cnrs

9th edition of the CNRS Thematic School

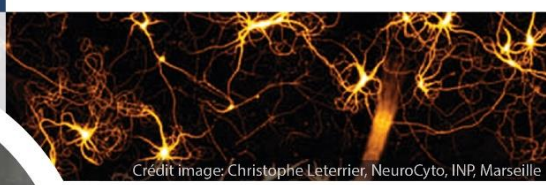
ImaBio

MiFoBio

Functional Microscopy for Biology

Presqu'île de Giens (Var), France

5 - 12 Nov 2021



Crédit image: Christophe Leterrier, NeuroCyto, INP, Marseille

Courses Seminars

Round tables

Workshops

Fablab Opticslab

Labelling, probes and constrasts.

New imaging for life

AI for bioimaging.

Molecular dynamics and interactions : *experiments and modeling.*

Quantification in nanoscopy.

Cellular signalization, Mecanobiology, Mecanotransduction.

Multicellular imaging : *organoids, tissue, embryos.*

SS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dépasser les frontières
ImaBio
Inserm
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INSTITUT FRESNEL
FRANCE-BIOIMAGING
aviesan
alliance nationale
pour les sciences de la vie et de la santé
GDR Groupement de recherche
ISIS Information Signal
Image viSion
COLLÈGE DE FRANCE
1530
CIIL
CENTER FOR INFECTION
& IMMUNITY OF LILLE
Société Française
des Microscopies
sbcf
Société de Biologie Cellulaire de France
www.sbcf.fr
IBDM
INSTITUT DE BIOLOGIE
DU DÉVELOPPEMENT
DE MARSEILLE
approches
quantitatives
du vivant
emipai
Microscopie de super-résolution sur virus et bactéries en BSL3
PSL
UNIVERSITÉ PARIS
igbmc
institut de génétique et de
biologie moléculaire et cellulaire
BioImaging
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BIOLOGIE MOLÉCULAIRE
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ET CELLULAIRE
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ÉCOLE
DOCTORALE
340
MRI
Montpellier Ressources
Imagerie

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REMERCIEMENTS

2021 le retour…

Après le report de 2020, l'équipe d'organisation est heureuse de vous accueillir dans un nouveau site pour cette nouvelle édition 2021 de l'école MiFoBio du CNRS.

Comme pour les précédentes éditions depuis 2004, cette formation est portée par le GDR ImaBio (imagerie et microscopie pour la biologie) dans le cadre des actions des Écoles Thématiques de la Formation Permanente du CNRS en collaboration avec les formations permanentes de l'INSERM, et avec le compagnonnage du réseau technologique de microscopie de fluoresence multidimentionelle (RTmfm). Cette école MiFoBio 2021 est organisée sous le patronage de la société de biologie cellulaire française (SBCF) et de la société de microscopie (SFµ). Nous remercions ces deux sociétés pour leur soutien. Elle est également soutenue par l'AVIESAN (ITMO BCDE) et France BioImaging (FBI) depuis plusieurs années, qu'ils en soient remerciés. MiFoBio bénéficie du soutien de différentes structures et laboratoires : Collège de France (Paris), Institut Fresnel (Marseille), CIIL (Lille), IBDM (Marseille), Q-life/PSL, MRI (Montpellier), BiCel (Lille) et CEMIPAI (Montpellier), Ecole doctorale BMIC (Lyon). D'autres GdR sont aussi partenaires scientifiques du projet : GdR Onde (INSIS), GdR ISIS (INS2I), GdR ADN&G (INP) et GDR AQV (INSB). Nous remercions ces différents partenaires et soutiens pour l'aide financière et organisationnelle précieuse apportée à MiFOBio 2021.

Nous souhaitons remercier les personnels des formations permanentes et des services administratifs du CNRS DR 18 : Claire Dufossé, Fabienne Lebleu, Nathalie Vialette, Pierre Silveira, Delphine Alexandre, Justine Thirion, Nathalie Dugautier, Alexandre Masure et Sonia Duval pour leur aide dans la mise en place de cette école. Nos remerciements vont plus particulièrement à Claire Dufossé, Annabelle Vandermoere, Joellie Omara-Besson, pour l'aide importante apportée cette année à la gestion de MiFoBio. Nous remercions également Fatima Hammadi pour son aide et le CIIL qui a accepté de libérer du temps de gestion pour l'école MiFOBio. Nous avons une pensée amicale pour Stéphanie Costeur qui n'aura pas pu se joindre à nous pour cette école (nous lui donnons rendez-vous en 2023…). Nous remercions la formation permanente de l'INSERM en la personne de Dorothée Terryn, pour l'aide financière et le soutien apporté au projet MiFoBio.

Que Jean Marc Blondy et son équipe qui assurent la gestion au niveau national des écoles thématiques du CNRS, trouvent ici l'expression de nos remerciements pour leur travail à long terme pour rendre possible de tels événements hors normes. Nous remercions également Jean-Claude Pommier, Chargé de mission pour la formation permanente à l'INSIS, pour son soutien permanent et son accompagnement dans ce projet et au-delà.

Nous exprimons nos sincères remerciements aux intervenants pour leur participation, la qualité de leurs cours et conférences, et pour leur enthousiasme. De même, nous remercions ceux qui ont accepté d'animer les pré-modules, les modules avancés et les tables rondes. Que les responsables de modules qui ont largement contribué à cette école par leur engagement et leur temps, trouvent aussi ici l'expression d'une gratitude collective.

L'ensemble de l'équipe tient à remercier tous les collègues impliqués dans la mise au point et la réalisation de chaque atelier, table-ronde, cours (malgré une année 2020 grise), dont la variété est une richesse pour l'école, mais aussi ceux qui ont assuré la mise à disposition des modèles cellulaires et animaux. Nous remercions aussi chaleureusement les organisatrices et organisateurs des deux symposium satellites. Le but de ces symposiums est de permettre d'aller plus loin sur deux thématiques plus transversales.

Nous tenons à saluer l'effort des équipes qui apportent à MiFoBio des systèmes expérimentaux "home made". Nous savons que c'est un investissement en temps et moyens important. Ces ateliers sont des « locomotives » pour les développements instrumentaux à venir et de formidables supports pour la transmission de savoir pratique. Un grand merci à celles et ceux qui les portent.

Nous remercions chaleureusement les partenaires industriels de cette école pour leur engagement dans cette aventure, leur fort soutien et la mise à disposition d'un parc technologique exceptionnel, qui nous permet de réaliser les ateliers pratiques dans d'excellentes conditions. C'est avec plaisir que nous les retrouvons après 3 ans et quelques confinements. Cette collaboration académique-industriel est une fierté de notre communauté tout autant qu'une formidable occasion de rencontre, de promotion et développement des technologies de l'imagerie en biologie. Nous remercions Virginie Georget, Olivier Renaud et Frédéric Brau pour le formidable travail qu'elle et ils ont réalisé depuis des mois pour gérer le club partenaire du GdR ImaBio, et rendre possible l'école MiFoBio 2021.

Il n'y aurait pas de MiFoBio sans un formidable collectif pour l'organiser et l'animer. Plus de 30 personnes sont impliquées à différents niveaux et degrés dans ce projet collectif. Que l'ensemble des membres du comité d'organisation et plus généralement tous ceux et celles qui ont mis la "main à la pâte" depuis 2x12 mois trouvent ici l'expression de nos sincères remerciements.

Nous tenons à remercier les auteurs de ce fascicule et en particulier Sandrine Lévêque-Fort pour son travail en amont de coordination des modules et de l'ensemble des enseignements avec de nombreuses nuits de travail pour tout boucler. Merci à Guillaume Baffou pour l'affiche, reprise en couverture de ce fascicule.

Héritière d'une encore jeune aventure dans le GdR ImaBio, nous remercions Hana Valenta pour son implication dans l'animation du groupe des doctorants et post-doctorants. C'est à MiFoBio 2018 que ce groupe IYSN (ImaBio Young scientist Network) a vu le jour sous l'impulsion de Marie Fournier et Laura Caccianini (toutes deux alors doctorantes). Le but de cette initiative est de permettre aux jeunes scientifiques de notre communauté de se connaître et organiser tous les ans des rencontres scientifiques en toute autonomie et ainsi créer un réseau socio professionnel solide.

Nous remercions le comité scientifique de MiFoBio qui a rendu possible ce projet, ainsi que les comités des sections scientifiques du CNRS qui nous ont fortement soutenus lors de leur évaluation du projet.

Nous remercions également le personnel du centre de vacances Belambra, pour son accueil et son aide, la mise à disposition de locaux et le soutien technique qu'il nous a apporté.

Merci à toutes et tous pour avoir rendu possible cette exceptionnelle aventure humaine et technologique.

Sandrine Lévêque-Fort, Serge Monneret, Tristan Piolot, Laurent Héliot

Pour le comité de pilotage

L'ESPRIT MIFOBIO

L'école MiFoBio vise à favoriser des ruptures scientifiques et technologiques permettant de progresser dans la compréhension des différents niveaux d'organisation du vivant, de la cellule jusqu'aux organismes. Elle constitue donc une porte d'entrée majeure sur la microscopie fonctionnelle de manière intégrative et interdisciplinaire et plus largement sur l'ingénierie biologique associée en amont et les méthodes d'analyse développées en aval.

L'école MiFoBio assure la formation à l'utilisation de différentes technologies de microscopie, d'analyse des images et de modélisation au travers d'une pédagogie participative organisée en modules thématiques comprenant le triptyque : cours, tables rondes et ateliers. Ce triptyque recouvre une autre dimension : nous sommes tous acteurs de la formation en apportant nos savoirs et questionnements.

L'ensemble des stratégies de formation mises en place repose sur l'incitation au partage des savoirs et savoir-faire entre tous les acteurs de cette formation (intervenants, participants, organisateurs, industriels). La grande interdisciplinarité du domaine, couplée à sa forte dynamique, font que chacun a des choses à transmettre et des choses à apprendre.

MIFOBIO 2021 : 9EME EDITION ET TOUJOURS DU NOUVEAU !

Pour cette édition 2021, l'école MiFoBio change de région. Après 10 ans dans l'Ouest, l'école a levé l'ancre et quitté les vagues de l'Atlantique pour venir en méditerranée se poser sur la presqu'île de Giens, revenant ainsi sur des rivages déjà fréquentés en 2008.
Mais l'école MiFoBio ne fait pas que bouger. Elle se réinvente à chaque édition, portée depuis presque 20 ans par la formidable dynamique interdisciplinaire de la biophotonique et des percées dans la compréhension du vivant.
MiFoBio2021 propose une formation de haut niveau couplant les approches théoriques et expérimentales, en rassemblant académiques et industriels, chercheurs, ingénieurs et étudiants de différentes disciplines. Elle constitue une porte d'entrée interdisciplinaire vers la biologie fonctionnelle pour ces scientifiques de différentes disciplines, de manière intégrative et sur un mode de co-production des savoirs. Elle a pour vocation d'apporter aux participants de différentes disciplines un socle commun de connaissances et savoir-faire, de leur permettre de se former aux nouvelles technologies du domaine et de partager leurs compétences.
MiFoBio est un véritable laboratoire interdisciplinaire temporaire qui offre aux participants une vision complète théorique et pratique allant de la question biologique à l'exploitation de l'image, de l'instrument à la modélisation et l'analyse. MiFoBio 2021, fidèle à sa tradition interdisciplinaire, est un lieu d'échange des savoirs où chacune et chacun apprend et enseigne, car nous sommes tous détenteurs d'un peu de savoir et de beaucoup d'ignorance.
Les participants sont donc appelés à devenir des acteurs de la formation sur un modèle participatif. En complément des cours qui permettent d'accéder aux concepts, et des ateliers formels qui apportent les savoir-faire fondamentaux, l'ensemble des participants est activement mobilisé lors des modules avancés, tables-rondes et ateliers innovants. L'organisation pratique de MiFoBio incite à un partage permanent entre les participants.

L'école MiFoBio permet à ses acteurs de différentes origines disciplinaires de se rapprocher pour initier de nouveaux travaux originaux et d'échanger et imaginer ensemble les solutions transdisciplinaires de demain. La formation par la pratique telle que portée par exemple au travers du FabLab et de l'OpticLab, participe très directement avec les ateliers à la construction de cette dynamique.
La période de confinement qui nous a conduit à reporter MiFoBio 2020 d'un an, a aussi conduit nombre de doctorants à soutenir en comité réduit. Nous avons fortement encouragé les doctorants et post-doctorants à participer à cette édition post-confinement. Dans ce but le groupe IYSN propose aux jeunes scientifiques d'animer plusieurs temps forts (chair(wo)man, poster-rencontre, rencontre, table-ronde, soirée, ...).

MiFoBio 2021 marque aussi une prise de conscience renforcée dans la prise en compte des changements climatiques et de nos responsabilités de scientifiques et citoyennes-citoyens dans la conversion de nos pratiques vers un développement plus durable. Ainsi nous vous proposons quelques petits gestes : fasicule en numérique, pas de gobelets jetables pour le café et utilisation de vos Mugs (offerts par la société Olympus) et vos Gourdes (Nikon), réutiliser des porte-badges, ... mais aussi contribuer financièrement à une action de reboisement de la forêt voisine des Maures qui a été victime d'un terrible incendie l'été dernier.
Avec MiFoBio 2021 nous marquons aussi un changement... Désormais MiFoBio aura lieu les années impaires tous les deux ans. En 2023 nous fêterons la 10ème édition de l'école. Nous profitons de cette occasion pour inviter les jeunes permanents ou non à prendre des responsabilités dans l'organisation de cette prochaine édition et ainsi préparer l'avenir…

Bon MiFobio 2021 à toutes et à tous !

Teaser MiFoBio: www.youtube.com/watch?reload=9&v=Bnpx5JGUlyA

AVEC MIFOBIO REBOISONS LES MAURES….

Avec **l'Office National des Fôret**

Agissons ensemble….

L'été dernier le massif des Maures (Var) a été violemment touché par un incendie. On dénombre plus de 7000 hectares partis en fumée au cours du mois d'août. En plus de la végétation calcinée, la tortue d'Hermann, une espèce protégée, n'a pas été épargnée...

Aujourd'hui, les surfaces brûlées ont laissé derrière elles des milliers d'arbres calcinés, impactant durablement la biodiversité. Parmi les écosystèmes touchés, on peut citer les pinèdes de pin parasol, des peuplements de chêne liège et de châtaignier, ainsi qu'une mosaïque de milieux naturels abritant une faune remarquable, telle que le lézard ocellé, les rapaces, ou bien encore la tortue d'Hermann… Un bilan terrible ! Face à l'urgence, aidez-nous à faire renaître de ses cendres ce site forestier emblématique !

Un massif à réhabiliter suite à l'incendie

Les premières interventions, dès l'automne 2021, visent à sécuriser les secteurs brûlés par l'abattage des arbres dangereux en périphérie des voies de communication et à prévenir l'érosion des sols, grâce à l'installation de fascines végétales réalisées à partir des bois brûlés. Il est en effet urgent d'agir avant de possibles pluies méditerranéennes, qui ne feraient qu'accroître la dégradation des milieux et handicaper la reprise végétale. Dans un second temps, des replantations localisées seront envisagées.

Le fonds de dotation ONF-Agir pour la forêt et le fonds RESPIR de la Région Sud s'associent, afin de récolter des dons, qui permettront de financer les travaux de réhabilitation post-incendie sur ce massif.

Avec MiFoBio Participons ensemble à la restauration du Massif des Maures !

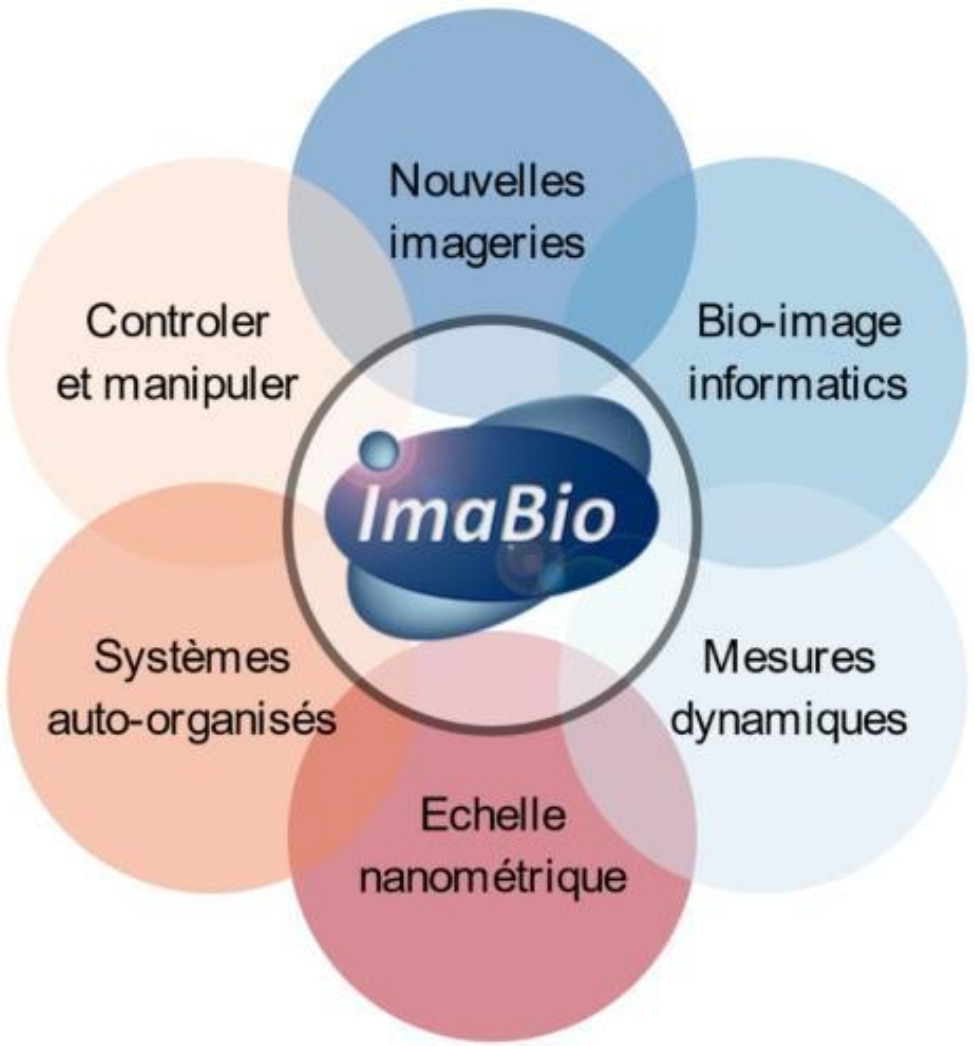
Faire un don : *Indiquer MiFOBIO dans votre adresse, pour le suivi de notre action.*
https://www.helloasso.com/associations/onf-agir-pour-la-foret/formulaires/2

GRANDS ENJEUX DE L'IMAGERIE EN BIOLOGIE ...

La compréhension des mécanismes moléculaires sous-jacents aux grandes fonctions du vivant et leurs régulations est un enjeu majeur en biologie. Une première approche consiste à réaliser des mesures des activités, forces et cinétiques au niveau moléculaire dans les cellules vivantes et de quantifier et modéliser les résultats à l'aide des lois de la physique et de la chimie. Cette approche réductionniste prise isolément, ne suffit pas à rendre compte de la complexité des observations et de l'organisation du vivant. ***Des approches plus intégratives et systémiques couplant des mesures, analyses et modélisations à différentes échelles spatiales et temporelles d'organisation du vivant sont aujourd'hui nécessaires.***

L'étude des grandes fonctions cellulaires au niveau d'organismes en conditions physiologiques ou pathologiques, ainsi que l'analyse des dynamiques moléculaires aux différentes échelles d'organisation du vivant, sont aujourd'hui possibles grâce aux récents développements réalisés en microscopie notamment depuis le début des années 2000. Ce champ de recherche interdisciplinaire est extrêmement dynamique grâce à des progrès conceptuels et technologiques successifs. L'attribution du prix Nobel de chimie 2014 pour la super-résolution en microscopie photonique et ses applications au vivant attribué à E. Betzig (qui a donné un séminaire à MiFoBio la veille de l'attribution de ce prix), S. Hell et W. Moerner est un des signes forts de ce dynamisme. La microscopie est entrée de l'observation (son sens étymologique) à la quantification. Cette extraordinaire évolution s'accompagne du dépassement de multiples verrous conceptuels (interaction photon/matière, dynamique moléculaire, mécanique cellulaire, physique des tissus, problèmes inverses, modélisation) et technologiques (sondes, ciblage, instrumentation, détection, photo-toxicité, analyse d'images). Ce dépassement exige de nouvelles stratégies co-produites de manière interdisciplinaire entre chimistes, physicien(ne)s, informaticien(ne)s, mathématicien(ne)s et biologistes, mais aussi le renforcement des travaux croisés entre expérimentateurs et théoriciens. Au niveau national, le GDR "Imagerie pour la biologie - ImaBio" regroupe cette large et dynamique communauté interdisciplinaire et inter-organismes (120 laboratoires, 1400 scientifiques). L'exploitation de la richesse de ces modalités d'imagerie permet d'accéder à une nouvelle compréhension de l'organisation du vivant, et de lier les mesures faites à l'échelle moléculaire avec les autres échelles fonctionnelles du vivant. Cette progression nécessite un véritable travail en équipe interdisciplinaire qui impose une complémentarité et une forte compréhension mutuelle, un dialogue permanent entre les disciplines et donc une base de connaissances partagées aux niveaux théorique et pratique. On arrive ainsi à la notion de "co-recherche" : l'imagerie fait avancer le questionnement biologique et les biologistes stimulent de nouveaux axes de développement et de recherche dans des disciplines non biologiques. Cette dynamique nécessite d'assurer de manière récurrente le transfert de nouveau savoir entre disciplines, mais aussi de former les jeunes générations à une base commune interdisciplinaire de concepts et outils de l'imagerie, tout en promouvant l'intégration de nouvelles compétences pour induire le dépassement des nouveaux verrous conceptuels et technologiques.

Maintenant au travail…


Nouvelles imageries
Controler et manipuler
Bio-image informatics
ImaBio
Systèmes auto-organisés
Mesures dynamiques
Echelle nanométrique

IMABIO YOUNG SCIENTIST NETWORK (IYSN)

http://imabio-cnrs.fr/presentation-iysn/

Current committee:
Hana Valenta[1], Valério Laghi[2], Samer Alhaddad[3], Clément Cabriel[3], Nathan Quiblier[4], Maciej Kerlin[5], Mohamad Jamal Wawi[6]

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In 2018, the GDR ImaBio created an associated network of young researchers (IYSN for ImaBio Young Scientists Network). IYSN aims to bring together PhD students and post-docs from the bio-imaging community in France and connect them around key topics. Our goal is to offer specific opportunities to young researchers in terms of knowledge-transfer, training, as well as interacting with the industrial partners and other laboratories of ImaBio. The network welcomes all young researchers whose interests fall within the topics of GDR ImaBio (microscopy for biology from the nano- to macro-scale).
To increase the communication within the community, the IYSN organizes an interactive conference every year.
We are recruiting new members to the team!
Contact us or Come to see our poster and discuss with us at MiFoBio!

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Fatima Hammadi

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PLANNING DES COURS

Lectures

Vendredi 5 novembre

9h00-12h00 Pré-modules
Les salles seront communiquées sur place.

15h-18h Module Fondamentaux et concepts
Salle Méditerranée

15h00-15h30
Basics of photochemistry for fluorescent microscopy Dominique Bourgeois
15h30-16h00
Concepts fondamentaux sous-jacents aux techniques de microscopie de fluorescence avancées. Guillaume Dupuis
16h00-16h30
La biologie par les nombres Gabriel Bidaux
16h30-17h00
Notions de base en apprentissage machine David Rousseau

Pause libre (17h00-18h)

18h-20h SEMINAIRES
Salle Méditerranée (retransmission live en Salle Porquerolles)
18h00-18h50
S1. Cytoplasmic forces functionally reorganize nuclear condensates in oocytes
Marie-Hélène Verlhac

18h50-19h40
S2. How do dyes get into cells?
Luke D. Lavis

20h00-21h00
Diner

21h30-0h00 Posters & Beer sessions
(organized by Imabio Young Scientist Network)

IYSN

Samedi 6 novembre Matin

Salle Méditerranée

Module 3 : Intelligence artificielle pour l'imagerie biologique

8h30-9h15 M3-1.
Microscopy Image Analysis: The Shift to Deep Learning?
Daniel Sage
EPFL, Laboratoire d'imagerie biomédicale.

9h20-10h05 M3-2.
Multiscale and multimodal registration: an overview of methods
Perrine Paul-Gilloteaux
Institut du thorax, SFR Santé François Bonamy, Université de Nantes, CNRS, INSERM, Nantes, France.

Salle Porquerolles

Module 1 : Sondes fluorescentes

8h30-9h15 M1-1.
Fluorescent-protein based biosensors to monitor biochemical activities and protein-protein interactions by FRET- FLIM
Marie Erard
Université Paris-Saclay, CNRS, Institut de Chimie Physique.

9h20-10h05 M1-2.
Systemic imaging with light sheet fluorescence microscopy
Kaspar Podgorsky
HHMI Janelia Research Campus.

10h10-10h40 Coffe Break sponsored by TreeFrog

Salle Méditerranée (retransmission live en Salle Porquerolles)

Module 3 : Intelligence artificielle pour l'imagerie biologique

10h40-11h25 **M3-3**
Self-Supervised Deep Learning for Fluorescence Imaging and nD Image Viewing with Napari
Loïc Alain Royer
Chan Zuckerberg Biohub, *San Francisco, USA.*

11h30-12h15 M3-4.
Microscopy image analysis with machine learning
Martin Weigert
EPFL, Lausanne, Switzerland.

Samedi 6 novembre Après-midi

Salle Méditerranée

Module 2 : Le défi de la quantification en nanoscopie

16h10-16h55 M2-1.
Structured illumination microscopy (SIM) for high-speed super-resolution fluorescence imaging of living cells
Alexandra Fragola
Laboratoire Physique et Étude de Matériaux UMR 8213, ESPCI Paris-PSL, CNRS, Sorbonne Université.

17h-17h45 M2-2.
3D single molecule localization microscopy
Sandrine Lévêque-Fort
Institut des Sciences Moléculaires d'Orsay, CNRS, Université Paris Saclay, 91405 Orsay.

Salle Porquerolles

Module 3 : Intelligence artificielle pour l'imagerie biologique

16h10-16h55 M3-5.
Deep Learning with Medical Images: learning with small datasets and few annotations
Diana Mateus
Centrale Nantes/LS2N.

17h00-17h45 M3-6.
Extracting the invisible from live cell microscopy
Assaf Zaritsky
Department of Software and Information Systems Engineering, Ben-Gurion University of the Negev, Israel.

18h00-18h15 pause

Salle Méditerranée (retransmission live en Salle Porquerolles)

Module 2 : Le défi de la quantification en nanoscopie

18h15-19h00 M2-3.
Superresolution microscopy for structural cell biology
Jonas Ries
Cell Biology and Biophysics unit, European Molecular Biology Laboratory (EMBL).

19h05-19h50 M2-4.
Quantification of filament structures in superresolution and expansion microscopy
E.A. Katrukha
Cell Biology, Department of Biology, Faculty of Science, Utrecht University, Netherlands.

Dimanche 7 novembre Matin

Salle Méditerranée

Module 1 : Sondes fluorescentes

8h30-9h15 M1-3.
Fluorescent dyes and nanoparticles as bright probes for advanced bioimaging.
Andrey Klymchenko
Laboratory of Bioimaging and Pathologies.

9h20-10h05 M1-4.
Labeling strategies to visualize the inner life of microbes by single-molecule localization microscopy – a practical guide
Ulrike Endesfelder
Institut für Mikrobiologie und Biotechnologie, Universität Bonn.

10h10-10h40 Coffe Break sponsored by Telight

Salle Porquerolles

Module 2 : Nanoscopies

8h30-9h15 M2-5.
From images to information: enhancing resolution and improving accuracy in SMLM
VISIO
Susan Cox
King's college, London.

9h20-10h05 M2-6.
Computational microscopy by PSF engineering - or – how and why to ruin a perfectly good microscope
Yoav Shechtman
Department of Biomedical Engineering, Technion – Israel Institute of Technology.

Salle Méditerranée (retransmission live en Salle Porquerolles)

Module 1 : Sondes fluorescentes

10h40-11h25 M1-5.
Switchable Organic Dyes: the Photophysics of STORM
Mark Bates
Department of NanoBiophotonics, Max Planck Institute for Biophysical Chemistry.

11h25-12h10 Seminar 3
Force transmission at cell adhesions and the nucleus
Nicolas Borghi,
Institut Jacques Monod, CNRS, Université de Paris, Paris, France

19h10-20h05 S.3 VISIO
Building the next generation of genetically encoded probes and actuators
Nathan C. Shaner
Department of Neurosciences, University of California San Diego School of Medicine.

18h15 Seminar 4
3D super-resolution imaging of living systems using Multifocus Microscopy and Structured Illumination Microscopy
Sara Abrahamson,
University of California Santa Cruz, , USA; National Microscopy Infrastructure, KTH, Stockholm, Sweden

Lundi 8 novembre Matin

Salle Méditerranée

Module 5 : Ondes sur le vivant (avec GDR Ondes)

8h30-9h15 M5-1.
Measuring and shaping the phase of light: key applications in biology
Pascal Berto
Institut de la vision, Sorbonne Université, INSERM, CNRS.

9h20-10h05 M5-2.
X-ray coherent diffraction imaging: 3D exploration of biologically relevant hard and soft tissues
Virginie Chamard
Aix-Marseille Université, CNRS, Centrale Marseille, Institut Fresnel, Marseille.

Salle Porquerolles

Module 6 : Dynamique et interactions moléculaires en cellules vivantes : expérimentation et modélisation

8h30-9h15 M6-1.
Revealing spatial and kinetic details of life processes by analyzing live cell single molecule tracking data
J. Christof M. Gebhardt
Institute of Biophysics, Ulm University, Germany.

9h20-10h05 M6-6. VISIO
***E. coli* chromosome dynamics and the cell cycle**
Marco Cosentino Lagomarsino
Department of Physics, University of Milan, Italy

10h10-10h40 Coffe break sponsored by

Salle Méditerranée (retransmission live en Salle Porquerolles)

Module 5 : Ondes sur le vivant (avec GDR Ondes)

10h40-11h25 M5-3.
Imaging the brain at high spatiotemporal resolution with wavefront shaping
Na Ji
Department of Physics, Department of Molecular & Cell Biology, University of California, Berkeley.

11h25-12h10 M5-4.
Volumetric imaging at high speeds
Jérome Mertz
Biomedical Engineering Department, Boston MA.

18h15 Seminar S
Super-resolution microscopy: Challenges and Potentials in biomedical research
Christian Eggeling,
Institute for Applied Optics and Biophysics, Friedrich-Schiller-University Jena; Leibniz Institute for Photonic Technology e.V., Jena, Germany; MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom

Mardi 9 novembre Après-midi

Salle Méditerranée

Module 6 : Dynamique et interactions moléculaires en cellules vivantes : expérimentation et modélisation

14h00-14h45 M6-3.
Imaging DNA repair at the single molecule level
Judith Miné-Hattab
Inst Curie, PSL University, UMR3664, France.

