outil, nous pourrons visualiser les colocalisations entre différentes protéines avec une résolution sous-mitochondriale supérieure aux techniques de microscopie de fluorescence conventionnelles. Ainsi, cette technique nous permettra de séparer des protéines qui se trouvent à une distance d'environ 10 nm. Nous analyserons la colocalisation entre deux couples de protéines : ATP5A et MIC60, ATP5A et PHB2. Nous les comparerons entre elles et avec un couple contrôle, ATP5A et TOMM20, où la colocalisation sera très limitée. Nous aborderons la problématique biologique et les différentes étapes de préparation des échantillons spécifiques à l'imagerie mitochondriale. Puis, nous effectuerons l'acquisition d'images et, à la fin, nous présenterons des perspectives pour l'analyse de colocalisation avec la solution GcoPS.

Educational goal

Le principal objectif est de pouvoir analyser et comparer les distances entre deux protéines en utilisant la structure de la mitochondrie comme support.
La mise en contexte de cette analyse sera effectuée par une présentation d'une quinzaine de minutes en amont de la prise d'image. Cela permettra de présenter la mitochondrie dans nos recherches, échanger autour de la distribution des protéines dans les crêtes et donc potentiellement ouvrir une discussion avec les chercheurs spécialistes de la mitochondrie. L'apport éducationnel se fait autant par l'expérience en elle-même que par l'échange d'idées et d'expériences entre nous. Cet atelier pourra donc profiter à tout le monde (y compris les intervenants).
Ceci ne bénéficiera pas uniquement aux personnes intéressées par la mitochondrie, mais donnera un cadre général pour toute application biologique qui requiert une résolution spatiale améliorée. Donc, ce sera sans doute un atelier à la fois adressé aux spécialistes de la mitochondrie, mais aussi généraliste pour tous biologistes avec des thématiques impliquant une résolution spatiale très élevée.
Ensuite, une session d'analyse d'image sera proposée afin de découvrir ou compléter ses connaissances en colocalisation par dSTORM. Nous souhaitons utiliser l'approche GcoPS (https://icy.bioimageanalysis.org/plugin/gcops/) pour les analyses de colocalisation. Ceci ouvrira, sans doute à une plus large discussion concernant les différents outils de colocalisation existants (par ex. Icy SODA STORM…).
Un temps sera dédié à la prise d'image faite par les participants. Pratiquer reste le meilleur moyen d'apprentissage et d'échange. Nous allons montrer aux participants la méthode avec un premier couple de protéines, puis ils pourront directement essayer sur l'autre couple de protéines ou bien reproduire la première prise d'image. En plus des discussions et du « hands-on » pour la prise d'images, un quizz sera effectué au fur et à mesure de l'atelier afin d'en conserver l'esprit dynamique.
La discussion pourrait s'articuler également autour de l'utilité d'utiliser telle ou telle approche pour étudier cet organite ou, à l'inverse, donner la possibilité d'utiliser cette technique pour l'étude d'autres structures. L'ouverture d'un débat constructif sur la technique dSTORM serait la bienvenue. A nouveau, la discussion sera un élément clé d'apprentissage pendant toute la durée cet atelier, que nous envisageons comme participatif.
Le protocole qui servira à la réalisation de cette expérience pourra être fournis pour que les participants puissent repartir avec. Cela donnera la possibilité aux personnes présentent de reproduire ce qu'ils auront appris. Le PowerPoint pourra aussi être mis à disposition.

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A073-Smart Microscopy: optimizing microscope usage by autonomous operations.

Proposer/Coanimator

Steven Nedellec
Philippe Labrot

Abstract

Adaptive Feedback Microscopy is a powerful tool in order to identify specific events in hetergenous samples. Linking image analysis with fully motorized and computer-controlled microscopes makes possible the generation of automated and adaptive imaging workflows.

In classical microscopy, generating large, quantifiable datasets can be a time-consuming process. The operator must first locate the desired targets and then acquire data using experiment-specific settings, which restricts the number of observations recorded and can prevent reproducibility,.

To address these issues in quantitative biology, developements have been made to integrate bioimage analysis, which is used to identify specific events in microscopy images, with motorized and computer-controlled microscopes through various software packages. This creates automated and adaptive imaging workflows designed in order to very significantly optimize image recording process on the basis of a software based selection of the events of interest.

Educational goal

At the end of a workshop on smart microscopy, the participants will likely be able to:

- Understand the principles and applications of smart microscopy and advanced technologies used in microscopy (e.g. driving hardware microscope parameters on the basis of on-the-fly image analysis).

- Acquire and analyze images using smart microscopy techniques, including the use of computer algorithms (and maybe machine learning). The sample will be fluorescent fixed cells on multiwell plates and/or stained tissues sections.

- Evaluate the advantages and limitations of smart microscopy compared to traditional microscopy techniques.

- Apply smart microscopy techniques to address specific scientific questions.

The specific learning outcomes aim is to provide the participants with the knowledge and skills they need to effectively use smart microscopy in their own research or work.

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A074-Integrated machine learning for scanned slides analysis with Qupath

Proposer/Coanimator

Pierre Hener
Rémy Flores-flores

Abstract

Since the last five years, various machine learning tools dedicated to the classification of pixels or objects have emerged (Ilastik, Trainable Weka Segmentation…). These tools are powerful but until now, it remains very difficult to analyze whole slide images or big tile scan images with these software.

Few years ago, Qupath, an open source software dedicated to whole slide images was released. It integrates visualization and annotation tools, but also machine learning modules without the need of specific plugins installation or coding, helping users to simplify some image analysis workflows. These integrated modules are the pixels classifier for object detection and the objects classifier. They are easy to handle, efficient and versatile.

This workshop will focus on these modules and how to use them on whole slide images. For the complete analysis workflow, we will as well explain how to display and export the parameters measured automatically by Qupath.

Educational goal

A the end of the workshop the participants will be able to use Qupath integrated machine learning modules to perform quantitative image analysis of a whole slide image. They will discover good practices for organizing their projects and avoiding the pitfalls that can occur when using these tools.

The workshop will consist in pixels and objects classification application on different projects.

The pixels classification will be used for automatic whole tissue detection and staining quantification, as well as for creating segmented cells annotation. Workshop will continue with objects (cells) classification of a whole brain region according to three targets labelled with fluorescence. Finally, the participant will leave the workshop with the projects use for the workshop (exercise and results) and a power point with steps explanations.

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A075-Les bases du FRET FLIM par la méthode comptage de photons corrélé dans le temps.

Proposer/Coanimator

Claire Dupont
Carine Alcon

Abstract

Cet atelier s'adresse aux personnes qui débutent ou qui veulent se lancer dans les mesures de FRET (FÖrster Resonance Energy Transfert) par FLIM (Fluorescence Lifetime Imaging Microscopy).
Dans cet atelier, nous nous intéresserons au principe de FRET-FLIM, au matériel nécessaire pour réaliser les mesures, quels peuvent être les avantages et les limitations, et son application pour des approches biologiques. Pour illustrer, nous prendrons comme exemple l'étude de la proximité des nucléosomes à l'échelle nanométrique en mesurant le FRET entre des histones marquées par fluorescence. Nous montrerons comment détecter, quantifier et cartographier spatialement des états distincts de la chromatine par l'analyse.

Cet atelier se déroulera en plusieurs étapes :
1) Une introduction par la présentation des intervenants mais également des participants et un recueil de leur attente.

2) Théorie sur le principe du FRET, du FLIM, le matériel nécessaire pour faire les mesures et son application pour des approches biologiques. Discussion : quels peuvent être selon vous les biais d'une telle approche ? Quels sont les contrôles à effectuer ?
3) Test de la sensibilité de l'approche par des mesures sur différents contrôles négatifs et positifs par des fluorophores liés ou non par différent linkers.
4) Démonstration d'une application biologique : mesure de la proximité entre nucléosomes dans des cellules HeLa exprimant des histones marquées par GFP ou mCherry
5) Initiation à l'analyse des mesures
6) Clôture de l'atelier par un Quizz pour tester les connaissances acquises et un récapitulatif avec les « take home » messages.

A l'issue de l'atelier, les participants seront familiarisés avec l'approche de FRET FLIM. Ils seront capables de planifier une expérience de FRET-FLIM en utilisant les outils moléculaires les plus adaptés et en anticipant les contrôles à effectuer.

Keywords: FLIM, FRET, nanoscale proximity, GFP, mCherry,

Educational goal

L'objectif de l'atelier est de donner les bases théoriques et pratiques de la technique FRET-FLIM en mode comptage de photons nécessaires pour la mise en œuvre d'une expérimentation.
Ils seront capables de planifier une expérience de FRET-FLIM en utilisant les outils moléculaires les plus adaptés et en anticipant les contrôles à effectuer. Ils auront des notions pour analyser et interpréter les résultats.

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A078-STED-FLIM, apport du temps de vie de fluorescence dans les techniques de STED au niveau multi-couleur, 3D et vivant

Proposer/Coanimator

Pierre Bourdoncle
Julie Lesieur

Abstract

La technique de FLIM (Fluorescence Lifetime Imaging Microscopy) mesure le temps de vie des molécules fluorescentes, permettant d'obtenir des informations sur l'environnement des molécules et sur les interactions entre elles. La technique STED (Stimulated Emission Depletion) permet de réduire la taille de la région d'émission de fluorescence à l'aide d'un laser de déplétion, ce qui permet d'obtenir une meilleure résolution spatiale. Cette résolution étant directement liée à la puissance de ce laser, il est très difficile d'envisager l'étude in-vivo en STED. En couplant le FLIM au STED, on peut ainsi réduire la puissance du laser de déplétion et utiliser cette technique de super-résolution sur des cellules vivantes.
En résumé, FLIM et STED sont des techniques complémentaires qui permettent d'obtenir des informations sur les molécules fluorescentes à la fois spatialement et temporellement. En utilisant de faibles puissances lasers le STED-FLIM permet l'approche super-résolution sur des cellules vivantes en multi-couleurs et en 3D.

Educational goal

L'atelier sera axé sur les avantages de la technique STED-FLIM (1,2)

- En rappelant les limites de la technique STED. Par la mesure des puissances d'irradiation des lasers d'excitation et de déplétion et ainsi montrer les effets de la phototoxicité sur la viabilité de fibroblastes et de macrophages

- En comprenant comment le FLIM permet de contourner les limites de la technique STED, par l'observation et la mesure de l'activité de fusion ou de fragmentation des mitochondries de fibroblastes et de macrophages en STED-FLIM.

1. Wang, L. et al. Resolution improvement in STED super-resolution microscopy at low power using a phasor plot approach. Nanoscale 10, 16252–16260 (2018).
2. Lanzanò, L. et al. Encoding and decoding spatio-temporal information for super-resolution microscopy. Nat Commun 6, 6701 (2015).

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A079-Patterning evanescent illumination with wavefront shaping

Proposer/Coanimator

Irène Wang
Marc Grosjean

Abstract

Evanescent waves are generally used in microscopy to confine light in a sub-wavelength-thick layer at the interface between two media, as in Total Internal Reflection Fluorescence (TIRF) microscopy. However, this type of illumination is homogenous in the (xy) plane. A number of applications, such as optogenetic photoactivation in living cells, would benefit from a 3 dimensional (3D) confinement of the excitation light field, since it would enable to activate subcellular regions with high spatial accuracy.
We propose a method to control evanescent field and create arbitrary intensity patterns in the xy plane, based on wavefront shaping in the super-critical ring of a high-NA objective pupil. A digital micromirror device (DMD) is conjugated to the objective pupil plane and fulfills two roles: i) reject incident light below the critical angle; ii) modulate the phase of the wavefront in the super-critical ring by holography to achieve the desired pattern in the sample plane.
In this workshop, we first show how to create an evanescent spot anywhere in the field-of-view and compare the resulting excitation volume to that generated by a standard focused spot. Then, we explain how to optimize the wavefront of the super-critical pupil region, in order to produce an arbitrary intensity pattern in the sample plane. This evanescent pattern is compared to a pattern formed with propagating light. Model samples (such as dye solutions, fluorescent beads) will be used.

Educational goal

At the end of this workshop, the participants would have learned:

- How to couple a digital micromirror device (DMD) to a microscope and use it for phase modulation.

Using a DMD with coherent light requires to take into account its diffraction and sets constraints on the possible incident and reflected directions. This issue will be illustrated on the setup. Moreover, since the DMD is an on/off intensity modulator, shaping the phase of the wavefront is not straightforward. This is performed using Lee holography. The principle of this strategy will be explained, as well as the limits set by this encoding method on the accessible field-of-view.

- How to create and scan an evanescent spot using a DMD.

In order to create an evanescent spot, the DMD blocks the light in the central part of the pupil that is responsible for propagating waves into the sample. By displaying a binary grating on the DMD, this spot can be moved in the field-of-view. The size and shape of this spot will be discussed.

- How to create an intensity distribution in the object plane by controlling the phase of the super-critical in the Fourier plane.

We will present an iterative optimization approach to determine the DMD state which produced a desired intensity distribution in the sample plane. The resulting pattern will be observed and evaluated.

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A080-Photoacoustic and fluorescence imaging through a single multimode fiber using wavefront shaping

Proposer/Coanimator

Irène Wang
Léo Djevahirdjian

Abstract

The photoacoustic effect is the generation of a sound wave upon absorption of a time-varying light beam. It has raised a lot of interest for biomedical applications, since it allows background-free absorption imaging of endogenous molecules (haemoglobin, melanin, lipids) or exogenous probes inside living organisms. One limitation of photoacoustic imaging is its depth-to-resolution ratio of around 200 (i.e. 1 µm resolution is only attainable at depth below 200 µm). Endoscopy provides a way to achieve high resolution imaging at larger depths. To avoid inflicting damage to the tissue upon insertion of an endoscopic probe, single multimode fibers (typical diameter of 125 µm) have been proposed as minimally invasive probes. However, when light propagates in a multimode fiber, it is projected into a large number of components (or modes) that undergo unpredictable phase delays, so that the output pattern is usually speckle-like and the spatial information is scrambled.
In this workshop, we will show how to use wavefront shaping to compensate for mode scrambling and scan a focused laser spot at the output of a multimode fiber. Then we obtain a small cross-section endoscope that allows to acquire both photoacoustic and fluorescence images point-by-point. A Q-switched laser delivering nanosecond pulses at 532 nm is used to generate the photoacoustic signal. A digital-micromirror device (DMD) is used as a phase modulator for wavefront manipulation. The acoustic signal will be detected by a fiber-optic hydrophone, which measures the acoustic pressure optically using a Fabry-Pérot interferometer at the tip of the fiber. Images of absorbing patterns and red blood cells, as well as fluorescent beads, will be acquired.

Educational goal

At the end of this workshop, the participants would have learned:

- How to perform wavefront shaping using a digital-micromirror device (DMD). To obtain the desired optical field at the output of a multimode fiber, we first characterize the fiber transmission matrix: for each input mode, the output field is measured by off-axis holography in both amplitude and phase, yielding one column of the transmission matrix. Then using the transpose conjugate of this matrix, we can design the input wavefront for any desired output pattern (in our case, a focused spot). The same method could be applied to image through a complex medium, such as a scattering layer.
- How to detect an acoustic signal with a fiber-optic hydrophone, which can fit inside a small cross-section endoscope, and acquire photoacoustic images.

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A081-Fast imaging of 3D models for Hight Content Screening quantitative analysis in 3D

Proposer/Coanimator

Jacques Rouquette
Margot Tertrais

Abstract

High-throughput imaging, which allows automated, rapid, quantitative and time-dependent analysis of events at the cellular level on 2D but also 3D models (organoids, etc.), has developed considerably. This technology has become central to a large number of projects to quantitatively phenotype different cell types from animal or human origin. For this, HCS (High Content Screening) imaging is a major asset allowing on the same system the imaging and the analysis of the sought phenomena.
In this context, we would like to image and characterized large organoids with cellular and subcellular resolutions. The goal will be to demonstrate, characterize and quantify cell differentiation within a 3D organoid. The biological question is to identify and quantify different cell types inside spheroids:
- Spheroids after differentiation: lipids-containing cells were stained with bodipy; endothelial cells and pericytes were revealed by specifics staining and Draq5 staining for cell nuclei.

For this we would like to use a high throughput imaging system allowing fluorescence imaging of organoids in 3D on fixed samples from setting up an acquisition protocol to analyzing images and evaluating results.
In order to facilitate questions, understand expectations, lead discussions, users will respond throughout the workshop to mini-quiz in real time via application on smartphones.

Educational goal

At the end of this workshop the participants will be able to:

1- Better envision if HCS can be a complementary solution for their research projects or not.
2- Understand how to acquire images for 3D visualization, reconstruction and quantitative analysis. Understand the value of advanced features, like “intelligent acquisition” with a prescan to find events (here, spheroids) of interest (in x, y and z) at low magnification, and rescan them at high magnification.
3- Understand the principle of image analysis, either by using common assays with ready-made solutions, or by creating their own with simple image-analysis building blocks, know that machin learning analysis tool will also be understood when applied to segmentation and object classification.

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A082-How to reach mitochondrial inner membrane remodeling by super resolution live imaging

Proposer/Coanimator

Arnaud Chevrollier
Pauline Teixeira

Abstract

Mitochondria are intracellular organelles surrounded by a double membrane. The inner mitochondrial membrane consists of subcompartiments called cristae and the inner boundary membrane. The main function of mitochondria is to generate energy, in the form of adenosine triphosphate (ATP). This is achieved by the

oxydative phosphorylation, OXPHOS, located in the cristae. Mutations in the OXPHOS subunits lead to extreme heterogeneity of inherited mitochondrial disorders. Mitochondria are organized in a dynamic connected network within the cells, constantly adapting to cellular requirements by changing its shape and position through processes of fission and fusion. The coordination of the assembly of proteins complexes such as OXPHOS influences the architecture of the mitochondrial cristae and so, the efficiency of mitochondrial ATP production. The key players in cristae morphology include the MICOS complex and the mitochondrial ATP synthase, whose oligomers are retrieved on the edges of the cristae and contribute to define cristae curvature.
The number of cristae per mitochondrion, the shape of the cristae, the number of cristae junctions with the peripheral inner mitochondrial membrane are essential structural information we need to assess mitochondrial phenotype. Furthermore, the inner membranes of mitochondria are continuously undergoing remodelling and this new area, recently accessible with the help of super-resolution microscopy, raises many questions regarding the relationship between inner membrane dynamics and the efficiency of ATP production by mitochondria. Mitochondria defects have been involved in many human diseases, such as neurodegenerative disorders, cardiomyopathies and also in cancer. Super-resolution imaging opens up a new field of research by exploring inner membrane remodelling and the content and distribution of inner membrane proteins in a pathological context.

Educational goal

Structured Illumination Microscopy (SIM) and Stimulated emission depletion (STED) are the most promising tools to visualize nanoscale cellular structures and dynamics in living cells.
However, its practical utility is considerably limited by the rapid photobleaching and phototoxicity of fluorescent dyes.
Recent technologies allow the speed of the resonance scanning to be increased, which significantly reduces illumination times and improves the signal-to-noise ratio (SNR). This reduction in acquisition time reduces the photo-toxicity induced by fluorescence emission and new fluorescent probes with remarkably low photodynamic damage have also been developed and commercialised (cyclooctatetraene-conjugated cyanine dyes PK Mito Red). In addition, the increased sensitivity of the new detectors makes it possible to increase the number of images per minute and thus to image faster events. Using the new SIM and STED excitation and acquisition systems, we will present a visualization of the inner membrane dynamics of mitochondria with nanometer spatiotemporal resolution.

We propose to reach these acquisition limits in a biological context of mitochondrial phosphorylation complex alteration. Cell transfection of chimeric constructs addressed to mitochondrial outer and inner membranes will be used to specifically label these membranes. The acquisitions will show the relevance of the organization of mitochondrial cristae in the structure and dynamics of mitochondria.

Why our lab: Mitochondrial pathophysiology studies are the main focus of our lab, MITOVASC, Mitolab Angers. Mitochondrial imaging is our specialty, using widefield fluorescent microscopy and deconvolution, TIRF microscopy and dSTORM/sptPALM super resolution. We have recently developed the super-resolution markers using SNAP-tag technology to improve our ability to image the inner mitochondria by super-resolution live cell approaches (SIM and STED). Cell permeable red benzylguanine substrates (siR) are used for labeling SNAP-tag™.

The main objective is to explain to the participants the importance and the limits of the observation of mitochondria by fluorescence microscopy. The second objective would be to succeed in visualizing the cristae and their dynamics. Thus, mitochondria are a complex dynamic biological object with several possible levels of observation. In cultured cells, mitochondria typically form connected tubules 250 to 350 nm in diameter. These tubes are constantly changing shape, fusion, fission and displacement. Quantifying the organization and distribution of cellular mitochondria is already a complex first level to establish. Furthermore, the mitochondria are a complex three-dimensional organisation. In optical microscopy, it is difficult to correctly account for the superposition of mitochondrial signals, even within adherent cells of a few micrometers.
To be interested in mitochondrial internal membranes require to go below 100nm of resolution by taking into account the depth. In addition, the inner membrane faces the outer membrane and folds to the matrix side to form the cristae. The differential labeling of the cristae is not currently available, leading to an accumulation of signals coming from the peripheral inner membranes and the cristae. Using control cells and cell models with

strong mitochondrial structural alteration, we will attempt to distinguish the organization of cristae. These data will be compared to transmission electron microscopy images made in these models.

There are several approaches to super-resolution imaging but few systems are currently capable of imaging cristae in timelapse. Depending on the type of microscope, a time of explanation will be devoted to the presentation of the technological approach by the industrial partner.
We will then explain the labeling strategy and discuss the possibilities of labeling depending on the microscopes. Participants will be able to position the sample, visualize the whole mitochondrial network before focusing on an area of interest. We will take several image sequences. We will test the sensitivity to phototoxicity. Beyond the decrease of the signal intensity, the excitation of fluorophores generates free radicals that affect the mitochondrial structure. Swelling of the mitochondria is often a consequence of the observation. Some excitation systems preserve these effects.
We will also present our analysis approaches by fluorescent signal reconstruction and 3D visualization of mitochondrial dynamics.
We will make a summary about the observations : the link with the biological issue of OXPHOS deficit and mitochondrial structure, the assessment of the resolution, the time of possible acquisition. We can also discuss the comparisons with the Super resolution single particule tracking sptPALM.

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A083-Optimization of 3D High Content Screening (HCS) acquisitions on thick samples

Proposer/Coanimator

Laetitia Ligat
Romina D\'angelo

Abstract

In the last years, many devices dedicated to high content (HC) microscopy (imaging or screening) have been developed. These systems allow to measure in a fast, automated and reproducible way a large number of biological parameters, whether we work on thin (e.g. 2D cell culture) or thick samples (e.g. 3D cell culture). The main goal of this workshop is to give the necessary tools to improve 3D acquisitions in a HCS context. From samples' preparation to instrumental calibration, we will present all the key points to the successful implementation of a 3D HCS.
We will use as a 3D model spheroid of approximately 200µm in size that were obtained from a pancreatic tumor cell line.
Our workshop will address and illustrate efficiently the following issues:
A. On sample preparation, how to choose the: (i) Fluorescent probes and immunolabeling techniques, (ii) Clearing medium, and (iii) Mounting/Support.
B. On the instrumental calibration: (i) How to choose the objective (e.g. multi-immersion) accordingly to the refractive index of sample mounting medium (ii) The relevance of the disk (spinning) to improve speed, depth, and resolution.
Finally, we will also discuss the various existing softwares available for imaging processing.

Being able, for the different steps, to rethink what to do, or rather to avoid “stupid errors”, will be put forward. The participants will be actors and feel free to come and modify, propose an alternative approach/parameters on the machine that can improve the result.
At the end of the workshop, participants will acquire theoretical and practical knowledge to perform sample preparation (including clearing approaches and immunolabeling strategies) on spheroids and that will allow them to improve their 3D HCS experiments by making better choices on 3D sample preparation, HCS acquisition, and analysis.

Educational goal

The main goal of this workshop is to identify the best choices that can be made to optimize a 3D HCS experiment on thick samples.

1. Clearing approaches to improve depth imaging of thick samples
The participant will be able to compare Z-stack acquisition of cleared spheroids (using 2 protocols) vs non cleared, and visualize the image effects made in depth (signal loss and scattering).

2. Matching the mounting medium and lens immersion
We will perform a Z-stack on our cleared samples with different immersion objectives (dry, oil, water, and silicone immersion). The goal is to observe the impact on image quality (signal quantity and sharpness) in depth, depending on the index's matching.

3. Wavelength selection to improve depth imaging
We will compare on a Z-stack a Dapi against a FarRed marker to show the difference in depth detection as a function of wavelength.

4. Right choice of disk or spinning disk model according to the constraints of 3D HCS applications (speed and/or depth)
We will test a fast Z stack imaging and show the impact of choosing a speed optimized disk (close holes) in contrast to a depth optimized disk (spaced holes). This will allow us to visualize the technical limitations of the spinning disk itself.

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A085-Super-resolved Imaging based on RIM technology

Proposer/Coanimator

Thomas Mangeat
Léa Costes

Abstract

The goal of the workshop is to explain the new technologie of the RIM (Random Illumination Microscopy) and the interest for life science applications [1,2]. The principle of RIM is based of the knowledge of the statistical properties of the illumination.

RIM is a super-resolution technique that extends the resolution of a factor in 3 dimensions of space, in a robust and quantitative way.

RIM's inherent simplicity and extended biological applicability, particularly for imaging at increased depths, could help make SRM accessible to biology laboratories

A new real-time algorithm will be presented, making RIM compatible with medium-content applications in life imaging.

We illustrate the potential of RIM on diverse biological applications, from the mobility of chromatin in U2OS cells to the 3D motion of myosin minifilaments deep inside Drosophila tissues.

The system is compatible with magnifications from X20 to X100. All imaging modalities will be available to participants during the workshop.

[1] Mangeat, T., Labouesse, S., Allain, M., Negash, A., Martin, E., Guénolé, A., ... & Sentenac, A. (2021). Super-resolved live-cell imaging using Random Illumination Microscopy. Cell Reports Methods, 1(1), 100009.
[2] Labouesse, S., Idier, J., Sentenac, A., Mangeat, T., & Allain, M. (2021, January). Random Illumination Microscopy from Variance Images. In 2020 28th European Signal Processing Conference (EUSIPCO) (pp. 785-789). IEEE.

Educational goal

The objective is to explain some of the properties of speckle illumination and why the use of dynamic speckle illumination statistics allows to obtain super-resolution in three dimensions.

The second objective is to explain why RIM enables quantitative super-resolution imaging for tissue analysis of single cells or populations of cells : optical sectionning, fluorescence linearity, artefact free superresolved images.

Finally, we will explain the ease of use of RIM and make interaction with workshop participants problematic.

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A086-Eternity-Plus buffer for 3 color Nanoscale imaging in depth.

Proposer/Coanimator

Karine Monier
Céline Malleval

Abstract

Direct STochastic Optical Reconstruction Microscopy (dSTORM) is a single molecule localization microscopy (SMLM) approach, allowing to reach precision localization around 20 nm, thus providing a 10 time magnification compared to the classical conventional microscopy approach. However, accessing the world of SMLM requires a big step forward and is still paved with many challenges. Our goal was to democratize dSTORM, by developing solutions for a better and easier way to perform nanoscopy imaging. One challenge is the short life of aqueous blinking buffer required to discriminate individual fluorescent molecules. To tackle this, we developed a new buffer, called Eternity Plus, allowing imaging for several weeks and three colors. To evaluate buffer capabilities independently of biological variability, we developed 1 µm calibrated spheres, coated at their periphery with far red blinking fluorophores, known as FluoRef.
Imaging deep inside thick samples is challenging for many biological research fields.
We propose to challenge an SMLM microscope with non-TIRF illumination and high laser power to image tubulin and myofiber organelles, deep inside isolated muscular fibers.
Fine delineation of these structures in control conditions will allow a better comprehension of default structuration in centro-nucleated myopathies.
Our workshop will be focused on our recent breakthrough to expand the multicolor capabilities of Eternity-PLUS buffer: a new version applicable to the majority of green emitting fluorochromes opening the way to 3 color dSTORM nanoscopy.
Our workshop will be divided into three successive steps:
1- Demonstrate the superior blinking capabilities of our most recent Eternity-Plus buffer in comparaison to Glox buffer using 1 µm calibrated spheres coated with a green fluorophore

2- 3 color Deep SMLM imaging using Eternity-Plus buffer in isolated muscular fibers immuno-detected with microtubules, actinin and mitochondria.

Educational goal

At the end of this workshop the participants will have experienced the chromatic superiority of our Eternity-PLUS buffer for green emitting fluorophores compared to Glox buffer

At the end of this workshop the participants will have experienced the ability on a wide-field system for in depth dSTORM sample imaging with the capabilities of z-Stack integration to increase the thickness of the observed volume.

At the end of this workshop the participants will be able to understand that dSTORM microscopy with Everspark is easier, long-lasting (several weeks) and reproducible.

At the end of this workshop the participants will be able to understand that blinking calibration tools are efficient to evaluate the capabilities of a buffer even for a beginner, without being submitted to variability of biological structures.

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A087-DeepIcy: A user-friendly plugin to use latest Deep Learning methods in Icy

Proposer/Coanimator

Thibault Lagache
Vannary Meas-yedid

Abstract

Deep-learning is revolutionizing many fields in bio-image analysis such as cell segmentation and classification. Despite the rapid development of robust networks and the availability of many trained models (e.g. BioImage Model Zoo https://bioimage.io/#/), or at least annotated images, the use of DL methods is still restricted in the biological community as it still requires some advanced programming skills. Recently, user-friendly plugins such as DeepImageJ (https://www.nature.com/articles/s41592-021-01262-9) or online applications such as CellPose (https://www.cellpose.org/) have been proposed for the generic use of pre-trained deep learning models for biomedical image analysis. In this workshop, we will introduce the user-friendly solution that we are currently implementing for Icy (https://icy.bioimageanalysis.org/), a standalone application and online platform for advanced image analysis.

Educational goal

To involve the participant as acting people we will begin by a short oral survey (by raising their hand) asking them if they already had to deal with DL when analyzing their images
We will list with them, which solution they used or they know for now, and if they're able to use these solutions easily. A particular attention will be given to the user-friendluness and robustness of proposed solutions.
People will be installed on computers with maximum 2-3 persons by computers.
Since people will be able to manipulate the software themselves, we will alternate between showing procedure on screen and let them practicing directly on example pictures that we'll bring. They will try DeepIcy plugin on different microscope modalities (fluorescence microscope, histopathological images...).

We will keep time, for them to analyse their own pictures to appreciate the accuracy and user-friendliness of DeepIcy on their own biological images. We will be there, to help them and answer their specific questions related to their own biological question.
At the end of the workshop, the users should be able to use advanced DL algorithms with DeepIcy on their images.

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A089-Deep Learning for fluorescence lifetime imaging microscopy (FLIM)

Proposer

Aymeric Leray

Abstract

Fluorescence lifetime imaging microscopy (FLIM) is a powerful technique to probe the molecular environment of fluorophores. The analysis of FLIM images is usually performed with time consuming fitting methods. For accelerating this analysis, we propose a simple neural network formed only with fully connected layers able to analyze fluorescence lifetime images. This network is called Phasor-Net and it is based on the reduction of high dimensional fluorescence intensity temporal decays into four parameters which are the phasor coordinates, the mean and amplitude-weighted lifetimes. Phasor-net is able to determine quickly and accurately the bi-exponential parameters with a lower number of photons than standard fitting methods
The aim of this workshop is to introduce some deep learning algorithms used for analyzing FLIM images and to perform experimental FLIM acquisitions in living cells expressing fluorescent proteins: eGFP only for negative control and eGFP linked to mCherry for positive control. We will finally analyze these data with the Phasor-Net network implemented in a home-made software and discuss some strategies for improving the results.

Educational goal

At the end of this workshop the participants will be able to acquire FLIM images and to analyze them with both standard fitting methods and deep learning algorithms.

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A090-Batch processing images from OMERO in Fiji

Proposer/Coanimator

Pierre Pouchin
Frédéric Brau

Abstract

The Open Microscopy Environment Remote Objects (OMERO) is an open-source image manager used by many biologists to store, organize, view, and share microscopy images, while the open-source software ImageJ/Fiji is a very popular program used to analyze them.
However, there is a lack of an easy-to-use generic tool to run a workflow on a batch of images without having to download them to local computers, and to automatically organize the results in OMERO.

To offer this functionality, we have built an ImageJ/Fiji plugin to run a macro-program on a batch of images from OMERO, as well as a new set of Macro Functions ("OMERO Macro extensions"), dedicated to interact with OMERO in macro-programming.
In this workshop, we will demonstrate how to use the "Batch OMERO plugin", and, more importantly, how macros should be adapted to fully benefit from its functionalities. We will also outline the limits of this plugin and see how we can circumvent this using the macro extensions.

Attendees should have at least a basic understanding of OMERO and ImageJ macro programming.

Educational goal

A rapid introduction will first summarize the challenges faced when processing images from OMERO on Fiji and explain how the plugins can be installed.
Then, using sample images, we will see how to store segmentation results in two different ways on OMERO (labels and ROIs, especially in 3D) and how to adapt macros to best generate OMERO tables from ImageJ results. These operations will also be repeated on images stored locally.
Finally, after a brief overview of the "Batch OMERO plugin" limitations, we will interact directly with the server using OMERO Macro extensions.
At the end of this workshop the participants will be able to modify ImageJ macros to use them efficiently when processing images in batch from OMERO.