14h50-15h35 M6-4.
Investigating reaction-diffusion dynamics of proteins in the nucleus of living cells using fluorescence-based methods
Sébastien Huet
Institut de Génétique et Développement de Rennes, France.

15h40-16h10 pause

Salle Porquerolles

Module 5 : Mécanobiologie cellulaire et tissulaire : expérimentation et modélisation

14h00-14h45 M5-5.
Gouy and Brown to the rescue: Label-free virus detection and virus-antibody interaction monitoring by common-path interferometry
Ignacio Izeddin
Institut Langevin, ESPCI Paris, PSL University, CNRS.

14h50-15h35 M5-6.
Polarized microscopy resolves protein's organization in cells
Sophie Brasselet
Aix Marseille Université, CNRS, Centrale Marseille, Institut Fresnel.

Salle Méditerranée (retransmission live en Salle Porquerolles)

Module 6 : Dynamique et interactions moléculaires en cellules vivantes : expérimentation et modélisation

16h10-16h55 M6-5.
Understanding the molecular assembly of the cell contractile machinery
François Robin
CNRS UMR7622 and Inserm ERL 1156, Institut de Biologie Paris-Seine (IBPS), Paris, France.

16h55-17h40 M6-6.
Lighting up the central dogma in living embryos to uncover how genomic sequence encodes cell fate decisions
Jacques Bothma
Hubrecht Institute for Developmental Biology and Stem Cell Research, Utrecht, The Netherlands

18h15 Seminar S6
Metal- and Graphene-Induced Energy Transfer Imaging
Jörg Enderlein, Institute of Physics – Biophysics, Göttingen, Germany.

19h15 Sminar 7
Towards quantitative correlative microscopy
Lucy Collinson, Francis Crick Institute, 1 Midland Road, London NW1 1AT, UK

Mercredi 10 novembre Matin

Salle Méditerranée

Module 4 : Imagerie multicellulaire : organoïdes, tissus, embryons

8h30-9h15 M4-1. VISIO
Sometimes, there IS a free lunch: How to get twice the resolution from your microscope, without (serious) drawbacks
Andrew York
Calico Life Sciences LLC, South San Francisco, CA, USA.

9h20-10h05 M4-2.
3D printing and bioprinting for the development of microenvironment and tissue models VISIO
Laurent Malaquin
ELiA team, LAAS – CNRS UPR 8001, Toulouse, France.

Salle Porquerolles

Module 7 : Signalisation cellulaire, mécanobiologie, mécanotransduction

8h30-9h15 M7-1.
Spatial regulation of exocytosis
Stéphanie Miserey-Lenkei
Institut Curie, PSL Research University, Sorbonne Université, CNRS, UMR 144, Molecular Mechanisms of Intracellular Transport laboratory.

9h20-10h05 M7-2.
Clathrin plaques from mechano-transducting platforms.
Stéphane Vassilopoulos
INSERM UMRS_974, Sorbonne Universités, UPMC Univ Paris 06, Institute of Myology, Paris, France.

10h10-10h40 coffe break sponsored by:

Salle Méditerranée (retransmission live en Salle Porquerolles)

Module 4 : Imagerie multicellulaire : organoïdes, tissus, embryons

10h40-11h25 M4-3.
Organ on chip, a new generation of in vitro models.
Descroix Stéphanie,
Institut Curie UMR 168- Institut Pierre Gille de Gennes.

11h25 Siminar S8
Synaptic vesicle pools under the nanoscope
Frédéric A. Meunier, Clem Jones Centre for Ageing Dementia Research (CJCADR), Queensland Brain Institute (QBI), University of Queensland, St Lucia Campus, Brisbane, QLD, 4072, Australia.

18h15-19h10 M4-4. VISIO
SCAPE microscopy for high-speed 3D imaging.
Elizabeth M. C. Hillman,
Mortimer B. Zuckerman Mind Brain Behavior Institute, Professor of Biomedical Engineering and Radiology, Columbia University, Jerome L. Greene Science Center

19h15 Seminar S9
The Mechanobiology of Cell Growth and Shape control
Nicolas Minc, Institut Jacques Monod, CNRS UMR 7592, Université de paris.

Jeudi 11 novembre Matin

Salle Méditerranée

Module 7 : Signalisation cellulaire, mécanobiologie, mécanotransduction

8h30-9h15 M7-3.
Impact of physical forces of the gut on pathogen infection using organ on ship (OOC).
Nathalie Sauvonnet,
Intracellular trafficking and tissue homeostasis, department Cell Biology and Infection, Institut Pasteur.

9h20-10h05 M6-6.
Mechanisms and mechanics driving composite morphogenesis.
Matteo Rauzi,
Université Côte d'Azur, CNRS, Inserm, iBV, Nice, France.

Salle Porquerolles

Module 4 : Imagerie multicellulaire : organoïdes, tissus, embryons

8h30-9h15 M4-5.
Quantifying transport and efficacy of therapeutics in spheroids.
Charlotte Rivière,
Université Claude Bernard Lyon 1, CNRS, Institut Lumière Matière ; Institut Convergence Plascan, CRCL ; Institut Universitaire de France (IUF).

9h20-10h05 M4-6. VISIO
3D-printed minimally assembled interchangeable LSFM chamber for serial imaging of organoids and spheroids.
Francesco Pampaloni,
Buchmann Institute for Molecular Life Sciences (BMLS) Germany; Institute for Research in Biomedicine, IRB Barcelona.

10h10-10h40 coffe break sponsored by:

Salle Méditerranée (retransmission live en Salle Porquerolles)

Module 7 : Signalisation cellulaire, mécanobiologie, mécanotransduction

10h40-11h25 M7-5.
Correlated super-resolution light and electron microscopy reveals a novel actin-driven mechanism of nuclear envelope rupture in starfish oocytes.
Peter Lenart,
Cell Biology and Biophysics Unit, European Molecular Biology Laboratory (EMBL), Max Planck Institute for Biophysical Chemistry, 37077 Göttingen, Germany.

11h25-12h10 M7-6.
Dissecting the link between signaling and cell mechanics using optogenetics and AFM.
Guillaume Charras,
University College London, UK.

18h15 Seminar 10
Médiation technique et conception : une création orientée ?
Claude Paraponaris,
DR CNRS, Professeur des universités Aix Marseille, Anthropologue et philosophe de la technique, organologie de la création industrielle

PRE-MODULES

Vendredi 5 novembre Matin Lectures

8h30-12h00 Pré-modules

Les salles seront communiquées sur place

Bases en Biologie Cellulaire

Delphine Muriaux

L'organisme qu'il soit végétal ou animal est constitué de milliards de cellules de nature et de fonctions différentes dont la différenciation apparaît au cours du développement suite à la fécondation. Dans ce cours, nous allons porter une attention particulière à cette unité qu'est la cellule, en définissant de manière générale ses aspects moléculaires. Nous décrirons les différences entre cellules eucaryotes et procaryotes, leurs compartiments intracellulaires (Noyau, Reticulum, Golgi, Mitochondries, Endosomes …) et les notions de Membranes, Protéines, ARN et ADN, en taille, en nombres et en couleur. Puis, pour finir nous aborderons les notions de transfection, transduction, et infection, comme outils afin d'exprimer les protéines fluorescentes dans ces cellules et les visualiser par microscopies.

Gabriel Bidaux

L'organisation des organismes vivants est contrainte par les lois de la chimie et de la physique et son étude requiert des outils dont les gammes dynamiques et les limites doivent être ajustées aux bonnes dimensions. Ce cours introduit les notions d'espace et de temps des processus de l'échelle moléculaire à l'échelle de l'organisme.

Bases en analyse d'image

Bertrand Simon & David Rousseau

Ce prémodule reprendra la notion d'une image numérique, les problématiques liées au format et les techniques de base de prétraitement et segmentation des images (seuillage, filtres, frontières, binarisation & analyses morphologiques).

Optique pour les nuls

Olivier Haeberlé

Ce prémodule propose de reprendre des notions de base de la formation des images dans un microscope optique et de les replacer dans le contexte des différentes technologies qui seront abordées plus en profondeur lors des modules.

(Optique géométrique élémentaire, la diffraction, la phase et la PSF, les techniques de superrésolution et de microtomographie)

Bases de la fluorescence (public cible : biologistes, mathématiciens, informaticiens). *Dominique Bourgeois*

Beaucoup de participants à l'école MiFoBio utilisent la microscopie de fluorescence. Cependant, les propriétés des marqueurs fluorescents sont parfois méconnues. Ce prémodule propose de faire un point sur les différents processus liés à la fluorescence et sur les propriétés des fluorophores (spectres, conversions radiatives et non-radiative, fluorescence, phosphorescence, états singulets, triplets, réactions avec l'environnement, bleaching, blinking, coefficient d'absorption molaire, section croisée, rendement quantique, brillance, FRET, notion de flux de photon en microscopies de fluorescence, durée de vie). Nous donnerons aussi une ouverture sur les processus non fluorescents (génération d'harmonique par exemple).

MODULES FONDAMENTAUX

L'objectif de ce module est de mettre en place les concepts et connaissances fondamentales nécessaires pour l'intégration du contenu des différents modules. En particulier l'objectif est de replacer correctement les vocabulaires propres à chaque discipline ou domaine. Ce module assurera à chacun les bases interdisciplinaires nécessaires. Pour faciliter la compréhension des concepts entre discipline ces cours fondamentaux seront en français (avec traduction du vocabulaire spécifique Anglais).

The *objective of this module is to put in place the fundamental concepts and knowledge necessary for the integration of the content of the different modules. In particular, the objective is to correctly place the vocabulary specific to each discipline or field. This module will provide each student with the necessary interdisciplinary foundation. To facilitate the understanding of concepts between disciplines, these fundamental courses will be in French (with translation of specific English vocabulary).*

Lectures

Basics of photochemistry for fluorescent microscopy

Dominique Bourgeois , Institut de Biologie structurale, Grenoble
dominique.bourgeois@ibs.fr

Fluorescence microscopy always starts with the choice of a suitable fluorescent marker. Beyond live-cell compatibility, labeling specificity, preservation of target biological function, minimization of cyto- and photo-toxicity, suitable emission color and maximization of fluorescence brightness, the performance of all fluorescence microscopy techniques, and notably advanced methods such as super resolution or FRET-based imaging strongly depends on the highly complex photochemical properties of the chosen markers or sensors. In this lecture, after reviewing the essential vocabulary, I will recapitulate the main photochemical and photophysical parameters to consider when selecting fluorophores and I will introduce the different families of fluorescent markers, from fluorescent proteins to organic dyes and nanoparticles, listing their main advantages and drawbacks.

Concepts fondamentaux sous-jacents aux techniques de microscopie de fluorescence avancées.

Guillaume Dupuis, Institut des Sciences Moléculaires d'Orsay, Université Paris Saclay
guillaume.dupuis@universite-paris-saclay.fr

L'imagerie de fluorescence est un outil de référence dans l'étude des systèmes biologiques parce qu'elle offre simultanément la possibilité de l'observation en milieu vivant à haute résolution spatiale et la spécificité moléculaire de la fluorescence. Cela étant, ses champs d'investigation ont longtemps été restreints par une limite fondamentale de tout système optique : la limite de diffraction. Depuis une dizaine d'années, des développements instrumentaux alliant de nouvelles approches optiques et l'ingénierie des transitions moléculaires des fluorophores ont réussi à complètement dépasser la limite de diffraction et offrent dorénavant accès à l'échelle nanoscopique des systèmes biologiques. L'idée de ce module fondamental d'optique est de décrire les concepts fondamentaux sous-jacents à ces techniques de microscopie de super-résolution, dans le but de mieux appréhender les principes des méthodes et des instruments qui seront présentés et utilisés au cours de cette école thématique. Cette présentation orale sera faite en Français avec un support

en Anglais. L'intervenant s'efforcera de rappeler les acronymes et mots-clés d'usage liés à ces concepts.

La biologie par les nombres

Gabriel Bidaux , Laboratoire Carmen, INSERM, Université Lyon 1
gabriel.bidaux@univ-lyon1.fr

« Je dis souvent que lorsque vous pouvez mesurer ce dont vous parlez et l'exprimer en chiffres, vous en savez quelque chose » (William Thomson, alias Lord Kelvin, 1883). De nos jours, les scientifiques seraient majoritairement d'accord avec cette formule tant la « biologie quantitative " est utilisée pour mesurer et comparer les réactions chimiques à toutes les échelles dans les organismes vivants. Les nombres sont non seulement utiles aux biologistes pour mieux comprendre les mécanismes du vivant mais aussi aux chimistes, physiciens et mathématiciens pour caractériser et modéliser ces mécanismes, ainsi qu'aux ingénieurs pour calibrer leurs outils et ainsi améliorer la précision de la mesure. Regardons donc ces nombres de l'échelle atomistique à celle du vivant.

Notions de base en apprentissage machine

David Rousseau , Laboratoire Laris, Université d'Angers
david.rousseau@univ-angers.fr

MIFOBIO propose un parcours IA couvrant les aspects pratiques de mise en œuvre pour les débutants et les initiés ou encore des focus plus méthodologiques sur les outils les plus récents, et enfin des tables rondes sur des sujets spécifiques qui appellent à débat. Dans ce cours fondamental, pour permettre à chacun de suivre tout ou partie de ce parcours IA, nous passerons en revue les principales notions de base en apprentissage machine. Nous montrerons comment les méthodes de vision artificielle les plus en vue (deep learning) peuvent être comprises dans une continuité des méthodes plus traditionnelles et nous discuterons enfin les raisons du succès de ces méthodes mais aussi leurs limites

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MODULES

MODULE 1 : SONDES FLUORESCENTES

Lectures

Coordination : Marie Erard, Andrey Klymchenko et Dominique Bourgeois

Introduction

Any fluorescence microscopy technique is fundamentally based on the use of adapted and efficient fluorescent markers. Whether they are localization or functional markers, whether the target is a protein, a nucleic acid, a carbohydrate or a lipid, whether the biological object of study is fixed or living, a single cell or a multicellular organism, each project requires an elaborate optimization of the labeling process, which can be based on the use of genetically encoded fluorescent proteins, organic fluorophores, or nanoparticles. This module aims to highlight the crucial importance of fluorescence labeling, present the diversity of state-of-the-art fluorescent probes, and make the audience aware of the complexity of the biochemical, biophysical and photophysical mechanisms that must be considered when deciding on a fluorescent marker and labeling strategy.

Samedi 6 novembre - 8h30

Fluorescent-protein based biosensors to monitor biochemical activities and protein-protein interactions by FRET- FLIM

Marie Erard
Université Paris-Saclay, CNRS, Institut de Chimie Physique, UMR CNRS 8000, 91405, Orsay, France.
marie.erard@universite-paris-saclay.fr
Salle Porquerolles – Cours parallèle

Förster resonance energy transfer (FRET) between spectrally adapted fluorescent proteins gave rise to a large variety of biosensing modalities to elucidate cellular events at the molecular level with unprecedented spatial and temporal resolution. In FRET-based biosensors, the two FPs sandwich a sensing protein module, whose conformation changes upon a biological stimulus such as a post-translational modification consecutive to a signaling event or the variation of a metabolite concentration. This conformational change modulates the FRET efficiency between FPs that is indeed the optical readout of the biosensor. The combination of the right FPs with a detection by Fluorescence Lifetime Imagéing (FLIM) allows the detection of one to multiple biological parameters at the same time. It is also possible to fuse the two FPs to different proteins to probe their interaction and the FRET efficiency being its readout. The combination of FRET detected by FLIM with other imaging modalities such as Fluorescence Cross Correlation Spectroscopy (FCCS) allows a quantitative analysis of the interaction. Furthermore, if structural data obtained *in vitro* are available on the same proteins of interest, FRET become a powerful tool to propose topological models valid in live cells.

The lecture will contain the following points:

- The fundamentals - including the choice of the probe-, to design a FRET strategy for biosensing compatible with a FLIM detection
- Intramolecular FRET biosensors and FLIM, from design to multiplexing

- Intermolecular FRET probed by FLIM and quantitative analysis of protein-protein interactions

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A third-generation glutamate indicator optimized for synapses

Abhi Aggarwal[1], Rui Liu[2], Yang Chen[3], The GENIE Project Team[1], Amelia Ralowicz[4], Samuel J Bergerson[4], Filip Tomaska[1], Tim Hanson[1], Ronak Patel[1], Paul Tilberg[1], Boaz Mohar[1], Loren Looger[1], Jonathan Marvin[1], Michael Hoppa[4], Arthur Konnerth[3], David Kleinfeld[2], Eric Schreiter[1], Kaspar Podgorski[1,5]

[1]HHMI Janelia Research Campus, Ashburn, VA, USA

[2]University of California, San Diego, CA, USA

[3]Technical University of Munich, Munich, Germany

[4]Dartmouth University, Hanover, NH, USA

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Salle Porquerolles – Cours parallèle

The glutamate indicator iGluSnFR enables high-resolution imaging of neurotransmission with genetic and molecular specificity. A primary motivation for iGluSnFR's development and application has been to monitor synaptic communication between neurons. However, we found that existing iGluSnFR variants poorly distinguish synaptic from extrasynaptic glutamate transients due to kinetic saturation. Using a multi-assay screen, we generated novel variants with improved kinetic properties and display vectors that improve surface trafficking and nanoscopic localization to postsynapses. The resulting indicator, iGluSnFR3, exhibits rapid non-saturating kinetics, higher signal-to-noise ratios in vitro and *in vivo*, and readily reports single-vesicle release. Manipulations in cultured neurons show that iGluSnFR3 quantitatively reports glutamate release at synapses and that the relative contribution of extrasynaptic signals is strongly reduced. In the visual cortex of mice, we mapped the tuning of dendritic spines and demonstrated reliable detection of glutamate release at individual boutons triggered by single electrophysiologically-observed action potentials. In Layer 4 of barrel cortex, we used iGluSnFR3 to characterize the distinct activity patterns of thalamic axons and dendritic spines evoked by whisker stimulation, demonstrating the sensitivity and synaptic specificity of iGluSnFR3 *in vivo.*

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Dimanche 7 novembre - 8h30

Fluorescent dyes and nanoparticles as bright probes for advanced bioimaging.

Andrey Klymchenko

Nanochemistry and Bioimaging group, Laboratory of Bioimaging and Pathologies, CNRS UMR 7021, University of Strasbourg, Illkirch-Strasbourg, France.

andrey.klymchenko@unistra.fr

Salle Méditerranée – Cours parallèle.

Advanced fluorescence imaging relies on the performance of fluorescent tools – probes, which light up biomolecular processes and cellular structures. Of particular interest are fluorescent molecular probes that change their color (solvatochromic dyes) or intensity (fluorogenic dyes) in response to cellular targets.[1] Probes based on solvatochromic dyes enable super-resolution imaging of plasma membrane organization[2] and polarity mapping of a cell and its organelles in response

stress conditions.[3] On the other hand, specially designed fluorogenic probes allow background free imaging of target G protein coupled receptors[4] and intracellular RNA.[5]

To go beyond the limits of brightness of organic dyes, luminescent nanoparticles are an attractive alternative.[6] In particular, it concerns dye-loaded fluorescent polymeric nanoparticles.[7] Their size can be tuned from 7 till 100 nm[8] and their brightness can be 100-fold higher compared to semiconductor quantum dots of similar size.[9] Small size of nanoparticles below 23 nm is found essential for their free diffusion inside live cells.[8] Their high brightness enable single-particle tracking in mice brain.[10] Moreover, using nanoparticles of different color, a technique for long-term barcoding of living cells (chemical analogue of Brainbow) is introduced, which allows tracking multiple cell populations *in vitro* and *in vivo*.[11] Finally, based on these ultrabright nanoobjects as light-harvesting nanoantennas,[12] FRET-based color switching nanoprobes are designed for detection and imaging target nucleic acids with single-molecule sensitivity[13] and compatibility with RGB camera of a smartphone.[14]

ERC consolidator grant BrightSens 648528 is acknowledged for the financial support.

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Labeling strategies to visualize the inner life of microbes by single-molecule localization microscopy – a practical guide

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Microbes, as single-celled organisms, are important model systems for the study of cellular mechanisms and functions. In recent years, immense progress has been made using advanced fluorescence microscopy techniques to characterize and quantify the behavior of single bacterial cells based on molecular interactions and assemblies in the complex environment of living cultures. Importantly, single-molecule imaging enables *in vivo* determination of the stoichiometry and molecular architecture of subcellular structures, providing detailed, quantitative, spatially and temporally resolved molecular maps and deciphering dynamic heterogeneities and subpopulations at the subcellular level.

However, there are still many open challenges. Choosing an experimental strategy for a particular microbial organism and biological question in mind is not routine work and one usually encounters several (unexpected) obstacles [1, 2]. Here I briefly review the past and current state of the field and discuss examples of probe and labeling applications from our work - e.g., establishing SMLM imaging for archaea [3] - all of which can serve as practical examples for designing your own study.

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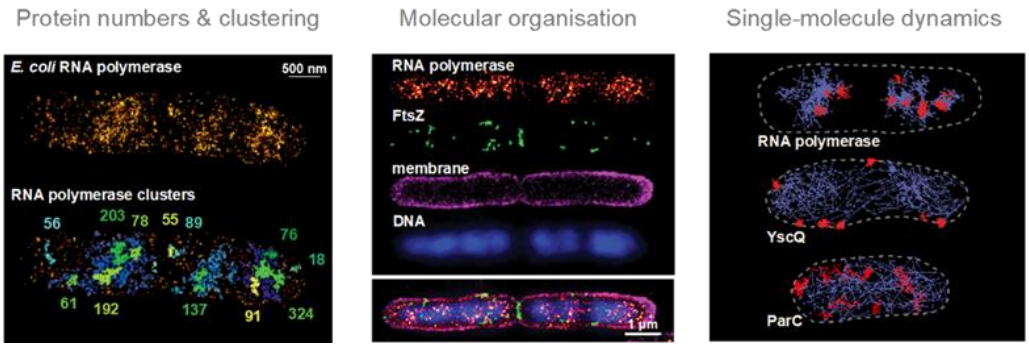
[3] Turkowyd, B., Schreiber, S., Wörtz, J., Segal, E.S., Mevarech, M., Duggin, I.G., Marchfelder, A. and Endesfelder, U., 2020. Frontiers in microbiology, 10.3389/fmicb.2020.583010



Quantitative single-molecule microscopy in microbiology (i) Clustering of RNA polymerase in *E. coli* (Endesfelder BIOspektrum 2016, 22(2)); (ii) correlative multi-color imaging of DNA, membrane, RNA polymerase and FtsZ in *E. coli* (Virant *et al.* Int J Mol Sci 2017, 18(7)); (iii) Dynamic maps of RNA polymerase in *E. coli*, YscQ (T3SS protein) in *Y. enterocolitica* and ParC in *V. parahaemolyticus*. Trajectories are classified into fast (blue) and slow (red, diffusion coefficient $D^* \leq 0.03$ µm²/s) diffusing proteins (RNAP (Virant *et al*. Int J Mol Sci 2017, 18(7)), YscQ and ParC unpublished).

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Switchable Organic Dyes: the Photophysics of STORM.

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Super-resolution fluorescence methods such as PALM, STORM, and MINFLUX have re-defined the boundaries of possibility for biological imaging with optical microscopes. Intrinsically, all of these concepts rely on a highly non-linear property possessed by certain fluorescent molecules: optically controlled ON-OFF switching. In this lecture I will review the discovery and characterization of synthetic fluorescent probes bearing this property, and how they are used to create images of biological samples with spatial resolution reaching single-nanometer length scales. By understanding the physical mechanisms underlying optical switching, we can consider which factors limit the performance of these dyes in imaging applications, and how they could be improved. As important as the fluorophore itself, we will further discuss how the choice of labeling method influences the result. Finally, we examine state of the art examples of what is presently possible with STORM microscopy and look to future directions for this class of imaging methods.

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Building the next generation of genetically encoded probes and actuators.

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Salle Méditerranée – Cours plénier VISIO 19h15

Genetically encoded optical probes are the cornerstone of modern biological imaging. For nearly three decades, mutants of the green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* have been the most commonly used genetically encoded probes for imaging. The remaining FPs regularly used in laboratories come from just a handful of species, despite the near ubiquity of FPs in many classes of marine invertebrate. My group's current research focuses on exploring the ocean for new FP genes with improved or novel optical and photophysical properties and engineering the most promising clones to produce next-generation tools for fluorescence microscopy in living cells. Bioluminescence, the production of biological light from the oxidation of a luciferin substrate by a luciferase or photoprotein enzyme, is emerging as a promising new tool for imaging living systems. However, the ability to generate photons inside a living organism also lends itself to the development of tools for many non-imaging applications. Another major focus of our research group is to engineer tools to probe and manipulate cells inside intact organisms by adapting bioluminescence as both an imaging and chemogenetic platform. For example, linking bioluminescent proteins to optogenetic actuators allows us to non-invasively activate or silence specified subpopulations of neurons in a behaving animal simply by injecting luciferin. Another modification to this approach allows the generation of "optical synapses" between specified neurons when luciferin is present. Bioluminescence generates light selectively and non-invasively in deep tissues, a property we are currently exploiting to engineer a chemogenetic protein-protein interaction probe (a signal integrator) based on FRET between bioluminescent donors and a highly unusual fluorescent protein we discovered in a reef invertebrate. Here, I present the story of genetically encoded optical probe engineering, from the ocean to the microscope, and discuss the newest developments in my group's research with views to the future.

Dans la même thématique

Tables rondes :

TR1.03 -Emerging chemical tools, techniques, and methods for the realization of competitive biological projects

Ateliers :

A023-Real-time mitochondrial Ca2+ and ATP measurements in mammalian cells using single excitation wavelength dual colour FLIM

A034-3D FRET biosensor measurements using light sheet microscopy.

A039-Imagerie 3D de la vascularisation par feuille de lumière pour évaluer l'évolution d'une pathologie et/ou l'efficacité d'un traitement

A117-Fluorescent imaging and techniques to study lignocellulosic biomass at the nanoscale

A121-CentrO2 : Influence de la concentration en oxygène pour l'imagerie dSTORM multi-couleur du cil primaire

A136-Biosensing the cell: FRET by FLIM using AurkA kinase activation biosensor

MODULE 2 : LE DEFI DE LA QUANTIFICATION EN NANOSCOPIE

Coordination : Sandrine Lévêque-Fort, Christophe Leterrier, Jean-Batiste Sibarita

Introduction

In less than 20 years after their discovery, super-resolution microscopy (SRM) techniques have reached the required maturity for biologists to produce images in routine, contributing to major discoveries in cell biology, neuroscience and developmental biology. They are however still limited in speed, penetration depth, and in their capability to provide reliable quantitative information at the nanoscale. This module aims at presenting how the combination of optics and computerized methods allows pushing forward the limits of 3D quantitative super-resolution microscopy.

Samedi 6 novembre 16h10

Structured illumination microscopy (SIM) for high-speed super-resolution fluorescence imaging of living cells.

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The observation of living cells at the molecular level is now possible thanks to the development of super-resolved microscopy techniques over the last twenty years. Structured illumination microscopy is a technique of choice for high spatial and temporal resolution 3D fluorescence imaging of living cells, as it allows a gain in spatial resolution of a factor of 2 (in its linear version) from a small number of image acquisitions, compatible with the study of dynamic phenomena. This course will detail the principles of structured illumination microscopy and its implementation, from the point of view of the instrument but also of the image reconstruction, as well as the characterization of its performances and the preparation of samples. Applications to dynamic cell imaging will be presented. Finally, we will see how non-linear structured illumination microscopy allows to obtain a gain higher than 2 and thus to observe living cells with a spatial resolution of a few tens of nanometers.

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3D single molecule localization microscopy.

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Single molecule localization microscopy (SMLM) is now a widely used tool for various biological applications. Although SMLM initially allowed the localization of molecules in 2D with a precision of a few nanometers, the inherent 3D organization of the samples triggers the development of complementary strategies to retrieve the axial information with ultimate precision. There are several challenges in achieving near-isotropic accuracy but also in accessing deep imaging. This not only has an impact on the detection strategy but may also require an alternative excitation configuration.

In this lecture we will review the different optical methods to retrieve the axial position of single molecule with their optical implementations and performances[1]. Commonly used PSF engineering approaches (astigmatism, double helix, …) that introduce an axial dissymmetry will be presented. We will also see that by exploiting interferometry [2-3] or intrinsic fluorescence emission property (supercritical angle fluorescence)[4], axial information can also be retrieved. In addition to all these approaches which are based on a spatial analysis of the PSF which is sensible to defocus or aberrations, we will discuss the introduction of a temporal parameter in the localization process. In particular by replacing the uniform excitation by a shifting structured excitation over the entire field of view, this induces a time modulated emission of the fluorophores whose phase directly encodes its position. This modulated excitation improves the lateral precision by a factor 2, but provides unique advantages when applied along the axial direction. This technique called ModLoc [5] thus offers an axial precision below 7 nm, uniform over several micrometres, and images up to 40 µm in depth can be retrieved.

[1] A von Diezmann, Y Shechtman, WE Moerner, Three-dimensional localization of single molecules for super-resolution imaging and single-particle tracking, Chemical reviews 117 (11), 7244-7275

[2] G. Shtengel *et al.* , Interferometric fluorescent super-resolution microscopy resolves 3D cellular ultrastructure, PNAS March 3, 2009 106 (9) 3125-3130

[3] P. Bon *et al.*, Self-interference 3D super-resolution microscopy for deep tissue investigations, Nature Methods volume 15, pages 449–454 (2018)

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[5] Jouchet *et al.* Nanometric axial localization of single fluorescent molecules with modulated excitation. Nat. Photonics 15, 297–304 (2021).

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Superresolution microscopy for structural cell biology.

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Salle Méditerranée – Cours plénier

Superresolution microscopy, such as single-molecule localization microscopy (SMLM), is becoming a key technique for structural cell biology, ideally complementing electron microscopy.

I will discuss projects in my group in which we contributed to this aim to develop technologies to image the structure and dynamics of molecular machines in cells. We a) pushed the 3D resolution in multi-color towards the nanometer scale, b) increased throughput and imaging speed of the notoriously slow SMLM, c) developed reference standards for quality control and for counting of protein copy numbers in complexes and d) developed software to extract specific and quantitative information from SMLM data for biological interpretation. I will then show, how these new technologies enabled us to gain mechanistic insights into the structural organization of a complex protein machine, namely the machinery involved in clathrin-mediated endocytosis. We developed a high-throughput superresolution microscope to reconstruct the nanoscale structural organization of 23 endocytic proteins from over 100,000 endocytic sites in yeast. This allowed us to visualize where individual proteins are localized within the machinery throughout the endocytic process and resulted in a model of how the force is produced to pull in the membrane and form a vesicle. In mammalian cells, we could address a long-standing question how the clathrin coat is formed and the membranes are deformed during vesicle formation.

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Quantification of filament structures in superresolution and expansion microscopy.