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A092-Single Molecule Localization Microscopy and use of calibration tools to unravel EVs composition and 3D morphology at a single-vesicle level

Proposer

Daniele D\'arrigo

Abstract

Single Molecule Localization Microscopy (SMLM) allows to overcome the light's diffraction limit, offering typically a localization precision lower than 20 nm. This is particularly important for the imaging and the characterization of extracellular vesicles (EVs). These lipid nanovesicles play a crucial role in cell communication process and they have a typical size below 200 nm. Thanks to the use of large unilamellar vesicles (LUVs), synthetic nanometric vesicles with comparable size that mimic natural EVs, we optimized the 3D and dual-color imaging of the EVs. Currently, different techniques are used to characterize their size and concentration (light scattering, tunable resistive pulse sensing and electron microscopy), morphology (electron microscopy and atomic force microscopy) and surface markers (western blot, confocal and fluorescent microscopy, ELISA and flow cytometry). However, SMLM could own the potential to provide all these information, allowing to reduce the variability connected with the use of several analytical techniques. During this workshop, we show an SMLM approach to image and characterize both LUVs and EVs, including the size, the 3D morphology, and the surface markers. Our experimental set-up includes dedicated capture surfaces that enables both a targeted (tetraspanins) and an untargeted capture of LUVs and EVs isolated from human mesenchymal stem cells, their 3D and dual-color imaging (using the spectral demixing approach) with a super-resolution microscope and an optimized proprietary software to extract quantitative data. In particular, we will present the theoretical basis and the sample preparation approaches for the imaging of EVs with SMLM, then we will perform a practical demonstration about the optimization and the imaging process and, lastly, the analysis of the obtained data. After this demonstration, the participants will actively participate in the acquisition of super-resolved EV images and in the subsequent data analysis.

Educational goal

During this workshop we will :
- remind the alternative strategies currently used to characterize extracellular vesicles (EVs)
- present the EV structure, morphology and function
- preparation of the samples for the SMLM
- acquire 3D and dual-color images (spectral demixing) of EVs and LUVs with a description of the 3D and dual-color strategy
- analyze the images with a proprietary software developed by Abbelight company

At the end of the workshop, the participants will be able to evaluate the best approach to image and characterize their EV samples, to prepare the vesicles for a SMLM session and to optimize the acquisition. They will be more familiar with a 3D and multi-color imaging setup (spectral demixing), and how to process the data obtained from the pictures.

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A094-Tissue multiplexing and whole section imaging for quantitative histopathological analysis

Proposer/Coanimator

Laurence Dubreil
Julien Pichon

Abstract

Quantitative analysis of immune cells in a tissue section is essential for the characterization of adaptive immunity of host against pathogens and/or cancer. During this workshop, we will focus on the key steps for performing multilabelling of lymphocyte T cytotoxic, Treg, Thelper, B cells and macrophages (6 fluorescent immunolabelings) on spleen section for quantitative histopathological analysis. The first objective of the workshop will be to define a multifluorescent labelling techniques adapted to paraffin sections with choice of fluorophores, amplification, unmasking techniques ect. The second objective of the workshop will be to assess the potentialities of basic equipment of platform microscopy as confocal microscopy and spinning disk to image multiplexed tissue sections. In addition, correlative microscopy will be done to image same ROI of tissue section from the two different microscopes in order to perform comparative quantitative analyses.
The quantitative analysis of multiplexed images will be briefly discussed during the workshop and treated in more depth during the QuPath workshop led by Thierry Pecot.

Educational goal

The objective of this workshop is firstly to discuss about fluorescent multilabeling technics compatible with the analyse immune cells response on Formalin-Fixed Paraffin-Embedded (FFPE) tissue sections and secondly to evaluate the potential of basic equipment of platform microscopy as spinning disk and confocal spectral microscopy to image multiplexing instead to use a dedicated system. In addition, correlative microscopy will be performed to image same ROI from different setup.
At the end of the workshop, the participants will know the different steps that are necessary to performed multiplexing of immune cells on tissue section. They will be able to select fluorescent probes compatible with the system used for tissue section imaging. They will be able to evaluate two systems of acquisition using (i) a high-speed optical sectioning technic (spinning disk) and (ii) a spectral unmixing from spectral confocal microscopy to separate fluorescent probes used for the multiplexing. Correlative microscopy solution will be assess to image same region on tissue section from both spinning disk and confocal microscope in order to do comparative quantitative analysis of immune cells (Image analysis will be done with QuPath, workshop Thierry Pecot).

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A095-Contribution of expansion microscopy for studying organs: Example of lymph node

Proposer

Samira Benadda

Abstract

This workshop aims to present an imaging approach for a high-resolution study of organs.
The expansion microscopy method increases the resolution by 4 and circumvent the lack of funding to acquire a super resolution system.
Clearing is the most common method for studying organs.
This workshop proposes to show the contribution of expansion microscopy in comparison to clarification for studying organs.
We are using clearing and expansion microscopy to image whole mouse lymph nodes by light sheet microscopy.
Expansion microscopy allows us to reach a resolution to visualize structures in the whole organ in a way that we were unable to achieve with clearing.
In this workshop, we show that microscopic expansion could be adaptable to whole organs, more specifically to the lymph node.

Educational goal

Number of participants: Engineers, biologist researchers limited to a maximum of 10 so that everyone can see and participate.
The goal of this workshop is to give participants all the thoughts, tips, technical tools around the expansion microscopy of organs.
It will detail the principle of expansion, the preparation of samples and the fluorophores compatibles.
In this workshop, we will explain the different steps of the expansion microscopy protocol for lymph node, the manipulation and mounting of the sample on the sample holder in the lightsheet, the acquisition of images of the organ in 3D.
We will discuss the comparison with the clearing protocols by highlighting the benefits and drawbacks of the expansion microscopy of lymph nodes.
At the end of this workshop, participants will be able to follow a protocol for the expansion microscopy of lymph nodes which might be adapted to other organs

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A098-Optimizing fluorophores and data acquisition parameters in SMLM and sptPALM in silico using the SMIS simulator.

Proposer/Coanimator

Dominique Bourgeois
Jip Wulffelé

Abstract

Advanced fluorescence imaging techniques such as single-molecule localization microscopy (SMLM) fundamentally rely on the photophysical behavior of the employed fluorophores. This behavior is generally complex and impacts data quality in a subtle manner. SMIS (Single-Molecule Imaging Simulator) is a simulation software that simulates a widefield microscope and incorporates fluorophores with their spectral and photophysical properties. With SMIS, data collection schemes combining 3D, multicolor, single-particle-tracking or quantitative SMLM can be implemented. The influence of advanced fluorophore characteristics, imaging conditions and environmental parameters can be evaluated. In this workshop, participants will learn how to use SMIS. This will help them to design real experiments and to properly interpret them.

The workshop stands at the frontier between 3 modules: Labeling strategies and probes; Nanoscale quantification; Molecular dynamics and interactions.

Reference: Bourgeois, D. Single molecule imaging simulations with advanced fluorophore photophysics. Commun Biol 6, 53 (2023). https://doi.org/10.1038/s42003-023-04432-x

Educational goal

The goal of the workshop is to make participants familiar with SMIS, so that they can use it as a tool to:
=> choosing adequate fluorophores for their SMLM or sptPALM experiments.
=> choosing adequate data aquisition parameters
=> becoming aware of the complex photophysics of fluorophores employed in SMLM
=> becoming aware of the artifacts that can arise due to this complex photophysics (e.g. crosstalk effects in multicolor experiments, incomplete effective labeling due to photobleaching ..., interrupted tracks and confinement effects in sptPALM)

At the end of this workshop the participants will be able to implement their own planned experiments in SMIS and run them virtually. They will be able to process the produced stacks of images, as they would for real data sets, and compare the results with the ground truth.

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A100-Optimizing imaging schemes for PALM and single particle tracking in bacteria

Proposer/Coanimator

Oleksandr Glushonkov
Jip Wulffelé

Abstract

The goal of our workshop is to introduce Photoactivated Localization Microscopy (PALM) and single particle tracking (sptPALM) imaging techniques, which are based on the sequential detection and localization of genetically encoded photo-transformable fluorescent proteins, allowing non-invasive labeling of a target protein. Collected data are used for a reconstruction of better-resolved images or protein trajectories, and with a further in-depth analysis can provide insights into protein distribution, clustering, dynamics, and binding-unbinding events.

However, PALM/ sptPALM imaging and data analysis are not always straightforward. Experiments are complicated by motion blur, the relatively low photon budget of fluorescent proteins and their complex photophysics/blinking. These challenges as well as specific technical requirements for successful imaging (e.g.

use of a sCMOS camera, advanced control of laser pulse sequence) will be addressed and discussed during the workshop.

We will perform PALM and sptPALM acquisitions to unveil the localization and dynamics of the nucleoid associated protein HU from Deinococcus radiodurans. HU is one of the most conserved proteins in bacteria. In our group, we are interested in its role in nucleoid reorganization during recovery from DNA damage. To localize HU we labeled it with mEos4b, one of the best performing photoconvertible fluorescent protein. Furthermore, we will discuss strong and weak points of PALM versus other Single Molecule Localization Microscopy technics and how to adapt and optimize the experimental protocol for particle tracking. In particular, we previously showed that addition of low intensity 488 nm light reduces the blinking duration of mEos4b and thereby can increase the track length in sptPALM experiments.

Who should attend:
Our workshop is aimed at students and researchers who are new to the field of PALM and sptPALM imaging and researchers who want to optimize their imaging conditions.

Educational goal

At the end of this workshop the participants will be aware of the technical requirements and differences between PALM and sptPALM imaging. With this knowledge, participants will be able to optimize their imaging schemes and workflows for their experiments.

To make the workshop interactive, we will call few volunteers from the participants to let them make acquisitions under our guidance, change the laser sequence and intensity while monitoring in real time the number of localizations and the length of tracks (observation of the 405 and 488 lasers effect).

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A101-Slide scanner use case: from the acquisition to the analysis of a stack of 2D slices registered onto a 3D reference brain atlas

Proposer/Coanimator

Marine Breuilly
Bruno Chapuis

Abstract

Slide scanner offers now the possibility to quickly and automatically digitize sections mounted onto up to 100 slides. This gives the opportunity to acquire many images with robust and reproducible parameters, with a wide range of fluorescence wavelengths, brightfield or polarization settings. It can run 24/7 and quickly produce thousands of images to be analyzed. For instance, this type of microscope is particularly suited to the field of neurosciences, since it makes it possible to image serial brain sections to compute whole brain estimates of the density of selected cell types.
After acquisition, biologists end up with stacks of 2D images to be analyzed. This analysis can be performed with QuPath [1]. This open-source software is indicated for cell detection and classification in whole-slide image analysis. Among other extension, QuPath offers a bridge to the Fiji plugin ABBA [2]. Using this plugin, neuroscientists can register stacks of 2D images onto 3D reference atlases such as the ALLEN mouse brain atlas [3]. Those can be imported back to QuPath and all the brain atlas regions become available for analysis of cell counts per brain regions.

Together, this approach allows neuroscientists to perform analysis on large number of brain sections and to figure out several quantitative parameters (cell density per subject/condition/brain region) thanks to R and python scripting. By the mean of the python library “brain render” [4], they will be able to display their results on a 3D view of the ALLEN mouse brain atlas.

In this workshop, we will teach the entire pipeline from acquisition to image analysis (see below "educational goal" for the details).

Educational goal

At the end of this workshop, participants will have the knowledge of the entire process from acquisition to image analysis, with a list of open-source tools and softwares for each step. An active discussion will be held to address all the drawbacks of the technics and the tips and tricks one should know in order to avoid/compensate them. We will make sure the participant will manipulate QuPath and ABBA plugin. Given a small effort, this workflow is dedicated and accessible to beginners and intermediate in image processing / analysis. Ideally, participants who already have an experience with QuPath are welcome.

In this workshop, we will teach the entire pipeline from acquisition to image analysis with the following steps:

1) the acquisition of 4-5 slices sampled every 150-200 um (e.g. characteristic rostral-caudal hippocampal brain region) with the slide scanner. One volunteer (ideally with low experience) will perform the settings and acquisition under the supervision of the trainers;
2) the analysis part: All participants will be guided through the different tools and softwares
a) the project management in QuPath;
b) the registration of a small stack of consecutive slices onto the ALLEN mouse brain atlas with ABBA plugin. The registered brain atlas regions will be imported back to QuPath;
c) the cell detection and classification in all brain regions in QuPath;
d) the final analysis and 3D representation of the "most active" regions with Python script using brainrender library.

References

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[2] Fiji plugin “ABBA” developed by BioImaging And Optics Platform (BIOP), EPFL Lausanne: https://biop.github.io/ijp-imagetoatlas/
[3] 3D ALLEN mouse brain atlas: https://atlas.brain-map.org/atlas?atlas=602630314#atlas=602630314&plate=576989940&structure=549&x=5280.000145755596&y=3743.8806334538244&zoom=-3&resolution=11.93&z=3
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A102-Guidelines to probe protein-protein interactions by FRET-FLIM

Proposer/Coanimator

Marie Erard
Dounia Zamiati

Abstract

Forster resonance energy transfer (FRET) between two fluorescent proteins (a donor and an acceptor) fused to proteins of interest is an efficient approach to monitor their proximity at the nanometer scale. In this workshop, we will focus on the observation of membrane contact sites (MCS) between the Endoplasmic Reticulum and the plasma membrane. We will use fluorescent proteins-tagged membrane-bound proteins known to form MCS as a FRET pair. We will show how the direct measurement of donor fluorescence lifetime (FLIM) is a powerful method to assess FRET efficiency. This workshop will present how to design such a FRET-FLIM experiment. We will discuss the choice of fluorescent proteins, the required controls, and the various options to analyze FLIM data.

Educational goal

By the end of this workshop, participants will know the main guidelines for designing a robust FRET-FLIM experiment and interpret their results.

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A103-Single-shot polarimetric and quantitative phase imaging

Proposer/Coanimator

Baptiste Blochet
Anis Aggoun

Abstract

The polarization and the phase of a beam can carry information about objects the light encountered. Quantitative phase imaging typically allows high-contrast imaging of transparent object such as cultured cells and can extract important biophysical parameters. Polarimetric imaging provides complementary information about nanoscopic molecular ordering both through birefringence or fluorescence polarization.
Wavefront sensors (WFS) are simple and compact optical devices providing both the intensity and the wavefront (or spatial phase) of a light beam, from a single image acquisition. The phase information is encoded at a camera plane by placing a so-called Hartman mask upstream. Contrary to phase imaging, polarimetric imaging still typically requires several (≥4) sequential measurements involving several dynamic polarization optics.

Relying on the principle of WFS, we have developed a system allowing to provide all at once: high-resolution intensity, phase, and polarization images, in a single acquisition step, and in real time. Our system is based on the use of an optimized polarization-modulating Hartmann mask.

In this workshop, we will introduce the principle and illustrate the performances of a single-shot high-resolution polarimetric wavefront sensor prototype that we developed in our laboratories. We will discuss the information that these two contrast modalities (phase and polarization) can bring to bioimaging: label free imaging, sensitivity to the nanoscopic molecular orientation, application to fluorescence imaging.
Proof-of-principle samples (ranging from soft matter to biomimetic and fixed fluorescent cells) will be available to illustrate the principle and potentialities of the system. Our real-time monitoring and acquisition software will allow participants to bring their own samples and to experiment themselves.
The participants will then learn both basic knowledge about phase and polarization imaging as well as about potential bio-applications.

Educational goal

At the end of this workshop the participants will be able to understand:
- the principle of single-shot wavefront and polarimetric sensing to get phase, intensity, and polarization state images (oral explanation + experimental demonstration)
- the typical properties of a sample that may be deduced from the properties of a light beam that originates from or travels through a sample (oral explanation + experimental demonstration)
- how polarization-resolved fluorescence imaging can recover the orientation of the excited fluorophores (experimental demonstration)

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A104-Microfluidic circuits under the microscope

Proposer/Coanimator

Benoit Charlot
Sakina Bensalem

Abstract

This workshop aims to provide an overview of microfluidics and the use of microfluidics in microscopy. After a presentation of microfluidics both in terms of microfabrication technologies and practical applications, we will show some experiments of flow in microfluidic circuits with red blood cell suspensions as well as experiments of cell trapping with microalgaes. The experiments will be performed with standard microfluidic circuits connected with pressure controllers and observed under a microscope. The microscope used is a home made setup with an inverse configuration, transmission light and 3 color LED light source for fluorescence.

Educational goal

This workshop aims to familiarise an audience outside the world of microfluidics research with the use of microfluidic circuits for microscopy experiments. We will show the techniques for making the circuits and their use in microscopy experiments. We will also show how to use fluidic transport equipment, such as syringe pushers and pressure controllers, to apply controlled pressure to the inlet of a microfluidic circuit. Experiments will be done with cell suspensions (red blood cells and microalgae) to observe the transport dynamics but also how it is possible to trap single cells in microfluidic structures. The observation of red blood cells flowing into a replica of blood microcirculation network is really amazing.

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A105-FRET SRRF: where super-resolution meets protein activation.

Proposer/Coanimator

Xavier Pinson
Giulia Bertolin

Abstract

Super resolution techniques are becoming increasingly popular but are often incompatible with live imaging. Moreover, their combination with the implementation of advanced quantitative is challenging.

In this workshop, we aim at providing a straightforward protocol to estimate FRET in the context of super-resolution images obtained with SRRF (Super Resolution Radial Fluctuations). As a biological paradigm, we will work with a well-established FRET biosensor detecting the activation of the AURKA kinase. The activation of the sensor will be followed in a particular subcellular compartment of interest such as mitochondria, but the protocol can be applied to other subcellular compartments or other biosensors of interest. Users attending the workshop will be able to detect the activation of AURKA by measuring FRET of 3 constructs: an active biosensor, an inactive kinase-dead variant or a donor-only "FRETless" counterpart. FRET will be measured in the ratiometric mode.

We are confident that this workshop will provide the attendees with the conceptual and practical tools to readily adapt FRET measurements on the widefield or spinning-disk setups available in their closest facility.

Educational goal

The participants will be able to:

- Understand the information they can get from FRET. Understand how to properly design a FRET measurement experiment with the right controls.

- Directly discriminate between cells expressing the biosensor or its donor-only counterpart.

- Directly discriminate between cells expressing the WT biosensor or its kinase-dead version by exploring the probe distribution in cells, and the morphology of the mitochondrial network.

- Measure FRET and calculate FRET efficiency during the workshop.

- Use SRRF to create super-resolution images of the probe and of the mitochondrial network

- Discuss the applicability of the approach to their own biological question and, more broadly, to the analysis of FRET to determine protein activation and/or protein-protein interactions coupled to an improved spatial solution

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A106-Quantitative phase imaging with a diffuser

Proposer/Coanimator

Anis Aggoun
Baptiste Blochet

Abstract

Quantitative Phase Imaging (QPI) is a label-free approach which not only provides an enhanced contrast on transparent objects, but also enables the extraction of important biophysical cell parameters, such as the dry mass. In this workshop, we will explain how a quantitative phase camera (a.k.a. High-Definition Wavefront Sensor) can be simply implemented by placing a thin diffuser (for instance a piece of scotch-tape) in front of any standard cameras. The diffuser generates a speckle pattern (random distribution of light) on the camera. A fascinating property of thin diffusers, the "memory effect" allows, through a fine tracking of the speckle grains, to precisely quantify the phase distortion at rates compatible with real-time imaging (40Hz). As the method only requires cost-effective, off-the-shelf components, it has the potential to further ease the dissemination of QPI.
We will illustrate the implementation of this QPI technique on a commercial microscope and will use it for cells imaging and characterization. We will inspect the acquired QPI images and illustrate their relevance for cells monitoring (and classification).

Educational goal

At the end of this workshop, many physical concepts such as the phase, or the memory effect of diffusers will have been demystified through didactic experiments.
The participants will be acquainted with a low-cost, highly performant, quantitative phase imaging technique. The implementation of such a system will also be demystified as the participants will have hands on deck at every step of the process.
A better understanding of the data it provides will also be unlocked through experimenting will the various post processing tools we developed for bio-imaging.

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A107-Roboscope prototype for smart automated microscopy

Proposer/Coanimator

Marc Tramier
Jacques Pécréaux

Abstract

In conventional microscopy, complex microscopy methods or detection of rare event needs supervision. The microscopist is in front of the microscope to choose the adequat cell and/or to set up the acquisition. On the other hand, biology needs high-content screening (HCS) to understand the complexity of live, microscopy using HCS are usually only basic unsupervised acquisition. Smart automated microscopy will open new avenue to combine complex acquisition or detection of rare event with HCS in an unsupervised manner.
The Roboscope project aims to develop sequence-driven acquisition on an automated fluorescence microscope by integrating real-time image analysis by artificial intelligence to implement feed-back loop. The objective is to allow unsupervised acquisitions of advanced microscopy methods both to track rare events or to acquire high through-put. This collaborative project involves the teams of Jacques Pécréaux and Marc Tramier at the IGDR with the company Inscoper. The technological choice of our project to achieve the real-time constraint is to develop embedded solutions for image acquisition, image analysis, and feed-back loop automation.

In this workshop, we will show how an embedded AI algorithm enables real-time execution in an automated microscopy servo sequence. We will then apply it to detect automatically mitotic cells from a mosaic acquisition of chromatin label in order to choose the best cell to follow dynamics of mitotic spindle using a second fast sequence with another objective and another color.

Educational goal

At the end of this workshop the participants will be able to understand the advantage of real-time embedded systems to perform smart microscopy.
We will set up our Roboscope prototype at Mifobio.
We will describe in detail the technological choices that governed the assembly of this prototype.
We will present the methodological choices for the deployment of embedded real-time AI solutions.
We will demonstrate the potential of our approach by an automated acquisition sequence on biological samples of interest (mosaic to find metaphase/anaphase transition to start acquisition of fast mitotic spindle dynamics).

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A108-Simultaneous multiplexed staining of the infarcted area and Connexin-43 on wide thick heart sections

Proposer/Coanimator

Guillaume Pidoux
Matthieu Cortes

Abstract

Myocardial infarction (MI) is one of the major causes of death in industrialized countries and is characterized by a prolonged cardiac ischemia usually triggered after blood clot, coronary artery constriction or atherosclerosis. This leads to irreversible cardiomyocyte death and impairs cardiac function. Ischemia will engender the cardiac scar in acute phases of MI and affects proteins expression and localization at the proximal area (ie. area at risk). In this context, the expression et subcellular location of the gap junction connexin-43 (Cx43) protein are affected in cardiomyocytes after MI, which trigger arrythmias occurrence and fatal death. However, the full characterization of these changes between each areas remain elusive. It is challenging to image and reconstruct in 3D an entire heart section with a structural study of the infarcted area in classical histology and simultaneously combine multiplexing fluorescence analyses of proteins of interest behavior and their subcellular distribution in the proximal and healthy zones. To do so, we will be utilizing the latest generation of spinning disk technology, which boasts a wide field of view and modular pinholes. This cutting-edge technology is ideal for rapidly acquiring and reconstituting deep tissue mosaics, providing a real field of 25mm and homogeneous illumination. Our focus will be on imaging sections of infarcted hearts using a customizable spinning disk with custom pinhole sizes and various pattern distributions. Moreover, we will explore various options for acquiring mosaics, including low magnification techniques, with the goal of reconstructing our samples in 3 dimensions while minimizing any overlapping areas. By varying the magnification levels and the resolution, we will be able to study two critical aspects: i) the size of the infarct using TUNAL and ii) a precise Cx43 localization and expression in all areas of the heart. Through these efforts, we hope to gain new insights into the propagation

Educational goal

The aim of the workshop is to show the possibilities of acquiring at high-speed tissues of several mm2 with a very good resolution and without any edge effect, the reconstruction of several hundred mosaic images taking only a few minutes. This technology makes it possible to explore high resolution 3D tissues fixed in multi-markings with very reasonable acquisition times compared to the surface of the acquired sample.

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A109-Microscopie d'expansion: Trucs et astuces pour l'analyse des compartiments cellulaires dans les cellules de mammifères et dans la levure

Proposer/Coanimator

Jim Dompierre
Manuel Rojo

Abstract

Contrairement aux autres techniques d'imagerie optique de super-résolution, qui améliorent la résolution par le développement des microscopes eux-même, la microscopie d'expansion (ExM) consiste à agrandir physiquement l'échantillon de 4 à 10 fois, de façon isotrope et dans les 3 dimensions au moyen d'un hydrogel « gonflable ». Ainsi, cette technique permet d'acquérir facilement des images en super-résolution, 3D, multicolores, avec des fluorophores conventionnels (protéines fluorescentes et les colorants organiques) avec des microscopes à champ large. En outre, il est possible de coupler l'ExM aux techniques instrumentales de super-résolution pour améliorer plus encore la résolution finale.
Nous avons optimisé l'ExM pour étudier la nanostructure et l'organisation des membranes mitochondriales et du cytosquelette et développé un protocole permettant l'ExM sur la levure bourgeonnante S. cerevisiae.
Au cours de cet atelier, nous décrirons les trucs et astuces que nous avons developpé pour adapter l'ExM à l'étude des interactions entre les mitochondries et d'autres compartiments membranaires de la cellule tels que le reticulum endoplasmique, les peroxisomes et les lysosomes ainsi que des composants du cytosquelette (actine et microtubules).
Nous nous concentrerons sur l'optimisation de l'utilisation des sondes vitales fluorescentes pour marquer de façon simple et efficace les compartiments cellulaires d'intérêt. Nous verrons quelles sont les prérequis à l'utilisation de ces sondes en ExM (famille de fluorophores, résistance à la polymérisation, ancrage au gel).
Les participants seront amenés à effectuer eux même les expériences pour les étapes critiques : polymérisation de gels de différentes compositions, comparaison de différents fluorophores, manipulation, montage et imagerie de l'hydrogel expansé.
À la fin de cet atelier, les participants seront en mesure de reproduire facilement les protocoles et de l'adapter à leur propre thématique.

Educational goal

Plusieurs protocoles d'ExM existent aujourd'hui suivant l'organisme ou tissu, la structure subcellulaire, le facteur d'expansion et les fluorophores que l'utilisateur souhaite observer.
Nous nous focaliserons sur l'imagerie spécifique à la mitochondrie et au cytosquelette dans les cellules de mammifères en culture et sur les techniques propres aux levures.
Le protocole complet que nous souhaitons présenter prenant plusieurs heures, nous nous attarderons sur les étapes clés de la technique: fixation des échantillons, ancrage des protéines au gel, gélification et manipulation. Les utilisateurs qui le souhaitent pourront pratiquer eux même les étapes de gélification et de manipulation du gel.
L'atelier se déroulera comme suit :
1) Description brève des spécificités liée aux mitochondries, aux compartiments membranaires et au cytosquelette (fixateurs et tampons à utiliser…). Spécificité de l'expansion des lipides (ancrage au gel, tampon d'homogénisation de l'échantillon). Protocoles spécifiques aux levures (digestion de la paroi, préservation des structures membranaires).
2) Comparaison théorique et pratique des différentes méthodes d'ExM, celles des équipes de Boyden (l'originale) et de Vaughan, adaptées aux protéines fluorescentes et aux anticorps appliqués avant gélification), et celle de l'équipe de Guichard (U-ExM) et de ses dérivés (TREx, MAGNIFY), appliquant les anticorps après gélification et expansion. Les participants manipuleront les gels polymérisés précédemment et suivront l'expansion des gels dans de l'eau. Ils pourront ainsi appréhender la manipulation des gels. Nous comparerons

les gels en suivant leur facteur d'expansion, leur solidité et la facilité à les manipuler. Nous verrons quelles sondes vitales commerciales sont les mieux adaptées à l'observation des compartiments membranaire en ExM, telles que Mito-Tracker, ER-Tracker ou LysoTracker... Nous ferons de même avec les sondes fluorescentes de suivi du cytosquelette (phalloïdine, SiR-actine, SiR -tubulin...) et l'immunomarquage.

3) Enfin, les participants seront amenés à monter les gels dans une chambre d'observation et à imager au microscope. Ils pourront alors observer et comparer des échantillons préparés par les différents protocoles.

Suite à cet atelier, les participants seront amenés à débattre des différents protocoles abordés lors d'une table ronde prévue à la fin du parcours ExM.

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A111-Imagerie de fluorescence par microscopie confocale spinning-disk : une exploration des avantages et limitations techniques sur 2 systèmes

Proposer/Coanimator

Baptiste Monterroso
Sameh Ben aicha

Abstract

La microscopie de fluorescence conventionnelle consiste à éclairer un échantillon dans son ensemble pour collecter la fluorescence émise. La contribution de la fluorescence émise hors foyer de l'objectif, et collectée par le détecteur, est une limitation fondamentale à ce type d'imagerie. Dans le cadre d'échantillons épais et diffusants, ce problème est exacerbé. L'avènement de la microscopie confocale de fluorescence (LSCM – Laser Scanning Confocal Microscopy), grâce au couplage foyer d'excitation / sténopé à la détection, a permis de palier, en partie seulement, cette limitation.

La microscopie confocale à disque tournant (SDCM – Spinning-Disk Confocal Microscopy) représente une alternative à la LSCM. Cette technique a l'avantage de balayer l'échantillon à grande vitesse avec un faisceau laser découplé en plusieurs centaines de points focaux. En théorie, l'utilisation d'un microscope confocal multipoint de type SDCM améliore considérablement la vitesse d'acquisition d'images (permettant l'imagerie de processus dynamiques rapides et d'échantillons vivants) et réduit considérablement les photo-dommages.

Les participants à cet atelier découvriront ou approfondirons leurs connaissances sur cette technique. Les notions de résolutions, d'échantillonnage, de vitesse d'acquisition, d'homogénéité de champ et de diaphonie en milieux diffusant seront abordées, parmi d'autre. L'aspect de suivi dans le temps des performances d'un tel système sera également évoqué. L'intérêt sera également de comparer les différentes propositions technologiques retenues par les constructeurs de SDCM, et d'évaluer leurs avantages/inconvénients en fonction des échantillons et problématiques scientifiques. En ce sens, la participation et l'interaction avec les fournisseurs de système d'imagerie sera cruciale. Toutes les questions seront les bienvenues dans le but de repousser les limites de cette technique devenue courantes dans nos laboratoires.

Educational goal

Les ateliers proposés ont pour but d'explorer la technique SDCM de la théorie jusqu'à la comparaison d'images acquises sur divers échantillons biologiques ou métrologiques. Nous aurons l'occasion unique de travailler sur 2 systèmes différents, ce qui permettra de comprendre les différentes approches appliquées par les différents constructeurs. En s'approchant des limitations de ces microscopes, nous tenterons de mieux appréhender les applications qui leurs sont dédiées.

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A113-Multiplexed biosensor imaging to visualize and quantify signaling pathways in 2D and 3D cellular models.

Proposer/Coanimator

Francois Sipieter
Philippe Girard

Abstract

Cell-cell communication plays a pivotal role in coordination and function of biological systems. In this context, we want to investigate the relationships between the dynamics of kinase activities and molecular mechanics in living tissues with high sensitivity and spatiotemporal resolution. To this aim, we will use an approach relying on multiplexed functional live cell imaging with various genetically-encoded Kinase Translocation Reporters and custom genetically-encoded FRET biosensors. We will first use this approach in 2D tissues and then in 3D cellular models, which provide venues to investigate morphogenesis and for drug discovery, as their architecture mimics native in vivo microenvironments.
At the end of this workshop, participants will be able to generate rich multiparametric images of basic biological processes. We will also perform on-the-fly quantitative image analysis of generated datasets by using custom-made routines developed on ImageJ to automatically segment cells and track their biosensors outputs over time.

Educational goal

Cell-cell communication is fundamental to multicellular life and to the emergence of higher-order functions in a wide variety of tissues and organs. It involves the coordination of cell movements and cell functions at the tissue scale. This emerges from the integration of local interactions and global signals. How cells integrate these multiple cues to elicit a specific cellular response is unclear.

Culturing cells on 2D surfaces has been the foundation of in vitro cell culture for decades and recapitulates some in vivo environments. In many other in vivo situations, tissue extend in 3D, with specific cellular behaviors. To address this, we will investigate the spatiotemporal relationships between the dynamics kinase activities and adhesion protein tensions (Aoki et al., 2017; Lee et al., 2018, Gayrard et al., 2018) in cultured epithelia of Madin-Darby Canine Kidney cells (MDCK) cells and human mesenchymal stem cells spheroids (hMSC).

To make these activities and their relations visible, we will use an approach relying on functional live cell imaging with genetically encoded biosensors. Most biosensor studies are restricted to the visualization of one signal by one biosensor per experiment. The need for better spatiotemporal resolution in signal correlations has led over the recent years to the development of approaches to image multiple biosensors in the same cell. This “multiplexed” detection poses the issue of spectral compatibility. To overcome such limitations, we used biosensors with spectrally compatible fluorophores that allow for time-lapse imaging of others reporters in the same cell on a standard confocal microscope.

We will discuss the rationale for the choice of fluorescent proteins with respect to the choice of imaging instrument, and assess the possibility to further multiplex one FRET biosensor, with spectral acquisition, with one or two additional fluorescent Kinase Translocation Reporters.

This workshop will allow participants to appreciate the requirements to measure multiplexed biosensors, and provide them a framework to adapt this approach to their biological question using instruments generally available on imaging facilities.

Then, participants have the opportunity to analyze their images with a dedicated workflow based on custom routines within commercial software and freeware.

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A114-Exploring the Impact of Laser Power and Pulse Duration on Two-Photon Microscopy

Proposer/Coanimator

Elric Esposito
Julien Fernandes

Abstract

Many two photon users have a large span of settings available to them that results from supplier implementation based on academic advancement in the field. However, optimized parameters is seldomly used du to the lack of knowledge and transmission towards non-expert users. This workshop aims to address one of those gaps by exploiting a common feature available on modern ultrafast laser allowing pre-compensation of pulse duration. We will investigate the effect of group delay dispersion (GDD) compensation on a thick sample at various depths and its impact on collected fluorescent signal, photobleaching and phototoxicity and compare with a more conventional laser power compensation. We will further investigate this method's relevance on second harmonic generation signal.