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Salle Méditerranée – Cours plénier

A wide variety of microscopy images in cell biology contain dense networks of curvilinear structures. Examples include images of cytoskeleton filaments (microtubules, intermediate filaments), filopodia, neurites, as well as kymographs of timelapse videos of intracellular transport. Precise quantitative measurement of parameters of these networks is an important step for understanding the architecture and structure of cells and the underlying dynamic processes. However, frequent intersections of lines represent the most challenging part in tracing these dense networks. A typical approach for resolving intersections consists in an application of a sophisticated linking algorithm, connecting free ends of line based on a set of rules and penalty functions. Here, we apply Fourier transform decomposition based on curvelets for filtering of an original image and extracting its directional components. It removes intersection problem and substantially simplifies the linking algorithm. We apply the developed approach for tracing microtubules in superresolution and expansion microscopy images and for tracking movements of neuronal cargo in live-cell movies. Our results show that this approach outperforms current tracing methods and has a variety of applications for microscopy image analysis.

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Dimanche 7 novembre 8h30

From images to information: enhancing resolution and improving accuracy in SMLM.

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Salle Porquerolles – Cours parallèle – VISIO

SMLM microscopy can provide huge amounts of high resolution information about a sample. Deducing whether that information is accurate, and making best use of the information, is a major challenge. Here we discuss two major challenges. First, assessing the quality of information, and in particular the potential presence of sharpening artifacts. We have developed HAWKMAN, an image assessment tool which allows the lengthscale of sharpening artifacts to be identified. Second, it is possible to synthesise information from multiple images to improve the quality of reconstructed structures. We will discuss the potential of deep learning to accelerate model-free fitting.

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Computational microscopy by PSF engineering – or – how and why to ruin a perfectly good microscope.

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Salle Porquerolles – Cours parallèle

In localization microscopy, the positions of individual nanoscale point emitters (e.g. fluorescent molecules) are determined at high precision from their point-spread functions (PSFs). This enables highly precise single/multiple-particle-tracking, as well as super-resolution microscopy, namely single molecule localization microscopy (SMLM). To obtain 3D localization, we employ PSF engineering – namely, we physically modify the standard PSF of the microscope, to encode the depth position of the emitter. In this talk I will describe how this method enables unprecedented capabilities in localization microscopy; specific applications include dense emitter fitting for super-resolution microscopy[1,2], multicolor imaging from grayscale data[3], volumetric multi-particle tracking/imaging[2], dynamic surface profiling[4], and high-throughput in-flow colocalization in live cells5. We often combine the optical encoding method with neural nets (deep-learning) for decoding, i.e. image reconstruction; however, our use of neural nets is not limited to image processing - we use nets to design the optimal optical acquisition system in a task-specific manner[2,3].

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2. Nehme, E. *et al.* DeepSTORM3D: dense 3D localization microscopy and PSF design by deep learning. Nat. Methods 17, 734–740 (2020).
3. Hershko, E., Weiss, L. E., Michaeli, T. & Shechtman, Y. Multicolor localization microscopy and point-spread-function engineering by deep learning. Opt. Express (2019). doi:10.1364/oe.27.006158
4. Gordon-Soffer, R. *et al.* Microscopic scan-free surface profiling over extended axial ranges by point-spread-function engineering. Sci. Adv. 6, eabc0332 (2020).
5. Weiss, L. E. *et al.* Three-dimensional localization microscopy in live flowing cells. Nat. Nanotechnol. 15, 500–506 (2020).

Dans la même thématique

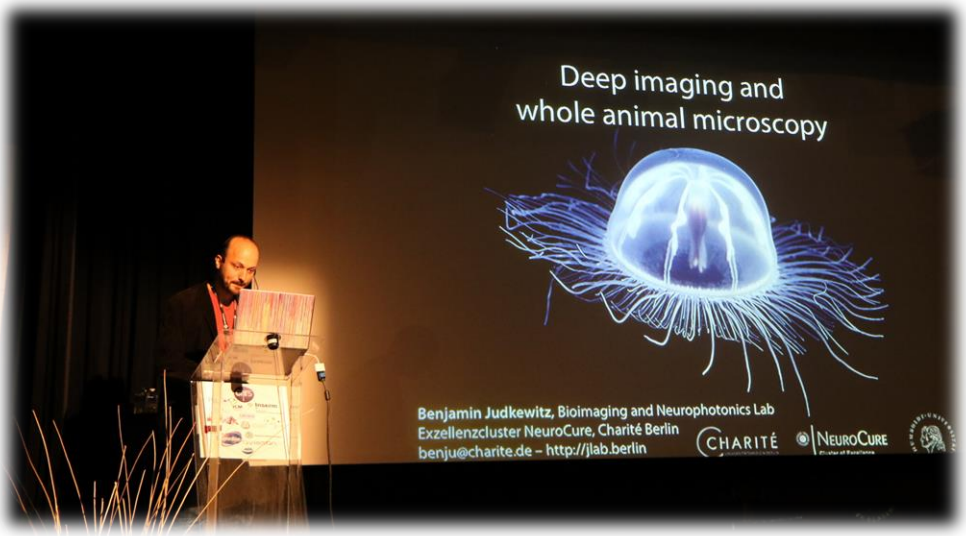
Tables rondes :

TR2.02 Bilan du module 2 Nanoscopie avec les intervenants
TR2.12 SMLM au-delà de l'esthétique ?

Ateliers :

A002-3D STED microscopy for nanoscopic imaging of virus-host cell interactions.
A003-Multicolor and 3D STORM to study localization of proteins adressed to apical membrane of polarized epithelial cells
A006-AFM on microbial surfaces : from imaging to single-cell force measurements
A013-Comparing multicolor Single-Molecule Localization Microscopy strategies: Application to the neuronal cytoskeletonA020-Expansion microscopy imaging with a lattice light-sheet microscope
A029-Imagerie ultrastructurale 3D par ultramicrotomie in-situ (technique SBF-SEM)
A045-Ultrastructure cellulaire par microscopie d'expansion
A046-Comment adapter son microscope TIRF pour faire du STORM!
A056-Microscopy on thin resin sections: multimodal and correlative approaches using scanning electron microscopy.
A057-Introduction to single molecule localization super-resolution microscopy (SMLM)
A062-Contrôle qualité des données brutes de SMLM en temps réel
A071-Microscopie d'expansion : stratégies et astuces pour l'analyse des cellules de mammifères en culture, de la levure S. cerevisiae et pour la visualisation de l'organisation mitochondriale

A074-3D High Resolution imaging by PSF engineering using ZOLA-3D
A076-Alternative strategies to image multiple proteins in single molecule localization microscopy
A094-Modulated excitation for enhanced localization : ModLoc
A097-Nuclear Pores Complexe : a tool for metrology in Single Molecule Localization microscopy
A118-Relative localization of dendritic spine proteins in mouse brain tissue using 3D-STED microscopy and deconvolution.
A123-Atomic Force Microscopy analysis of SARS-CoV-2 virus-like particles and producing cells: nanoscale imaging to mechanical characterization
A128-Mechanical characterisation by AFM of murine oocytes to predict their fitness
A129-AFM on microbial surfaces: basics of force spectroscopy measurements
A132-Structured Illumination Microscopy : SIM on cells !
A135-Imaging multiprotein complexes in the cytosol by super-resolution fluorescence. Introduction to lattice and dual iterative SIM
A138-Colocalisation de complexes macromoléculaires en super-résolution PALM/STORM
A139-Confronting Lattice SIM imaging to various scattering samples of different thickness



MODULE 3 : INTELLIGENCE ARTIFICIELLE POUR L'IMAGERIE BIOLOGIQUE

Coordination : Thomas Walter, Perrine Paul-Gilloteaux et David Rousseau

Introduction

Artificial intelligence, and in particular deep learning, is becoming an important part of our analysis methods. Its applications cover the tasks of image restoration, segmentation and analysis of extracted data. During this module we will try to have an overview of the developments in this direction, the efforts of the community to make these methods more accessible, but also the current limitations and points of attention. We will also compare these methods with analytical methods, for a reasoned and controlled use of artificial intelligence in our community. Numerous workshops will also be proposed during an associated thematic course

Samedi 6 novembre 8h30

Microscopy Image Analysis: The Shift to Deep Learning?

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The quantification of microscopy images requires automatic tools to extract relevant information from complex data. To tackle this task, numerous image analysis algorithms have been designed, commonly based on prior knowledge and on physical modeling. However, the recent success of the deep learning (DL) in computer science have drastically changed the bioimage analysis workflows to a data-centric paradigm. While this DL technology remains relatively inaccessible to end-users, recent efforts has been proposed to facilitate the deployment of DL for some bioimage applications through new open-source software packages.

Here, we present a set of user-friendly tools that allows to test DL models and to gain proficiency in DL technology: the centralized repository of bioimage model (Bioimage Model Zoo), the ready-to-use notebooks for the training, and the plugin deepImageJ that can run a DL model in ImageJ.

We provide also good practice tips to avoid the risk of misuses. We address some practical issues such as the availability of massive amount of images, the understanding of generalizability concept, or the selection of the pre-trained models. The shift to deep learning also questions the community about the trust, the reliability and the validity of such trained deep learning models.

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Multiscale and multimodal registration: an overview of methods.

Perrine Paul-Gilloteaux,
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Registration in image analysis and computer vision is the process of finding and applying the transformation linking two images/volumes, by optimizing the matching between either intensity, either known matching structures. It has a large panel of application in microscopy (time drift compensation, tomographic reconstruction, stitching for mosaicking …). In this talk I will focus on its usage in correlated multimodal imaging [1]. Two main approaches can be considered. First, considering the full content of the images and trying to find a common representation intensity space, by known filters or deep learning, to be able to use monomodal approaches and metrics, or to define a metric that would take into account the possible discrepancy [2]. The second approach is considering elements of interest extracted from both modalities, for example anatomical landmarks (points or shape of interest) or multimodal markers visible in both modalities [3]. These approaches, generally called feature-based registration, are of particular interest when the relation between content is unknown or cannot be taken as an assumption (for example for the validation of a new probe or a new imaging modality). Some approaches mixed both feature-based and full registration by restraining the learning data set to registered features [4]. Additional questions that will be tackle in this talk are the way to estimate errors in registration [5], but also how to exploit the information brought by the fusion of two or more modalities at very different scales.

[1] A. Walter *et al.*, « Correlated Multimodal Imaging in Life Sciences: Expanding the Biomedical Horizon », Front. Phys., vol. 8, p. 47, avr. **2020**, doi: 10.3389/fphy.2020.00047.
[2] X. Cao, J. Yang, L. Wang, Z. Xue, Q. Wang, et D. Shen, « Deep Learning Based Inter-modality Image Registration Supervised by Intra-modality Similarity », in Machine Learning in Medical Imaging, vol. 11046, Y. Shi, H.-I. Suk, et M. Liu, Éd. Cham: Springer International Publishing, 2018, p. 55-63. doi: 10.1007/978-3-030-00919-9_7.
[3] P. Paul-Gilloteaux *et al.,* « eC-CLEM: flexible multidimensional registration software for correlative microscopies », Nat. Methods, vol. 14, no 2, p. 102-103, 31 2017, doi: 10.1038/nmeth.4170.
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[5] G. Potier, F. Lavancier, S. Kunne, et P. Paul-Gilloteaux, « A Registration Error Estimation Framework for Correlative Imaging », in 2021 IEEE International Conference on Image Processing (ICIP), Anchorage, AK, USA, sept. 2021, p. 131-135. doi: 10.1109/ICIP42928.2021.9506474.

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Self-Supervised Deep Learning for Fluorescence Imaging and nD Image Viewing with Napari.

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Deep learning has ushered a revolution in the way that we process and analyse images. However, this novel technique has an Achilles heel: it typically requires ground-truth and yet this ground truth is often difficult to obtain in the bioimage domain. In our team we have developed techniques to avoid the need for ground truth: Noise2Self for blind image denoising, SSI for self-supervised deconvolution, and Cytoself -- a new approach to discover and classify the rich landscape of protein subcellular localisation. In addition, I will present Napari, an nD image viewer in Python that that I co-created with Juan Nunez Iglesias and currently further developed by Nick Sofroniew at CZI. Napari aims to become the platform of choice for image visualisation, processing and analysis for Python with a standalone app, plugin infrastructure and a large inclusive open source community.

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Microscopy image analysis with machine learning.
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Modern microscopy modalities produce increasingly large volumetric image datasets which are difficult to interpret without the help of computational methods. In the last years machine learning (deep learning) based methods have seen widespread use in image analysis applications for microscopy and for the automatic detection and segmentation of cells, nuclei, and organelles in light and electron microscopy images. In my lecture I will give an overview of modern machine learning approaches to these common problems such as image restoration and nuclei/cell segmentation. Furthermore, I will give an introduction to different software frameworks that we develop to make these methods available to the microscopy research community and which we demonstrate on examples ranging from nuclei detection in fluorescence microscopy, tissue classification in histopathology to the comprehensive organelle reconstruction of whole cells in 3D electron microscopy.

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Samedi 6 novembre 16h10

Deep Learning with Medical Images: learning with small datasets and few annotations.
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This talk will describe some of today's bottlenecks when designing deep learning approaches for medical image analysis and review recent alternatives to overcome them. The main focus will be on image classification and segmentation problems confronted with small datasets and few or noisy annotations. In the past years, our team has proposed different advances towards addressing the above issues. The first alternative incorporates prior domain knowledge, which helps design a curriculum to guide the optimization[1][2], or allows simulating data for self-supervised pre-training [3]. We also explore spatial and temporal dependencies in sequential data [4][5] or rely on anatomical consistencies across image modalities through domain adaptation [6]. These methodological contributions will be illustrated in the context of biomedical applications. The talk will close with remarks about current open challenges and ongoing works.

[1] A Jiménez-Sánchez, D Mateus, S Kirchhoff, C Kirchhoff, P Biberthaler, Nassir Navab, Miguel A González Ballester, Gemma Piella. Medical-based Deep Curriculum Learning for Improved Fracture Classification. Int. Conference on Medical Image Computing and Computer-Assisted Interventions (MICCAI). Springer, Cham, 2021.
[2] Amelia Jiménez-Sánchez, Diana Mateus, Sonja Kirchhoff, Chlodwig Kirchhoff, Peter Biberthaler, Nassir Navab, Miguel A. González Ballester, Gemma Piella. Curriculum learning for annotation-efficient medical image analysis: scheduling data with prior knowledge and uncertainty. arXiv:2007.16102 (under revision)
[3] Tardy M, Mateus D. Looking for abnormalities in mammograms with self-and weakly supervised reconstruction. IEEE Trans Med Imaging. 2021 Jan 8
[4] Duque, Vanessa Gonzalez, et al. "Spatio-temporal consistency and negative label transfer for 3d freehand us segmentation." International Conference on Medical Image Computing and Computer-Assisted Intervention. Springer, Cham, 2020.

[5] Al Chanti, Dawood, et al. "IFSS-Net: Interactive Few-Shot Siamese Network for Faster Muscle Segmentation and Propagation in Volumetric Ultrasound." IEEE Transactions on Medical Imaging (2021).
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Extracting the invisible from live cell microscopy.

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Cell imaging has entered the "big data" era with high content and multidimensional data sets encapsulating complex and dynamic patterns that are inaccessible by human visual observation and that are absolutely necessary for taking our understanding of the cell's structure and function to the next level[1]. Motivated by fundamental questions in cell biology my lab produces biological insights along with specialized analytic tools that reveal hidden patterns in dynamic cell imaging data. I will demonstrate these concepts with several specific projects on interpretable Artificial Intelligence in the context of cancer metastasis[2] and multicellular information processing[3-5].

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[2] Zaritsky, A., Jamieson, A.R., Welf, E.S., Nevarez, A., Cillay, J., Eskiocak, U., Cantarel, B.L. & Danuser, G. Interpretable deep learning uncovers cellular properties in label-free live cell images that are predictive of highly metastatic melanoma. *Cell Syst* (2021).
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[4] Zamir, A., Li, G., Chase, K., Moskovitch, R., Sun, B. & Zaritsky, A. Emergence of synchronized multicellular mechanosensing from spatiotemporal integration of heterogeneous single-cell information transfer. *BioRxiv* (2020).
[5] Nahum, A., Koren, Y., Natan, S., Goren, S., Lesman, A. & Zaritsky, A. Quantifying cell-cell mechanical communication through fibrous environments. *BioRxiv* (2020).

Dans la même thématique

Tables rondes :

TR-3-01-Retour sur l'ANF du RT-MFM « Deep-learning pour les microscopies »;
TR-3-10-Comment l';IA impacte-elle notre travail ?
TR-3-13-Clôture du parcours thématique : Deep learning pour l'analyse d'images de microscopie

Symposium :

S02-03 - Organisation et dynamique moléculaire : qu'apporte le deep learning aux analyses ?

Ateliers :

A005-Deep learning avec les doigts dans le moteur (2/2)
A012-3D quantitative analysis of colocalisation or spatial coupling in conventional and super resolution microscopy.
A014-Deconvolution 3D

A015-Deep learning sans se salir les doigts (1/2)
A016-Deep bar à images
A018-Obtenir la PSF d'un système de microscopie de fluorescence
A050-Multiplexed FRET biosensor imaging to quantify the dynamic coordination between cell signaling and mechanics during collective cell migration (part II).
A052-Quantitative 3D Spatial Analysis of multicellular specimens (Organoids w/o clearing)
A054-Le machine learning au service la déconvolution
A055-Image Analysis Flash Tutorials
A058-Probabilistic pipeline to extract reliable information from single molecule microscopy data.
A059-Coordinate-based quantification of multidimensional and multicolor single-molecule localization microscopy data.
A061-Acquisitions intelligentes sous micromanager
A077-BIAFlows
A078-BioImage-IT: Implement image processing workflows with tools from multiple software
A102-Publishing FAIR-ly with OMERO
A112-Virtual reality for multidimensional data visualization and analysis
A113-An example of feedback microscopy: Developing a High Content Screening (HCS) Optogenetics experiments.
A114-Deep learning made easy for microscopy: an introduction to ZeroCostDL4Mic and DeepImageJ
A116-Bioimage Analysis: Practice Deep Learning Without Coding
A124-QuPath: pyramid image analysis for everyone. Case study: Deep-learning cell counting and quantification of histological slides.
A125-Speckle-based computational microscopy : harnessing scattering for enhanced imaging of tissues
A133-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
FT1-3D Reconstruction, visualisation and movie creation
FT2-Cell count and cell spatial arrangement
FT3-Cell volumetric and morphology
FT4-Colocalisation
FT5-Neuron tracing and Spine Classification
FT6-Tracking biology over time
FT7-Object classification with Machine Learning
MA-3-01-Strengths and Limits of Deep Learning for Image Restoration in Microscopy

MODULE 4 : IMAGERIE MULTICELLULAIRE : ORGANOÏDES, TISSUS, EMBRYONS

Coordination : Gaelle Recher et Lydia Danglot

Introduction

Whether natural (embryos of model species, tissue explant, live animal) or synthetic (engineered cell assemblies as spheroids, organ-on-chip or bioprinted tissues), imaging multicellular cell assemblies is especially cumbersome. Approaches require to take into account light penetration in depth, suitable staining or contrast generation methods as well as robust 3D optical sectioning with low cytotoxicity.

The question of scale is as well at stake. A holistic approach has to be implemented, because no matter the structure of interest being at the scale of the molecule, the cell or a cell population: the entire tissue structure has to be considered as a whole. Conciliation of irreconcilable dimensions is a permanent challenge: spatial resolution, field of view, multimodalities, temporal resolution, observation length and possibly high-throughput. And this is also even more complex when live imaging has to be considered since multi-color imaging and multi-dimensional illumination is a source of cytotoxic free radicals.

In this module, speakers will present how they tackle the question of imaging multicellular samples. They will focus on the design of the samples themselves, with microfluidics, directed tissue fabrication, 3D-printing and hydrogel customisation as well as on the development of new microscopy concepts from the use of structured illumination and computational approaches to the implementation of ultrafast and low toxicity imaging.

Mercredi 10 novembre 8h30

Sometimes, there IS a free lunch: How to get twice the resolution from your microscope, without (serious) drawbacks.

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Salle Méditerranée– Cours parallele **VISIO**

Structured Illumination Microscopy (SIM) with stripes [1, 2] or spots [3, 4] gives a modest but useful improvement in resolution: about 2x. Unlike other "superresolution" techniques, SIM doesn't rely on unusual photophysics, so it works for a wide range of samples, and doesn't substantially degrade speed, penetration depth, gentleness, or signal-to-noise ratio. Originally, SIM was a computational technique, fusing multiple raw camera exposures into a single resolution-doubled image. However, in ~2013, several groups [5, 6, 7] realized that this image fusion algorithm could also be implemented by optics instead of computers! This all-optical processing allows "instant" structured illumination microscopy in a single camera exposure (or even viewed by the human eye through eyepieces), yielding the first superresolution technique with no drawbacks. Many modern microscopes now include this idea, including the Yokogawa SoRa, the VisiTech iSIM, and the Confocal.nl RCM.

[1] https://doi.org/10.1046/j.1365-2818.2000.00710.x
[2] https://doi.org/10.1117/12.336833
[3] Optik 80.2 (1988): 53-54.
[4] https://doi.org/10.1103/PhysRevLett.104.198101
[5] https://doi.org/10.1038/nmeth.2687
[6] https://doi.org/10.1364/BOE.4.002644
[7] https://doi.org/10.1186/2192-2853-2-5

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3D printing and bioprinting for the development of microenvironment and tissue models.

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Salle Méditerranée– Cours parallèle VISIO

A major challenge in the field of biology and medicine is the development of artificial matrices and in vitro models allowing the growth of cells and tissues in controlled microenvironments. The systems most currently used in research are far from the level of heterogeneity of the natural microenvironments of cells, which are organized in heterogeneous three-dimensional architectures. Novel strategies based on bio-printing technologies (also referred to as additive manufacturing) appear as a new paradigm in this field. Current strategies are devoted either to the fabrication of artificial scaffolds acting as a mechanical support for soft tissues or either to the direct printing of cells seeded within an extracellular matrix. This latter approach is intriguing as it can be used to mimic the topography of the natural cell microenvironment together with its cellular heterogeneity (i.e. controlling the position of cells in the 3D structure). The targeted goal consists in organizing directly differentiated cells and ultimately undifferentiated stem cells on a 3D biodegradable scaffold that will direct their fate: the scaffold should induce cell spreading and differentiation while promoting the formation of a functional tissue. As a major advantage of such bio printing approach, tissues can be directly fabricated using numerical data obtained directly from real tissue samples through advanced imagery techniques (e.g. CT scans, histopathology or confocal images of tissues, etc). After introducing the basic concepts of bioprinting, this presentation will give an overview of the latest technologies and developments performed in the field of 3D printing and bioprinting for the creation of tissue models. Strategies based on the creation of high-resolution scaffolds, porous bioreactors will first be addressed through several examples of application in the field of cancer study and regenerative medicine. Then the presentation will be focused on the use of bioprinting for the construction of tissue models with a special focus on the reconstruction of heterogenous and self-evolving tissues using various building blocks including stem cells, cell aggregates, spheroids and organoids.

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SCAPE microscopy for high-speed 3D imaging.

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Salle Méditerranée – Cours plenier **VISIO**

In growing numbers of model organisms, fluorescent indicators of cellular activity can enable optical monitoring of large populations of neurons. However, capturing this activity *in-vivo*, in 3D and in real-time has remained a significant challenge for conventional point-scanning microscopes.

SCAPE microscopy is a high-speed light-sheet imaging method that permits cellular-level 3D imaging at over 100 volumes per second[1,2]. Combining low phototoxicity with a simple, single stationary objective lens, SCAPE permits high-speed microscopy that is well-suited to the study of behaving small model organisms such as *C. elegans* worms, zebrafish larvae and adult and larval Drosophila. The method can also be applied to high-speed, 3D functional imaging of the intact, living rodent brain, achieving 400 micron penetration depth when using two-photon excitation. By capturing real-time activity across large volumes of the brain during natural behaviors, without needing averaging over multiple repeated trials or tasks, SCAPE microscopy permits the application of modelling and analysis techniques to understand network-wide neural representations of complex and spontaneous behaviors, and could potentially provide new insights into the brain's representation of internal state. This approach also enables high-throughput structural imaging of fresh, fixed, cleared of expanded tissues of unlimited lateral extent.

1. Bouchard, M.B. *et al.* Swept confocally-aligned planar excitation (SCAPE) microscopy for high speed volumetric imaging of behaving organisms. *Nature photonics* **9**, 113-119 (2015).
2. Voleti, V. *et al.* Real-time volumetric microscopy of in vivo dynamics and large-scale samples with SCAPE 2.0. *Nature methods* **16**, 1054-1062 (2019).

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Organ on chip, a new generation of in vitro models

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Stephanie.Descroix@curie.fr

Salle Méditerranée – Cours plenier

The development of a new generation of *in vitro* models is of interest in different fields such as basic research in life science to decipher physiological and patho-physiological mechanisms or in industry to drastically improve drug screening process. Organ on chips aim at recapitulating on chip the main features of an organ. Microfluidics thanks to its remarkable properties and versatility is now considered as key technology for the development of such miniaturized microphysiological systems. In this presentation, we will demonstrate how microfluidics and microfabrication can be used to develop new relevant *in vitro* models and how imaging is pivotal to characterize such devices. We will in particular focus on a gut on chip model that integrates both the epithelial and stromal compartments.

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Jeudi 11 novembre 8h30

Quantifying transport and efficacy of therapeutics in spheroids.

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Salle Porquerolles– Cours parallèle

Despite a growing number of innovative therapeutic strategies and a better understanding of cancer cell biology, the vast majority of drug candidates fail in clinical trials. This failure is partially due to the fact that it remains difficult to model and predict the transport and accumulation of therapeutics *in vivo*, within the tumour. To move beyond the classical 2D plastic dishes, different 3D

in vitro models have been developed to try to better replicate *in vivo* the complexity of the tumour microenvironment. Among them, multicellular tumour spheroids recapitulate many tumour features. However, the classical approaches to produce spheroids suffer from low yield, slow process, difficulties in spheroids manipulation and compatibility with high-magnification fluorescence optical microscopy. On the other hand, spheroid-on-chip set-ups developed so far require a practical knowledge of microfluidics difficult to transfer to a cell biology laboratory. We have developed a simple yet highly flexible 3D model microsystem consisting of agarose-based microwells[1]. Fully compatible with the multi-well plate format conventionally used in cell biology, our simple process enables the formation of hundreds of reproducible spheroids in a single pipetting. Immunostaining and fluorescence imaging including live high-resolution optical microscopy can be performed *in situ*, with no manipulation of spheroids.

As a proof of principle of the relevance of such an *in vitro* platform, I will first present results where the kinetics of transport and localisation of nanoparticles were investigated within colorectal cancer spheroids[2]. Our approach is also fully compatible with primary cells from patients, and was recently used to screen patient-derived Gonadotroph Tumours (GoTs). I will then introduce a new collaborative work where we have integrated our agarose-based microsystems in a microfluidic platform enabling the electroporation of a large number of spheroids in parallel.

Combined with optical and digital clearing[3], our approach opens up the possibility to resolve tumour heterogeneity, at the single cell level, in a physiological context.

1. C. Riviere *et al.*, Plaques de micropuits en hydrogel biocompatible. *Patent FR3079524A1*, https://patents.google.com/patent/FR3079524B1/en. (2018)
2. Goodarzi *et al*., Quantifying nanotherapeutic penetration using a hydrogel-based microsystem as a new 3Din vitroplatform. *Lab-on-chip,* **21 (13)**, 2495-2510 (2021).
3. A. Ahmed *et al*., Clearing spheroids for 3D fluorescent microscopy: combining safe and soft chemicals with deep convolutional neural network. *bioRxiv*, (2021)

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3D-printed minimally assembled interchangeable LSFM chamber for serial imaging of organoids and spheroids.

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Salle Porquerolles – Cours parallèle **VISIO**

Light sheet fluorescence microscopy (LSFM) is the technology of choice for imaging large organs and three-dimensional cell cultures. However, the geometry of conventional LSFM set-ups does not allow operations that are common in microscopy, such as exchanging the objective lens, or quickly remove a substrate plate and serially replacing with other ones. Although Oblique Plane Microscopy (OPM) solves many of these issues by integrating light sheet illumination in an inverted microscope, it requires a very precise alignment to not compromise the overall NA. Moreover, OPM does not allow for a straightforward multiangle imaging, which is quite important for the analysis of large specimens *in toto*. Thus, finding a way to easily change chamber or objective lens in a conventional LSFM set-up for the serial imaging of multiple specimens is a highly desirable feature for biologists. We present a new LSFM chamber that is easily detached from the set-up without removing neither the immersion water nor the specimen inside. The chamber with its specimen can be placed back in the incubator and replaced in the microscope with another one. This allows serial

imaging of different specimens during the same experiment. The construction of the chamber itself exploits several innovative approaches: fully 3D printed as a block, “minimally assembled” (meaning that no screws, O-ring, or other parts are used to assemble and seal the chamber), “panoramic” observation window. We demonstrate the application of the new chamber for the imaging of organoids and spheroids.

Dans la même thématique

Modules avancés /Tables rondes :

TR-4-04-Which light-sheet microscope for gentle live imaging ?
TR-4-11-Imagerie multicellulaire : organoïdes, tissus, embryons, quelles nouveautés pour l’imagerie des tissus épais

Ateliers :

A011-Etude de la vascularisation sanguine et lymphatique de l’épididyme : clarification d’organe, microscopie à feuille de lumière et quantification en trois dimensions
A025-Exploitation combinée d’une sonde et d’une caméra Infra rouge (SWIR) pour imager le réseau vasculaire d’un muscle entier de souris dans la fenêtre NIR II.
A032-Immunomarquage et transparisation de tissus entiers, acquisition avec système home-made vs commercial
A035-Studying organogenesis of the pronephros of Xenopus tadpole early stages using light sheet and confocal microscopy
A037-Imaging transcription at a high temporal resolution in a living organism.
A063a-Transparisation, acquisition au microscope à feuillet de lumière et post traitement de sphéroïdes
A063b-Transparisation, acquisition au microscope à feuillet de lumière et post traitement de sphéroïdes
A065-Getting the most out of 3D pheroids by combining microfabricated wells, clarification techniques, standard confocal imaging and deep learning image processing
A072-Evolution of brain morphology from invertebrates to mammals. Everything we can learn from in toto 3D imaging of autofluorescence signals ?
A073-Microscopie plein champ « haute résolution » et traitement numérique sur échantillon épais
A075-Adaptive optics fluorescence microscopy for biological imaging
A079-Bioprinting as a solution for recreating a physiological environment on a slide
A100-Préparation d’échantillon, acquisition et analyse d’image pour les applications de culture cellulaire 3D
A106-Serial Block Face Imaging: imagerie 3D sans marquage de gros échantillons
A107-Standardization of organoids culture allowing high throughput 3D live imaging using sospim technology
A119-L’autofluorescence chez les plantes: adversaire ou alliée?
A120-Imagerie sur petit organoides 3D, Troubleshooting
A127-Microscopie large champ sur mésoscope homemade
A131-Imagerie 3D d’organes transparisés par microscopie à feuille de lumière
A137a-Imagerie de fluorescence par microscopie confocale spinning-disk : une exploration des avantages et limitations techniques sur 4 systèmes (en 2 parties)

A137b-Imagerie de fluorescence par microscopie confocale spinning-disk : une exploration des avantages et limitations techniques sur 4 systèmes (en 2 parties)
A140-Du plus petit échantillon au plus gros, la microscopie à feuille de lumière face à l'enjeu du multi-échelle
A141-Medium throughput imaging of thick samples: a practical comparison of different samples (Drosophila tissues and encapsulated spheroids) in native opaque state and after light-penetration facilitation
A142-Microscopie à feuillet de lumière pour l'imagerie volumique
A143-Imagerie à feuillet de lumière des échantillons 3D montées d'une manière peu contraignante

Teaser MiFoBio: www.youtube.com/watch?reload=9&v=Bnpx5JGUlyA

MODULE 5 : ONDES SUR LE VIVANT (AVEC GDR ONDES)

Coordination Sophie Brasselet, Pierre Bon et Olivier Haeberle

Introduction

Within this module coupled with the GDR "Waves", we will explore the interest of measuring and controlling the physical properties of light for imaging in biology, as well as the methods to manipulate them. This approach is indeed one of the key research and development avenues to design the imaging systems of tomorrow. In particular: accessing quantitative, deep, fast information, detecting ever smaller events/objects, doing without labeling, adapting to situations where the amount of information is reduced or the signal is weak... are some of the paths that will be described during this module.