Educational goal

At the end of this workshop the participants will be able to correctly apprehend trade offs regarding instrument settings at desired imaging depth depending of desired modality (Fluorescence Vs Second harmonic generation). Students will further gain a hands-on understanding of the principles of two-photon microscopy and second harmonic generation imaging.

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A115-Monitoring calcium responses of moving T cells in fresh tissue slices by multiphoton microscopy

Proposer

Lene Vimeux

Abstract

In many cell types the intracellular calcium (Ca2+) acts as a second messenges controlling important cell functions.
Ca2+ responses are usually measured in vitro on cultured cells. Our lab has developed a model based on thick slices made from fresh tissues.
By using fluorescent imaging microscopy it is possible to measure several functional responses of T cells after adding them onto fresh tissue slices. Depending on the timescale of the response, we have 3 standard experiments :

- Intracellular Ca2+ as a proxy of cell activation
- Localization, mobility and interaction with other cells
- Cell death

Here, I want to focus on the initial activation of T cells when they recognize their target in tissue slices. In our lab we focus on CAR-T cells in tumor slices. The advantage of the model is the intact environment with physiological expression of the target.

The different steps of the protocol are the following:

1) The fresh tissue sample is embedded in agarose and cut in thick slices(400 µm). To make the workshop feasible, we will use tissue slices made from a tumor that has been frozen and thawed, or fixed tumor slices.
2) Immunostaining of the tissue slices (structural organization) and loading of immune cells with the calcium specific dye.
3) Loading of CAR-T cells on the slice directly beneath the microscope (upright multiphoton microscope) and the calcium responses of single cells are measured immediately and during the first 10-20 minutes after plating the cells.
4) Tracking of the loaded cells and extraction of the Ca2+ concentration in each T cell within the microscopic field over time (ImageJ or Icy followed by RStudio).
a. We will distinguish different calcium profiles which encode information controlling cell activation.
b. From these individual cell data, we can synchronize the responses and calculate the average Ca2+ responses.
The main outputs are the maximal response, the Ca2+ level at a later timepoint and the percentage of responding ce

Educational goal

At the end of this workshop the participants will have a more detailed notion about:
1) How to set up a microscopy experiments to analyze the calcium signaling in tissue slices. When working with alive tissues and cells, one must take into consideration physiological parameters as temperature, oxygenation and CO2. The first step of the experiment will be in the culture room and participants will be able to manipulate the tissue slices and see the setup for the staining of the tissue.

2) Acquisition of timelapse sequences of the intracellular calcium levels in immune cells in tissue slices.
When imaging alive cells and tissue, the experimental set-up must take into account limitations such as photobleaching, the balance of timescale of the response that we anticipate, the duration of acquisitions and the right temperature for the physiological events to happen. In front of the microscope the participants will realize how to set up with these parameters to reach the required conditions.

3) The calcium signaling to monitor the activation of immune cells.
This last steps is the analysis of the calcium signaling.
First is the reconstitution and observation of the movies, followed by tracking and extraction of intensity profiles. Thereafter a more global analysis include the typical groups of profiles and comparison of T cells of different affinity.

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A117-Orchestrating complex bioimage workflows-leading up to smart microscopy

Proposer/Coanimator

Johannes Roos
Christer Lohk

Abstract

Bioimage workflows have transformed quite dramatically over the last year, making once thought impossible challenges like 3D segmentation of complex data possible. Meanwhile, new imaging modalities are breaking records in both resolution and acquisition speed, generating gigabytes if not terabytes of data.
This transition is powered by a new generation of tools like Napari (for visualising big datasets), and deep learning-based methods such as CARE or Stardist, which are co-existing with proven apps like ImageJ or MicroManager.

However, with this shift in bioimage workflows comes the burden of orchestration and data management, that is hindered not only by the variety of software platforms these tools are developed in but also by the requirement for dedicated computing resources (GPUs, High-Performance Computing). This causes existing methods to be still limited in their interoperability.
In this workshop, we will explain the challenges of modern bioimage workflows, especially real-time data analysis and management. Furthermore, we will introduce our solution to this problem: Arkitekt - a powerful middleman between users and bioimage apps for building and orchestrating real-time analysis and microscopy workflows. The workshop will rely on conventional bioimage software (ImageJ, Napari, Micro-Manager) and modern deep learning frameworks (CARE, StarDist) to build advanced data pipelines that can go from acquisition to statistics. It will also demonstrate Arkitekt's capability to set up a "Smart Microscopy" workflow by modifying microscope acquisition parameters in real time according to on-the-fly detection of specific events.

Educational goal

The workshop will be divided into three parts. In the first part, participants will get to know the challenges of orchestrating modern bioimage workflows, illustrated by several simple examples that require multiple steps performed in different environments (ImageJ, Napari, …). It will be followed by an introduction to Arkitekt, our solution for bridging different bioimage apps in a single and unified workflow. In the second part, participants will get to understand how Arkitekt can allow them to orchestrate popular tools like Napari, Fiji and No-Code Deep Learning in real-time and batch workflows. In the third and last part, the participants will be shown that through real-time workflows it is easy to bridge the microscope and their analysis, opening the way to the implementation of "Smart Microscopy".

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A118-Clearing and 3D imaging of mesenteric adipose tissue of rat

Proposer/Coanimator

Laurence Dubreil
Chloé Chaumeton

Abstract

Clearing technics are used in biology to make initially opaque samples transparent and allow their three-dimensional imaging in depth. Many clearing protocols have been developed over the past twenty years, however these methods are continually modified according to the nature of the samples, their size, the fluorescent markings used and the imaging techniques used to analyze structures or follow the biodistribution of agents at the organ level. Most of clearing methods remove lipids stored in the tissue which is not compatible with 3D investigation of adipose tissue. In this workshop we will propose a method to clear adipose tissue and with the preservation of lipids. Dual fluorescent labeling of adipocytes and extracellular matrix will be developed before clearing and we demonstrate the high potentialities of combination of clearing and light-sheet fluorescence microscopy to image 3D structure of an entire adipose tissue.

Educational goal

The objective of this workshop is first to discuss on different clearing methods and to learn how to select method of clearing in relation to the tissue composition and biological questions. The choice of fluorescent probes used for adipose tissue labeling and of the clearing method compatible with lipids will be described and explained. At the end of the workshop, the participants will know the different steps that are necessary to performed clearing on adipose tissue. They will be able to select fluorescence probes compatible with the clearing method and compatible with the light sheet set up used for sample imaging. They will assess light sheet imaging of adipose tissue and get some notions of 3D image analysis.

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A119-The TriScan: a fast 3D fluorescence microscope with single molecule sensitivity

Proposer/Coanimator

Robin Van den eynde
Wim Vandenberg

Abstract

Fast high-sensitivity fluorescence imaging of optically large 3D samples continues to pose challenges in the lab. The gold standard methodology, confocal laser scanning, offers a high resolution but requires a long duration to image large samples such as multicellular organisms, tissue slices, organoids, etc., especially when a high sensitivity is required. Parallelized confocal methodologies such as spinning-disk offer a strong speed-up, but suffer from high complexity and optical issues such as cross-talk and/or low light-coupling efficiencies. Light-sheet microscopy offers an exciting breakthrough, but can be difficult to combine with sample formats such as well plates unless more complex instrumentation is used.
In this workshop, our goal is to introduce the 'TriScan', an all-optical line-scanning confocal developed in our research group. The 'TriScan' can in principle deliver up to kHz full field-of-view imaging rates while having a sensitivity that is far higher than that of a classical confocal microscope and achieving a similar optical resolution. Due to its all-optical synchronization and very fast scanning rates, the system offers the convenience and performance of a widefield microscope but the depth-resolved imaging of a confocal. Combined with its modular design, the system can in principle be directly integrated within conventional instruments, while not requiring modifications to the acquisition software. The system is also simple and requires few optical components, allowing the setup-specific components (excluding always-needed components such as a light source, camera, objective etc.) to be purchased for approximately 5,000 euro in total. By delivering a very strong imaging performance within a self-contained and inexpensive package, the TriScan offers exciting opportunities as a workhorse system for the fast and sensitive imaging of 3D samples.

Educational goal

In this workshop, we will discuss and demonstrate the TriScan microscope together with the participants. We will present the basic concept of the system and discuss how this translates into the selected optical layout. Because we will be using a home-built prototype, the internals of the microscope can be readily seen and inspected, and we will discuss how our optical design results from theoretical and practical considerations. Together, we will apply the system to the measurement of different samples ranging from single-molecule imaging to multicellular systems, exploring the performance of the system and how this compares to other imaging modalities.
This workshop is useful not only for those interested in the TriScan, but also more generally for researchers interested in developing a better understanding of the functioning and engineering of (confocal-like) microscopes, because we will discuss the instrument design and implementation in an accessible but thorough manner.

The goal of this workshop is to introduce the audience to our new TriScan technology. As part of this demonstration, we in particular hope to familiarize the audience with:
*The performance of the instrument, showcased via the test measurements. The goal of this will be to educate the audience regarding the performance implications associated with a confocal microscope based on line scanning, and on how these compare to other imaging modalities.
*The basic operating principle of the microscope, explaining how our strategy distinguishes itself from other confocal-like and widefield systems and what the associated trade-offs and consequences for this are.
*The optical design of the microscope, and how the overall layout dictates the choices of particular components. We have created a full optical-ray tracing calculation of the system, which we can use to explain to the audience how the optical train affects the imaging quality and presence of aberrations.

*The controlling electronics hardware and software, and particularly how our designs allow these to be very strongly simplified.
Overall, we intend that we will be able to demonstrate an attractive instrument and at the same time dive deep, yet in an accessible format, into the overall design of the system itself, how this determines the achievable performance, and the design and implementation trade-offs that are made.
However, it is very important for us that the principles and insights discussed during this training will be sufficiently general and accessible so that the audience will gain basic skills or at least an enhanced confidence in discussing and evaluating their own imaging hardware of choice. By opting for an accessible format that nevertheless covers fundamental optomechanical considerations, our workshop will offer education well beyond 'merely' the system in question. In that sense, we specifically envision that our educational strategy and level will be useful also for those researchers who would like to go beyond the TriScan instrument itself.

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A120-Nanoscale imaging of the Toxoplasma gondii microtubules network using Expansion microscopy and 3D-STED

Proposer/Coanimator

Bastien Touquet
Eric Denarier

Abstract

Toxoplasma gondii is an elliptical shaped-cell of about 5 to 7 µm length in the major axis and 2 µm in the minor one, featured by a distinctive apical and basal pole. In particular, the apical pole comprises a structure known as the conoid, a truncated cone complex of 280 nm in length and 380 nm in diameter composed of curved tubulin fibers, with apical rings in its basis. Given its size, to characterize the architecture of this structure, super-resolution techniques are necessary.

Between those techniques, Expansion microscopy (ExM) has emerged as a low-cost and powerful option. ExM consists in physically expanding a swellable gel-embedded biological specimens to overcome the resolution limit of light microscopy. Several protocols are now available wherein the sample can be expanded by 4 to 10 fold to obtain a resolution between 70 to 25 nm. The 4-fold expansion protocols are the more extended and easy to use and recent combination with optical super-resolution like STED show a resolution gain below 10 nm.

In this workshop, through the tubulin labeling of the conoid structure, we will show two types of 4-fold ExM protocols (ProExM from Boyden Lab and U-ExM from Guichard Lab) with technical manipulations for the participants. The attendees will critically analyze the samples with respect to labeling quality of both protocols, to later discuss and select one ExM sample for optical super resolution acquisition (STED) and appreciate the resolution gain between non expand and expand biomolecules.

Educational goal

1-Brief presentation of the Toxoplasma microbe structure and ExM techniques will be provided.

2-Expanded sample preparation:
We will bring materials and reagents to demonstrate the requirements and preparation of an ExM sample. Throughout the workshop (see timeline), participants will perform 3 important technical steps: Gelation, expansion in water, and gel mounting on a coverslip. (Slides will run parallel to the practical demo to underline each part of the preparation with protocol steps and photos).

3- Participants will observe the results on ExM samples by epifluorescence microscopy.

4- After confocal acquisition participants will be able to identify the best-expanded sample for STED acquisition and discussion to demonstrate their advantages

5-STED acquisition: During this time a brief presentation of the tool will be done.

6-Image analysis: Participants will determine expansion factor from an expanded versus non expanded images using scale bar. Furthermore, they will compare the resolution between
confocal and STED images of expanded gel using a cross-section and FMWH calculation-10min.

7-3D reconstruction: Participants will be able to reconstruct in 3D the images acquired through open source software FIJI and ChimeraX.

At the end of the workshop the participants will be able to understand the major advantages and limitations of the two expansion techniques presented with direct comparison between each. They will also be able to appreciate the gain of ExM and STED as compared to confocal and then using ExM and STED alone or in combination. They will determine the expansion factor of the sample and gain knowledge on 3D reconstruction using open source software.

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A122-COLORME/FLUOGAN : Super-resolution in fluorescence microscopy with standard setup.

Proposer/Coanimator

Sebastien Schaub
Luca Calatroni

Abstract

Many super-resolution techniques have been developed, each having its advantages and drawbacks (specific dyes, specific mounting medium, complex optic devices).
Since few years, some techniques, SRRF is the most well known, use the fluorescence fluctuations to improve the resolution. In our projects we developed two different approaches based on fluctuations : COLORME [1] based on variational approach and FluoGAN [2], an unsupervised hybrid approach combining generative adversarial learning and physical modelling. Both provide substantial gain in resolution and have the advantage to be quantitative in term of fluorescence intensity.
One goal for this workshop is to show the versatility of the COLORME/FLUOGAN : indeed, the strength of fluctuation super-resolution methods is that we don't require fancy microscope, fluorophore or sample preparation. So to demonstrate how it works, we will use standard sample slides with 2-4 stainings of fibrillar structures (i.e. microtubules) in cell cultures, but it's for simplicity. As today, the bottle neck is the analysis time, we only show it on ROI.
To first goal of this atelier will be to provide a demonstration of the pipeline and of how it works from acquisition processing to image analysis. To demonstrate the gain in resolution, we'll collaborate with Argolight to use their calibration slide phantom which provides a reference for the setup. The second objective of the atelier will be challenging the algorithm on "real samples", hopefully coming from some users. We'll compare SRRF algorithm (being widely diffused as a plugin in ImageJ) with our own algorithm to see advantages and defaults of both approaches.

Educational goal

At the end of this workshop the participants will be able to...
- know the principle of fluctuation-based super resolution techniques,
- set a microscope to get suited raw data (we'll test various frame rate/ duration)
- use ArgoLight slide to quantify resolution. Our goal is not to
- have an overview of the specificities of different approaches (SRRF and COLORME/FLUOGAN)

About how to make active the audience that's the hard part we are working on it. The best approach we reached today (still working on it...) is to get acquisition with different parameters (ie. many noisy images or few good images) and playing with the reconstruction parameters (how smooth is my sample to distinguish between signal and out of focus part) to feel how it induces varation in the reconstruction

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A123-A simple way to enhance TIRF microscopy using dedicated coverslips

Proposer

Cyril Favard

Abstract

High numerical aperture objective based Total Internal Reflection Fluorescence Microscopy (TIRF-M) is widely used thanks to its simplicity of implementation (dedicated commercial microscopes) and its improved axial resolution, down to hundreds of nanometers. However, TIRF-M suffers from various limitations; in particular, its signal-to-noise ratio is intrinsically not sensitive and different sources of noise often make its optical contrast poor. An interesting solution is to increase the fluorescence signal without increasing the noise linked to the excitation laser, by acting on the evanescent field i.e. the fluorescence excitation field.
In addition, although theoretically the evanescent excitation should appear at the critical angle, the angular divergence of the excitation filed due to the use of high NA objective is generating far field propagation, preventing the "TIRF" effect to be correctly achieved.
This can be rescue by increasing the incident angle of excitation above the critical angle, however, this lead to strong decrease in fluorescence signal. To circumvent that, using dielectric multilayers (DM) we have designed coverslips that generate a strong evanescent field at high angle. These coverslips are fully biocompatible and reusable. The workshop will illustrate how using such coverslips on a commercial TIRF microscope strongly enhance the evanescent imaging.

Educational goal

We will start by illustrating the physical concepts, ie, the principle of evanescent waves, the depth of field of an evanescent wave and the different issues that can be encountered in objective based TIRF microscopy. This will be complemented by the physical concepts of evanescent filed enhancement, and introduction to metal vs DM surface enhancement. We will finish this introduction with explanations on how we designed the DM coverslips to make them biocompatible.
Then comes the hands-on section:
It will be divided into two main parts, the first one conducted on classical microscope coverslips, the second using our specially designed DM-coverslips.
We will first illustrate the notion of contrast of the TIRF at the critical angle thanks to a sample containing either virus like particles (100 nm particles, low intensity emitters) or fluorescent beads (200 nm, high intensity emitters), immerged in increasing concentrations solutions of a fluorescent dye. This will be compared to measurements of the same sample at a higher excitation angle (decreased penetration depth).

Because the beam divergency is never controlled in TIRF performed on commercial microscopes, we will then show how this contrast is modulated as a function of the beam divergency. We will then perform the same experiments to show how the contrast changes with increasing angle and in the meantime, illustrate the loss in fluorescence signal.
The second part will address the same type of measurements using the DM coverslips. Depending on the available time, we will show two types of DM coverslips, one optimized for low beam divergence, one for high divergence (more useful for facilities microscopes). Finally, we will illustrate the benefit of such coverslips in cellular imaging.
At the end of the workshop, participants will have an idea of the real penetration depth and efficiency of a TIRF microscope depending on the beam divergency of the setup.

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A126-Cell and tissue manipulation with ultrashort infrared pulses in multi-view light sheet microscopy

Proposer/Coanimator

Matteo Rauzi
Abdul basith Tanari

Abstract

Fast 3D live imaging with high resolution has become an indispensable technique in the field of cell and developmental biology to characterize the biological dynamics from the molecular scale up to the entire organism. Nevertheless, live imaging is often not sufficient: precise spatio-temporal manipulation of biological components is required to directly test the underlying mechanisms. Here we present an integrated framework that combines three-dimensional light-sheet imaging in time with precise spatio-temporal photo-manipulations induced by short infrared laser pulses. As a proof of concept, we will use the living Drosophila embryo as a model system and perform in toto imaging in tandem with two-photon optogenetics and multiphoton plasma ablation of the actomyoin network with subcellular precision to test cell and tissue mechanics. Finally, with this hands-on workshop the participants will have the opportunity to learn more about and test on living samples multi-view light sheet microscopy coupled to multiphoton laser manipulation: a powerful tool in modern biology.

Educational goal

At the end of this workshop the participants will be able to: (1) understand the functioning of multi-view light sheet microscopy and the working principles of multiphoton laser-tissue interaction. The participants will also be able to appreciate how the two techniques can be coupled together. (2) The participants will also be able to mount a living sample and control the system via a user interface to perform in toto multiu-view light sheet imaging and multiphoton laser manipulation.

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A127-CDK5 conformational FRET biosensor to study allosteric inhibitors in living cells by fastFLIM acquisition.

Proposer/Coanimator

Marc Tramier
Shruti Singh

Abstract

FLIM is a powerful method for quantitative fluorescence microscopy unmixing concentration and fluorescence quantum yield of the probe in imaging mode. In particular, FRET by FLIM permits the direct quantification of transfer efficiency measuring the fluorescence lifetime of the donor in living cells independent of the donor and acceptor quantity. But the main limitation of FLIM is generally the speed of acquisition which is not adequat to follow localized transient and dynamic molecular processes. By combining wide field illumination of pulsed laser, time-gated camera and online mean lifetime image calculation, we have developped a fastFLIM. A TIRF version is now setup in Grenoble and the team of Olivier Destaing will present this prototype at mifobio. Taking advantage of this system, we will present our development of a new CDK5 conformational FRET biosensor and its use to screen allosteric inhibitors in living cells.

Educational goal

In this workshop, we will present user-friendly approaches of FLIM to monitor FRET biosensors. As an example, we will determine CDK5 activity in living cells and allosteric inhibitors effects. FLIM is a very robust method to measure FRET but its main difficulties comes from the analysis. We will show that real time FLIM calculation is possible and make the method easier to handle.

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A135-Single Molecule Orientation and Localization Microscopy (SMOLM) using polarized super resolution imaging and PSF engineering

Proposer/Coanimator

Charitra sree Senthil kumar
Isael alejandro Herrera hernandez

Abstract

Understanding the way proteins are organized in cells is an important element to answer key questions in biology. Super resolution fluorescence imaging has brought a considerable step in this direction; Stochastic optical reconstruction microscopy (STORM) and Photoactivation light microscopy (PALM) are able to provide a spatial resolution down to tens of nanometers, exploiting the high precision of single molecule's point spread function (PSF) localization, even in dense samples. While these methods measure single molecule's positions, they however do not give access to their orientation, which is required to investigate organization, alignment and conformational changes. Reporting single molecule's orientation, at the same time as their position, is challenging : in this workshop we will describe and demonstrate different approaches to solve this problem, based on polarized fluorescence imaging and splitting of polarized detection channels [1] or PSF engineering [2].

Combining orientation and localization super resolution imaging opens new directions towards structural imaging of complex proteins assemblies in cells. We will discuss how to access to 3D orientation information based on these schemes.

[1] C. Rimoli, C. Valades Cruz, V. Curcio, M. Mavrakis, S. Brasselet. 4polar-STORM polarized super-resolution imaging of actin filament organization in cells. Nat. Communications 13, 301 (2022)
[2] V. Curcio, et al. Birefringent Fourier filtering for single molecule Coordinate and Height super-resolution Imaging with Dithering and Orientation (CHIDO), Nat. Communications 11 (1) (2020)

Educational goal

The training related to this workshop is dedicated to the understanding of the added value of manipulating polarization of light in a microscope.
The participants will first learn about the basics on polarization : what is light polarization, what is a polarizer, what is a phase plate and how we can use it to encode light polarization properties in a fluorescence microscope.
The participants will then learn about the relation between polarization and the orientation of a single molecule, when radiation from a single oriented molecule propagates through a microscope.
The participants will then learn about how to measure the orientation of a single molecule using the polarization optics tools that they'll have learnt about at the beginning of the workshop.
At all time, the teachers will make the connexion between what is a single emitter's orientation and how does this relate to important biological questions related to molecular organization in a cell or in a tissue.
At the end of this workshop the participants will go back home with a better idea of what light polarization can bring as additional information on a fluorescent cell sample, how to exploit it, and how to identify possible artifacts that come from polarized effects in a microscope.

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A136-Coupling fastFLIM and TIRF imaging for optogenetics multiplexing and measuring fluorescence-live time at the substrate vicinity.

Proposer

Olivier Destaing

Abstract

Coupling the live cell imaging and FRET-biosensors allows to measure the dynamics of a specific biological activity. Evanescent field excitation of biosensors presents important advantages while targeting peri-substrate structures: excellent axial resolution, low photo-toxicity and bleaching, due to the total internal reflection (TIRF) of the excitation light. However, quantifying FRET in TIRF mode presents a challenge with classical ratiometric methods based on fluorescence intensities since poorly compatible with the exponentially decaying of evanescent excitation. Fluorescence lifetime (FLIM) allows the alternative readout of FRET efficiency, independent of intensity. Here, we propose to perform fastFLIM in TIRF mode since highly compatible with optogenetics applications and the analysis of new FLIM-based probes (FLIPPER-TR…). Indeed, our challenge is now to combine FRET-biosensors and optogenetic approaches. This implies mastering of the spectral multiplexing of optogenetics and biosensors.
Most of these FRET-based biosensors reporting systems are based on a dual-color ratiometric FRET detection, using fluorophore couples efficient in 400-550 nm range. Moreover, the detection of both donor and acceptor fluorescence needs two distinct spectral bands. Optogenetics actually also uses the activation light in the same spectral range, which makes the simultaneous observation of biosensors reporting concomitant biological activities extremely difficult. To be compatible with the optogenetic systems, FLIM can overcome this issue by

using single emission FRET probes using only a fluorescent donor associated with a dark acceptor (a light-absorbing but non-fluorescent or “shadowed” protein), allowing the multiplexed FRET-based biosensors. We thus propose to combine the fastFLIM imaging with the TIRF microscopy in order to multiplex optogenetics with FRET-based biosensors at the vicinity of the substrates where numerous

Educational goal

At the end of this workshop the participants will be able to understand the principles of measuring FRET by fluorescence life time imaging (FLIM), to acquire the concepts-advantages-limits behind fastFLIM detection. The attendees will compare the advantages-limits of fastFLIM detection associated with TIRF illumination, understand the problematic of multiplexing biosensors and optogenetics, determine the limits and controls for a trustfull FLIM analysis, to see the possibility of fastFLIM imaging with high-temporal resolution and live optogenetics. In particular, will be discussed the advantages of the non-fitting FLIM data analysis, optimal at low photon budget and taking advantage of high spatial statistics.

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A137-Multitemporal imaging of red blood cell transit through biomimetic splenic slits

Proposer/Coanimator

Emmanuèle Helfer
Simon Regal

Abstract

Splenic filtration of red blood cells through narrow inter-endothelial slits remains poorly understood despite its physiological significance as experiments and imaging of red blood cells passing through the slits are lacking. To understand the physical mechanisms at play, we combine physics of living systems and cell biology approaches in collaboration with physicians. Strikingly, the 8-µm diameter cells are able to pass through slits as narrow as 0.3 µm at body temperature. To do so, they undergo extreme deformations in the slit. Numerical simulations predicted that the cell adopts the morphology of two spheres connected by a thin neck that does not fill the slit. This was experimentally evidenced using confocal microscopy .
In this workshop we will perform live imaging of red blood cells transiting through biomimetic spleen-on-chip. We will observe the red blood cell transit through biomimetic slits at multitemporal resolution: from confocal imaging to get high resolution of the 3D-shape in the slit to live imaging (wide-field deconvolution) of the transiting cell to get the dynamics of the process.

Educational goal

This workshop aims at teaching the handling of a sophisticated microfluidic device with very narrow constrictions, which is not commonly used in both physics and biology labs. Our biological system is a suspension of red blood cells. The device can be used with other biological objects such as bacteria or parasites.

The use of narrow constrictions (less than 1 µm) creates high hydrodynamic resistance and high probability of clogging, thus making the control of the flow tricky. The microfluidic approach is combined with a benchtop confocal microscope embedded in an incubator. This microscope allows adaptation of the imaging modes upon varying flow conditons.

At the end of this workshop the participants will know how to handle the flow and manipulate biological objects in complex microfluidic conditions.

A141-Label-free, non-invasive microscopy using digital holography for real-time observation.

Proposer/Coanimator

Nicolas Guigui
Paul Balondrade

Abstract

Imaging living matter, whether 2D cell cultures or organoids and thick tissues, represents the initial stages of biological research. The ultimate objective is to obtain images of tissues at multiple scales ranging from nanometers to millimeters and milliseconds to days in time. This type of imaging involves visualizing both the structure of the sample and specific features. Although many techniques have been developed over the past 50 years, from confocal microscopy to super-resolution and holography, achieving this goal remains highly complex.

Currently, digital holography is limited to real-time imaging of the morphological structure of 2D cell cultures due to technical constraints in the source-to-camera pipeline and computational capacity. Despite these limitations, digital holography shows great promise for non-invasive, label-free imaging of living cells and tissues in real-time.

We would like to demonstrate this potential to revolutionize thick tissue imaging by offering label-free, real-time, and quantitative information on 3D samples.

The workshop will be divided into two parts. First, the fundamentals of digital holography will be presented with the aim to familiarize attendees with laser manipulation, the concept of spatial coherence of the source, and the basics of optical alignment.

Secondly, numerical propagation of the electric field will be conducted on the computer. The objective is to demonstrate that it is feasible to perform a variety of operations digitally, which is crucial for real-time imaging as it transfers the complexity of hardware to the software component.

Educational goal

Microscopy is an essential technique used to investigate the intricate details of biological samples at a microscopic level. However, microscopy poses a significant challenge as it aims at capturing images with high resolution and contrast while preserving the sample's structural and functional integrity. Two primary approaches to microscopy are fluorescence and label-free microscopy, each measuring different aspects of the sample. In this introduction to microscopy, we will briefly compare these techniques and outline the challenges associated with them.

Moving on to label-free microscopy by introducing digital holography, we will present its basics principles. We will start with a theoretical section to introduce fundamentals concepts of field propagation using Fourier optics framework.

This theoretical introduction will be followed by setting up the equipment to measure the electrical field backscattered from a resolution target via an interferogram in a standard Michelson configuration. We will explore different parameters to acquier distinct interferometric figures on the camera. This will be followed by the principle of field acquisition using an off-axis configuration. The idea is to use a particular type of interferogram to modulate the intensity to numerically recover the electric field by demodulation.

We will also introduce the fundamental principle of Fourier optics to demonstrate the possibility of performing the same operations numerically as experimental light propagation. The idea is to compare light propagation through a simple lens with numerical 2D Fourier transform. For all these steps, the audience will perform the experiments both on the optical setup and the computer. Simulations will be prepared in case of experimental failure.

Then, we will investigate the free-space propagation of the field. To do so, we will explain the impact of fundamental parameters on the resolution and intentionally defocus the target resolution from the microscope objective's focal plane. The objective is to identify the appropriate propagators to be applied digitally to the measured electrical field to restore resolution. Using a contrast cursor, we will select the appropriate depth of focus.

As we transition into the challenges of imaging biological samples at depth, we will present aberrations and multiple scattering in a practical manner using everyday examples such as fog or light passing through skin. We will show the effect of aberrations on the field propagation by adding opaque layers before the sample. The goal here is to show the audience the need for aberration correction when imaging in depth.

The take-home messages are the following:
- Label-free imaging allows to image any type of samples.
- Label-free microscopy allows real-time imaging and drastically simplifies the study process.
- Holography is a very powerful solution as all the complexity of imaging is set in the algorithms.
- Algorithms based both on Physics and AI can lead to great performances in describing tissues in 3D.

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A143-Coupling Expansion to STED microscopy for centriole protein imaging

Proposer/Coanimator

Camilla Luccardini
Véronique Morel

Abstract

Proposer/Coanimator
Camilla Luccardini
Véronique Morel

Abstract: Light microscopy is very helpful to give information on protein localization at the cellular scale, but investigation of protein interactions at the subcellular, nanometric scale is prevented by the diffraction limit.
To decipher sub-cellular ultra-structure biologists have long used electron microscopy (EM), which has a higher resolving power (up to 10-10m) since it uses an electron beam to illuminate the sample. However, EM is still limited in the choice of antibodies as protein reporters. This dead end has been solved with the development of expansion microscopy, a break through technique of sample preparation allowing to increase sample size by at least 4x and push the resolution limits of microscopes.
We developed a high-resolution microscopy approach by combining expansion with STED microscopy. This strategy allows us to separate a highly dense molecular complex such as the centriole and its associated proteins. The aim of this workshop is to share our “savoir faire” with the audience. We will discuss how to perform expansion on cultured cells and Drosophila ciliated tissues, giving you all the tips and tricks that you need when you are a beginner. We will talk about the benefits and the limits of this technique and will compare confocal vs STED imaging on expanded samples.

Educational goal

Our power point presentation will begin by giving the definition of the resolution of an optical microscope. We will show what can be observed with such a microscope, its limitations and advantages. We will shortly introduce super-resolution imaging techniques, by focusing on STED and expansion microscopy. We will discuss the material needed, protocols, how to evaluate sample expansion, sample mounting and observation.

At the end of the workshop, participants will have acquired a theoretical and practical knowledge of STED (what it involves and which fluorescent probes are suitable for this type of microscopy) and expansion microscopy (its principle, how to prepare, handle, observe and store a gel). Participants should be able to reproduce the manipulations shown, i.e. know how to handle a gel, know how to mount a sample, know how to set up a STED and confocal acquisition.

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A144-Imaging contact sites in neuronal and epithelial cells by multicolor STED microscopy

Proposer/Coanimator

Paul Nazac
David Boulet

Abstract

Les cellules sont des unités fonctionnelles hautement complexe constituée de différents organelles assurant des fonctions spécialisées (métabolisme énergétique, production de protéine, transport, dégradation, …). Ces divers compartiments cellulaires ont une répartition propre en fonction du type cellulaire rencontré (cellules épithéliales, immunitaires ou neuronales) ce qui nous donne cette grande variété de distribution au sein des cellules.

La microscopie à fluorescence a révolutionné l'analyse de la composition intracellulaire. Cela a notamment permis d'étudier la localisation et la dynamique des organelles de façon spécifique mais aussi de mettre en évidence la communication entres ces petits compartiments. La microscopie confocale, grâce à l'illumination laser en un plan focal, donne accès à une image plus nette avec une résolution de l'ordre de 200 à 250 nm de résolution.
Cependant, l'étude de ces petits organelles nécessitent de s'affranchir de cette barrière de diffraction et d'aller encore plus loin dans la résolution. En effet certaines vésicules ou compartiments font de l'ordre de 50 nm à 200 nm de diamètre. Certaines observations ont été rendu possibles récemment grâce aux techniques de Super-résolution (SIM, STED et STORM). Nous proposons ici de présenter les avantages de la microscopie STED qui permet de descendre jusqu'à une résolution de 40nm à 80nm.

Dans cet atelier les participants pourront observer en microscopie confocale et en microscopie STED, la communication entres différents organelles (Réticulum endoplasmique-appareil de Golgi puis réticulum endoplasmique –Endolysosomes) dans différents type cellulaire. Afin de présenter les spécificités liées à la polarité cellulaire, nous présenterons deux types cellulaires à géométrie très différentes : les cellules épithéliales de type HeLa et les neurones d'hippocampe, connus pour avoir de long prolongements où le trafic cellulaire intense permet des transports rapides sur de longues distances.