Lundi 8 novembre 8h30

Measuring and shaping the phase of light: key applications in biology.

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Salle Méditerranée– Cours parallèle

In recent decades, the advent of spatially-resolved techniques to control and image the phase of light waves has deeply transformed microscopy. In this talk, I will first demystify the notions of optical phase or wavefront, and present several important examples of phase shaping in microscopy (Light-sheet, STED, ...), with a focus on the use of holograms for neurons stimulation (optogenetics) and temperature control at the microscale [1] After discussing some limits of the standard phase shaping techniques, I will introduce a novel concept where the transmitted light is shaped using thermo-optics.[2] I will explain how this simple concept can provide arrays of electrically tunable lenses, but also complement the existing optical shaping toolbox by offering low-chromatic-aberration, polarization-insensitive micro-components. We will finally discuss the potential of this technique for multiplane Ca^{2+} or Voltage Imaging.

The second part of this talk will be dedicated to phase imaging, and particularly Quantitative Phase Imaging (QPI). Phase-contrast microscopies are well known for their ability to reveal rich images related to refractive index inhomogeneities, and thus improve contrast in cells, which are essentially transparent and barely visible in brightfield microscopy. I will describe the recent research field of Quantitative Phase Imaging, which has proposed valuable label-free biomedical methods to measure biophysical cell parameters, including its dry mass. In particular, I will describe how a broadband and cost-effective quantitative phase camera can be simply implemented by placing a thin diffuser in front of a standard camera.[3] I will discuss the potential of the technique for quantitative phase imaging of biological samples and for 3D nanoparticles super-localization and tracking.

[1] Liu, C., Tessier, G., Flores Esparza, S. I., Guillon, M. & Berto, P. *Reconfigurable Temperature Control at the Microscale by Light Shaping*. ACS Photonics 6, (2019).
[2] Berto, P., Philippet, L., Osmond, J., Liu, C. F., Afridi, A., Montagut Marques, M., Molero Agudo, B., Tessier, G. & Quidant, R. *Tunable and free-form planar optics.* Nature Photonics 13, 649–656 (2019).
[3] Berto, P., Rigneault, H. & Guillon, M. *Wavefront sensing with a thin diffuser*. Opt. Lett. 42, 5117 (2017).

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X-ray coherent diffraction imaging: 3D exploration of biologically relevant hard and soft tissues.

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Imaging tri-dimensional complex materials at the nanoscale is a major challenge of nanoscience, which calls for a microscopy method combining sensitivity to density distribution, in situ compatibility and high spatial resolution. In this context, the recent advents of x-ray lens-less imaging methods, based on coherent diffraction, have opened promising perspectives[1] as they fill the gap between direct microscopies (AFM, SEM, TEM) and reciprocal-space based x-ray scattering or Bragg diffraction analysis. This family of modalities have been proposed to circumvent the lack of efficient focusing optics in the x-ray regime. They are based on the acquisition of far-field coherent intensity patterns, from which the phase of the diffracted field is retrieved with inversion iterative algorithms, providing access to truly quantitative information in the object plane, such as the density distribution inside the sample or the lattice distortions in a crystalline material.
In this presentation, I will review two major modalities of coherent diffraction imaging (CDI) methods: finite-sized sample CDI[1, 2] and ptychography[3, 4]. Their principles will be introduced, together with their implementations at synchrotron sources. Finally, a series of recent examples will be described to illustrate the interest in life science related problems.

[1] D. Shapiro *et al.* Biological imaging by soft x-ray diffraction microscopy. Proc. Natl Acad. Sci. USA. 102, 15343-15346 (2005).
[2] M. M. Seibert *et al*. Single mimivirus particles intercepted and imaged with an X-ray laser. Nature 470, 78–81 (2011).
[3] H. Tri Tran *et al.*, Alterations in Sub-Axonal Architecture Between Normal Aging and Parkinson's Diseased Human Brains Using Label-Free Cryogenic X-ray Nanotomography. Front. Neurosci. 14, 570019 (2020)
[4] F. Mastropietro et al., Revealing crystalline domains in a mollusc shell single-crystalline prism. Nat. Mater. 16, 946-952 (2017).

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Imaging the brain at high spatiotemporal resolution with wavefront shaping.

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To understand computation in the brain, one needs to understand the input-output relationships for neural circuits and the anatomical and functional properties of individual neurons therein. Optical microscopy has emerged as an ideal tool in this quest, as it is capable of recording the activity of neurons distributed over millimeter dimensions with sub-micron spatial resolution. I will describe how we use concepts in astronomy and optics to develop next-generation microscopy methods for imaging the brain at higher resolution, greater depth, and faster speed. By shaping the

wavefront of the light, we have achieved synapse-level spatial resolution through the entire depth of the primary visual cortex, optimized microendoscopes for imaging deeply buried nuclei, and developed video-rate volumetric and kilohertz functional imaging methods. We apply these methods to understanding neural circuits, using the mouse brain as our model system.

1. Rodríguez C, Chen A, Rivera JA, Mohr MA, Liang Y, Sun W, Milkie DE, Bifano TG, Chen X, Ji N. An adaptive optics module for deep tissue multiphoton imaging in vivo. *Nature Methods*, in press. (Also see *BioRxiv* (2020) https://doi.org/10.1101/2020.11.25.397968.)
2. Meng G, Liang Y, Sarsfield S, Jiang WC, Lu R, Dudman JT, Aponte Y, Ji N. High-throughput synapse-resolving two-photon fluorescence microendoscopy for deep-brain volumetric imaging in vivo. *Elife* (2019) e40805.
3. Lu R, Sun W, Liang Y, Kerlin A, Bierfeld J, Seelig J, Wilson DE, Scholl B, Mohar B, Tanimoto M, Koyama M, Fitzpatrick D, Orger MB, Ji N. Video-rate volumetric functional imaging of the brain at synaptic resolution. *Nature Neuroscience* (2017) **20**, 620-628.
4. Lu R, Liang Y, Meng G, Zhou P, Svoboda K, Paninski L, Ji N. Rapid mesoscale volumetric imaging of neural activity with synaptic resolution. *Nature Methods* (2020) **17**, 291–294.
5. Wu J, Liang Y, Chen S, Hsu C-L, Chavarha M, Evans SW, Shi D, Lin MZ, Tsia KK, Ji N. Kilohertz two-photon fluorescence microscopy imaging of neural activity in vivo. *Nature Methods* (2020) **17**, 287–290.

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Volumetric imaging at high speeds.

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Obtaining high-resolution images from extended 3D volumes has been a longstanding challenge in the microscopy community. This challenge is particularly acute when high-speed imaging is required, such as when performing voltage imaging in brain tissue. I will describe different techniques we have developed to address this challenge, for both scanning and camera-based microscopes.

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Mardi 9 novembre 14h00

Gouy and Brown to the rescue: Label-free virus detection and virus-antibody interaction monitoring by common-path interferometry.

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Salle Porquerolles – Cours parallèle

In this talk, we will present a new, sensitive, interferometric, and non-destructive optical approach to detect, count and sort different types of label-free, biotic and non-biotic single nanoparticles (NPs) in aqueous media [1]. In short, we measure the light scattered by the nanoparticles and obtain an interferometric signal arising due to the Gouy phase shift and related to the NP size and refractive index; this measurement is complemented with single particle tracking analysis of their Brownian motion in order to determine the NP size and nature, allowing to discriminate objects of the same size like for example viruses and membrane vesicles.

Thanks to the common-path and transmission configuration of our microscope, the detection of small virus and NPs is robust and insensitive to spherical aberrations produced by the

index mismatch of the environment. This opens important perspectives to study virus infection and target-search strategies without the need of fluorescence labelling. With this approach, we have developed an assay based on antibody recognition of targeted virus in which we associate changes in diffusion to antibody recognition and possibly aggregation. We have applied this approach to different bacteriophages and mammalian virus, with antibodies targeting different surface proteins, using only primary antibodies or a combination of primary and secondary antibodies. In all cases, we have observed a significant change in diffusion and an increase in signal strength and number of detected particles at the time mark of around one minute, and a decrease of number of detected particles at longer time scales indication aggregation (tens of minutes), validating thus our assay as a label-free tool for rapid virus identification or to study antibody-antigen interactions.

1. Boccara *et al*, Biomed. Opt. Exp. 2016

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Polarized microscopy resolves protein's organization in cells.

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Salle Porquerolles – Cours parallèle

Fluorescence and nonlinear microscopy imaging reveal important biological processes occurring in cells and tissues from fixed situations to in vivo dynamics. Optical imaging cannot however directly access the way molecules are organized in specific structures occurring at the molecular scale. This property, which is important in many fields in cell biology, embryogenesis or biomechanics, is today most often studied using electron microscopy (EM) or X ray diffraction, which are not compatible with real time imaging. We will show that reporting molecular orientation and protein's organization in protein filaments, aggregates or lipid membranes down to the nano-scale is made possible using polarization resolved optical microscopy, which takes advantage of the orientation-sensitive coupling between optical excitation fields and molecules.

We will show an overview of polarization sensitive approaches in fluorescent microscopy, with a particular focus on super resolved methods. We will discuss the advantages of transposing polarized methodologies to scanning nonlinear optical microscopy, as well as the challenges still to overcome with respect to real time polarized live imaging.

Dans la même thématique

Ateliers :

A042-Remember your wavefront: adaptive optics and memory effect in different regimes
A090-Label-free virus detection and sorting with full-field interferometric microscopy
A093-Measuring protein's orientation and organization by polarized fluorescence and polarized super resolution imaging
A096-Multimode fiber based-endoscope for fluorescence imaging using wavefront shaping
A145-OpenUC2 – Modular Optics with a "Click"

MODULE 6 : DYNAMIQUE ET INTERACTIONS MOLECULAIRES EN CELLULES VIVANTES : EXPERIMENTATION ET MODELISATION

Coordination Cyril Favard, Ignacio Izeddin et Antoine Coulon

Introduction

This module covers the latest developments in the field of quantification, by optical microscopies, of the dynamics and interactions of biological molecules in their cellular context. In the module, these experimental approaches are paralleled by theoretical approaches that aim to model the hypotheses and/or observations.

Beyond new technologies, this module is therefore at the interface between theoretical (bio)physics, instrumentation and experimental biology (biophysics) and aims to have an integrated approach to a biological problem.

Lundi 8 octobre 8h30

Revealing spatial and kinetic details of life processes by analyzing live cell single molecule tracking data.

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Salle Porquerolles– Cours parallèle

In a living organism, numerous inanimate biomolecules and their inherently stochastic interactions are precisely orchestrated and organized in space and time, together enabling the enigma of life. By using single molecule fluorescence microscopy, we are able to monitor single molecules in their natural environment of a live cell or organism and thus obtain an exceptionally detailed view on life processes including transcription. Tracking individual molecules reveals subpopulations with distinct behaviour and yields quantitative information such as reaction rates, bound fractions, diffusion coefficients and concentrations and the spatial distribution and stoichiometry of molecules and cellular structures. Thus, we obtain information necessary for a deep understanding and modelling of life processes.

In the presentation, I will give an overview over state-of-the-art methodologies to image single molecules in live cells and organisms, i.e. zebrafish embryos, and discuss the spatial and kinetic information that can be extracted from such data. In addition to basic concepts, I will highlight some insights we obtained on the kinetic regulation of transcription and the activation of transcription in zebrafish development. For example, perturbing the interaction kinetics between a transcription factor (TF) and DNA enabled us to expand the common two-state model of gene transcription, in which the active gene releases a burst of transcripts before turning inactive again, to a model in which TF binding triggers multiple successive steps before the gene transits to the active state. Using single molecule methods, we comprehensively quantified the transition times of the TF and the gene, including the TF target search and residence times and the delay between TF binding and the onset of transcription.

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***E. coli* chromosome dynamics and the cell cycle.**

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Salle Porquerolles– Cours parallèle **VISIO**

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Mardi 9 octobre 14h00

Imaging DNA repair at the single molecule level.

Judith Miné-Hattab[1], Mathias Heltberg[2,1], Marie Villemeur[1], Chloé Guedj[1], Thierry Mora[2], Aleksandra Walczak[2], Maxime Dahan[3], Angela Taddei[1], Fabiola Garcia Fernandez[1], Sébastien Huet[4]
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[3] *Institut Curie, PSL University, Sorbonne Université, CNRS, Physico Chimie Curie, France;*
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Our genome is constantly damaged by a variety of exogenous and endogenous agents. Among the various forms of DNA damage, double-strand breaks (DSBs) are the most cytotoxic and genotoxic for the cell. Eukaryotic organisms use several mechanisms to repair DSBs among them non-homologous end-joining (NHEJ) and homologous recombination (HR).

Here, **we investigate the molecular mechanisms of HR proteins inside cells at the single molecule level**. In response to DSB, repair proteins colocalize from a diffuse distribution to repair foci located at the damaged DNA site. An enduring question in the DNA damage field is how do repair proteins find their correct target, accumulate within repair foci and disassemble with the proper time-window? Despite their functional importance, the physical nature of repair sub-compartments remains unclear. To answer these questions, we use **single particle tracking** (SPT) and **PALM** (Photo Activable Localization Microscopy) to assess the physical properties underlying repair foci formation and the internal dynamics of these membrane-less sub-compartments. Using this approach in *Saccharomyces cerevisiae* yeast, we found that **Rad52 share many properties characterizing Liquid Liquid Phase Separation** including: sharp change in diffusion coefficient while entering or escaping foci, fusion of multiple foci, existence of a potential attracting molecules to the center of foci. Furthermore, I will present a recently developed set-up combining laser micro-irradiation and single molecule microscopy. Using this set-up in living human cells, we visualize the changes in proteins dynamics just after damage in living human cells

1. Heltberg Mathias, Miné-Hattab Judith, Taddei Angela , Walczak Aleksandra M. , Mora Thierry. Physical observables to determine the nature of membrane-less cellular sub-compartments. BioRxiv **(2021).**
2. Judith Miné-Hattab*, Mathias Heltberg, Marie Villemeur, Chloé Guedj, Aleksandra M. Walczak, Thierry Mora, Maxime Dahan, Angela Taddei*, * corresponding authors. Single molecule microscopy reveals key physical features of repair foci in living cells ***eLife***, 10: e60577, (**2021)**.
3. Judith Miné-Hattab***** & Irène Chiolo, * corresponding authors. Complex Chromatin Motions for DNA Repair. ***Frontiers in Genetics***, 11: 800, (**2020)**.
4. Camille Clément, Guillermo Orsi, Alberto Gatto, Ekaterina Boyarchuk, Audrey Forest, Bassam Hajj, Judith Miné-Hattab, Mickaël Garnier, Zachary Gurard-Levin, Jean-Pierre Quivy, and Geneviève Almouzni. High-resolution

visualization of H3 variants during replication reveals their controlled recycling", ***Nature Communication***, 9;9(1):3181, **(2018).**

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Investigating reaction-diffusion dynamics of proteins in the nucleus of living cells using fluorescence-based methods.

Sébastien Huet,
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In contrast to the cytoplasm, which is subdivided into mutiple compartments due to the presence of membrane-surrounded organelles, nearly all the nuclear volume is readily accessible to proteins by diffusion processes. Nevertheless, this nuclear environment is extremely dense, containing complex multiscale structures such as the chromatin. Yet, proteins involved in essential physiological functions such as transcription or DNA repair need to efficiently navigate through this environment to find their target on the DNA within a reasonable time frame. Improving our characterization of the diffusion-reaction dynamics displayed by the nuclear proteins is therefore essential to better understand how these proteins reach their targets to fulfill their functions. In this course, I will describe how methods such as fluorescence recovery after photoperturbation (FRAP) or fluorescence correlation spectroscopy (FCS), can be used to assess the mode of diffusion-reaction displayed by a given nuclear protein and estimate quantitatively the parameters that describe its dynamics.

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Understanding the molecular assembly of the cell contractile machinery.

Shashi Kumar Suman[1], Serena Prigent[1], Vlad Costache[1], Pierre Mahou[2], Camille Plancke[1], Emmanuel Beaurepaire[2], François Robin[1]
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Salle Méditerranée – Cours plénier

Cell morphogenesis relies on a tight spatial and temporal control of cell mechanics by the actomyosin machinery. Understanding how these mechanical properties are controlled by a combinatorics of biochemical properties and tuning of concentration and activity remains a major question in modern cell biology. Looking at the assembly of the actomyosin cytoskeleton in the C. elegans, I will show how we can explore the dynamics of these molecular assemblies in live cells, revealing the complex architecture of this force generating machinery.

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Lighting up the central dogma in living embryos to uncover how genomic sequence encodes cell fate decisions.

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Salle Méditerranée – Cours plénier

Unraveling the mystery of how a single embryonic cell gives rise to the menagerie of differentiated cell types that build an animal will not only reveal the organizing principles of life, but will yield new insights into disorders of development and cancer. Even though classic genetic screens and functional genomic approaches have uncovered essentially all the transcription factors and enhancers involved in specifying cell identity, we still don't understand how these molecular players work together to choreograph development. Thanks to a recent suite of innovations in live imaging and computational analysis methods the time is ripe for the introduction of a new quantitative paradigm in the study of development based on how cell fates are established in living animals as development is actually taking place. Indeed, we can now visualize transcription, translation, and even the binding of a single TF molecule to DNA in living Drosophila embryos. I will discuss the technologies we developed to enable this and what we learned by applying them, focusing on a new protein tag, LlamaTag, which makes it possible to visualize transcription factor concentration dynamics in live embryos. Using LlamaTags we discovered stochastic bursts in the concentration of transcription factors that are correlated with bursts in transcription. We further used LlamaTags to show that the concentration of protein in a given nucleus depends heavily on transcription of that gene in neighboring nuclei and show that this short range inter-nuclear coupling is an important mechanism for coordinating gene expression across many nuclei to delineate straight and sharp boundaries of gene expression.

Dans la même thématique

Tables rondes :

TR-6-08-Table ronde de clôture du module 6 Dynamique et intéraction moléculaires

TR-T-06-Suivi de qualité d'un microscope photonique dans le temps - dialogue avec les constructeurs

Ateliers :

- A004-Molecular dynamics, the challenges of live cytoskeletal microscopy in vivo.
- A036-Lattice light-sheet microscopy for fast 3D time lapse of live samples
- A048-Mécanobiologie de cellules tumorales circulantes
- A126-Microscopie quantitative : dynamique moléculaire par Spectroscopie de Corrélation de Fluorescence - Mise en oeuvre, calibration et analyse
- A144-Use of detrended Fluorescence Lifetime Correlation Spectroscopy (dFLCS) to assess protein dynamics in the cell nucleus
- MA-5-01- Phase and index imaging for biology

MODULE 7 : SIGNALISATION CELLULAIRE, MECANOBIOLOGIE, MECANOTRANSDUCTION

Coordination : Marie-Emilie Terret et Laetitia Kurzawa

Introduction

Mechanobiology studies how biological materials respond to mechanical forces exerted on them. This biological material includes a great diversity of scales that can go from the microscopic scale (cytoskeleton, nucleus, organelles) to the macroscopic scale (organs, whole organisms) through intermediate scales (cells, tissues). A great heterogeneity is also encountered at the level of the mechanical forces exerted by their different intensities, their nature (shear forces, viscoelastic, osmotic pressure, rigidity, stretching, compression, gravity...), or their origin (extra or intracellular environment...). Finally, the response of the biological material is also complex and involves a large number of interconnected phenomena that may involve mechanisms of mechano-transduction, reorganization of the cytoskeleton, biochemical reactions, collective migration, change of polarity... In view of this great diversity, measuring these mechanical forces, understanding their integration at different scales and analyzing the response of biological material is often a challenge, relying in particular on the development of new technologies and on a strong interaction between biology and physics. Mechanobiology is therefore of major importance in many fields of research, such as the study of embryonic development, the fight against cancer and tissue engineering. The invited speakers of the mechanobiology session reflect this diversity of scales, models and applications.
Guillaume Charras will focus on cell mechanotransduction by showing how signaling proteins control cell mechanics at the cortical level. Peter Lenart will present the mechanical response of the starfish oocyte nucleus leading to the rupture of the nuclear envelope under the effect of an original intrinsic piercing mechanism resulting from the forces exerted by the actin cytoskeleton. Stéphane Vassilopoulos will explain how clathrin patches at the cell plasma membrane constitute a mechanotransduction crossroads, relaying mechanical stimuli by remodeling the cytoskeleton to modulate gene expression. Stéphanie Miserey-Lenkei will show how Golgi-dependent protein secretion is controlled by mechanical forces, potentially via microtubules that interrogate the extracellular environment through focal adhesions. Matteo Rauzi will present his work on the mechanics and mechanisms governing tissue folding and extension during embryonic gastrulation and neurulation in Drosophila and sea urchin. Finally, Nathalie Sauvonnet will talk about how pathogens adapt their virulence to mechanical forces encountered in host tissues, particularly in the gut.

Mercredi 10 novembre 8h30

Spatial regulation of exocytosis.

Miserey-Lenkei S.,
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To reach the cell surface, secreted proteins are transported along intracellular routes from the endoplasmic reticulum through the Golgi complex, which acts as the main sorting station. Cargos exit the Golgi complex in transport carriers that use microtubules to be addressed to the plasma membrane before exocytosis. Using a synchronized secretion assay in 2D, we have recently highlighted that exocytosis is not random and occurs at hotspots juxtaposed to focal adhesions (FAs). The Golgi-associated small GTPase RAB6 acts as a general regulator of post-Golgi secretion, irrespectively of the cargo. The RAB6-dependent machinery plays an essential role in the restricted exocytosis *(Fourriere, Kasri, JCB, 2019)*. However, several questions remain unanswered, especially regarding the mechanical influence from the cell environment on protein secretion. To address this point, we aim actually at understanding how microtubules sense extracellular environment through FAs and how Golgi-dependent protein secretion is controlled by mechanical forces.

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Clathrin plaques form mechanotransducing platforms.

Stéphane Vassilopoulos,
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Salle Porquerolles – Cours parallèle

Large flat clathrin plaques are stable features of the plasma membrane associated with sites of strong adhesion suggesting that they could also play a role in force transduction. Here, we analyzed their response to mechanical cues and their association with the cytoskeleton. Flat clathrin plaques and surrounding branched actin filaments sequestered YAP/TAZ mechanotransducers at the plasma membrane and were required for efficient nuclear translocation in response to cyclic stretching. Branched actin filaments surrounding clathrin plaques also formed anchoring points for intermediate filaments and were required for organization of the intermediate filament subcortical web. Thus, clathrin plaques act as molecular platforms conveying mechanical cues which integrate cell signaling with cytoskeletal regulation.

Moulay G, Lainé J, Lemaître M, Nakamori M, Nishino I, Caillol G, Mamchaoui K, Julien L, Dingli F, Loew D, Bitoun M, Leterrier C, Furling D, Vassilopoulos S. Alternative splicing of clathrin heavy chain contributes to the switch from coated pits to plaques. J Cell Biol. 2020 Sep 7;219(9):e201912061. doi: 10.1083/jcb.201912061.
Vassilopoulos S. Unconventional roles for membrane traffic proteins in response to muscle membrane stress. Curr Opin Cell Biol. 2020 Mar 19;65:42-49. doi: 10.1016/j.ceb.2020.02.007.
Vassilopoulos S, Gibaud S, Jimenez A, Caillol G, Leterrier C. Ultrastructure of the axonal periodic scaffold reveals a braid-like organization of actin rings. Nat Commun. 2019 Dec 20;10(1):5803. doi: 10.1038/s41467-019-13835-6.

Franck A, Lainé J, Moulay G, Lemerle E, Trichet M, Gentil C, Benkhelifa-Ziyyat S, Lacène E, Bui MT, Brochier G, Guicheney P, Romero N, Bitoun M, Vassilopoulos S. Clathrin plaques and associated actin anchor intermediate filaments in skeletal muscle. Mol Biol Cell. 2019 Mar 1;30(5):579-590. doi: 10.1091/mbc.E18-11-0718. Epub 2019 Jan 2.
Vassilopoulos S, Gentil C, Lainé J, Buclez PO, Franck A, Ferry A, Précigout G, Roth R, Heuser JE, Brodsky FM, Garcia L, Bonne G, Voit T, Piétri-Rouxel F, Bitoun M. Actin scaffolding by clathrin heavy chain is required for skeletal muscle sarcomere organization. J Cell Biol. 2014 May 12;205(3):377-93. doi: 10.1083/jcb.201309096

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Jeudi 11 novembre 8h30

Impact of physical forces of the gut on pathogen infection using organ-on-chip (OOC).

Nathalie Sauvonnet,
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Salle Méditerranée – parallèle

Mechanical forces trigger many cellular functions and change the geometry and physical properties of a tissue. Very few studies address the role of physical inputs during infectious processes, and an important field of investigation remains on how pathogens have adapted their virulence to the mechanical forces encountered in the host tissues[1]. The intestine, and in particular the colon, comprising a mucus blanket, an epithelial monolayer and the lamina propria rich in immune cells, is constantly subjected to two main forces induced by the fluid-flow (shear stress) and the peristaltic motion (cyclic stretching), both forces being important for the tissue organization and renewal. These cells layers cooperate to form a protective barrier facing the external side that provides a wealth of "ecological niches" permanently exposed to microorganisms that can be beneficial (microbiota) or damageable (pathogens). Recently, we have shown using gut-on-a-chip technology recapitulating the 3D tissue architecture of the intestine and its physical forces, that this gut surrogate allows the highly efficient invasion of the human restricted pathogen *Shigella*, directly from the apical side of the epithelial monolayer, thereby shifting the paradigm on the early stages of invasion[2]. Furthermore, by modulating the flow rate and stretching conditions, and implementing a framework to reconstructing dynamic OOC imaging in 4D live, we observed that both the 3D topology of the monolayer and the peristaltic motion greatly enhance the colonization rate, the bacterial virulence, its invasion and cell-to-cell spreading throughout the barrier. Our results reveal that *Shigella* takes advantage of the gut morphology and physical forces to disrupt the intestinal barrier[2]. More recently, we also used OOC to investigate enteric infections by two other pathogens, the parasite *Entamoeba histolytica* and the virus SARS-CoV-2. Once again, the 3D topology and mechanical stimulation of the gut enhance the infection, but each pathogen adapted differently to gut physical cues, highlighting the essential role of mechanical forces in host-pathogen interactions.

1. Feaugas T and N Sauvonnet. Organ-on-chip to investigate host-pathogens interactions. *Cell Microbiol*., 23(7) (2021).
2. Grassart A, Malardé V, Gobaa S, Sartori-Rupp A, Kerns J, Karalis K, Marteyn B, Sansonetti PJ and N Sauvonnet. Bioengineered Human Organ-on-Chip Reveals Intestinal Microenvironment and Mechanical Forces Impacting Shigella Infection. *Cell Host and Microbe.* **26(3)** :435-444 (2019)

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Mechanisms and mechanics driving composite morphogenesis.
Matteo Rauzi,
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Salle Méditerranée – Cours parallèle

Tissue shape changes drive morphogenesis. During embryo development, a tissue can undergo multiple simultaneous changes in shape resulting in a composite morphogenetic process. While much work has been undertaken to unravel the mechanisms responsible for simple morphogenetic processes, how *composite morphogenesis* is controlled and driven is poorly studied. We focus on the process of concomitant tissue folding and extension that is vital since it can initiate embryo gastrulation or neurulation. To tackle this, we use two complementary model systems: the *Drosophila* and the sea urchin *Paracentrotus lividus* embryos. While the former provides cutting edge genetic tools, the latter is ideal for *in toto* imaging and direct tissue mechanical measurements. Finally, by using advanced multi-view light sheet microscopy coupled to infrared femtosecond laser manipulation, optogenetics, µ-pipette aspiration, µ-indentation, and quantitative big data analysis, we aim to unravel the signaling pathways, the mechanisms and mechanics controlling and driving composite morphogenetic transformations.

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Correlated super-resolution light and electron microscopy reveals a novel actin-driven mechanism of nuclear envelope rupture in starfish oocytes.
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Salle Méditerranée – Cours plénier

The nucleus of oocytes (germinal vesicle) is unusually large and its nuclear envelope (NE) is densely packed with nuclear pore complexes (NPCs) stockpiled for embryonic development. We used correlated super-resolution light microscopy (AiryScan imaging in live samples and STED microscopy in fixed probes) and electron microscopy to address how this exceptionally large nucleus is ruptured upon entry to meiotic divisions.

We found in starfish oocytes that breakdown of this specialized NE is mediated by an Arp2/3-nucleated F-actin 'shell', in contrast to microtubule-driven tearing in fibroblasts. We show that actin is nucleated within the lamina sprouting filopodia-like spikes towards the nuclear membranes. These F-actin spikes protrude pore-free nuclear membranes, whereas the adjoining membrane stretches accumulate packed NPCs associated with the still-intact lamina. NPC conglomerates sort into a distinct membrane network, while breaks appear in pore-free regions.

Together, we reveal a novel function for Arp2/3-mediated membrane shaping in NE rupture that is likely to have broad relevance in diverse other contexts such as nuclear rupture frequently observed in cancer cells.

[1] Wesolowska, N., Avilov, I., Machado, P., Geiss, C., Kondo, H., Mori, M., & Lenart, P., Actin assembly ruptures the nuclear envelope by prying the lamina away from nuclear pores and nuclear membranes in starfish oocytes. *eLife*, **9**, e49774. https://doi.org/10.7554/eLife.49774

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Dissecting the link between signalling and cell mechanics using optogenetics and AFM.

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Salle Méditerranée – Cours plénier

The submembranous actin cortex is the main determinant of cell shape. During mitosis, spatiotemporal changes in cortex mechanics give rise to a series of marked shape changes: rounding at metaphase, elongation in anaphase, and finally furrowing in cytokinesis. Mitotic morphogenesis results from tightly orchestrated global and local changes in RhoGTPase activity regulated by recruitment of RhoGEFs and RhoGAPs to the cortex. Despite its importance, little is known about how signalling controls cell mechanics.

I will present two studies examining the link between signalling and cell mechanics. First, we characterise the mechanical changes that accompany the onset of cytokinetic furrowing using AFM and determine how RhoGEFs and RhoGAPs contribute to cell mechanics and the rate of furrowing. Next, we use optogenetics to control the activity of RhoGTPases by relocalising a RhoGEF to the cortex and investigate the resulting temporal changes in surface tension.

Dans la même thématique

S07-SYMPOSIUM : - module 7 Mécanobiologie

Ateliers :

A026-Multiplexed FRET biosensor imaging to visualize the dynamic coordination between cell signaling and mechanics during collective cell migration (part I).
A040a-Coupling High Resolution Traction Force Microscopy with protein dynamics measurements
A040b-Coupling High Resolution Traction Force Microscopy with protein dynamics measurements
A041-Soft cell confiner development to decipher the impact of mechanical stimuli on cell
A044-étude de l'organisation des composants du cytosquelette et de l'autophagie dans un cadre infectieux sur des cellules à géométrie contrôlée
A099-Practical considerations for reporting change in cell membrane tension using FLIM
A111-Optogenetic control and measurement of cell contraction 1/2
A122-Mapping elasticity of micro-patterned living cells by AFM
A130-Optogenetic control of 3D micro- tissue 2/2
A134-Measuring turgor pressure of living plant cells with an Atomic Force Microscope

SÉMINAIRES

Vendredi 5 novembre

Lectures

S1 – 18h15

Cytoplasmic forces functionally reorganize nuclear condensates in oocytes

Marie-Hélène Verlhac,
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Salle Méditerranée – Séminaire

Cells remodel their cytoplasm with force-generating cytoskeletal motors. Their activity generates random forces that stir the cytoplasm, agitating and displacing membrane-bound organelles like the nucleus in somatic and germ cells. These forces are transmitted inside the nucleus, yet their consequences on membraneless organelles with liquid-like properties such as intranuclear biomolecular condensates remain unexplored. Here, we probe experimentally and computationally diverse nuclear condensates, that include splicing speckles, Cajal bodies, and nucleoli, during cytoplasmic remodeling of female germ cells named oocytes. We discover that growing mammalian oocytes deploy cytoplasmic forces to timely impose multiscale reorganization of condensates inside the nucleus. We determine that cytoplasmic forces accelerate nuclear condensate collision-coalescence and molecular kinetics within condensates. Inversely, disrupting the forces decelerates nuclear condensate reorganization on both scales. We link the molecular deceleration found in mRNA-processing splicing speckles to reduced and altered splicing of mRNA, which in oocytes impedes fertility. We establish that different sources of cytoplasmic forces can reorganize nuclear condensates and that this cytoplasmic aptitude for subnuclear reorganization is evolutionary conserved in insects. This study implies that cells evolved a mechanism, based on cytoplasmic force tuning, to functionally regulate a broad range of nuclear condensates across scales.

S2 – 19h15

How do dyes get into cells?

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Salle Méditerranée – Séminaire

Specific labelling of biomolecules with bright, photostable fluorophores is the keystone of fluorescence microscopy. An expanding method to label cellular components utilizes genetically encoded self-labelling tags, which enable the attachment of chemical fluorophores to specific proteins inside living cells. This strategy combines the genetic specificity of fluorescent proteins with the favourable photophysics of synthetic dyes. In addition to brightness and photostability, a key factor in this labelling scheme is the bioavailability of the fluorophore ligands. We discovered that incorporation of four-membered azetidine rings into many fluorophores improves brightness and photostability while also allowing fine-tuning of chemical properties.1,2 This work led to a general rubric to explain dye bioavailability,3 yielding a collection of dyes for advanced imaging experiments in increasingly complex biological samples.

1. Grimm, J. B.; English, B. P.; Chen, J.; Slaughter, J. P.; Zhang, Z.; Revyakin, A.; Patel, R.; Macklin, J. J.; Normanno, D.; Singer, R. H.; Lionnet, T.; Lavis, L. D. A general method to improve fluorophores for live-cell and single-molecule microscopy. Nat. Methods, 12, 244–250 (2015).
2. Grimm, J. B.; Muthusamy, A. K.; Liang, Y.; Brown, T. A.; Lemon, W. C.; Patel, R.; Lu, R.; Macklin, J. J.; Keller, P. J.; Ji, N.; Lavis, L. D. A general method to fine-tune fluorophores for live-cell and in vivo imaging. Nat. Methods, 14, 987–994 (2017).
3. Grimm, J. B.; Tkachuk, A. N.; Xie, L.; Choi, H.; Mohar, B.; Falco, N.; Schaefer, K.; Patel, R.; Zheng, Q.; Liu, Z.; Lippincott-Schwartz, J.; Brown, T. A.; Lavis, L. D. A general method to optimize and functionalize red-shifted rhodamine dyes. Nat. Methods, 17, 815–821 (2020).

Dimanche 7 novembre

S3 – 11h25-12h10

Force transmission at cell adhesions and the nucleus

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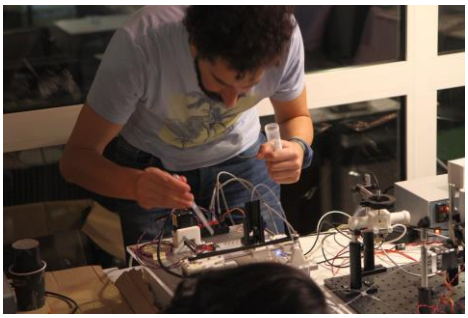
Salle Méditerranée – Séminaire

In multicellular organisms, cells generate and undergo mechanical forces that may shape cells, tissues and organs, but also regulate genetic programs. The recent advent of genetically-encoded sensors of molecular tension has allowed to provide quantitative, molecular-scale and molecular-specific information on these forces. We have leveraged these tools in proteins of cell adhesion and of the nuclear envelope to address how forces transmit across scales and regulate signaling pathways during model morphogenetic processes in cell culture. Our results reveal non-trivial relationships between molecular-scale and tissue-scale forces, and show how signaling pathways targeting gene transcription involve mechanotransduction events at multiple levels.

1. Déjardin T, Carollo PS, Sipieter F, Davidson PM, Seiler C, Cuvelier D, Cadot B, Sykes C, Gomes ER, Borghi N. Nesprins are mechanotransducers that discriminate epithelial-mesenchymal transition programs. The Journal of Cell Biology, (2020) 219:e201908036.
2. Audugé, S. Padilla-Parra, M. Tramier, N. Borghi, M. Coppey-Moisan. Chromatin condensation fluctuations rather than steady-state predict chromatin accessibility. N. Nucleic Acids Research, (2019) 47:6184-6194
3. C. De Pascalis, C. Pérez-González, S. Seetharaman, B. Boëda, B. Vianay, M. Burute M, C. Leduc, N. Borghi, X. Trepat, S. Etienne-Manneville. Intermediate filaments control collective migration by restricting traction forces and sustaining cell-cell contacts. The Journal of Cell Biology, (2018) 217:3031-3044.
4. C. Gayrard, C. Bernaudin, T. Déjardin, C. Seiler, N. Borghi. Src- and confinement-dependent FAK-activation causes E-cadherin relaxation and beta-catenin activity. The Journal of Cell Biology, (2018) 217:1063-1077.
5. Sarangi BR, Gupta M, Doss BL, Tissot N, Lam F, Mège RM, Borghi N, Ladoux B. Coordination between Intra- and Extracellular Forces Regulates Focal Adhesion Dynamics. Nano Lett. (2017) 17:399-406.



S4 – 18h15

3D super-resolution imaging of living systems using Multifocus Microscopy and Structured Illumination Microscopy

Sara Abrahamson,
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Salle Méditerranée – Séminaire

Forming a 3D image is a classical problem in Optics. Classical image formation yields a 2D image of the world with a depth of field/focus determined by the aperture of the optical systems. Microscopes can be used to record "focal stacks" consisting of a series of 2D images with very short depth of field recorded while scanning through focus through the depth of a specimen. Using the diffractive Fourier optics method Multifocus Microscopy[1], it is possible to record entire focal stacks simultaneously, without need for focus scanning or sequential recording of images. This allows us to image living specimens in 3D without the classical conflict between spatial (3D) and temporal (time) resolution. Structured Illumination Microscopy[2] can extend the resolution of the microscope image to visualize structures smaller than the classical ~200nm limit of resolution, down to ~100 nm and below. Combining Multifocus and Structured Illumination Microscopy, we can increase the acquisition speed of 3D SIM data by an order of magnitude. We are currently employing this technique to study sister chromatids and their associated biomolecules during meiosis[3] (unpublished).

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2. Gustafsson, M.G.L. *et al.* Three-dimensional resolution doubling in wide-field fluorescence microscopy by structured illumination, Biophys J. (2008) doi: 10.1529/biophysj.107.120345
3. MacQueen, A.J. Phillips, C.M., Bhalla, N., Weiser, P., Villeneuve, A.M., Dernburg, A.F., Chromosome sites play dual roles to establish homologous synapsis during meiosis in C. elegans, Cell, 123,1037-1050, 2005, https://doi.org/10.1016/j.cell.2005.09.034.

Lundi 8 novembre

S5 – 18h15

Super-resolution microscopy: Challenges and Potentials in biomedical research

Christian Eggeling,
Institute for Applied Optics and Biophysics, Friedrich-Schiller-University Jena; Leibniz Institute for Photonic Technology e.V., Jena, Germany; MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom
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Salle Méditerranée – Séminaire

Understanding the complex interactions of molecular processes underlying the efficient functioning of the human body is one of the main objectives of biomedical research. Scientifically, it is important that the applied observation methods do not influence the biological system during observation. A suitable tool that can cover all of this is optical far-field fluorescence microscopy. Yet, biomedical applications often demand coverage of a large range of spatial and temporal scales, and/or long acquisition times, which can so far not all be covered by a single microscope and puts some challenges on microscope infrastructure. Taking immune cell responses, virus infection, and

plasma membrane organization as examples, we outline these challenges but also give new insights into possible solutions and the potentials of these advanced microscopy techniques, e.g. for solving long-standing questions such as of lipid membrane organization and dynamics (e.g. lipid rafts).

Mardi 9 novembre

S6 – 18h15

Metal- and Graphene-Induced Energy Transfer Imaging

Jörg Enderlein,
Institute of Physics – Biophysics, Georg August University, 37077 Göttingen, Germany; Cluster of Excellence "Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells" (MBExC), Georg August University, 37077 Göttingen, Germany.
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Salle Méditerranée – Séminaire

Metal-Induced Energy Transfer (MIET) Imaging is a recently developed method [1] that allows for nanometre resolution along the optical axis. It is based on the fact that, when placing a fluorescent molecule close to a metal, its fluorescence properties change dramatically, due to electromagnetic coupling of its excited state to surface plasmons in the metal. This is very similar to Förster Resonance Energy Transfer (FRET) where the fluorescence properties of a donor are changed by the proximity of an acceptor that can resonantly absorb energy emitted by the donor. In particular, one observes a strongly modified lifetime of its excited state. This coupling between an excited emitter and a metal film is strongly dependent on the emitter's distance from the metal. We have used this effect for mapping the basal membrane of live cells with an axial accuracy of ~3 nm. The method is easy to implement and does not require any change to a conventional fluorescence lifetime microscope; it can be applied to any biological system of interest, and is compatible with most other super-resolution microscopy techniques that enhance the lateral resolution of imaging [2-3]. Moreover, it is even applicable to localizing individual molecules [4], thus offering the prospect of three-dimensional single-molecule localization microscopy with nanometre isotropic resolution for structural biology. I will also present latest developments of MIET where we use a single layer of graphene instead of a metal film that allows for increasing the spatial resolution down to few Ångströms (Graphene-Induced Energy Transfer or GIET) [5,6]. In combination with single-molecule localization microscopy methods such as dSTORM or PAINT, MIET/GIET imaging offers nanometric isotropic resolution for bioimaging of molecular complexes and cellular structures.

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4. Isbaner, S., Karedla, N., Kaminska, I., Ruhlandt, D., Raab, M., Bohlen, J., ... & Tsukanov, R. „Axial Colocalization of Single Molecules with Nanometer Accuracy Using Metal-Induced Energy Transfer" Nano Lett. 18 (2018) 2616-2622.
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6. Ghosh, A., Chizhik, A. I., Karedla, N., & Enderlein, J. „Graphene-and metal-induced energy transfer for single-molecule imaging and live-cell nanoscopy with (sub)-nanometer axial resolution" Nature Protocols (2021) 1-21.

S7 – 19h15

Towards quantitative correlative microscopy

Lucy Collinson
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Salle Méditerranée – Séminaire **VISIO**

Correlative light and electron microscopy (CLEM) combines the benefits of fluorescence and electron imaging, allowing researchers to track rare biological events in the context of cell structure. Depending on the biological question, correlative workflows can be tailored to incorporate almost any imaging modality. For example, in collaboration with Dr Eva Frickel and Dr Serge Mostowy, we imaged Toxoplasma parasites in zebrafish brain, using fluorescence microscopy, microCT, transmission EM, Serial Block Face SEM and Focused Ion Beam SEM to locate and image the 'needle in the haystack'[1].

However, correlative microscopy tends towards 'n=1', largely due to the complexity of the experimental workflows and the lack of automated image analysis algorithms. To overcome this bottleneck, our technology development work has focused on improving the speed, accuracy, and accessibility of CLEM. In this talk, I will discuss the benefits of preserving fluorescent proteins in cells and tissues prepared for electron microscopy[2,3], which enables 'smart tracking' correlative workflows[4], and our collaboration with astrophysicists, citizen scientists and machine learning specialists[5] to automate segmentation and visualisation of cell organelles at the nanoscale for large-scale downstream quantitative analysis of cells in health and disease.

1. Yoshida N, Domart MC, Peddie CJ, Yakimovich A, Mazon-Moya MJ, Hawkins TA, Collinson L, Mercer J, Frickel EM, Mostowy S. The zebrafish as a novel model for the in vivo study of Toxoplasma gondii replication and interaction with macrophages. Dis. Model Mech. 13(7): dmm043091 (2020).
2. Brama E, Peddie CJ, Jones M, Domart MC, Snetkov X, Way M, Larijani B, Collinson LM. Standard fluorescent proteins as dual-modality probes for correlative experiments in and integrated light and electron microscope. JoCB. DOI:10.1007/s12154-015-0147-3 (2015).
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Mercredi 10 novembre

S8 – 11h25

Synaptic vesicle pools under the nanoscope

Frédéric A. Meunier,
Clem Jones Centre for Ageing Dementia Research (CJCADR), Queensland Brain Institute (QBI), University of Queensland, St Lucia Campus, Brisbane, QLD, 4072, Australia.

Salle Méditerranée – Séminaire **VISIO**

Synaptic vesicles (SVs) are highly enriched in the presynapse of neurons and contain the neurotransmitter which can be released by exocytic fusion to mediate neuronal communication. These vesicles are 45 nm in diameter and organised into distinct functional pools based on their availability to release neurotransmitters. SVs are highly clustered facing their release sites at the active zone. Their exclusive concentration at the presynapse suggests an anchoring mechanism preventing them from spreading throughout the neuron. To undergo fast recycling following fusion, SVs can also move although it is currently unknown how presumably mobile SVs can remain clustered.

In this talk, I will discuss our attempts to image SVs using various single-molecule imaging techniques1-5 specifically designed to unravel the dynamic nanoscale organisation of distinct pools of SVs in live hippocampal neurons.

1 Joensuu, M. *et al.* Visualizing endocytic recycling and trafficking in live neurons by subdiffractional tracking of internalized molecules. Nat Protoc 12, 2590-2622, (2017).
2 Joensuu, M. *et al.* Subdiffractional tracking of internalized molecules reveals heterogeneous motion states of synaptic vesicles. J Cell Biol 215, 277-292, (2016).
3 Padmanabhan, P. *et al.* Need for speed: Super-resolving the dynamic nanoclustering of syntaxin-1 at exocytic fusion sites. Neuropharmacology, (2019).
4 Vanhauwaert, R. *et al.* The SAC1 domain in synaptojanin is required for autophagosome maturation at presynaptic terminals. EMBO J 36, 1392-1411, (2017).
5 Wallis, T. P. *et al*. Molecular videogaming: Super-resolved trajectory-based nanoclustering analysis using spatio-temporal indexing. bioRxiv, 2021.2009.2008.459552, (2021).

S9 – 19h15

The Mechanobiology of Cell Growth and Shape control

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Salle Méditerranée – Séminaire

Walled cell exhibit a remarkable range of size and growth properties, which are largely determined by the mechanics of their cell wall. The cell wall is a thin and rigid layer which provides mechanical integrity by opposing large turgor pressure derived stresses. By developing super-resolution imaging methods to map Cell Wall thickness all around live and growing yeast and fungal cells, we deciphered the mechanisms controlling CW dynamics during growth and shape changes. We uncovered a mechanical feedback system controlling wall thickness at growing cell tips, which impinge on cell viability and growth regulation, and relies on mechanosensing activities in the cell wall. Mechanosensation is mediated by a class of surface sensors which can detect local mechanical

stresses on the cell wall surface and adapt wall synthesis and mechanics. Finally, using a combination of wall thickness measurement and laser ablation, we also screened libraries of yeast mutants, and fungal cells with large range of sizes, shapes and growth modes, and established large-scale correlations between mechanical properties of the cell wall, growth and morphologies in these cells. These data impact our current understanding of the mechanobiology of the cell wall and its impact on cell growth and shape control.

Jeudi 11 novembre

S10 – 18h15

Médiation technique et conception : une création orientée ?

Claude Paraponaris,
DR CNRS, Professeur des universités Aix Marseille, Anthropologue et philosophe de la technique, organologie de la création industrielle
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Salle Méditerranée – Séminaire

MODULES AVANCES / TABLES RONDES

Module **A**vancé ou **T**able **R**onde- /-Numéro module associé ou **T**ransversal-/-Numéro **MA** ou **TR**

Round tables

Timeslot	Sat 06 Nov	Sun 07 Nov	Mon 08 Nov	Wed 10 Nov	Thu 11 NOV
14:00-15:45			**SYMPOSIUM** Organisation et dynamique moléculaire: qu'apporte le deep learning aux analyses? (Amphi Méditerranée)		**SYMPOSIUM (13:45-16:00)** Mécanobiologie (Amphi Méditerranée)
16:15-18:00					
21:30-00:00	**MA-3-01** Strengths and Limits of Deep Learning for Image Restoration in Microscopy (Amphi Porquerolles)			**MA-5-02** Imagerie de phase et d'indice pour la biologie (Amphi Porquerolles)	

Timeslot	Sat 06 Nov	Sun 07 ov	Mon 08 Nov	Tue 09 Nov	Wed 10 Nov	Thu 11 Nov
14:00-15:45	**TR-3-01** Retour sur l'ANF du RT-MFM Deep Learning pour les microscopies (Amphi Porquerolles)	**TR-2-02** TR du module Nanoscopie (Haut-Lounge)	**TR-T-06** Suivi de qualité d'un microscope photonique dans le temps (Amphi Porquerolles) **TR-T-07** De la découverte à la valorisation (Haut-Lounge)		**TR-3-9** Comment l'IA impacte-t-elle notre travail ? (Amphi Porquerolles)	**TR-4-11** Imagerie multicellulaire : organoïdes, tissus, embryons (Amphi Porquerolles)
16:15-18:00		**TR-1-03** Emerging chemical tools, techniques, and methods for the realization of competitive biological projects (Amphi Porquerolles) **TR-4-04** Which Light Sheet for gentle imaging (Haut-Lounge)	Tables-Rondes Aditionnelles Proposées durant l'école		**TR-2-10** Microscopies du Vivant sur virus et bactéries en BSL3 (Haut-Lounge)	**TR-2-12** SMLM au delà de l'esthétique (Amphi Porquerolles) **TR-3-13** Clôture du parcours thématique deep learning (Haut-Lounge)
21:30-00:00		**TR-T-05** IYSN Career development perspectives for young scientist (Amphi Porquerolles)		**TR-6-08** TR du Module 6 Dynamique et intéraction moléculaires (Haut-Lounge)		

MODULES AVANCÉS

MA-3-01 - Strengths and Limits of Deep Learning for Image Microscopy

Round tables

Samedi 6 novembre -21h30-00h00- Amphi Porquerolles

Proposer/Coanimator
Loic A. Royer
Martin Weigert

Abstract

In this advanced module we will discuss the strengths and weaknesses of deep learning for image restoration in Microscopy. We will first review the classical methods for image restoration and review some of the most popular algorithms for deconvolution and denoising.
We will then review recent deep learning methods in that context to highlight their advantages. Importantly, we will discuss their limits and remind ourselves of what can go wrong and why. Finally, if there is time we will discuss more generally the pitfalls of deep learning beyond image restoration.

***

MA-5-02 - Phase and index imaging for biologie

Mercredi 10 novembre -21h30-00h00- Amphi Porquerolles

Proposer/Coanimator
Pierre Bon
Olivier Haeberlé

Abstract

The objectives of this advanced module are: presenting the basics of image formation in the least invasive unlabeled microscopy modalities (i.e. not involving non-linear techniques like Raman, second harmonic…). We will discuss in this module quantitative phase microscopy and optical-echography (wavefront sensing, holography, OCT…). Highlights will be made on the recent biological applications and on the remaining challenges to extend these methodologies to instrumental solution-free biological questions. Mainly, we will insist on 3D imaging (tomography), thick sample imaging, resolution enhancement, and also on specific and/or functional information retrieval.
Part 1: Quantitative phase imaging: Principle, interest and applications on biological specimens, coupling to other imaging modalities.
Part 2: Current limit, identified technological issues, work in progress and potential applications unlocked.
Discussion time will then be devoted to identify the expectations of users (mainly biologists) who can't use or are willing to avoid fluorescence labeling.

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TABLES RONDES

TR-3-01 - Retour sur l'ANF du RT-MFM "Deep-learning pour les microscopies"

Samedi 6 novembre 14h00-15h45 Amphi Porquerolles

Proposer/Coanimator

Fabrice Cordelières
Christian Rouvière

Abstract

L'idée est avant tout de faire un retour d'expérience suite à formation et de partager les pratiques qui en ont émergé.
Vous avez entendu parler de la révolution du machine learning, mais ne savez pas par où commencer pour vous y mettre ? Vous avez tenté d'utiliser des logiciels clé-en-main mais peinez à comprendre comment ils fonctionnent ? Vous suivez avec minutie les tutoriels disponibles sur le net mais n'avez pas réussi à installer cette *** de bibliothèque qui débloquerait votre problématique de segmentation ? C'est à ces défis que se sont heurtés les 15 participants de l'ANF "Deep-learning pour les microscopies". Des spécialistes du DL les ont guidés dans l'acquisition des bonnes pratiques, et dans l'utilisation d'outils open-source. Nos participants sont à présent à même de diffuser la connaissance auprès d'un public plus large, et prêts à échanger avec vous sur leurs déboires mais surtout sur leurs success stories.

***

TR-2-02 - Table ronde de clôture du module 2 Nanoscopie

Dimanche 7 novembre 14h00-15h45 Haut-Lounge

Proposer/Coanimator

Jean-baptiste Sibarita
Sandrine Lévêque-Fort
Christophe Leterrier

Abstract

The objective of this round table is to close the nanoscopy module, by gathering the workshop leaders, the speakers of the module and the participants particularly interested in this topic, and to offer the opportunity of informal exchanges around the challenges to come for the field.

***

TR-1-03- Emerging chemical tools, techniques, and methods for the realization of competitive biological projects

Dimanche 7 novembre 16h15-18h00 Amphi Porquerolles

Proposer/Coanimator

Dominique Bourgeois
Andrey Klymchenko
Marie Erard
Ludovic Jullien

Abstract

The objective of this round table is two-sided, to close the module 1 "Probes", by bringing together workshop leaders, module speakers and participants particularly interested in this theme, and to define the plan of action of the work package “Probe development, Optomanipulation & Optogenetic“ from the national infrastructure France Bio Imaging -- open to new contributors.
This round table will be the occasion to discuss current challenges in the field of fluorescent labelling in biology:
(i) at different levels from single molecules to cells, tissue and small animals;
(ii) multiple targets to be labelled (proteins, nucleic acids, lipids, etc);
(ii) fluorescent probes for advanced microscopy techniques, including super-resolution imaging.
It will be the occasion to discuss emerging approaches in the field, which include, new chemistry, new molecular biology methods and combination of chemistry with molecular biology. It will also try to define the current needs in the new probes from biology and advanced microscopy.
FBI WP 3 (Probe development, Optomanipulation & Optogenetics) takes advantage of MiFoBio2021 to lead a discussion in which representatives of the WP will interact with the event participants to define a plan of action for the next two years. The goal of this discussion is to discuss how to provide access and training to emerging chemical techniques and methods for the realization of competitive biological projects.

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TR-4-04 Which light-sheet microscope for gentle live imaging ?

Dimanche 7 novembre 16h15-18h00 Haut-Lounge

Proposer/Coanimator

Alexandra Fragola
Mathieu Ducros
Rémi Galland

Abstract

L’objectif de cette table ronde est double :
- pour les utilisateurs : il s’agit de choisir le microscope à feuille de lumière le plus adapté à l’imagerie live de leurs échantillons d’intérêt (grâce à un arbre de décision)
- pour les développeurs : l’objectif est d’identifier de nouveaux besoins en microscopie à feuille de lumière.
Pendant cette table ronde, nous présenterons rapidement les différents systèmes optiques et les méthodes de préparation des échantillons en microscopie à feuille de lumière du vivant, et ferons un focus sur trois modalités avancées de microscopie à feuille de lumière: imagerie dynamique rapide à haute résolution en lattice, imagerie en profondeur grâce à l’optique adaptative, imagerie à haut débit d’images.
Les échanges entre les participants seront tournés autour de leurs problématiques.

***

TR-T-05 IYSN Career development perspectives for young scientist

Dimanche 7 novembre 21h00-00h00 Amphi Porquerolles

Proposer/Coanimator

Clément Cabriel
Hana Valenta

Abstract

This round table is organized by the Imabio Young Scientists Network (IYSN), who will chair the discussion. Academic researchers as well as industry and startup scientists will be invited to present their professional experience. The informal discussion will allow young scientists to interact with the invited people to provide them with their personal points of view.
The objectives are to:
- Assess the needs of the Imabio young scientists in terms of career development
- Make them aware of possible career pathways in academic research, companies and others
- Give them the opportunity to discuss this with more senior members of the community, as well as with other young scientists
- Help them build and use their professional network efficiently

***

TR-T-06- Suivi de qualité d'un microscope photonique dans le temps - dialogue avec les constructeurs

Lundi 8 novembre 14h00-15h45 Amphi Porquerolles

Proposer/Coanimator

Thomas Guilbert
Aurélien Dauphin

Abstract

La microscopie est à notre connaissance un des seuls domaines technologiques où l'achat d'un système vient sans mode d'emploi d'entretien, indice de points critiques, fichier de calibrations mesurées, document d'aide à la maintenance. Le groupe de travail 3M du RT-mfm souhaite entamer un dialogue avec les différents constructeurs de microscopes pour échanger sur les méthodes, les outils et les protocoles de mesures de l'état de santé d'un microscope photonique dans le but commun d'améliorer le suivi de performance de nos systèmes, en harmonisant les procédures entre SAV et utilisateurs. Une conséquence directe pourrait être une meilleure communication entre ingés de PF et SAV pour, par exemple, faciliter la validation du bon fonctionnement du système après l'achat. Le groupe de travail 3M du RT-mfm souhaite préciser que dans le cadre des discussions entamées avec les constructeurs, l'utilisation du terme « métrologie en microscopie de fluorescence » fera en réalité référence au suivi de qualité de fonctionnement d'un microscope dans le temps. Ensuite, nous souhaitons également préciser qu'à aucun moment il s'agira de comparer des mesures effectuées sur différents microscopes en termes de performance, mais bien de suivi dans le temps de l'état de santé d'un système par des mesures bien précises, issues du protocole établi par le GT3M.

***

TR-T-07- De la découverte à la valorisation

Lundi 8 novembre 14h00-15h45 Haut-Lounge

Proposer/Coanimator

Marc Tramier

Abstract

L'objectif de cette table ronde est le partage d'expériences enrichissantes et de répondre à toutes les questions que les participants pourraient se poser sur la valorisation sans jamais avoir osé le demander.

Nous présenterons des exemples d'histoires de valorisation avec leur succès et échecs (5 min par histoire) et donnerons des informations et astuces aux chercheurs désirant se lancer dans l'aventure.

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TR-6-08 - Table ronde de clôture du module 6 Dynamique et intéraction moléculaires

Mardi 9 novembre 21h00-00h00 Haut-Lounge

Proposer/Coanimator

Cyril Favard
Ignacio Izeddin
Antoine Coulon

Abstract

The objective of this round table is to close Module 6, by bringing together the workshop leaders, the speakers of the module and the participants particularly interested in this theme, and to offer the opportunity for informal exchanges around the challenges to come for the field.

***

TR-2-09 - Microscopies du Vivant sur virus et bactéries en BSL3

Mercredi 10 novembre 16h15-18h00 Haut-Lounge

Proposer/Coanimator

Delphine Muriaux
Sébastien Lyonnais

Abstract

La table ronde permettra la discussion sur le thème de l'installation de microscope en P3, de la formation des individus en P3 et des questions pratiques soulevées.

Les virus et bactéries sont des petits pathogènes, parfois en dessous de 100 nm en taille, et souvent de risque de niveau 3. Ceci implique de les étudier en laboratoire de confinement de niveau 3 (BSL3) qui nécessite des instruments et manipulations particulières. Nous présenterons ici comment manipuler ces pathogènes dans un BSL3 afin de les imager vivant ou en infectant la cellule hôte. Nous présenterons comment nous avons introduit ces microscopes (Bio-AFM, Spinning-disk ou encore en TIRF-PALM) dans le BSL3 afin de les étudier par imagerie du vivant infectieux.

***

TR-3-10 - Comment l'IA impacte-elle notre travail ?

Jeudi 11 novembre 14h00-15h45 Haut-Lounge

Proposer/Coanimator

Cédric Matthews
Guillaume Gay
Claude Paraponaris

Abstract

Depuis ces 5 dernières années, nous vivons une évolution du métier en plateforme par l'apport de l'intelligence artificielle. Nous devons repenser tant l'organisation des données que les méthodologies d'acquisition d'images pour répondre au mieux à de nouvelles contraintes que nous imposent les thématiques scientifiques complexes, les images massives et le big data. Nous découvrons aussi au fil de l'eau que le cadre règlementaire nous contraint dans la gestion de ces données, nous amenant à repenser nos applications dans le monde de l'open data. Le métier d'ingénieur en plateforme, poussé par l'évolution des techniques, doit à nouveau se projeter dans un monde technique qui a évolué, ici avec une rupture qui touche tant tous les domaines scientifiques que la vie de chacun dans notre société.

***

TR-4-11 - Imagerie multicellulaire : organoïdes, tissus, embryons, quelles nouveautés pour l'imagerie des tissus épais

Jeudi 11 novembre 14h00-15h45 Amphi Porquerolles

Proposer/Coanimator

Gaëlle Recher & Lydia Danglot (Module 4)
Morgane Belle et Pierre BON (Parcours Milieux épais)

Abstract

Chacun des objectifs cités ci-dessous sera abordé au cours de la table ronde. Nous pensons lancer la discussion en abordant chacun des sujets par une courte présentation d'un des participants (parmi les ateliers associés) afin de lancer un problème bio ou un verrou technologique non résolu. Les participants à la table ronde pourront ainsi rebondir sur le sujet afin de partager leur expérience et de proposer d'éventuelles solutions :

- Aborder les contraintes techniques et difficultés rencontrées lors du marquage et du montage des échantillons épais fixés ou vivants (stratégie de marquage, chambre imprimée, fraisage de verre ou de plastique, moule 3D).
- Aborder les contraintes techniques et difficultés rencontrées lors clearing des tissus clarifiés ou préparés pour l'expansion microscopie.
- Aborder et discuter les avantages et inconvénients des techniques actuelles (optique adaptative, feuille de lumière, confocal, spinning,)
- Aborder les contraintes techniques et difficultés rencontrées lors de l'analyse quantitative des données de larges volumes issues de l'imagerie des tissus, organoïdes ou tissus "expansés" (expansion microscopie).
- Permettre à des communautés d'échanger des protocoles et des stratégies lors de la table ronde (nouvelles sondes, préparation d'échantillons, nouvelles méthodes pour suivre rapidement les déformations, technologies 3D, imagerie multi couleur).