Educational goal

Sur le plan biologique, cet atelier permettra d'observer la diversité morphologique et structurale entres différentes cellules (HeLa, neurones). De plus les participants pourront observer différents compartiments cellulaires (RE, Appareil de Golgi, Endolysosome) au microscope et ainsi voir la proximité et la communication potentielle qui existe entre eux.
Concernant les techniques, les utilisateurs pourront faire de la microscopie confocale multi-couleur (4 à 5 couleurs en fonction du système) puis s'initier à la microscopie à haute résolution STED 1 couleurs dans un premier temps puis 2 ou 3 couleurs à la fin de l'atelier. Nous discuterons des fluorophores optimaux en fonction des raies de déplétions utilisées, des constructions plasmidiques et des méthode de transfection idéales pour

les cellules épithéliales et les neurones qui sont des cellules très fragiles et nécessitant des conditions particulières pour être doublement transfectées.

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A145-Label-free imaging of entire organisms using a mobile optical coherence microscopy platform

Proposer

Robert Prevedel

Abstract

Optical coherence microscopy is a label-free imaging modality that operates similar to ultrasound but based on light. OCM obtains label-free, cross-sectional images of microstructure in biological systems with high optical resolution by measuring the echo time delay of backscattered light. In general, OCM allows 3D imaging with high speed, high resolution and over relatively large (up to 4 mm depending on the magnification factor of the objective lens) field-of-views. These features make it a powerful intravital imaging tool whose contrast can give complementary information to fluorescence imaging. The Prevedel lab has built a spectral domain OCM system based on a spectrometer armed with a high-speed, 250kHz line camera and miniaturised it for direct deployment in the field. In this workshop, we aim to showcase this new, mobile imaging platform that can be utilised for mapping and screening a variety of samples, such as the morphological diversity found in marine organisms (such as sponges, plankton, etc.) but also other sample types such as plants. We will work together with the conference organizers to ensure a wide range of different samples to be imaged and showcased on our minimised OCM instrument.

Educational goal

At the end of this workshop the participants will be able to:

*) understand the technical concept behind Optical Coherence Microscopy

*) realize the potential for label-free imaging in surveying biological samples that can not be fluorescently labelled

*) prepare samples for OCM imaging

*) be able to visualise 3D OCM volumes and perform basic image analysis on them

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A146-Label-free imaging of tissues and collagen with high-definition quantitative phase imaging

Proposer/Coanimator

Julien Savatier
Cassandra Borgane

Abstract

We combine a high-definition wavefront sensor (SID4-sC8, Phasics S.A.) and a polarization-resolved quantitative phase imaging (QPI) modality to discriminate fine birefringent structures within cells or tissues (ex: collagen fibers), without labeling and at 1 Hz. This is based on quadriwave lateral shearing interferometry (QLSI) phase imaging, combined with light polarization. We place a wavefront analyzer measuring an optical path difference (OPD = dn e) on a video port of a microscope. We add a system that linearly polarizes the light and rotates it to illuminate the sample with a series of given linear polarization. The refractive index of the birefringent components varies according to the direction of polarization of the light and its direction of propagation relative to their optical axis. A series of images of an area at different polarization angles gives OPD images, where all the elements are contrasted, even the isotropic ones. Digital processing creates two images of only the birefringent components, in retardance (Δn e, with Δn = birefringence of the anisotropic element) and orientation.

This technique gives information about the local retardance and structure of anisotropic components. In addition to bringing morphological information on unstained biopsies, it can reveal tumor development grades thanks to specific fiber organization imaging. This information can be used as a tumor-associated signature. We show the capability of our approach on mouse skin and human breast tissues, with automatic mosaic acquisition and analysis.

Educational goal

At the end of the workshop, the participants will be able to understand the way phase and polarization of light can be analyzed together to reveal properties of biological matter, i.e. the presence and organization of birefringent structures within biological tissue, that you can image with high contrast. For that, we will use a two-part device that is quite simple compared to an SHG setup, a standard microscope, and a simple image analysis algorithm.

Quantitative phase imaging is now a well-known technique to image biological samples without labeling. It detects and quantifies mass density within samples. This way denser parts are revealed, including nuclei, membranes, bones, or fibers (actin or collagen). However, this technique is not molecule specific, like fluorescence can be. This means the phase value is similar in a nucleus and in a collagen fiber. But if we consider the phase accumulated for different incident linear polarization, we can compute the so-called phase retardance, which is specific to fiber bundles. This means that in quantitative retardance images, isotropic components such as nuclei, vesicles, or cytoplasm have negligible signal whereas actin or collagen are clearly visible. This is because they contain birefringent molecules. This property of fibers is already exploited in fiber imaging with SHG. In the case of the use of quantitative phase imaging, the setup is much simpler and cost-expensive since it does not require a high-power short-pulse laser and sensitive sensors.

To produce quantitative retardance images (QRI), we introduce a varying linear polarizer in the microscope illumination path, so that the light propagates through the sample with a linear polarization, which direction changes. We record quantitative phase images with a quadriwave lateral shearing interferometer, a wavefront sensor that produces QPI. The QRI is determined as the amplitude of the phase values for each pixel, whereas using the mean value generates an unpolarized quantitative phase image (uQPI), which reveals all the objects in the sample. For a sack of precision and robustness, the QRI and uPQI values are deduced from a sinusoidal fit of the pixel values. This way we can also deduce uncertainties in the measurement and determines whether a QRI signal is significant or not.

During the workshop, we are going to observe tissues that contain collagen or actin fibers. We will discuss the preparation protocol for such samples. Since QPI is a label-free technique, tissue preparation, and mounting are easier and faster than for observation with dyes or fluorophores. However, some molecules often use in histology, such as paraffin, have a strong signal in phase and should be cleared off the sample before observation to avoid artifacts.

We will discuss the underlying model of Quantitative Retardance Imaging, how data is treated, and how we can extract uncertainty information from the study of the residual noise in the sinusoidal fit used to determine the phase-amplitude. We will apply this approach to the determination of a detection limit and to the decision of whether a value is a signal, a quantitative signal, or just noise.

We will scan the tissue samples to obtain an overview of the tissue and detect any changes in the fiber structure for instance. It is known that tumors use collagen to protect themselves from immune cells or drugs. Therefore the presence of organized collagen areas reveals the presence of well-installed tumor cells, around which oriented fibers are visible. We will present how image stitching is done with QPI and QRI. Mostly, it is slightly

different from gray-level image stitching because it contains quantitative information. This means that values renormalization is not necessary.
After all the tools are explained, we will use the QRI microscope for various tissue samples, such as mouse skin and human breast tissues.
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A147-Multi-target imaging in 3D single molecule localization microscopy with a single laser : Spectral vs Flux demixing

Proposer/Coanimator

Laurent Le
Sandrine Lévêque-fort

Abstract

Single-molecule localization microscopy has become a reliable and go to technique for super-resolution imaging in biological samples. Biological issues owing to their complexity, demand for techniques that allow multiple localizations of several types of biomolecules simultaneously. The use of single excitation wavelength, allows one to observe dye with spectral overlap, but which minimize chromatic aberrations. We will show during the workshop that we can also use the flux analysis that is based on alternative intrinsic properties that can be used to differentiate fluorophores, especially with overlapping absorption-emission spectra. This presents the major advantage of being directly compatible with a single-camera configuration. We will show the benefits and constraints of this approach on DNA-PAINT imaging, and evaluate its performances compare to traditional spectral demixing strategy for multiple targets imaging. As an example, we will do demixing on COS-7 fixed cells with alpha-tubulin and clathrin-coated pits labelling. Furthermore, as this photon flux technique doesn't require any modification of the optical implementation, its combination with 3D super-resolution localization microscopy is straightforward. In particular, we will present the combination of the photon flux demixing with supercritical angle fluorescence to achieve axial super-resolution, known as DONALD.

Educational goal

At the end of this workshop the participants will be able to:
- be familiar with Single Molecule Localization Microscopy (SMLM) and DNA-PAINT imaging
- 2-colors imaging with Flux demixing and Spectral demixing
- 3D SMLM with Supercrital-angle fluorescence measurement (SAF, known as DONALD)

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A148-Identification de nanoparticules et de virus-like-particles avec la microscopie de phase quantitative

Proposer/Coanimator

Minh-chau Nguyen
Peter Bonnaud

Abstract

L'imagerie de phase peut décrire l'interaction entre le milieu et des échantillons : la transmittance et la différence de chemin optique. En appliquant aux cas de particules sous-résolues, la technique permet de déterminer les caractéristiques et d'identifier sa nature. Dans ce TP, nous présenterons une version compacte de microscopie de phase qui adapte pour des expériences dans un laboratoire biologique de haute-sécurité. La démonstration de fonctionnement du système sera effectuée avec des échantillons non-risqués, e.g. nanoparticules (NPs) simples et/ou virus-like-particles (VLP). Nous discuterons également des architectures de l'apprentissage profond pour l'identification. L'objectif pédagogique de ce TP est de montrer : 1) que détecter et résoudre sont 2 choses différentes. 2) L'observation sans marquage peut être suffisante même sur des objets nanométriques lorsque les conditions d'imagerie sont réunies. 3) L'automatisation par apprentissage machine est une solution robuste pour la reconnaissance d'objets biologiques.

Educational goal

L'atelier sera conçu de manière à expliquer et à informer un large public sur le fonctionnement de la microscopie de phase quantitative et de son application pour des échantillons biologiques. Il comprendra deux parties principales pendant lesquelles nous amènerons plusieurs points de discussion :

1) Microscopie de phase : le principe de fonctionnement et la capacité à caractériser des nanoparticules et des virus même s'ils sont théoriquement plus petit que le limite de diffraction

2) Apprentissage profond : la conception d'un modèle cohérent avec l'expertise terrain. L'intérêt de développer une architecture adaptée aux informations spécifiques récupérées par une méthode d'imagerie (ici l'imagerie de phase quantitative). Nous montrerons la capacité à identifier la nature d'une nanoparticule en temps quasiment réel.

Durant le TP, une série d'expérience sera menée avec notre version de microscopie de phase compacte afin de démontrer les performances du système tant en terme de sensibilité de détection qu'en terme d'insensibilité au « bruit » extérieur : vibrations, lumière parasite… afin de démontrer l'intérêt de ne pas toujours avoir recours à la fluorescence lorsque l'on peut s'en passer.

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A150-Adaptive optics two-photon fluorescence microscopy for in depth bioimaging

Proposer/Coanimator

Alice Guillaume-manca
Mathias Mercier

Abstract

This workshop aims to explain and demonstrate the gain of adaptive optics fluorescence microscopy for biological samples imaging. Indeed, image quality deep inside a sample is degraded by the inhomogeneities of the biological tissues which strongly distort the phase of optical waves, i.e. the wavefront of the light of interest, and thus limit contrast, spatial resolution and sensitivity. In the recent years, adaptive optics, which has been initially developed for astronomy, has shown its ability to significantly increase signal and resolution deep inside biological tissues, by correcting the optical aberrations induced by the sample itself. This is achieved through 1) wavefront sensing and 2) correction using optical wavefront modulators like deformable mirrors. Two main adaptive optics approaches have been proposed in microscopy, based on two methods of wavefront estimation before correction. This workshop will present the physical concepts underlying adaptive optics with simple experiments and explain the existing strategies to implement it in microscopy, with their benefits and constraints. We will then focus on an example of an adaptive optics loop implementation in two photon microscopy based on a novel wavefront measurement approach, with an extended source Shack-Hartmann sensor. This original approach leads to an efficient and fast correction
in scattering samples and allows to correct aberrations up to several hundred microns deep inside a fixed mouse brain tissue, proving large gain in intensity and resolution. At the end of this workshop, the participants will be able to evaluate if adaptive optics can bring image enhancement regarding the microscopy technique they use and their bioimaging application.

Educational goal

The goal of this workshop is to allow participants to understand how adaptive optics can enhance image quality in optical microscopy for biological samples imaging. To this aim, the participants will answer the following questions with a practical approach and experiments on both test sample (like fluorescent beads) and biological samples:

- what is an optical aberration ? what is a wavefront ?
- how optical aberration degrade image quality ?
- do aberrations constitute a limitation for all biological samples ? for every microscopy technique ?
- how to measure aberrations ? how to correct them ?
- how to implement adaptive optics on my microscope ?

At the end of this workshop, the participants will be able to evaluate if adaptive optics can bring image enhancement regarding the microscopy technique they use and their bioimaging application.

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A151-Hands-on light sheet microscopy with Flamingo, the shareable custom research microscope

Proposer

Gesine Müller

Abstract

In the early 21st century, light sheet microscopy was introduced to the biological sciences and is now accepted as the new standard for fast and gentle 3D fluorescence imaging in living multicellular specimens. The core of our lab evolves around custom-built light sheet microscopy technology for challenging biological multiscale imaging projects. To streamline custom microscope development we developed Flamingo, a powerful and modular microscope framework that is also compact enough to be quickly deployed in collaborators' labs. Over the last two years, the Flamingo has enabled novel biological experiments not only inside, but also outside our optics
lab. As a significant part of the scientific community continues to struggle to get access to a light sheet microscope tailored to their individual needs, the Flamingo gives more researchers the chance to utilize this powerful technology for their research.

In this workshop, we will introduce the concept of shared custom microscopy technology and demonstrate the modularity of Flamingo. We will provide you with insights into light sheet microscopy and give you hands-on time with the microscope to gain a deeper understanding of both the optics and the modes of operation. We will explain the relevant optics, discuss opportunities and challenges in light sheet microscopy, illustrated with image data recorded on the Flamingo. Live zebrafish embryos and larvae will be available to demonstrate and test Flamingo's capabilities in living specimens.

Educational goal

At the end of this workshop the participants will know the basic components and light paths of a light sheet microscope, as well as alignment principles. They will have got an insight into sample preparation for light sheet microscopy and all the parameters that go into setting up a light sheet imaging experiment. Participants will know the ups and downs of light sheet microscopy, and what to expect from such a microscope in terms of performance. Based on preview and recorded data, they will be able to determine if a light sheet microscope is well aligned and performs as expected. As a side effect, participants will learn aspects of zebrafish development and biology.

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A153-Customized Mounting of Cleared Organs (Brain, Ovary and Placenta) and organoids using CUBIC and Lighsheet imaging

Proposer/Coanimator

Vlad Costache
Matthieu Simion

Abstract

L'imagerie 3D d'échantillons intacts de grande taille, comme des organes ou des organismes entiers, est rendu possible grâce aux techniques de transparisation (tissue clearing).
Nous proposons cet atelier qui présentera la transparisation CUBIC, qui emploie des solutions aqueuses simples de mise en œuvre. Nous présenterons des techniques de montage d'échantillons à l'aide de supports fait maison par impression 3D, qui rendent possible leur observation entière à 360°.
Nous utiliserons pour la partie pratique de l'atelier, l'étude du système sérotoninergique du cerveau de souris, et montrerons à titre d'exemple, l'application sur d'autres organes de mammifère (ovaire, placenta) ou encore, des organoïdes intestinaux.
La reconstruction du volume de l'échantillon à partir de la pile d'images acquise au microscope à feuille de lumière est également un challenge et nous discuterons de manière comparative des possibilités de traitement d'images à l'aide d'outils open source ou des logiciels propriétaires.

Keywords:
Tissue Clearing, CUBIC, Lightsheet Microscopy, Brain, Placenta, Ovary, Organoids, Custom Sample Mounting.

Educational goal

L'atelier a pour objectif de montrer les possibilités de transparisation, de montage et d'imagerie d'échantillons épais préparés par des techniques CUBIC et imagés sur un microscope à feuille de lumière horizontal.
L'accent sera mis sur leur inclusion en bloc d'agarose et l'utilisation de supports porte-objets personnalisés fabriqués par impression 3D qui facilitent leur observation à 360°: ils permettent la suspension des blocs d'agarose dans la cuvette d'imagerie du microscope. Nous comparerons avec le montage classique capillaire suspendu, mais qui convient seulement à de petits échantillons, tels que les organoïdes intestinaux.
Nous aborderons de manière comparée sur plusieurs logiciels (ZEN, Imaris, FIJI) les possibilités de manipulation des images produites.
Comme exemple d'applications, nous nous appuierons sur l'utilisation d'organes ou de parties d'organes de souris et autre mammifères modèles (lapin, bovin) dans le cadre de projets développés en partenariats sur la plateforme MIMA2 de Jouy en Josas (Microscopie et imagerie des Microorganismes, Animaux et Aliments).

Les échantillons de demi-cerveaux de souris marqués pour le système sérotoninergique, avec des anticorps spécifiques seront préparés en amont de l'atelier et montés et imagés pendant la partie pratique de l'atelier.

A titre comparatif, nous montrerons en parallèle le montage classique suspendu adapté aux échantillons plus petits (inférieur à 1 mm), comme les organoïdes intestinaux.