TR-2-12 - SMLM au-delà de l'esthétique ?

Jeudi 11 novembre 16h15-18h00 Amphi Porquerolles

Proposer/Coanimator

Céline Malleval
Karine Monier

Abstract

Le but de cette table ronde est de revenir sur le parcours STORM, d'échanger autour des retours d'expérience et d'élaborer une liste de recommandations pour le choix et l'utilisation des techniques de SMLM en abordant les points suivants :

- Accessibilité de l'outil
- Leviers techniques
  - o Préparation d'échantillon (fixation, choix fluorophores, tampons) incidence sur la qualité des données obtenues
  - o Paramètre d'acquisition
  - o Traitement d'images : Softwares, paramètres de détection et de visualisation
  - o Quantification : Softwares
- Forces et limites des techniques de SMLM
  - o Comparaison avec d'autres techniques

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TR-3-13 - Clôture du Parcours thématique : Deep learning pour l'analyse d'images de microscopie

Jeudi 11 novembre 16h15-18h00 Haut-Lounge

Proposer/Coanimator

Daniel Sage
Fabrice Cordelières

Abstract

Cette table ronde est le récapitulatif du parcours thématique "Deep learning pour l'analyse d'images de microscopie" La formation plus avancée d'un plus grand nombre de scientifiques est la clef pour permettre une diffusion réfléchie de ces techniques. Il faut équiper la communauté scientifique d'un bagage pour appréhender le potentiel du DL et pour en connaître les limites. C'est essentiel pour une intégration valide de ces techniques dans un protocole scientifique.
L'univers du DL reste encore largement réservé aux informaticiens tant la mise en œuvre requiert des compétences bien spécifiques (Python) et des calculateurs puissants (GPU). Le DL est donc relativement inaccessible à la plupart des chercheur·euse·s en biologie, contrairement aux méthodes algorithmiques pour lesquelles on trouve des softwares facilement utilisables (par ex. ImageJ/Fiji).
Ce nouveau paradigme entièrement centré sur les données soulève des questions plus « philosophiques ». Quelles quantités de données-exemples, quelle variabilité de données, quelles annotations ? peut-on vraiment faire une analyse scientifique en se basant sur l'exemple ? Ce sont autant de questions qui doivent être adressées de façon rigoureuse. L"'explicabilité" du système « blackbox » et l'interprétabilité des résultats sont d'autres questions plus délicates qui ne doivent pas être ignorées dans un contexte scientifique. Ce parcours thématique, avec ses ateliers, séminaires, rencontres, sera une occasion d'aborder toutes ces questions actuelles entre spécialistes et utilisateurs.

PARCOURS THEMATIQUES

Les parcours thématiques ont pour objectif de rassembler des ateliers, tables-rondes et séminaires sur des thèmes sélectionnés par l'organisation sur la base des propositions d'ateliers reçues au cours de l'année.

Pour MIFOBIO 2021, nous avons donc sélectionné 3 parcours :

- Machine Learning pour l'analyse d'images de microscopie
- Imagerie en milieux épais
- Imagerie en molécule unique (SMLM/STORM)

Vous trouverez dans les pages suivantes, le synopsis des parcours ainsi que les ateliers, tables-rondes et séminaires associés.

The objective of the thematic tracks is to gather workshops, round tables and seminars on themes selected by the organization on the basis of workshop proposals received during the year.

For MIFOBIO 2021, we have selected 3 tracks:
- Machine Learning for microscopy image analysis
- Imaging in thick media
- Single Molecule Imaging (SMLM/STORM)

You will find in the following pages, the synopsis of the tracks as well as the associated workshops, round tables and seminars

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Parcours thématique 1: *Machine Learning pour l'analyse d'images de microscopie*

Responsable : Daniel SAGE (daniel.sage@epfl.ch)

Abstract

Deep learning techniques (deep learning DL), resulting from work in artificial intelligence, are changing the practices of many disciplines based on data sciences. Microscopy image analysis, which tries to understand biological processes by quantifying images, is directly concerned by this evolution. Many (but not all) bioimaging applications can thus benefit from artificial neural networks to solve complex or difficult to model tasks. DL networks are progressively imposing themselves in the scientific community, which does not go without raising a large number of questions that mix hope and skepticism, belief and reasoning.

The more advanced training of more scientists is the key to allowing a thoughtful diffusion of these techniques. The scientific community must be equipped to understand the potential of DL and to know its limitations. This is essential for a valid integration of these techniques in a scientific protocol.

This thematic track "deep learning for microscopy image analysis" proposes a set of workshops that will allow beginners to get to grips with relatively accessible tools and more advanced users to implement neural networks on simple examples. Workshops are also proposed on concrete applications of deep learning.

The world of DL is still largely reserved for computer scientists, as its implementation requires very specific skills (Python) and powerful computers (GPU). DL is therefore relatively inaccessible to most biology researchers, unlike algorithmic methods for which there are easily usable software packages (e.g. ImageJ/Fiji).

This new paradigm entirely centered on data raises more "philosophical" questions. What quantities of example data, what variability of data, what annotations? Can we really do a scientific analysis based on the example? these are all questions that must be addressed in a rigorous way. The explicability of the "blackbox" system and the interpretability of the results are other more delicate questions that should not be ignored in a scientific context. This thematic course, with its workshops, seminars, meetings, will be an opportunity to address all these current issues between specialists and users.

Pedagogical objectives of the course

- Understand the potential and limitations of DL techniques for microscopy imaging
- Implement a DL image analysis with simple tools or be able to design a DL image analysis
- Acquire the methodology to determine the relevance of DL and evaluate the risks of using a DL system
- Enable users to exchange DL developers and remove blockages

Identification sheet

Related modules: **1, 4 and 5**

Course:

Assaf Zaritsky, Ben-Gurion University of the Negev, Israel
Extracting the invisible from live cells microscopy

Loïc Royer, Chan Zuckerberg Biohub, San Francisco, USA
Self-supervised deep learning for fluorescence imaging and nD image viewing with Napari

Martin Weigert, EPFL Lausanne, Switzerland
Microscopy image analysis with machine learning

Perrine Paul-Gilloteaux, Université de Nantes, France
Multiscale and multimodal registration: an overview of methods

Diana Mateus, Université de Nantes, France
Deep learning with medical images: learning with small datasets and few annotations

Daniel Sage, EPFL Lausanne, Switzerland
Microscopy image analysis - The shift to deep learning?

Workshop :
A015
David Rousseau david.rousseau@univ-angers.fr Pejman Rasti pejman.rasti@univ-angers.fr
Deep learning sans se salir les doigts (1/2)

A005
David Rousseau david.rousseau@univ-angers.fr Pejman Rasti pejman.rasti@univ-angers.fr
Deep learning avec les doigts dans le moteur (2/2)

A016
David Rousseau david.rousseau@univ-angers.fr Pejman Rasti pejman.rasti@univ-angers.fr
Deep Bar à images

A114
Guillaume Mougeot guillaume.mougeot@uca.fr
Deep learning made easy for microscopy: an introduction to ZeroCostDL4Mic and DeepImageJ

A116
Anaïs Badoual anais.badoual@inria.fr
Daniel Sage daniel.sage@epfl.ch
Bioimage analysis: practice deep learning without coding

A065
Charlotte Riviere charlotte.riviere@univ-lyon1.fr
Ali Ahmad ali.ahmad@insa-lyon.fr
Getting the most out of 3D pheroids by combining microfabricated wells, clarification techniques, standard confocal imaging and deep learning image processing

A124
Estelle Anceaume estelle.anceaume@college-de-france.fr
Philippe Mailly philippe.mailly@college-de-france.fr
QuPath: pyramid image analysis for everyone. Case study: Deep-learning cell counting and quantification of histological slides

Parcours thématique 2 : *Imagerie en molécule unique SMLM (Single Molecule Localization Microscopy) et STORM (STOchastic Reconstruction Microscopy)*

Responsables : Béatrice Durel (beatrice.durel@inserm.fr)
et Lydia Danglot (lydia.danglot@inserm.fr)

Abstract
Single molecule imaging has experienced a considerable growth in recent years and is one of the 3 main techniques of "Super-resolution" microscopy with SIM (Structured Illumination Microscopy) and STED (STimulated Emission Depletion Microscopy).
As the number of publications concerning single molecule imaging and the installation of systems within platforms is increasing, we decided to establish a SMLM/STORM pathway within Mifobio in order to help newcomers to orient themselves in all the activities associated with Mifobio. This path has been established jointly with the Pointillism working group of the RTmfm Technology Network (Magalin Mondin and Karine Monier).
You will find here all the courses, seminars, symposiums, roundtables and workshops dealing with SMLM techniques.

Pedagogical objectives of the course
- To address the technical constraints and difficulties encountered during sample preparation (fluorophores, blinking buffer, mounting in chamber or on slides).
- To expose the different techniques that can be used on fixed (STORM or PAINT) or live samples (PALM) and the feasibility of their implementation on home made or commercial systems.
- To expose the different 2D or 3D techniques that can be used for multicolor imaging (sequences of images at different excitation wavelengths or mixing of wavelengths after a single excitation).
-To address the technical constraints and difficulties encountered in the quantitative analysis of SMLM data (particle density, fluorescence intensity per blink, imaging frequency).
- To open perspectives on new tools developed and to be developed in this field.
- To allow communities to exchange protocols and strategies during the round table (new probes, sample preparation, new methods to rapidly follow deformations, 3D technologies, multicolor imaging).

Seminars and courses related to the thematic pathway :

Super-resolution microscopy: Challenges and Potentials in biomedical research.
Christian Eggeling,
Institute for Applied Optics and Biophysics, Friedrich-Schiller-University Jena; Leibniz Institute for Photonic Technology e.V., Jena, Germany; MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom.

Synaptic vesicle pools under the nanoscope
Frédéric A. Meunier[1]
[1]Clem Jones Centre for Ageing Dementia Research (CJCADR), Queensland Brain Institute (QBI), University of Queensland, St Lucia Campus, Brisbane, QLD, 4072, Australia.

Module 1 : sondes fluorescentes :

Labeling strategies to visualize the inner life of microbes by single-molecule localization microscopy – a practical guide
Ulrike Endesfelder,
Institut für Mikrobiologie und Biotechnologie, Universität Bonn.

Switchable Organic Dyes: the Photophysics of STORM
Mark Bates,
Department of NanoBiophotonics, Max Planck Institute for Biophysical Chemistry.

Module 2 : Le défi de la quantification en nanoscopie

3D single molecule localization microscopy.
Sandrine Lévêque-Fort,
Institut des Sciences Moléculaires d'Orsay, CNRS, Université Paris Saclay, 91405 Orsay.

Superresolution microscopy for structural cell biology.
Jonas Ries,
Cell Biology and Biophysics unit, European Molecular Biology Laboratory (EMBL).

Quantification of filament structures in superresolution and expansion microscopy.
E.A. Katrukha, L.C. Kapitein,
Cell Biology, Department of Biology, Faculty of Science, Utrecht University, Netherlands.

From images to information: enhancing resolution and improving accuracy in SMLM.
Susan Cox,
King's college, London.

Computational microscopy by PSF engineering - or – how and why to ruin a perfectly good microscope.
Yoav Shechtman,
Department of Biomedical Engineering, Technion – Israel Institute of Technology.

Module 5 : Ondes sur le vivant (avec GDR Ondes)

Polarized microscopy resolves protein's organization in cells.
Sophie Brasselet,
Aix Marseille Univ, CNRS, Centrale Marseille, Institut Fresnel, F-13013 Marseille, France.

Module 6 : Dynamique et interactions moleculaires en cellules vivantes : expérimentation et modélisation avec GDR DNA

Revealing spatial and kinetic details of life processes by analyzing live cell single molecule tracking data.
J. Christof M. Gebhardt,
Institute of Biophysics, Ulm University, Germany.

***E. coli* chromosome dynamics and the cell cycle.**

Marco Cosentino Lagomarsino,
Department of Physics, University of Milan, Italy.

Imaging DNA repair at the single molecule level.
Judith Miné-Hattab,
Institut Curie, PSL University, Sorbonne Université, CNRS UMR3664, Nuclear Dynamics, France.

Attached workshops:

A003-Multicolor and 3D STORM to study localization of proteins adressed to apical membrane of polarized epithelial cells anne.cantereau@univ-poitiers.fr
A012-3D quantitative analysis of colocalisation or spatial coupling in conventional and super resolution microscopy. lydia.danglot@inserm.fr ; thibault.lagache@pasteur.fr
A013-Comparing multicolor Single-Molecule Localization Microscopy strategies: Application to the neuronal cytoskeleton christophe.leterrier@univ-amu.fr ; karoline.friedl@etu.univ-amu.fr
A046-Comment adapter son microscope TIRF pour faire du STORM! beatrice.durel@inserm.fr ; audrey.salles@pasteur.fr
A057-Introduction to single molecule localization super-resolution microscopy (SMLM) magali.mondin@u-bordeaux.fr
A058-Probabilistic pipeline to extract reliable information from single molecule microscopy data. alhassan.casse@sanofi.com; jean-baptiste.masson@pasteur.fr
A059-Coordinate-based quantification of multidimensional and multicolor single-molecule localization microscopy data. florian.levet@inserm.fr
A062-Real-time single molecule localization-based raw data acquisition, analysis and quality control mailfert@ciml.univ-mrs.fr ;nicolas.bertaux@fresnel.fr
A074-3D High Resolution imaging by PSF engineering using ZOLA-3D: Nuclear Pore complexes imaging mickael.lelek@pasteur.fr
A076-Alternative strategies to image multiple proteins in single molecule localization microscopy sandrine.leveque-fort@u-psud.fr
A094-Modulated excitation for enhanced localization : ModLoc
abigail.illand@u-psud.fr ; max.lengauer@gmx.net
A097-Nuclear pores complexe : a tool for for metrology in single molecule lancelot.pincet@free.fr
A121-CentrO2 : Influence de la concentration en oxygène pour l'imagerie dSTORM multi-couleur du cil primaire karine.monier@univ-lyon1.fr
A138-Colocalisation de complexes macromoléculaires en super-résolution PALM/STORM xavier.marques@sorbonne-universite.fr

Table ronde

A066-SMLM au-delà de l'esthétique ? celine.malleval@inserm.fr
TR-2.02 Bilan du module nanoscopie

Mini-symposium rattaché Lundi 8 novembre de 14h à 18h:
Organisation et dynamique moléculaire : qu'apporte le deep learning aux analyses ? juliette.griffie@epfl.ch & hverdier@pasteur.fr

Parcours thématique 3 : *Imagerie des échantillons épais*

Coordination : Morgane BELLE (morgane.belle@inserm.fr***) et Pierre BON (***pierre.bon@cnrs.fr***)***

Abstract :
Today we are interested in the single molecule but also in the whole network! A network is by definition in 3 dimensions and therefore relatively large and of millimetric or even centimetric size. The thickness of the sample and its intrinsic opacity often prevent in-depth analysis. Physicists and biologists are working to counteract the phenomena of light diffraction in thick tissues through histological techniques, optical development, microscopy methods or post-acquisition image processing. In this course "Imaging in thick media" we would like to introduce you to the advances in allowing light to pass through a thick sample by revealing 3D gratings thanks to :
1) clarification techniques to homogenize the refractive indices
2) microscopy techniques optimized for this type of sample such as light sheet microscopy, OPT, holographic, wide field and confocal imaging
3) image corrections through adaptive optics and wavefront
4) expansion techniques that allow the visualization of fine and confined structures rendered up to 20x larger: a tissue "become" thick!

Pedagogical objectives of the course

- how to visualize in 3D a thick tissue (multiple specimens are to be discovered in the workshops: mice, fish, amphibians, insects, plants, reptiles, chickens, isolated organs, tumors, organoids...) with or without fluorescent labeling, transparent or not with a consequent spatial resolution by different imaging approaches: holographic imaging, OPT, confocal, wide field, HCS, light sheet imaging
- approach of different types of microscopy for the acquisition of "transparent" tissues and the problem of mounting these samples to observe them
- how to visualize in depth living tissues
- preparation of tissues for clarification (aqueous, organic, commercial solution or RIMS protocols) and in-toto immunostaining
- to become familiar with 3D observation of samples and the different image treatments possible via dedicated software or machine learning
- understand the interaction between light and the medium through which it passes and learn how to correct it using adaptive optics and wavefront analysis
- learn the expansion technique to visualize biological phenomena in super resolution using classical fluorescence microscopy: from preparation to 3D reconstruction of samples and their analysis

Module 1 : sondes fluorescentes :

A third-generation glutamate indicator optimized for synapses.
Kaspar Podgorsky,
HHMI Janelia Research Campus.
Building the next generation of genetically encoded probes and actuators.
Nathan C. Shaner,
Department of Neurosciences, University of California San Diego School of Medicine, La Jolla, CA USA ncshaner@health.ucsd.edu

Module 2 : Le défi de la quantification en nanoscopie

Structured illumination microscopy (SIM) for high-speed super-resolution fluorescence imaging of living cells.
Alexandra Fragola,
Laboratoire Physique et Étude de Matériaux UMR 8213, ESPCI Paris-PSL, CNRS, Sorbonne Université.

Quantification of filament structures in superresolution and expansion microscopy.
E.A. Katrukha, L.C. Kapitein,
Cell Biology, Department of Biology, Faculty of Science, Utrecht University, Netherlands.

Module 3 : Intelligence artificielle pour l'imagerie biologique

Microscopy Image Analysis: The Shift to Deep Learning?
Daniel Sage,
EPFL, Laboratoire d'imagerie biomédicale.
Multiscale and multimodal registration: an overview of methods.
Perrine Paul-Gilloteaux,
Université de Nantes, CHU Nantes, Inserm, CNRS, SFR Santé, Inserm UMS 016, CNRS UMS3556, Nantes, France ; Université de Nantes, CNRS, INSERM, l'institut du thorax, Nantes, France.

Self-Supervised Deep Learning for Fluorescence Imaging and nD Image Viewing with Napari.

Loïc Alain Royer, Chan Zuckerberg Biohub.

Module 4 : Imagerie multicellulaire : organoïdes, tissus, embryons

Organ on chip, a new generation of in vitro models.
Descroix Stéphanie, Institut Curie UMR 168- Institut Pierre Gille de Gennes.
3D printing and bioprinting for the development of microenvironment and tissue models.
Laurent Malaquin, ELiA team, LAAS – CNRS UPR 8001, Toulouse, France.
SCAPE microscopy for high-speed 3D imaging.
Elizabeth M. C. Hillman,
Herbert and Florence Irving Professor, Mortimer B. Zuckerman Mind Brain Behavior Institute, Professor of Biomedical Engineering and Radiology, Columbia University, Jerome L. Greene Science Center.
Sometimes, there IS a free lunch: How to get twice the resolution from your microscope, without (serious) drawbacks.
Andrew York,
Calico Life Sciences LLC, South San Francisco, CA, USA.
Quantifying transport and efficacy of therapeutics in spheroids.
Charlotte Rivière,
Université Claude Bernard Lyon 1, CNRS, Institut Lumière Matière ; Institut Convergence Plascan, CRCL ; Institut Universitaire de France (IUF).
3D-printed minimally assembled interchangeable LSFM chamber for serial imaging of organoids and spheroids.
Francesco Pampaloni,

Buchmann Institute for Molecular Life Sciences (BMLS) Germany; Institute for Research in Biomedicine, IRB Barcelona, Barcelona Institute of Science and Technology, BIST.

Module 5 : Ondes sur le vivant (avec GDR Ondes)

Measuring and shaping the phase of light: key applications in biology.
Pascal Berto,
Institut de la vision, Sorbonne Université, INSERM, CNRS.

X-ray coherent diffraction imaging: 3D exploration of biologically relevant hard and soft tissues.
Virginie Chamard,
Aix-Marseille Université, CNRS, Centrale Marseille, Institut Fresnel UMR 7249.

Imaging the brain at high spatiotemporal resolution with wavefront shaping.
Na JI, Department of Physics, Department of Molecular & Cell Biology, University of California, Berkeley.

Volumetric imaging at high speeds.
Jérome Mertz,
Biomedical Engineering Department, Boston MA.

Module 7 : Signalisation cellulaire, mécanobiologie, mécanotransduction

Impact of physical forces of the gut on pathogen infection using organ on ship (OOC).
Nathalie Sauvonnet,
Intracellular trafficking and tissue homeostasis, department Cell Biology and Infection, Institut Pasteur.

Mechanisms and mechanics driving composite morphogenesis.
Matteo Rauzi,
Université Côte d'Azur, CNRS, Inserm, iBV, Nice, France.

Ateliers rattachés

A011-Etude de la vascularisation sanguine et lymphatique de l'épididyme : clarification d'organe, microscopie à feuille de lumière et quantification en trois dimensions
Porteurs : *Meryem Meryem tardivel (meryem.tardivel@univ-lille.fr), Antonino Bongiovanni (antonino.bongiovanni@univ-lille.fr)*
A019-Trucs et Astuces ExM
Porteurs : *Sophie Abélanet (abelanet@ipmc.cnrs.fr), Bruno Mesmin (mesmin@ipmc.cnrs.fr)*
A020-Expansion microscopy imaging with a lattice light-sheet microscope
Porteurs : *Monica Fernandez monreal (monica.fernandez-monreal@u-bordeaux.fr), Mathieu Ducros (mathieu.ducros@u-bordeaux.fr)*
A021-Expansion Microscopy From theory to practice
Porteur : *Tudor Manoliu (tudor.manoliu@gustaveroussy.fr)*
A022-Bring your sample to experiment a very fast and universal clearing technique

Porteurs : *Jennifer Coridon (jennifer.coridon@hotmail.fr), Brigitte Delhomme (jennifer.coridon@parisdescartes.fr)*

A025-Exploitation combinée d'une sonde et d'une caméra Infra rouge (SWIR) pour imager le réseau vasculaire d'un muscle entier de souris dans la fenêtre NIR II.

Porteurs : *Laurence Dubreil (laurence.dubreil@oniris-nantes.fr), Xavier Le guevel (xavier.le-guevel@univ-grenoble-alpes.fr)*

A028-3D holography imaging of algae populations

Porteurs : *Audrey Audrey beaussart (audrey.beaussart@univ-lorraine.fr), Isabelle Bihannic (isabelle.bihannic@univ-lorraine.fr)*

A032-Immunomarquage et transparisation de tissus entiers, acquisition avec système home-made vs commercial

Porteurs : *Chloé Dominici (chloe.dominici@univ-cotedazur.fr), Sophie Abélanet (abelanet@ipmc.cnrs.fr)*

A035-Studying organogenesis of the pronephros of Xenopus tadpole early stages using light sheet and confocal microscopy

Porteurs : *Chloé Chaumeton (chloe.chaumeton@sorbonne-universite.fr), Thomas Panier (thomas.panier@sorbonne-universite.fr)*

A039-Imagerie 3D de la vascularisation par feuille de lumière pour évaluer l'évolution d'une pathologie et/ou l'efficacité d'un traitement

Porteurs : *François Michel (francois.michel@inserm.fr), Laurence Dubreil (laurence.dubreil@oniris-nantes.fr)*

A042-Remember your wavefront: adaptive optics and memory effect in different regimes Porteurs : *Claudio Moretti (claudio.moretti@lkb.ens.fr), Bernhard Rauer (bernhard.rauer@lkb.ens.fr)*

A045-Ultrastructure cellulaire par microscopie d'expansion

Porteurs : *Virginie Georget (virginie.georget@mri.cnrs.fr), Marie-pierre Blanchard (marie-pierre.blanchard@igh.cnrs.fr)*

A051-De la dynamique cellulaire au sein d'organoïdes jusqu'à l'étude morphologique des tissus/organes transparisés : approche multi-échelle sur un système unique d'imagerie à feuille de lumière.

Porteurs : *Jacques Rouquette (jacques.rouquette@itav.fr), Laetitia Pieruccioni (laetitia.pieruccioni@itav.fr)*

A052-Quantitative 3D Spatial Analysis of multicellular specimens (Organoids w/o clearing) Porteurs : *Sébastien Marais (sebastien.marais@u-bordeaux.fr), Sophie Allart (sophie.allart@inserm.fr)*

A063a-Transparisation, acquisition au microscope à feuillet de lumière et post traitement de sphéroïdes

Porteurs : *Chloé Dominici (chloe.dominici@univ-cotedazur.fr), Cédric Gaggioli (Cedric.GAGGIOLI@unice.fr)*

A063b-Transparisation, acquisition au microscope à feuillet de lumière et post traitement de sphéroïdes

Porteurs : *Chloé Dominici (chloe.dominici@univ-cotedazur.fr), Cédric Gaggioli (Cedric.GAGGIOLI@unice.fr)*

A065-Getting the most out of 3D pheroids by combining microfabricated wells, clarification techniques, standard confocal imaging and deep learning image processing

Porteurs : *Charlotte Riviere (charlotte.riviere@univ-lyon1.fr), Ali Ahmad (ali.ahmad@insa-lyon.fr)*

A071-Microscopie d'expansion : stratégies et astuces pour l'analyse des cellules de mammifères en culture, de la levure S. cerevisiae et pour la visualisation de l'organisation mitochondriale

Porteurs : *Jim Dompierre (jim.dompierre@ibgc.cnrs.fr), Manuel Rojo (manuel.rojo@ibgc.cnrs.fr)*

A072-Evolution of brain morphology from invertebrates to mammals. Everything we can learn from in toto 3D imaging of autofluorescence signals?
Porteurs : *Christelle Langevin (christelle.langevin@inra.fr), Morgane Belle (morgane.belle@inserm.fr)*
A073-Microscopie plein champ "haute résolution" et traitement numérique sur échantillon épais
Porteur : *Vicky Diakou-verdin (vicky.diakou@univ-montp2.fr)*
A075-Adaptive optics fluorescence microscopy for biological imaging
Porteur : *Alexandra Fragola (alexandra.fragola@espci.fr)*
A087-HCS sur sphéroïdes solides, transparisés ou non, avantages et inconvénients
Porteurs : *Laetitia Ligat (laetitia.ligat@inserm.fr), Romina D\'angelo (romina.dangelo@inserm.fr)*
A095-Multimodal imaging of biological tissues with the help of a miniature flexible endoscope
Porteur : *Vasyl Mytskaniuk (vasyl.mytskaniuk@fresnel.fr)*
A096-Multimode fiber based-endoscope for fluorescence imaging using wavefront shaping Porteur : *Irène Wang (irene.wang@univ-grenoble-alpes.fr)*
A098-OPenT - Bring your sample & learn how to build and use an OPT optical tomography mesoscope
Porteur : *Gabriel G. martins (gaby@igc.gulbenkian.pt)*
A100-Préparation d'échantillon, acquisition et analyse d'image pour les applications de culture cellulaire 3D
Porteurs : *David Akbar (david.akbar@icm-institute.org), Claire Lovo (claire.lovo@icm-institute.org)*
A120-Imagerie sur petit organoides 3D, Troubleshooting
Porteurs : *Adeline Boyreau (adeline.boyreau@u-bordeaux.fr), Laetitia Andrique (laetitiaandrique@hotmail.com)*
A125-Speckle-based computational microscopy : harnessing scattering for enhanced imaging of tissues
Porteur : *Alexandra D'arco (alexandra.darco@lkb.ens.fr)*
A131-Imagerie 3D d'organes transparisés par microscopie à feuille de lumière
Porteurs : *Sébastien Dupichaud (sebastien.dupichaud@inserm.fr), Louison Lallemant (louison.lallemant@gmail.com)*
A140-Du plus petit échantillon au plus gros, la microscopie à feuille de lumière face à l'enjeu du multi-échelle
Porteurs : *Julien Dumont (julien.dumont@college-de-france.fr), Astou Tangara (tangara@biologie.ens.fr)*
A141-Medium throughput imaging of thick samples: a practical comparison of different samples (Drosophila tissues and encapsulated spheroids) in native opaque state and after light-penetration facilitation
Porteurs : *Gaelle Recher (gaelle.recher@institutoptique.fr), Marilyne Duffraisse (Marilyne.Duffraisse@ens-lyon.fr)*
A142-Microscopie à feuillet de lumière pour l'imagerie volumique
Porteur : *Basile Gurchenkov (basile.gurchenkov@inserm.fr)*
A143-Imagerie à feuillet de lumière des échantillons 3D montées d'une manière peu contraignante
Porteur : *Basile Gurchenkov (basile.gurchenkov@inserm.fr)*

Table ronde

Imagerie multicellulaire : organoïdes, tissus, embryons. Quelles difficultés/nouveautés pour l'imagerie des tissus épais ? Gaëlle Recher & Lydia Danglot (pour le Module4) Morgane Belle et Pierre BON (pour le Parcours Milieux épais)

BAR A IMAGES

Workshops Round tables

Porteurs : Yves Usson, Arnold Fertin, Bertrand Simon, Sébastien Marais

- Avez-vous des questions ou besoins en analyse d'images ?
- Avez-vous ou envisagez-vous d'acquérir une quantité importante d'images dans le but de les analyser quantitativement ?
- Souhaiteriez-vous un (ou plusieurs) avis d'experts avant de vous lancer ?

Le bar à images est fait pour vous !

Le bar à images propose cette année **3 créneaux fixes** répartis sur la semaine dans un format type "portes ouvertes" : en vous y inscrivant, vous avez la possibilité de discuter avec un groupe d"experts", chacun spécialisé dans leur domaine, sur tout ce qui traite de l'utilisation des logiciels et algorithmes de visualisation et d'analyse d'images. Ces discussions se feront soit en tête à tête, soit en petits groupes, en fonction des besoins des participants et des compétences des animateurs présents à chaque créneau. Vous pouvez également apporter vos propres images, et vous aurez l'occasion de présenter votre problème (ou vos solutions !) d'analyse.
Pendant toute la durée de l'école, certains experts se rendront également disponibles pour des **rendez-vous impromptus** afin de répondre à vos questions de visualisation et d'analyse d'images en dehors des sessions prévues au planning ! Un tableau d'inscription sera accessible sur place pour vous mettre en contact avec ces experts.

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- Do you have questions or needs in image analysis?
- Have you or do you intend to acquire a large number of images in order to analyze them quantitatively?
- Would you like one (or more) expert advice before you start?

The "Bar à Image" is for you!

The "Bar à Image" proposes this year 3 sessions spread over the week in an "open house" type format: by registering, you have the opportunity to discuss with a group of "experts", each one specialized in their field, on everything related to the use of visualization and analysis software and algorithms. These discussions will be either face-to-face or in small groups, depending on the needs of the participants and the skills of the experts present at each session. You can also bring your own images, and you will have the opportunity to present your analysis problem (or solutions!).

Throughout the school, some experts will also be available for impromptu meetings to answer your image visualization and analysis questions outside of the scheduled sessions! A registration board will be available on site to put you in contact with these experts.

FABLAB

Round tables Lectures Workshops

Coordination : Christian Rouviére (christian.rouviere@univ-tlse3.fr)

Un espace "Fablab" vous est proposé cette année. Il s'agit d'un espace d'apprentissage et de réalisation de petits montages optiques, mécaniques et électroniques. Des ateliers pratiques encadrés ou libre (fiche TP) vous sont proposés. Le principe est que vous puissiez vous y rendre à tous moment hors des plages horaires occupées par des ateliers, et de pouvoir utiliser les outils mis à votre disposition (4 ordinateurs + soft, deux imprimante 3D (sous couvert d'un responsable), des composants, fer à souder, petits outillages. Nos experts, en fonction d'horaires à définir, seront aussi là pour vous guider, vous renseigner.

Des fiches préparées pour vous aider dans différents montages vous seront proposées, mais vous pouvez aussi profiter de cet espace pour développer un projet personnel et si besoin nous consulter, prendre un rdv et discuter avec les différents spécialistes.

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A "FabLab" space is a space of learning and realization of small optical and electronic assembly. Practical workshops supervised or free (worksheet) are offered. The principle is that you can go there at any time outside the time slots occupied by workshops, and can use the tools at your disposal (4 computers + soft, a 3D printer, components, soldering iron, small Workheets prepared to help you in different settings will be proposed, but you can also take advantage of this space to develop a personal project and need to consult us, take a meeting and discuss with the various specialists.

Cours/Courses :

A080 Conception de prototypes connectés sous rapsberry et **arduino (3 sessions indépendantes** : niveau débutant, intermédiaire et confirmé). Surveillance d'une expérimentation à distance (relevé de paramètres « connecté ») pour moins de 100€.

Certains microprocesseurs (Rapsberry, Arduino...) permettent aujourd'hui de développer des prototypes peu coûteux. Cependant leur mise en place demande l'apprentissage du langage de programmation et la compréhension de leur mise en œuvre. Nous proposons donc de lever cette barrière lors d'un atelier en trois modules (débutant à avancés). Nous assisterons les participants au cours de trois modules pour la fabrication d'un prototype fonctionnel et connecté, permettant d'envoyer sur un site web, les paramètres environnementaux et la distance entre deux capteurs en temps réel. Ces modules peuvent être réalisés comme un parcours ou se faire de manière indépendante selon trois axes
module 1 (débutant): connexion et programmation de deux capteurs (température et capteur de proximité) pilotés par un"Arduino".
module 2 (intermédiaire) pré requis: avoir déjà programmé afin de s'adapter à la compréhension du code que nous écrirons en Python) : génération d'une série de chiffres aléatoires et envois de ceux-ci sur un site web (Rapsberry PI)
module 3 (avancé) : pré requis: avoir déjà programmé et connaitre un microcontrôleur "Arduino" ou avoir assisté au premier atelier. Mis en œuvre de la communication entre deux microcontrôleur

(Arduino: plateforme de réception des mesures) et un Rapsberry PI par communication I2C ou série envoyant les informations recueillies vers le site web
A l'issue de ces modules les participants seront capables selon l'atelier suivi
Module 1: Mise en place d'un environnement de programmation Arduino : brancher et piloter deux capteurs (acquis général pour la mise en œuvre d'un autre type de capteur
Module 2 : Mise en œuvre un mini-ordinateur Rapsberry : installer un système d'exploitation de type Linux, programmer un script sous python pour générer un chiffre aléatoire et l'envoyer sur un site web
Module 3: Programmer en python un script de communication entre Arduino et Rapsberry pour le transfert de données et l'envoie sur un site web.

A084-Dessiner, paramétrez et Imprimer en 3D

Ludovic Leconte et Brice Detailleur. **(2 représentations)**

A061-Acquisitions intelligentes sous micromanager

Jérôme Mutterer, Christian Rouviere **(2 représentations).**

Une acquisition "intelligente" est un protocole d'acquisition d'images dont certaines étapes peuvent évoluer en cours d'exécution sur la base de critères propres au développement du spécimen ou de son environnement. Cette flexibilité n'est pas possible dans le cadre simple d'une acquisition de microscopie de fluorescence multidimensionnelle, mais nécessite de pouvoir évaluer des paramètres de l'échantillon par analyse d'image, ou des paramètres environnementaux (mesures externes issues de capteurs).

Nous utiliserons une extension du langage macro d'ImageJ qui permet un pilotage facile du logiciel d'acquisition Micro-Manager. (https://github.com/mutterer/MM2MacroExtensions)
Cette solution combine les avantages de Micro-Manager (richesse des pilotes de statifs et de périphériques, solution de choix pour le développement de périphériques home-made) et d'ImageJ pour le traitement et l'analyse des images acquises. Elle comble un espace en rendant accessible le pilotage de périphérique dans le langage macro d'ImageJ, sans doute le langage de programmation le plus accessible et le plus largement adopté dans la communauté des biologistes investis dans l'imagerie du vivant.

A l'issue de cet atelier les participants seront capables de programmer en langage macro d'ImageJ des scripts de pilotage de stations de microscopie et de les intégrer avec des éléments d'analyse d'image pour réaliser des boucles de rétroaction en fonction de paramètres extraits des images acquises.

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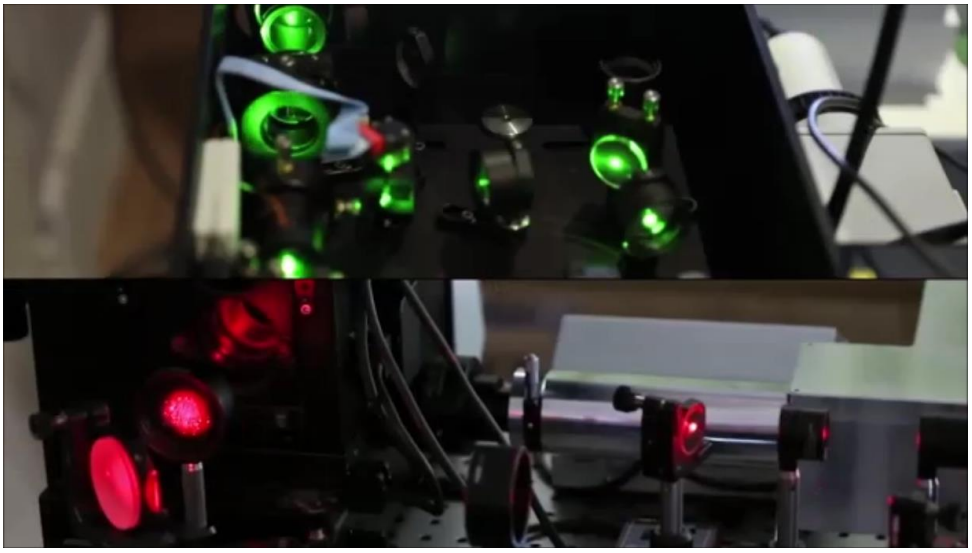
Planning des Ateliers de pratique dirigée/*Planning of practical workshops supervised***:**

FabLab- Hall	Sat 06		Sun 07		Mon 08		Tues 09	Wed 10		Thur 11
	14:00-16:00	21:30 00:00	14:00 15:45	16 :15 18 :00	14:00 16:00	16 :15 18 :00	21:30 00:00	14:00 15:45	21:30 00:00	14:00 15:45
A80		x	x			x			x	
A061				x				x		
A084			x				x			



OPTICLAB

Round tables Lectures Workshops

But de l'OpticLab

Le but de l'OpticLab est de permettre aux participants, débutants ou microscopistes plus expérimentés, d'apprendre à réaliser des mesures de performance sur des systèmes optiques et surtout de comprendre les principes physiques sous-jacents à travers des travaux pratiques réalisables de manière autonome (sans ou avec très peu d'encadrement).
L'OpticLab propose quatre « Parcours », comprenant chacun des stations avec bancs optiques, pour démontrer les principes d'optique (partie « Optique en pratique & Fabrique des images »), et des stations avec microscopes, pour apprendre les applications pratiques au quotidien, notamment entretien et réglage du microscope, diagnostic des problèmes, etc. (partie « Applications »). Les applications pratiques renvoient systématiquement à des éléments de la Valise Métrologie, que les participants pourront ainsi utiliser de manière autonome après leur formation au MIFOBIO.
Our goal is to help participants, from beginners to more experienced microscopists, to learn how to make different performance measurements on microscopes and more importantly to understand the underlying optical principles, thanks to small hands-on sessions, which can be followed independently, with no or very little supervision.
The OpticLab offers 4 "Parcours" (or "group of activities"), consisting each in exercises on optical benches on the one hand, to demonstrate some principles of Optics, and of exercises on microscopes on the other hand, to learn some basics of microscope performance assessment. All measurements refer to tools to be found in the "valise métrologique", which participants will be able to use independently after their training at MIFOBIO.

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The goal of the OpticLab is to allow participants, whether beginners or more experienced microscopists, to learn how to perform performance measurements on optical systems and, above all, to understand the underlying physical principles through practical work that can be done independently (with little or no supervision).
The OpticLab offers four "Courses", each including stations with optical benches, to demonstrate the principles of optics (part "Optics in practice & Image making"), and stations with microscopes, to learn practical applications in everyday life, including maintenance and adjustment of the microscope, diagnosis of problems, etc. (part "Applications"). ("Applications" section). The practical applications systematically refer to elements of the Metrology Suitcase, which the participants will be able to use independently after their training at MIFOBIO.
Our goal is to help participants, from beginners to more experienced microscopists, to learn how to make different performance measurements on microscopes and more importantly to understand the underlying optical principles, thanks to small hands-on sessions, which can be followed independently, with no or very little supervision.
The OpticLab offers 4 "Parcours" (or "group of activities"), consisting each in exercises on optical benches on the one hand, to demonstrate some principles of Optics, and of exercises on microscopes on the other hand, to learn some basics of microscope performance assessment. All measurements

refer to tools to be found in the "valise métrologique", which participants will be able to use independently after their training at MIFOBIO.

Parcours

Parcours 1 : Plans conjugués / Alignements Köhler et contraste de phase
Parcours 2 : Illumination et chemin optique / Mesures d'uniformité de champ
Parcours 3 : Diffraction et résolution / Mesures de PSF et préparation de lames de billes
Parcours 4 : Aberrations et chromatisme en imagerie / Alignements et corrections

Coordination

Laurent Gelman (laurent.gelman@fmi.ch)

Organisation et supervision

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PLANNING ATELIERS

Workshop	Sat 06 Nov		Sun 07 Nov			Mon 08 Nov		Tue 09 Nov	Wed 10 Nov			Thu 11 Nov	
	14:00-15:45	21:30-00:00	14:00-15:45	16:15-18:00	21:30-00:00	14:00-15:45	16:15-18:00	21:30-00:00	14:00-15:45	16:15-18:00	21:30-00:00	14:00-15:45	16:15-18:00
A002- 3D STED microscopy for nanoscopic imaging of virus-host cell interactions. (Muriaux D. . & Dibsy R)					BAS-SALLE 4			BAS-SALLE 4					
A003- Multicolor and 3D STORM to study localization of proteins adressed to apical membrane of polarized epithelial cells (Cantereau)	BAS-PORT-CROS					BAS-PORT-CROS							
A004- Molecular dynamics, the challenges of live cytoskeletal microscopy in vivo. (Robin & Reymann)								BAS-PORT-CROS				BAS-PORT-CROS	
A005- Deep learning avec les doigts dans le moteur (2/2) (Rousseau & Rasti)			BAS-MADRAGUE										BAS-MADRAGUE
A006- AFM on microbial surfaces : from imaging to single-cell force measurements (Audrey beaussart & El-kirat-chatel)			BAS-LES SALINS								BAS-LES SALINS		
A007- OpticLab (OpticLab)	BAS-AYGUADE		BAS-AYGUADE	BAS-AYGUADE		BAS-AYGUADE	BAS-AYGUADE	BAS-AYGUADE	BAS-AYGUADE	BAS-AYGUADE			
A011- Etude de la vascularisation sanguine et lymphatique de l'épididyme : clarification d'organe, microscopie à feuille de lumière et quantification en trois dimensions (Meryem tardivel & Bongiovanni)								BAS-SALLE 5			BAS-SALLE 5		
A012- 3D quantitative analysis of colocalisation or spatial coupling in conventional and super resolution microscopy. (Danglot & Lagache)			BAS-SALLE 4				BAS-SALLE 4						
A013- Comparing multicolor Single-Molecule Localization Microscopy strategies: Application to the neuronal cytoskeleton (Leterrier & Friedl)							BAS-PORT-CROS				BAS-PORT-CROS		
A014- Deconvolution 3D (Soulez & Sage)				BAS-ALMANARRE					BAS-ALMANARRE				
A015- Deep learning sans se salir les doigts (1/2) (Rousseau & Rasti)	BAS-MADRAGUE										BAS-MADRAGUE		
A016- Deep bar à images (Rousseau & Rasti)								HAUT-RESTAURANT ESTRADE		HAUT-RESTAURANT ESTRADE			
018- Obtenir la PSF d'un système de microscopie de fluorescence (Soubies & Sage)			BAS-ALMANARRE							BAS-ALMANARRE			
A021- Expansion microscopy imaging with a lattice light-sheet microscope (Fernandez monreal & Ducros)					BAS-LE LEVANT						BAS-LE LEVANT		
A023- Real-time mitochondrial Ca2+ and ATP measurements in mammalian cells using single excitation wavelength dual colour FLIM (Gouriou & Bidaux)								BAS-RIBAUD		BAS-RIBAUD			
A025- Exploitation combinée d'une sonde et d'une caméra Infra rouge (SWIR) pour imager le réseau vasculaire d'un muscle entier de souris dans la fenêtre NIR II. (Dubreil & Le guevel)			BAS-SALLE 2									BAS-SALLE 2	
A026- Multiplexed FRET biosensor imaging to visualize (part I) and quantify (part II) the dynamic coordination between cell signaling and mechanics during collective cell migration. (Sipieter & Moisan)									BAS-LE LEVANT			BAS-LE LEVANT	
A029- Imagerie ultrastructurale 3D par ultramicrotomie in-situ (technique SBF-SEM) (Erhardt & Genoud)		BAS-LE LEVANT			BAS-LE LEVANT		BAS-LE LEVANT					BAS-LE LEVANT	
A032- Immunomarquage et transparisation de tissus entiers, acquisition avec système home-made vs commercial (Dominici & Abélanet)				BAS-SALLE 5						BAS-SALLE 5			

Workshop	Sat 06 Nov		Sun 07 Nov			Mon 08 Nov		Tue 09 Nov	Wed 10 Nov			Thu 11 Nov	
	14:00-15:45	21:30-00:00	14:00-15:45	16:15-18:00	21:30-00:00	14:00-15:45	16:15-18:00	21:30-00:00	14:00-15:45	16:15-18:00	21:30-00:00	14:00-15:45	16:15-18:00
A035-Studying organogenesis of the pronephros of Xenopus tadpole early stages using light sheet and confocal microscopy (Chaumeton & Panier)					BAS-PORT-CROS						BAS-PORT-CROS		
A036- Lattice light-sheet microscopy for fast 3D time lapse of live samples (Ducros & Fernandez monreal)							BAS-LE LEVANT		BAS-LE LEVANT				
A037- Imaging transcription at a high temporal resolution in a living organism. (Bellec & Dufourt)			BAS-LE LEVANT				BAS-LE LEVANT						
A039- Imagerie 3D de la vascularisation par feuille de lumière pour évaluer l'évolution d'une pathologie et/ou l'efficacité d'un traitement (Michel & Dubreil)									BAS-TOUR FONDUE				
A040a- Coupling High Resolution Traction Force Microscopy with protein dynamics measurements (Ronde & Carl)		BAS-SALLE 3				BAS-SALLE 3							
A040b- Coupling High Resolution Traction Force Microscopy with protein dynamics measurements (Ronde & Carl)											BAS-TOUR FONDUE		
A041- Soft cell confiner development to decipher the impact of mechanical stimuli on cell (Riviere & Mouelhi)		BAS-RIBAUD						BAS-RIBAUD					
A042- Remember your wavefront: adaptive optics and memory effect in different regimes (Moretti & Rauer)	HAUT-SALLE 10					HAUT-SALLE 10			HAUT-SALLE 10				
A044- Etude de l'organisation des composants du cytosquelette et de l'autophagie dans un cadre infectieux sur des cellules à géométrie contrôlée (Salomé-desnoulez & Werkmeister)				BAS-LE LEVANT							BAS-LE LEVANT		
A045-Ultrastructure cellulaire par microscopie d'expansion (Georget & Blanchard)				BAS-SALLE 4							BAS-SALLE 4		
A046- Comment adapter son microscope TIRF pour faire du STORM! (Durel & Salles)				BAS-LE LEVANT					BAS-LE LEVANT				
A048- Mécanobiologie de cellules tumorales circulantes (Cinquin & Gasser)							BAS-SALLE 3						BAS-SALLE 3
A050- Multiplexed FRET biosensor imaging to visualize (part I) and quantify (part II) the dynamic coordination between cell signaling and mechanics during collective cell migration. (Girard & Borghi)										BAS-BERGERIE			BAS-BERGERIE
A052- Quantitative 3D Spatial Analysis of multicellular specimens (Organoids w/o clearing) (Marais & Allart)						BAS-BERGERIE	BAS-BERGERIE						
A055- Image Analysis Flash Tutorials (Marais & Goudin)													
A056- Microscopy on thin resin sections: multimodal and correlative approaches using scanning electron microscopy. (Canette & Boulogne)				BAS-LE LEVANT						BAS-LE LEVANT			BAS-LE LEVANT
A057- Introduction to single molecule localization super-resolution microscopy (SMLM) (Mondin)			BAS-LES SALINS					BAS-LES SALINS		BAS-LES SALINS			
A058- Probabilistic pipeline to extract reliable information from single molecule microscopy data. (Cassé & Masson)			BAS-PORT-CROS					BAS-PORT-CROS					
A059- Coordinate-based quantification of multidimensional and multicolor single-molecule localization microscopy data. (Levet)				BAS-BERGERIE							BAS-BERGERIE		
A061- Acquisitions intelligentes sous micromanager (Mutterer & Rouviere)				BAS-RIBAUD					BAS-RIBAUD				
A062- Contrôle qualité des données brutes de SMLM en temps réel (Mailfert & Bertaux)			BAS-LE LEVANT				BAS-LE LEVANT						
A063a- Transparisation, acquisition au microscope à feuillet de lumière et post traitement de sphéroïdes (Dominici & Gaggioli)							BAS-LES SALINS					BAS-LES SALINS	
A063b- Transparisation, acquisition au microscope à feuillet de lumière et post traitement de sphéroïdes (Dominici & Gaggioli)		BAS-LE LEVANT											
A065- Getting the most out of 3D pheroids by combining microfabricated wells, clarification techniques, standard confocal imaging and deep learning image processing (Riviere & Ahmad)					BAS-LE LEVANT					BAS-LE LEVANT			

A071- Microscopie d'expansion : stratégies et astuces pour l'analyse des cellules de mammifères en culture, de la levure S. cerevisiae et pour la visualisation de l'organisation mitochondriale (Dompierre & Rojo)						BAS-RIBAUD			BAS-SALLE 3				
Workshop	**Sat 06 Nov**		**Sun 07 Nov**			**Mon 08 Nov**		**Tue 09 Nov**	**Wed 10 Nov**			**Thu 11 Nov**	
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A072- Evolution of brain morphology from invertebrates to mammals. Everything we can learn from in toto 3D imaging of autofluorescence signals ? (Langevin & Belle)						BAS-SALLE 5						BAS-SALLE 5	
A073- Microscopie plein champ "haute résolution" et traitement numérique sur échantillon épais (Diakou-verdin)			BAS-RIBAUD									BAS-RIBAUD	
A074- 3D High Resolution imaging by PSF engineering using ZOLA-3D (Lelek)		HAUT-SALLE 8		HAUT-SALLE 8				HAUT-SALLE 8			HAUT-SALLE 8		
A075- Adaptive optics fluorescence microscopy for biological imaging (Fragola)													
A076- Alternative strategies to image multiple proteins in single molecule localization microscopy (Lévêque-fort & K-sreenivas)		BAS-SALLE 1									BAS-SALLE 1		
A077- BIAFlows (Bäcker)						BAS-ALMANARRE	BAS-ALMANARRE						
A078- BioImage-IT: Implement image processing workflows with tools from multiple software (Prigent & Ludovic.leconte)	BAS-BERGERIE											BAS-ALMANARRE	BAS-ALMANARRE
A079- Bioprinting as a solution for recreating a physiological environment on a slide (Furlan & Terrassoux)				BAS-PORT-CROS					BAS-PORT-CROS				
A080-Conception de prototypes connectés sous rapsberry et arduino Surveillance d'une expérimentation a distance (relevé de paramètres "connecté") pour moins de 100€. (Ronsin & Legou)					BAS-BUSINESS CENTER 1					BAS-BUSINESS CENTER 1			
A084- Dessiner, paramétrez et Imprimer en 3D (Detailleur & Ludovic.leconte)			BAS-BUSINESS CENTER 1					BAS-BUSINESS CENTER 1					
A090- Label-free virus detection and sorting with full-field interferometric microscopy (Alhaddad & Bey)	HAUT-SALLE 7			HAUT-SALLE 7									
A093- Measuring protein's orientation and organization by polarized fluorescence and polarized super resolution imaging (Munger & Sison)			HAUT-SALLE 7				HAUT-SALLE 7			HAUT-SALLE 7		HAUT-SALLE 7	
A094- Modulated excitation for enhanced localization : ModLoc (Illand & Lengauer)			BAS-SALLE 1					BAS-SALLE 1					
A096- Multimode fiber based-endoscope for fluorescence imaging using wavefront shaping (Wang)										HAUT-SALLE 6			HAUT-SALLE 6
A097- Nuclear Pores Complexe : a tool for metrology in Single Molecule Localization Microscopy (Pincet)		BAS-PORT-CROS			BAS-PORT-CROS								
A099- Practical considerations for reporting change in cell membrane tension using FLIM (Bun & Breton)			BAS-RIBAUD				BAS-RIBAUD						
A100- Préparation d'échantillon, acquisition et analyse d'image pour les applications de culture cellulaire 3D (Akbar & Lovo)				BAS-TOUR FONDUE				BAS-TOUR FONDUE					
A102- Publishing FAIR-ly with OMERO (Mateos langerak & Abélanet)		BAS-ALMANARRE	BAS-BERGERIE					BAS-ALMANARRE					
A106- Serial Block Face Imaging: imagerie 3D sans marquage de gros échantillons (Nedellec & Malloci)							BAS-SALLE 2			BAS-SALLE 2			
A107- Standardization of organoids culture allowing high throughput 3D live imaging using sospim technology (Galland)					HAUT-SALLE 6		HAUT-SALLE 6				HAUT-SALLE 6		
A111- Optogenetic control and measurement of cell contraction 1/2 (Artur Ruppel/Martial Balland) (Balland & Ruppel)					BAS-TOUR FONDUE							BAS-TOUR FONDUE	
A112- Virtual reality for multidimensional data visualization and analysis (Blanc)	HAUT-RESTAURANT ESTRADE		HAUT-RESTAURANT ESTRADE		HAUT-RESTAURANT ESTRADE		HAUT-RESTAURANT ESTRADE				HAUT-RESTAURANT ESTRADE		HAUT-RESTAURANT ESTRADE

A113- An example of feedback microscopy: Developing a High Content Screening (HCS) Optogenetics experiments. (Flores-flores & Sarthou)						BAS-LE LEVANT					BAS-LE LEVANT		BAS-LE LEVANT
A114- Deep learning made easy for microscopy: an introduction to ZeroCostDL4Mic and DeepImageJ (Mougeot)	BAS-ALMANARRE										BAS-ALMANARRE		
A116- Bioimage Analysis: Practice Deep Learning Without Coding (Badoual & Sage)		BAS-BERGERIE			BAS-BERGERIE			BAS-BERGERIE				BAS-BERGERIE	

Workshop	**Sat 06 Nov**		**Sun 07 Nov**			**Mon 08 Nov**		**Tue 09 Nov**	**Wed 10 Nov**			**Thu 11 Nov**	
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A117- Fluorescent imaging and techniques to study lignocellulosic biomass at the nanoscale (Habrant)	BAS-TOUR FONDUE		BAS-TOUR FONDUE										
A118- Relative localization of dendritic spine proteins in mouse brain tissue using 3D-STED microscopy and deconvolution. (Mazaud)	BAS-SALLE 4												BAS-SALLE 4
A119- L'autofluorescence chez les plantes: adversaire ou alliée? (Conéjéro & Jublanc)	BAS-TOUR FONDUE					BAS-TOUR FONDUE		BAS-LE LEVANT					
A120- Imagerie sur petit organoides 3D, Troubleshooting (Boyreau & Andrique)					BAS-SALLE 3					BAS-TOUR FONDUE			BAS-TOUR FONDUE
A121- CentrO2 : Influence de la concentration en oxygène pour l'imagerie dSTORM multi-couleur du cil primaire (Monier & Rousset)					BAS-LES SALINS			BAS-LE LEVANT					BAS-LE LEVANT
A122- Mapping elasticity of micro-patterned living cells by AFM (Janel)							BAS-LES SALINS			BAS-LES SALINS			
A123- Atomic Force Microscopy analysis of SARS-CoV-2 virus-like particles and producing cells: nanoscale imaging to mechanical characterization (Lyonnais & Arone)				BAS-LES SALINS		BAS-LES SALINS			BAS-LES SALINS				
A124- QuPath: pyramid image analysis for everyone. Case study: Deep-learning cell counting and quantification of histological slides. (Anceaume & Mailly)				BAS-SALLE 2							BAS-SALLE 2		
A125- Speckle-based computational microscopy : harnessing scattering for enhanced imaging of tissues (D'arco)					HAUT-SALLE 10							HAUT-SALLE 10	
A126- Microscopie quantitative : dynamique moléculaire par Spectroscopie de Corrélation de Fluorescence - Mise en oeuvre, calibration et analyse (Leclerc)						BAS-RIBAUD			BAS-RIBAUD				
A127- Microscopie large champ sur mésoscope homemade (Rogez)		HAUT-SALLE 9				HAUT-SALLE 9							
A128- Mechanical characterisation by AFM of murine oocytes to predict their fitness (Bulteau & Barbier)	BAS-LES SALINS	BAS-LES SALINS											
A129- AFM on microbial surfaces: basics of force spectroscopy measurements (Audrey beaussart & El-kirat-chatel)					BAS-LES SALINS								BAS-LES SALINS
A130- Optogenetic control of 3D micro- tissue 2/2 (Boudou & Méry)						BAS-TOUR FONDUE				BAS-TOUR FONDUE			
A131- Imagerie 3D d'organes transparisés par microscopie à feuille de lumière (Dupichaud & Lallemant)				BAS-LES SALINS		BAS-LE LEVANT			BAS-LE LEVANT				
A132- Structured Illumination Microscopy : SIM on cells ! (Dauphin & Salles)		BAS-LE LEVANT									BAS-LE LEVANT		
A133- 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 (Recher & Furlan)								BAS-LA CAPTE			BAS-LA CAPTE		
A134- Measuring turgor pressure of living plant cells with an Atomic Force Microscope (Bovio)								BAS-LES SALINS				BAS-LES SALINS	
A135- Imaging multiprotein complexes in the cytosol by super-resolution fluorescence. Introduction to lattice and dual iterative SIM (Valeva & Chatre)					BAS-LE LEVANT							BAS-LE LEVANT	
A136- Biosensing the cell: FRET by FLIM using AurkA kinase activation biosensor (Tramier)					BAS-RIBAUD						BAS-RIBAUD		
A137a- Imagerie de fluorescence par microscopie confocale spinning-disk : une exploration des avantages et limitations techniques sur 4 systèmes (en 2 parties) (Guilbert & Monterroso)				BAS-SALLE 3						BAS-SALLE 3			

A137b- Imagerie de fluorescence par microscopie confocale spinning-disk : une exploration des avantages et limitations techniques sur 4 systèmes (en 2 parties) (Guilbert & Monterroso)		BAS-PORT-CROS & BAS-TOUR FONDUE					BAS-PORT-CROS & BAS-TOUR FONDUE						
A138- Colocalisation de complexes macromoléculaires en super-résolution PALM/STORM (Marques)		BAS-LES SALINS				BAS-LES SALINS							
A139- Confronting Lattice SIM imaging to various scattering samples of different thickness (Bun & Danglot)	BAS-LE LEVANT									BAS-LE LEVANT			
A140- Du plus petit échantillon au plus gros, la microscopie à feuille de lumière face à l'enjeu du multi-échelle (Dumont & Tangara)			BAS-PORT-CROS			BAS-PORT-CROS							

Workshop	**Sat 06 Nov**		**Sun 07 Nov**			**Mon 08 Nov**		**Tue 09 Nov**	**Wed 10 Nov**			**Thu 11 Nov**	
	14:00-15:45	**21:30-00:00**	**14:00-15:45**	**16:15-18:00**	**21:30-00:00**	**14:00-15:45**	**16:15-18:00**	**21:30-00:00**	**14:00-15:45**	**16:15-18:00**	**21:30-00:00**	**14:00-15:45**	**16:15-18:00**
A141- Medium throughput imaging of thick samples: a practical comparison of different samples (Drosophila tissues and encapsulated spheroids) in native opaque state and after light-penetration facilitation (Recher & Duffraisse)					BAS-LES SALINS							BAS-LES SALINS	
A142- Microscopie à feuillet de lumière pour l'imagerie volumique (Gurchenkov)			BAS-LES SALINS							BAS-LES SALINS			
A143- Imagerie à feuillet de lumière des échantillons 3D montées d'une manière peu contraignante (Gurchenkov)	BAS-LES SALINS							BAS-LES SALINS					
A144- Use of detrended Fluorescence Lifetime Correlation Spectroscopy (dFLCS) to assess protein dynamics in the cell nucleus (Leray & Huet)				BAS-RIBAUD								BAS-RIBAUD	

FICHES DESCRIPTIVES DES ATELIERS

Workshops

Le concept original développé lors de la création de MiFoBio était de permettre aux participants de ne pas se limiter à des échanges théoriques, mais de se confronter avec l'instrument en conditions réelles de fonctionnement, sur des échantillons d'intérêt biologique. Ce concept, a priori risqué, s'est avéré extrêmement attractif et forme un des principaux attraits de l'Ecole. Il s'inscrit pleinement dans la pédagogie participative qui est au cœur du projet de l'école.

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The original concept developed at the time of the creation of MiFoBio was to allow the participants not to limit themselves to theoretical exchanges, but to confront themselves with the instrument in real operating conditions, on samples of biological interest. This concept, a priori risky, proved to be extremely attractive and forms one of the main attractions of the School. It is fully in line with the participatory pedagogy that is at the heart of the school's project.

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A002-3D STED microscopy for nanoscopic imaging of virus-host cell interactions.

Delphine Muriaux (delphine.muriaux@irim.cnrs.fr) and Rayane Dibsy

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. Since most of the viruses are around 30 to 140 nm in diameter, and associated host cell factors even smaller, super resolution microscopy becomes a tool of choices to study host cell factors - virus interactions at the nanoscale level, such as actin nanostructures at budding sites.