Objectifs pédagogiques de l'atelier : à l'issu de l'atelier, les participants disposeront de tous les outils pour préparer et imager leurs échantillons avec les techniques CUBIC. Dans le détail, les acquis pédagogiques des participants seront :
1- La connaissance des techniques de transparisation en solutions aqueuses CUBIC ;
2- Savoir inclure et manipuler des échantillons épais dans un bloc d'agarose avec CUBIC ;
3- Être sensibilisé à une démarche de prototypage pour le montage d'échantillons;
4- Appréhender l'impact des écarts d'indice de réfraction sur la qualité des images ;
5- Anticiper les besoins logiciels en matière de manipulation des images obtenues

A154-Adaptable excitation field for enhanced single-molecule regime in dSTORM

Proposer/Coanimator

Lancelot Pincet
Sandrine Lévêque-fort

Abstract

In Single Molecule Localization Microscopy (SMLM), the quality of the super-resolved image highly depends on the capacity to generate single molecule imaging conditions. dSTORM in particular strongly relies on the control of the fluorescent dye photophysics. A method, called ASTER (Adaptable Scanning for Tunable Emission Regions) has been recently proposed to generate an effective uniform excitation by introducing a fast scanning (kHz) of the gaussian LASER beam which creates dynamically a top hat excitation. We will see how this technology can achieve wide filed of views (150x150µm²) with several sectioning angles (EPI, HILO, TIRF).

We will then present how ASTER can be upgraded into IASTER (Intelligent ASTER) by coupling a dynamic zoom based on focal-tunable lenses. By simultaneously changing the width of the beam and its position, we can create an intelligent excitation pattern that would adapt to the imaged sample. Indeed, high labelling density is a known limitation that can deteriorate the single molecule regime. By previously studying the dye photoswitching response, we can efficiently adapt the scanning pattern of IASTER in these regions. This offers a smart way to redistribute the excitation photons in order to counteract high localizations density. We will show how this technique can be used in time, during an SMLM acquisition, to trigger an optimized and uniform dye photoswitching in the sample. We will finally show how the acquisition improvements can be quantified with Localisation density map, FRC, and SQUIRREL measurements.

Acquisitions will be made on homemade 1 color – 2D samples (ex. Microtubules), but other samples from the participants would be welcome.

Educational goal

At the end of this workshop the participants will be able to:
- Be familiar with Single Molecule Localization Microscopy (SMLM) on dSTORM imaging
- Generate and correctly tune a homogeneous illumination
- Find sectioning angles corresponding to EPI, HILO and TIRF
- Recognize a good single-molecule regime and a bad one
- Play with the beam size for irradiance control
- Quantify acquisitions quality with processing tools (Localisation density map, FRC, SQUIRREL)

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A155-Compact, simple and versatile light-sheet fluorescence microscopy module for long-term 1P/2P functional imaging.

Proposer

Antoine Hubert

Abstract

Light-sheet fluorescence microscopy (LSFM) is now a key method for high-speed volumetric imaging with applications in cell biology, neurosciences, developmental biology, organoids or plant research. Nevertheless, despite all these possibilities, problems such as alignment, sample adaptability and equipment control are often a barrier for potential users. Therefore, this complementary and easily adaptable imaging modality is not yet widespread over the bioimaging community.
This workshop aims to introduce participants to the assembly of a compact and versatile light sheet microscope that can be combined with standard imaging systems. Attendants will assemble from scratch a 3D printed analogue module from which they can target the critical points of the design of a light sheet microscope. A user interface will be provided to characterize the light-sheet profile and perform volumetric acquisitions of various biological samples.

Educational goal

The aim of this workshop is to teach the participants how to assemble a compact light-sheet microscope using 3D printed models, how to perform volumetric image acquisitions and how to characterize the microscope's resolution using beads. Additionally, we will record in larval zebrafish (3 to 5 dpf) brain-wide neuronal activity with single cell resolution that we will analyze and visualize in 3D.
After the workshop, participants will be able to use a new precise and efficient imaging modality on suitable samples. In addition, they will have realized the simplicity of such an accurate and efficient system and will have proven its value. This would encourage non-expert users to bring homemade light-sheet modules to their laboratories.
Such simple and compact modules promote the reuse of old system components or parts forgotten in our lab cupboards (a galvo or a piezo). In this way, it is possible to obtain a new relevant imaging modality at minimal cost and implementation complexity.

- Building a compact light-sheet microscope from scratch using a template (github of the lab, paper)

In order to understand a system that could be highly customizable we believe that it is important to build it from scratch. Participants should see that a light-sheet microscope can be built with many components already available in the lab. We will therefore introduce with 1 or 2 slides the basics of light-sheet microscopy, the typical architectures and how light-sheet microscopy could be relevant for participants' experiment according to their sample/study. A 3D printed model of the compact light-sheet system presented will be provided for attendants so they could mount the system and discuss the critical points. At the end of this part attendants will know the basics of LSFM, they will practice by mounting directly the mechanical parts and optics with 3D models using a github from LJP and real system (custom mechanical parts, excitation objectives, galvanometric mirror, electronics, piezo, fiber, coupling lens, sample stage).

- Sample mounting and chamber design

LSFM is adapted to transparent samples (e.g. small samples such as organoids, mutants that suppress pigmentation as the zebrafish Casper line, or cleared tissue). One slide of the presentation will discuss the type of samples typically used in light-sheet microscopy as well as the design of the sample chamber (e.g. either compatible with an immersion excitation objective or by imaging through a glass slide), and different samples holders (e.g. using a capillary or wedge slide...). Participants will practice to mount beads diluted in agarose in a

capillary. At the end of this step, participants will know for which type of sample LSFM is useful and how to design a suitable chamber adapted to the desired experiment.

- Key points in light-sheet microscopy alignment
For optimal image quality and resolution the light-sheet has to be aligned with the focal plane of the imaging objective and the sample has to be positioned at the waist of the light-sheet. Once the sample is mounted, the beam (before any scanning to create the LSFM) may be misaligned with the camera or tilted along the propagation axis. We will discuss how to recognize a misaligned system and how to perform the alignment steps, taking into account the degrees of freedom given by the system (e.g. automatic or not).

- Software to control the system
In this step, participants will learn the key parameters to drive a LSFM and will be able to generate 3D acquisition. We will present an open source light-sheet microscopy control software developed by the LJP that enables 4D volumetric imaging.

- Visualization using napari or Fiji
To view the 4D data sets, participants will either follow a jupyter notebook to guide them to use napari or will use Fiji.

- Characterization of the light-sheet profile
We will explain the parameters that characterize a light-sheet and that determine resolution and field-of-view.

- Run a volumetric manipulation on a live sample and 3D visualization.

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A156-Imaging FCS workflow to study membrane protein dynamics in living cells

Proposer
Thorsten Wohland

Abstract
The life of a cell is governed by highly dynamical microscopic processes. Fluorescence Correlation Spectroscopy (FCS) is a prominent method to evaluate molecular mobilitiy in living cells, providing information about dynamic cell processes at the molecular level. With the advent of fast and sensitive cameras, FCS can now also be conducted in an imaging mode (Imaging FCS) in which the correlation functions at each pixel of a camera are measured simultaneously increasing the amount of information available by orders of magnitude. In addition, as the information is measured at the same instance, spatial correlations between pixels increase the information content of these measurements even further. Imaging FCS can be implemented in total internal reflection fluorescence (TIRF) microscopes or single plane illumination microscope (SPIM).

In this workshop, I propose to use Imaging FCS in TIRF mode to monitor the mobility of membrane-associated proteins in cultured cells. I will demonstrate how to setup and perform Imaging FCS experiments and obtain reliable diffusion coefficient estimates in live cells. At the end of this workshop, the participants will have learned the fundamentals of Imaging FCS, the key experimental aspects of the technique and how to evaluate the data. Finally, the participants to this workshop will have the opportunity to delve into the details of data analysis and interpretation in Workshop “Imaging FCS data evaluation”, complementary to this one, which will use the data acquired here for a hands-on work on data analysis.

Educational goal

Imaging Fluorescence Correlation Spectroscopy (Imaging FCS) has emerged as a prominent method to evaluate molecular mobility in living cells, providing information about dynamic cell processes at the molecular level. Imaging FCS does not require customized setups but can be used with total internal reflection fluorescence (TIRF) microscopes or single plane illumination microscope (SPIM) as long as a fast ans sensitive camera is available, e.g. a sCMOS or EMCCD camera.

Although Imaging FCS is limited by diffraction, i.e. in the order of 100s of nm, it reaches a time resolution between 0.1 – 1 ms. Furthermore, it can be conducted simultaneously with computational super-resolution approaches to provide data with high spatiotemporal resolution.

However, FCS is conceptually difficult to understand and there is a large activation barrier to overcome for many researchers to employ FCS. However, as a matter of fact any undergraduate student can be taught within less than a week how to perform FCS measurements including basic interpretations.
In this workshop, we aim at performing an experimental workflow to measure the mobility of different membrane-associated proteins in living cells by Imaging FCS experiments. We will manipulate the cell state and monitor changes in diffusion coefficient of the proteins. This allows the participants to learn how to perform Imaging FCS efficiently.

At the end of the workshop, the aim is for the participants to have acquired the following skills:

i. Understanding FCS and Imaging principles as well as their underlying differences
ii. Ability to operate an Imaging FCS experimental set-up.
iii. Performing basic data analysis of Imaging FCS.
iv. Being aware of the subtleties of interpreting (and over-interpreting) molecular mobility data

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A157-Imaging FCS data evaluation

Proposer

Thorsten Wohland

Abstract

Imaging Fluorescence Correlation Spectroscopy (Imaging FCS or ImFCS) is a method to evaluate molecular mobilities in vivo or in vitro. FCS requires single molecule sensitivity for its measurement but provides ensemble interpretation of its data. It can measure in general in a wide range from about 0.1 nM to several ?M. In this workshop, we will use experimental raw data and demonstrate how the data can be used to obtain reliable diffusion coefficient estimates in live cell measurements. At the end of the workshop, the participants will have learned about the basics of Imaging FCS in terms of the experimental setup and sample requirements, about the theoretical underpinnings and about strategies how to treat Imaging FCS data.

This workshop would follow the experimental workshop(s) entitled "Imaging FCS workflow to study membrane protein dynamics in living cells".

Educational goal

Fluorescence Correlation Spectroscopy (FCS) is conceptually difficult to understand and there is a large activation barrier to overcome for many researchers to employ FCS. However, as a matter of fact, any undergraduate student can be taught within less than a week how to perform FCS measurements including basic interpretations. So why do so many researchers shun the use of FCS? There are at least three reasons for this: i) The mathematics behind FCS is complex and is not easily understood; ii) The curves and their fitting is complex and misinterpretations often happen at the level of data fitting; iii) FCS is influenced by a number of processes and has particular artefacts that make simple interpretations, especially in complex living systems, difficult.

Therefore, many researchers often find themselves stuck in the data processing and data analysis stages. The main reason to this roadblock is the correct analysis and interpretation of data, which often includes the choice of a sub-diffusion model, risking an (over)interpretation of the data which is not always justified.
In this workshop, we will introduce FCS on a conceptual level with a minimum of mathematics and will discuss problems of data fitting. We will also discuss the most common artefacts, how to identify them and how to correct for them. And the final aim will be to demonstrate how FCS can be reliably performed in biological systems.
Finally, we will discuss the results obtained in the FCS, and we will discuss the strengths and weaknesses of Imaging FCS in comparison to other approaches.

The aim of the workshop is to familiarize the participant with FCS data treatement procedures with the majority of the time spent on hands-on exercises. It will end with a discussion of different methods to measure diffusion in biological systems.

At the end of the workshop the participants should ...
1. ... be aware of different strategies how to measure diffusion in biological systems (FRPA, SPT, FCS).
2. ... have an understanding of FCS principles and judge data quality.
3. ... be able to treat imaging FCS and FCS data correctly in a quantitative manner
4. ... understand pitfalls in FCS data interpretation and identify artefacts correctly.

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A158-Fluorescence Lifetime DNA-PAINT for fast multiplexing

Proposer/Coanimator

Samrat Basak
Oleksii Nevskyi

Abstract

DNA-PAINT is one of the most powerful Single-Molecule Localization Microscopy (SMLM) techniques. It is capable of imaging with nanometer spatial resolution and is a perfect fit for multi-target super-resolution imaging of cells. The most common approach for this is Exchange-PAINT, which uses sequential imaging of targets. Exchange-PAINT does not have chromatic aberration, as the same fluorophore is used for imaging of all targets. However, its main disadvantage is its relatively long acquisition time, which scales linearly with the number of imaged targets, and the need for extensive sample washing during buffer exchange cycles. The elegant solution for fast multi-target super-resolution imaging is Fluorescence Lifetime DNA-PAINT (FL-PAINT). This technique enables parallel target acquisition and uses fluorescence lifetime information for targets identification. To do this, targets are imaged by a mix of different imagers labeled with fluorophores emitting in the same spectral region but having different lifetimes. FL-PAINT can be implemented both using wide-field and confocal FL-SMLM. The downside of FL-PAINT is a high background fluorescence level due to high concentration of imagers in the mix solution, which affects the reachable resolution. To address this limitation, we have implemented a speed-optimized DNA-PAINT (Fast-PAINT) introduced by Jungmann lab in 2020. This approach uses reduced concentration of imagers due to optimized imager/docking DNA sequences, thus reducing the background signal, and facilitates imager-docking binding kinetics thanks concatenated docking strands. The combination of Fast-PAINT with FL-PAINT allows for fast and simultaneous acquisition of multiple targets with improved resolution and minimal cross-talk between the targets.

Educational goal

The training will demonstrate fast and multiplexed super-resolution imaging. For the purpose, we will demonstrate Fluorescence Lifetime DNA-PAINT (FL-PAINT). The participant will learn and practice lifetime-based

multiplexed super-resolution imaging: selection of the fluorophores for optimal target detection with minimal cross talk; sample preparation protocols; experimental procedures; data analysis (analysis package is freely available on GitHub). The final goal of the workshop – a solid understanding of and hands-on fluorescence lifetime-based SMLM imaging.
Who should attend: Our workshop is aimed at students and researchers who want to explore the advantages of advanced FLIM in combination with SMLM, as well as researchers who are seeking to broaden their expertise in Fluorescence Lifetime SMLM/ MIET-SMLM.

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A159-Metal-Induced Energy Transfer Single-Molecule Localization Microscopy (MIET-SMLM)

Proposer/Coanimator

Samrat Basak
Oleksii Nevskyi

Abstract

Fluorescence lifetime imaging microscopy is an important technique that adds another dimension to intensity and color acquired by a conventional microscopy. Nowadays, single molecule localization microscopy (SMLM) techniques have become one of the most successful and widely applied methods of super-resolution fluorescence microscopy. Unfortunately, all SMLM techniques which utilize wide-field cameras completely lack the lifetime dimension. Here, we demonstrate the combination of fluorescence-lifetime confocal laser-scanning microscopy (CLSM) with popular single-molecule localization microscopy (SMLM) approaches such as dSTORM and DNA-PAINT. This combination enables the realization of single-molecule localization-based fluorescence-lifetime super-resolution imaging, which can be used for multiplexing on samples with different labels that differ only by fluorescence lifetime but not by their spectral properties. The technique is straightforward to be implemented on a commercial confocal scanning microscope setup with TCSPC capability and fast laser scanning unit. The method combines all the advantages of CLSM with those of SMLM: axial sectioning, shot-noise limited single-photon detection, pixel-free continuous position data, and fluorescence lifetime information acquired by CLSM with the exceptional spatial resolution and single-molecule identification of SMLM, moreover, method is conceptually free of chromatic aberrations. Additionally, we combine the extreme axial resolution of metal-induced energy transfer (MIET) imaging with the extraordinary lateral resolution of single-molecule localization microscopy utilizing confocal setup. This combination allows us to achieve isotropic three-dimensional super-resolution imaging of subcellular structures.

Educational goal

The training related to this workshop is dedicated to advanced FLIM in combination with SMLM. The participants will first learn about the basics of FLIM and SMLM. Then the participants will learn about possibilities of utilizing fluorescence lifetime for multiplexing and for high-precision 3D imaging with MIET. The participants will learn how to use the confocal microscope for SMLM, typical workflow and optimization of the imaging parameters.
At the end of this workshop the participants will go back home with a better idea of how a commercial confocal microscope could be used for a chromatic aberration free multiplexing and isotropic 3D imaging in various biological applications.
Who should attend: Our workshop is aimed at students and researchers who want to explore the advantages of advanced FLIM in combination with SMLM, as well as researchers who are seeking to optimize their imaging approaches with new methods.

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A194-Fifty shades of mounting: from zebrafish larvae to encapsulated spheroids, tie them tight, but gently.

Proposer

Gaelle Recher

Abstract

The aim of this workshop is to provide attendees general knowledge about light-sheet microscopy (history, principle, different configurations), but also to explore together different ways of mounting samples in the holder, depending on the sample itself (we will use fluorescent transgenic zebrafish larvae at different developmental stages and encapsulated spheroids of different size and shapes) and on the purpose of the experiment (mapping the expression of a maker over the whole sample, tracking cells during morphogenetic processes and self-organisation, or tracking fast-moving cells such as macrophages triggered by laser ablation). We would acquire some dataset on the fly, but also discuss and process some pre-baked datasets.

Educational goal

In the end, the attended would be confident in designing and planning their own experiment with their samples. They will have a mental toolbox of different products, configurations, and tricks to mount their samples. They will also be given advice and insights about acquisition parameters depending on their goal… Sometimes taking lots of blurry snapshots provides more information rather that taking a well contrasted one.

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