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 tool to obtained on the nanometer scale and now in 3D (70nm in the 3 directions) and in multi-color. Here, we will apply this 3D nanotechnology to very small biological objects like fluorescent HIV-derived virus-like-particles and on non infectious labelled virus expressing cells with labelled actin nano-filaments in fixed and living host cells.

Keywords : 3D STED, Multi-color STED, viruses, host cells, F-actin

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A003-Multicolor and 3D STORM to study localization of proteins adressed to apical membrane of polarized epithelial cells

Anne Cantereau (anne.cantereau@univ-poitiers.fr)

Abstract : STORM consists of high resolution imaging of biological sample stained with organic fluorophores generally coupled to antibodies. Incubation in oxygen-deprived buffer induces blinking and single molecule emission signals are recorded and analysed for spatial relocalisation under diffusion limited resolution. As far red fluorophores (i.e AF647) are more sensitive to this photoswitching compared to red fluorophore (i.e AF555) and quite impossible with green color, colocalization studies are difficult to conduct. Our workshop will show how 3 far red fluorophores with very close emission spectrum can be discriminated for multicolor imaging when using a combined system of dichroic mirror and of two cameras. Astigmatism lens will also be added to generate high resolution 3D imaging. This approach will be used to study localization of CFTR and associated proteins into membranes of epithelial cells cultured in 3D device.
This workshop is dedicated to novices, no prerequist are needed. Sample preparation and optical aspects will be explained. At the end, the participants will have anderstood the technical device necessary for 3D and multicolor STORM. They will be able to reproduce the experiment on any microscope equipped with appropriate optic elements. Because step of analysis is finally the most critical, we will discuss the critical points to improve signal.
Keywords : 3D STORM multicolor 3D cultured cells

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A004-Molecular dynamics, the challenges of live cytoskeletal microscopy in vivo.

François Robin (francois.robin@sorbonne-universite.fr) Anne-cécile Reymann (reymanna@igbmc.fr)

Abstract: In this workshop we propose to demonstrate the possibilities of live cell imaging techniques with super resolution using confocal spinning disk and Hilo TIRF microscopy in the context of molecular dynamics within the actin cytoskeleton of the C. elegans early embryo. We will perform high speed imaging of different actin binding proteins within the cortical meshwork bellow the cell membrane. By applying these two imaging techniques and comparing their performances on the same system, we aim at showing the limits and the advantages of each of these techniques in terms of resolution and sensitivity. Confocal spinning disk being more efficient for imaging larger motile structure such as filopodia. Hilo TIRF revealing the high density and fast dynamics of the single molecules within the underlying meshwork. We will present some image quantification that could be achieved, notably in terms of the possibilities these acquired data offer regarding single particle tracking.
In this workshop we will present and perform live imaging of cytoskeletal proteins in the early embryo from dynamic macroscopic architectures up to single molecule observation. The goal is to share with the participants the challenges of studying molecular dynamics in a live system and realize the expectations on the systems in order to move towards live super resolution microscopy techniques.
At the end of this workshop the participants will be able to:
- setup imaging protocols to observe molecular dynamics within a cytoskeletal meshwork;
- discuss possibilities and practical limitations for super-resolution imaging in live cells
- achieved some simple particle tracking quantification and analysis.
Keywords : actin dynamics, C. elegans embryo, Super Resolution live imaging, TIRF-Hilo microscopy, particle tracking analysis

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A005-Deep learning avec les doigts dans le moteur (2/2)

David Rousseau (david.rousseau@univ-angers.fr) Pejman Rasti (pejman.rasti@univ-angers.fr)

Abstract : Deep learning is currently used world-wide in almost all domains of image analysis as an alternative to traditional purely handcrafted tools. With their high capacity of tuning, deep neural networks are seemingly capable of solving informational tasks of any level of complexity provided enough annotated data are provided to them. However, this requested amount of data, typically 10000 instances, are overpassing most capacity of isolated human scientific consortium. A possible pathway though to apply deep learning without running the risk of overfitting is to proceed to data augmentation from an original small amount of significant, i.e. representative of the complexity of the expected data. In this tutorial we propose an overview of the different advanced techniques of data augmentation.

* Computer assisted annotation with Random-Forest
* Data augmentation by transfer learning
* Replication of instances after geometrical transformation
* Simulation of instances based on expert-based knowledge
* Simulation of instances based on neural networks via the so-called Generative adversarial networks.
* Illustration of these techniques are given in various bio-imaging problems including life sciences such as biology and medical imaging.
* Typical expected gain will be discussed

Keywords : Advanced deep learning, transfer learning, data augmentation, domain adaptation, fast annotation.

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A006-AFM on microbial surfaces : from imaging to single-cell force measurements

Audrey Audrey beaussart (audrey.beaussart@univ-lorraine.fr) Sofiane El-kirat-chatel (elkirat1@univ-lorraine.fr)

Abstract : AFM is a powerful tool to image and sense forces at the surface of living cells. In this practical, we will observe and qualify fine structures present at the surface of living microalgae (Chlorella vulgaris) with a few nanometers resolution using the latest developments in imaging techniques. In a first step, different techniques will be explained to immobilize microbes. Participants will then be trained to correctly mount the set-up, align the laser, and will have to possibility to adjust the parameters and sense which mode to use to optimise image quality (contact mode vs peak force tapping).

AFM can also be used to measure the adhesion of a whole living cell towards abiotic substrates or other cells.

For that, AFM tips need to be functionalized with a single microalgae which will then serve as a probe. Demonstration of cell attachment will be done during the workshop. For that, a single cell will be grabbed via the correlative functionality of the instrument combining AFM and optical/fluorescence and participants will be invited to measure forces toward abiotic samples.

Data treatment will be explained.

At the end of the practical, participants should be able to place the tip in the AFM set-up, align the laser, and acquire a simple image.

This workshop is open to biologists, chemists and physicists and competences in AFM or biophysics are not required.

Keywords : AFM, microorganisms, force spectroscopy, living cell, cell adhesion

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A011-Etude de la vascularisation sanguine et lymphatique de l'épididyme : clarification d'organe, microscopie à feuille de lumière et quantification en trois dimensions

Meryem Meryem tardivel (meryem.tardivel@univ-lille.fr)
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Abstract : L'épididyme est un organe du tractus génital male, accolé à la face postérieure du testicule et présentant une continuité anatomique. Il est composé d'un épithélium pseudo stratifié entourant une lumière dans laquelle les spermatozoïdes vont migrer pendant leur transit épididymaire. Le but de ces travaux est de mettre la lumière sur l'implication de cet organe dans les infertilités masculines et notamment l'implication de l'immunité qui représente 10-15% des cas d'infertilité. Les cellules de l'immunité sont les plus mobiles de l'organisme. Pour qu'elles puissent mener à bien leurs fonctions, elles ont besoin d'autoroutes pour circuler rapidement. Il existe deux voies de circulation rapides dans l'organisme ces sont les systèmes vasculaires sanguins et lymphatiques. Dans l'épididyme à ce jour il n'y a aucune étude dans laquelle les deux vascularisations sont abordées conjointement. Ce projet s'appuie sur l'utilisation de souris transgéniques VEGFR3-YFP. Le Vegfr3 est un récepteur transmembranaire de type tyrosine. C'est un régulateur clé du développement du système lymphatique. A travers cet atelier et ce projet, nous souhaitons démontrer que les techniques de clarification permettent de rendre transparent des organes à consistance plus dense comme l'épididyme.

Keywords : clarification, SPIM, épididyme, Imaris, organisation et visualisation spatiale, quantification

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A012-3D quantitative analysis of colocalisation or spatial coupling in conventional and super resolution microscopy.

Lydia Danglot (lydia.danglot@inserm.fr) Thibault Lagache (thibault.lagache76@gmail.com)

Abstract : The purpose of the workshop is to bring together acquisition tips and tricks for 3D quantitative imaging of colocalisation « per se » or coupling between 2 molecule indirectly associated without any necessary overlap but with correlated spatial distribution. We will take the example of localization of proteins at the synapse. Here the proteins can be physically colocalized (in the same cell, a receptor with its anchoring proteins) or can be apposed (2 proteins physically separated in 2 different cells but present at both side of the synaptic contact). We will begin with a session on microscope with acquisition of 3D stack imaging in conventional and multicolor STED microscopy to raise all the technical details that users have to bear in mind when comparing two biological conditions (z sampling, saturation, bleaching, compromise between x y and z resolution, matrix size and acquisition length). Then we will switch to computer rooms to process to image analysis to explain and handle Icy SODA suite (Standard Object Distance Analysis) on Mifobio acquired images (confocal and 3D STED). We will show how to identify coupled molecules and provide 3D distance, p value and color-coded localisation map within the cell on STED images. The 3D rendering map in color will show where are the coupled molecules and where are the isolated ones. Because of a lack of time, we won't be able to show image acquisition on all microscopy modalities, however this workshop is related both to the GT STORM « parcours » and to the SIM workshop proposed by A. salles. At the end of the practical on STED images, we will use images coming from other workshops (SIM and STORM or participant's own images), to show in computers' room to the candidate, how to analyse the proximity between 2 or 3 proteins. At the end of the workshop candidates will know how to acquire and analyse 3D images to detect colocalisation or coupling whatever the microscopy method that is used.

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A013-Comparing multicolor Single-Molecule Localization Microscopy strategies: Application to the neuronal cytoskeleton

Christophe Leterrier (christophe.leterrier@univ-amu.fr) Karoline Friedl (karoline.friedl@etu.univ-amu.fr)

Abstract : Pathology in neurons often goes hand in hand with the loss of cell structures that facilitate plasticity and compound transport. Furthermore, the requirements for these scaffolds are quite peculiar: on the one hand, the architecture of neurons must be stably maintained, on the other hand neurons have to allow for cellular plasticity and flexibility. In the NeuroCyto team, we are particularly interested in the organization and functions of axonal actin. The actin cytoskeleton is likely to provide both structural stability and plasticity to the exquisite arborization of axons. Single-Molecule Localization Microscopy (SMLM) has been instrumental in revealing new axonal actin structures such as periodic submembrane actin rings as well as intra-axonal actin hotspots and trails. Multicolor SMLM colocalizes actin with other components of the cytoskeleton with sub-diffraction precision, elucidating their structural and functional interplay. We are interested in using SMLM to look at multiple markers within thin biological objects such as neuronal axons and dendrites. However, obtaining multicolor images with the best resolution and quality remains challenging. The aim of the workshop will be to compare three different strategies for 2-color SMLM:

- Sequential detection of fluorophores with different excitation/emission spectra (i.e. CF568/AF647 in the red and far-red spectrum, respectively)
- Sequential detection of different targets through DNA-PAINT with imagers coupled to the same fluorophore
- Spectral unmixing of fluorophores with close excitation/emission spectra (AF647/CF680).

This workshop will show the participants how to use 3D multicolor Single Molecule Localization Microscopy (SMLM) on the example of the nanoscale architecture of the axonal cytoskeleton; they will discover the strength and requirements of the three different multicolor approaches possible on this setup.

Keywords : cytoskeleton, SMLM, 3D-STORM, DAISY, DNA-PAINT, axon, neuron, multiplexing, colocalization

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A014-Deconvolution 3D

Ferréol Soulez (ferreol.soulez@univ-lyon1.fr) Daniel Sage (daniel.sage@epfl.ch)

Abstract : Le but de la déconvolution est de compenser numériquement le flou introduit par le microscope. En microscopie 3D, la déconvolution permet d'améliorer les images sur plusieurs points:
- en améliorant la résolution (axiale en particulier),
- en diminuant le bruit (en particulier à faible flux),
- en améliorant le contraste.

Cela fait de la déconvolution un outil précieux pour améliorer les post traitements comme la segmentation.

Cet atelier propose de démystifier les méthodes de déconvolution et propose une prise en main des logiciels libres de deconvolution.

Il sera en 4 parties:
- une brève description théorique,
- les points importants pour une déconvolution réussie: conditions d'acquisition
- comment avoir une bonne PSF
- illustration des méthodes classiques avec le plugin DeconvolutionLab2 sur des données simulées et réelles

- dans le cas où la PSF n'est pas connue nous guiderons les utilisateurs dans l'utilisation de plugins de déconvolution aveugle ou myope

Keywords Deconvolution, Deconvolution aveugle

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A015-Deep learning sans se salir les doigts (1/2)

David Rousseau (david.rousseau@univ-angers.fr)
Pejman Rasti (pejman.rasti@univ-angers.fr)

Abstract : In this workshop we will introduce the basics of deep learning and illustrate them practically with examples in bioimaging. Hands on will be provided with software environments which do not require specific computing configuration nor programming skills.
Example will include classification of images and segmentation of objects in images. After this workshop you will be able to run deep learning for your own application and will be able to push forward to more advanced skills.

Keywords : deep learning

A016-Deep bar à images

David Rousseau (david.rousseau@univ-angers.fr)
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Abstract
* Vous avez un jeu d'images annoté. Vous avez un problème de classification d'images, de reconnaissance d'objet, segmentation, de débruitage d'image, de superrésolution ...
Venez tester les méthodes de deep learning les plus avancées sur une machine puissante.
* Vous ne disposez pas de données annotées mais seulement de données, nous vous montrons comment réaliser une annotation.

Keywords : Deep learning

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A018-Obtenir la PSF d'un système de microscopie de fluorescence

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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 ainsi que les différentes façons d'obtenir des PSF théoriques et expérimentales (i.e., à partir de microbilles fluorescentes). Les participants seront formés à l'utilisation d'outils open-source (sous-forme de plugins ImageJ) leur permettant de réaliser cette tâche.

Keywords : Estimation PSF, microscopie de fluorescence, microbilles, imagerie computationnelle

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A020-Expansion microscopy imaging with a lattice light-sheet microscope

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Mathieu Ducros (mathieu.ducros@u-bordeaux.fr)

Abstract : Expansion microscopy (ExM) consists in clearing and swelling a sample by a factor 4x, after fixation and staining. Expanded samples can then be imaged with conventional diffraction-limited fluorescent microscopes such as wide field, confocal or light-sheets. The resulting resolution is increased by the expansion factor, thus producing a super-resolution image without the need of a super resolution technique such as STORM, PALM or STED.

The lattice light sheet microscope (LLSM) is a very powerful fluorescent imaging technique that combines the advantage of conventional light-sheet fluorescence microscopes (optical sectioning, high temporal resolution and low photobleaching) with a specific beam shaping method to create a very thin and homogeneous planar illumination pattern (high spatial resolution).

Recently LLSM and ExM have been combined to produce high quality super resolution images of large samples at very fast rates (Gao et al, Science 2019). In this workshop we will explain the proExM protocol, the sample handling and mounting on the LLSM sample holder, the acquisition of mosaic of images, and how to stitch the tiled 3D acquisitions. The technique will be applied in neuronal cultures and brain slices expanded with the Boyden technique. The final objective will be to resolve postsynaptic and presynaptic compartments labeled with antibodies to specific markers, which distance (around 200 nm) cannot be distinguished by conventional techniques. This approach will help to investigate protein distribution in synapses at nanoscale level, and thus their role in synaptic transmission and plasticity.

At the end of this workshop the participant will be able to understand the main steps of sample preparation, and the handling and mounting of expanded samples in the LLSM. We will show the tuning parameters to acquire best quality LLSM images and reconstruction of a large field of view super-resolution image from a tiled LLSM acquisition.

Keywords : Expansion Microscopy, Lattice Light Sheet, Superresolution

A023-Real-time mitochondrial Ca2+ and ATP measurements in mammalian cells using single excitation wavelength dual colour FLIM

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Abstract : Monitoring dynamic fluctuations of both calcium and ATP homeostasis simultaneously in mitochondria using multiplex FRET biosensors provides an opportunity to study how calcium finely tunes the metabolic state of mammalian cells. Under physiological condition, mitochondrial calcium level has been proposed to modulate the ATP production in the mitochondria through the activation of dehydrogenases and ATP synthase. Attempts to correlate the rise of mitochondrial calcium and the ATP production has been performed indirectly in different models but it has never been done simultaneously what could demonstrate a timely causality between the two processes. Based on the strategy developed by the group of M. Tramier (Déméautis et al., Scientific Reports, 2017), we plan to use a 440 single excitation wavelength of the two donor mTurquoise2 (mTQ2) and LSSmOrange and a dual colour FLIM system to simultaneously measure two genetically encoded FRET biosensors. To avoid any spectral bleed through we use the non-fluorescent acceptor ShadowG for mTQ2 and red-shifted mKate2 for LSSmOrange. The original biosensors used for this strategy is 4mtD3cpv for calcium (Palmer et al Chem Biol. 2006) and Ateam mito for ATP (Imamura H,. PNAS. 2009). The aim is to detect fluorescence lifetime images of two donors in the same cellular localization by using FRET biosensors in mammalian cells.

At the end of this workshop the participants will be able to measure fluctuations of mitochondrial calcium and ATP simultaneously upon an IP3-agonist stimulation in different cellular compartments.

We will discuss the key role of mitochondrial calcium in the regulation of energy supply and demand. And finally, we will present the limitations of FRET biosensors and the controls that have to be done in order to perform real-time mitochondrial and ATP measurements in mammalian cells.
Keywords : FLIM, FRET, mTQ, mVenus, LSSmOrange, mKate

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A025-Exploitation combinée d'une sonde et d'une caméra Infra rouge (SWIR) pour imager le réseau vasculaire d'un muscle entier de souris dans la fenêtre NIR II.
Laurence Dubreil (laurence.dubreil@oniris-nantes.fr)
Xavier Le guevel (xavier.le-guevel@univ-grenoble-alpes.fr)
Abstract : L'UMR703 PAnTher INRAE/Oniris développe un programme de recherche en thérapie cellulaire pour traiter les dystrophies musculaires. L'équipe de Karl Rouger a identifié une population de cellules souches adultes du muscle comme possible candidat thérapeutique innovant pour traiter ces maladies. Afin d'évaluer son potentiel thérapeutique, il est primordial d'élaborer et de valider des outils pour permettre de qualifier les répercussions de son administration sur le tissu pathologique, notamment sur la vascularisation du muscle squelettique.
L'imagerie de la fluorescence dans la fenêtre NIR II entre 900 nm et 1700 nm appelée également SWIR (Short Wave Infrared Region) permet d'améliorer la détection des signaux en épaisseur et la résolution des images obtenues. Cette amélioration est due à une faible absorption des photons dans cette région du spectre, une faible autofluorescence et une réduction de la diffusion de la lumière dans les tissus comparé à ce qui est obtenu dans le visible ou la fenêtre NIR I (700-900 nm).

Des travaux ont été récemment publiés par l'équipe de Xavier Le Guével sur l'utilisation de l'imagerie SWIR afin de distinguer de façon non invasive les désordres vasculaires chez des souris transgéniques. Ces études ont été réalisées à l'aide de nouveaux agents de contrastes à base de nanoclusters d'or capables d'émettre au-dessus de 1250 nm (Musnier et al., Nanoscale (2019), 10.1039/c9nr04120f) et qui ont été administrée par voie systémique chez la souris. Il a été également démontré que la restauration des images SWIR par la méthode de Monte Carlo permettait une amélioration de la résolution spatiale et du contraste jusqu'à 4 mm en profondeur (Zhixi et al., ACS Nano (2020), doi.org/10.1021/acsnano.0c01174).

L'objectif de l'atelier est de montrer les atouts de l'imagerie SWIR non-invasive et en temps réel des tissus en profondeurs in vivo chez le petit animal (l'aide a la chirurgie ?) mais également après fixation pour la recherche préclinique.
Keywords : Fluorescence NIR II, imagerie SWIR, vascularisation, muscle, tumeur, tracking cellulaire

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A026-Multiplexed FRET biosensor imaging to visualize (part I) and quantify (part II) the dynamic coordination between cell signaling and mechanics during collective cell migration.

Francois Sipieter (francois.sipieter@ijm.fr)
Nicolas Moisan (nicolasmoisan@hotmail.com)

Abstract : Collective cell migration (CCM) plays a pivotal role in the formation of organs, tissue regeneration, and many disease processes. Cell migration relies on a continuous turnover of cells adhesions to their environment under the influence of biochemical signals and mechanical cues. How cells integrate signaling pathways and mechanics to coordinate their migration in a collective is poorly understood.
To address this issue, we want to investigate the spatiotemporal relationships between the dynamics of kinase activities and molecular mechanics that are involved in CCM.
To this aim, we will use an approach relying on multiplexed functional live cell imaging with custom genetically encoded FRET biosensors.
At the end of this workshop (Part I), participants will know how to acquire movies of multiplexed sensors in live cells and assess the acquisition quality in light of the specific requirements of FRET sensor image analysis. A complementary workshop (Part II) will focus on the quantitative image analysis, where we will detail step by step the analysis pipeline to automatically segment and track individual migrating cells and their biosensors outputs within the collective.

Keywords : Multiplexed FRET imaging, biosensors, mechanotransduction, collective cell migration, fluorescence.

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A029-Imagerie ultrastructurale 3D par ultramicrotomie in-situ (technique SBF-SEM)

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Abstract : L'ultramicrotomie in-situ aussi appelée Serial Block Face Imaging (SBF) est une technique d'imagerie 3D permettant d'analyser, à l'échelle nanométrique, de grands volumes de tissus biologiques. Dans cette approche, les échantillons sont préparés selon les techniques classiques de microscopie électronique à transmission, tout en veillant à optimiser le contraste introduit lors de la préparation des échantillons. La surface du bloc qui met en évidence les atomes lourds liés aux structures cellulaires sont visualisés grâce à un microscope électronique à balayage (SEM). Après chaque image une nouvelle surface du bloc est mise en évidence grâce à un ultramicrotome intégré dans la chambre du microscope qui ôte de la surface du block une fine couche de matériel d'épaisseur déterminée. Les images ultrastructurales 2D séquentiellement acquises sont utilisées pour construire le volume 3D de l'échantillon.
Cet atelier permettra aux participants de mieux appréhender la complexité de l'imagerie 3D par SBF. Les stagiaires seront amenés à réaliser des acquisitions 3D, d'analyser de grand volume, d'inspecter la connectivité des structures et de corréler ces images avec leurs connaissances en imagerie du vivant.

Keywords : imagerie 3D, Ultrastructure, microtomie, Volume

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A032-Immunomarquage et transparisation de tissus entiers, acquisition avec système home-made vs commercial

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Abstract : Durant ces dernières années, beaucoup de laboratoires se sont penchés sur différentes méthodes de transparisation afin de visualiser des tissus entiers permettant ainsi d'avoir accès à la structure spatiale complète des organes. Nous proposons, dans cet atelier, de faire un état des lieux des différentes techniques de transparisation disponibles et d'en connaitre leurs spécificités afin de pouvoir choisir la méthode la plus appropriée pour l'expérimentation. Nous reviendrons aussi sur les protocoles d'immunomarquages associés à chaque technique et à l'échantillon utilisé.
Par la suite, nous proposons d'acquérir les échantillons préalablement traités avec deux différents microscopes à feuillet de lumière : Un système commercial et un système homemade (Ultramacroscope modulaire, plate-forme MICA)
Pour finir, avec un logiciel adapté au traitement d'image 3D, nous étudierons les post traitements possibles sur les images 3D acquises avec un microscope à feuillet de lumière.
A la fin de cet atelier, les participants auront acquis des bases solides sur tout le processus de transparisation jusqu'aux post traitements des images et pourront ainsi être autonome afin de traiter leurs propres échantillons.

Keywords : Immunomarquage, transparisation, Microscope à feuillet de lumière, Imaris

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A035-Studying organogenesis of the pronephros of Xenopus tadpole early stages using light sheet and confocal microscopy

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Abstract : In this workshop we demonstrate how to study the organogenesis and 3D-morphology of early stages of the pronephros formation of xenopus tadpoles using the complementary performance of light sheet imaging and confocal imaging. We will use xenopus embryos at stage E21-E28 and developed a specific sample preparation protocol (clarification by refractive index matching) optimized for the tadpole and enabling combining the two microscopy techniques. We will perform 3D-imaging with light sheet microscopy in order to determinate the volume and cell number of the pronephros in whole embryos. Then we switch to confocal mode to investigate details of cell-to-cell and cell matrix interactions. We aim at showing the limits and advantages of each technique and compare their performances and possibilities in terms of resolution, sensitivity and imaging depth.

Keywords : Light sheet microscopy, confocal microscopy, 3D high resolution imaging, optical clearing, xenopus morphogenesis, pronephros organogenesis

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A036-Lattice light-sheet microscopy for fast 3D time lapse of live samples

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Abstract : The lattice light sheet microscope (LLSM) is a very powerful fluorescent imaging technique that combines the advantage of conventional light-sheet fluorescence microscopes (optical sectioning, high temporal resolution and low photobleaching) with a specific beam shaping method to create a very thin and homogeneous planar illumination pattern (high spatial resolution).

Due to its very low photobleaching/phototoxicity the LLSM is ideally suited to image live samples for long time-lapse at high spatiotemporal resolution. We will use live cells labeled with different cell markers to capture very fast events (i.e. lysosomal transport, membrane ruffles) in 3D+t.

At the end of this workshop the participant will:
- Understand the principles and main advantages of the LLSM
- Be able to mount a sample on the LLSM sample holder
- Optimize the acquisition parameters to perform best quality 3D imaging
- Visualize the 3D+t acquired with LLSM

Keywords : light-sheet microscopy, lattice, time lapse, photobleaching

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A037-Imaging transcription at a high temporal resolution in a living organism.

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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 Drosophila 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 on a specific slide 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. For this, we will use a LSM880 with Fast AiryScan module to be able to image at a high time frequency. To quantify transcriptional dynamics, we will present tools to extract intensity fluctuation of each transcriptional dots, at a single cell level and during time.
At the end of this workshop the participants will be able to prepare living Drosophila embryos, to image transcription with a high temporal resolution and extract intensities during time.

Keywords : live imaging, embryo, confocal microscopy, transcription, development, high temporal resolution.

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A039-Imagerie 3D de la vascularisation par feuille de lumière pour évaluer l'évolution d'une pathologie et/ou l'efficacité d'un traitement

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Abstract : Au cours de cet atelier, nous nous intéresserons à l'imagerie 3D de la vascularisation dans des tissus pathologiques en utilisant la feuille de lumière i) dans le muscle dystrophique ii) dans de la tumeur de cerveau chez le rat.

L'équipe de Karl Rouger (UMR703) conduit des recherches sur l'utilisation de cellules souches administrées à des modèles rongeurs atteints par la myopathie de Duchenne (MD) afin d'évaluer leur potentiel thérapeutique.
La caractérisation du réseau vasculaire pourrait renseigner sur l'évolution de la MD et/ou l'effet d'un traitement sur le muscle squelettique du rat DMD, animal modèle de la MD. Le réseau vasculaire du

muscle squelettique de rat myopathe sera imagé en 3D à partir du muscle gastrocnémien prélevé, fixé et transparisé suite à l'injection d'une sonde fluorescente dans la circulation sanguine de l'animal modèle. Le marquage des vaisseaux sera également réalisé post prélèvement. Les deux méthodes d'explorations seront comparées.

Yolanda Prezado et son équipe travail au sein de l'Institut Curie sur la mise au point d'une nouvelle méthode de radiothérapie des tumeurs de cerveau chez le rat en utilisant des mini faisceaux de protons. Cette méthode permet une plus grande préservation des tissus sains par rapport aux traitements conventionnels. L'imagerie 3D des vaisseaux au niveau de la tumeur et des régions saines du cerveau pourrait renseigner sur l'évolution de la tumeur et/ou l'efficacité du traitement. Le réseau vasculaire du cerveau et de la tumeur sera imagé en 3D à partir de tranches épaisses de cerveau, fixées et transparisées. Les deux méthodes de marquage des vaisseaux in vivo/ex vivo comme précédemment seront comparées.

A la fin de l'atelier, les participants auront acquis les connaissances nécessaires des étapes clés d'un marquage du réseau vasculaire, in vivo, ex vivo. Ils auront une connaissance opérationnelle de l'imagerie de gros volumes et des écueils à surmonter pour en faire une analyse scientifique rigoureuse.

Keywords : Marquage vasculaire ; échantillons transparisés ; microscope a feuille de lumière ;

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A040a / A040b -Coupling High Resolution Traction Force Microscopy with protein dynamics measurements

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Abstract : Cells adhering to the extracellular matrix can sense and respond to a wide variety of chemical and physical features of the adhesive surface. The response of the cell includes contractile force generation which plays a critical role in cell adhesion, migration, and extracellular matrix reorganization. Through their actomyosin machinery the cell generates an internal tension that contracts the cell body and thus exerts tractions on the underlying substrate. Theses tractions take place at subcellular structures called focal adhesions which physically link the actin cytoskeleton to the extracellular matrix. The TFM (Traction Force Microscopy) technique allows determining the tensile forces exerted by the cells on their substrate. For this purpose, cells are cultured on polyacrylamide hydrogels containing fluorescent microbeads as markers for the determination of stresses due to tensile forces. Nevertheless, to understand how adherent cells regulate and transduce traction forces, quantitative techniques are needed to measure forces at the cell–matrix interface while recording biological events. For this purpose, we first developed high-resolution TFM (hTFM) which combine the use of 2 colours nanobeads with image processing algorithms to specifically allocate vector forces to the different size of focal adhesions. Then, to correlate traction force with focal adhesion-related signalling, spectroscopy techniques such as FRAP (Fluorescence Recovery After Photobleaching) will be used to provide the diffusion time of fluorescent objects at focal adhesions.

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

- Set the acquisition conditions optimally on a multimodal microscope for the combination of high-resolution TFM and FRAP
- Acquire images of fluorescent beads in kinetics and at different positions.
- Measure the movement of beads using the PIV (Particle Image Velocymetry) technique.

- Determine from the displacements of the beads the traction forces usi

Keywords : Mecanobiology, Traction Force, TFM, FRAP, Adhesion, Migration

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A041-Soft cell confiner development to decipher the impact of mechanical stimuli on cell

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Abstract

Context:

Increasing evidences indicate that mechanical cues from cell micro-environment are impacted many critical cellular functions and are involved in a number of human diseases such as ageing, and cancer progression.

The effect of matrix stiffness has been and is still extensively studied in the context of stem cells and tumor progression. On the other hand, limited studies have focused on the role of mechanical stresses. Such limitation is largely coming from the lack of standard in vitro assays enabling extended and repeated mechanical stimulations, and compatible with dynamic quantification of changes in cell phenotype and genotype occurring during such imposed stress.

None of the set-ups currently used to apply a defined stress on an entire cell population fulfills all the criteria needed for long-term dynamic analysis (well-control applied stress, compatibility with high-resolution microscopy and video-microscopy, efficient medium and oxygen renewal, easy cell retrieval after the imposed compressive stress).

In this workshop, we will present how to use hydrogels to submit cells to a defined stress, in a 2D or 3D environment, for several days, with no impact on cells by other means.

In particular, we will present an agarose-based microsystem that we have developed that enable precise control of cell confinement for a defined cell population. The set-up rigidity matches physiological conditions and enable passive medium renewal. It is compatible with time-lapse microscopy, in-situ immunostaining, and classical molecular analysis.

Objective:

The aim of the workshop would be to present how to use such confining systems, and what kind of dynamic analysis could be done with it. At the end of this workshop the participants will know how to perform simple confining experiments in their lab. They will also know if more sophisticated agarose-based microsystem could be of interest for their research project.

Keywords : Mechanobiology, hydrogel, Microsystems, cell biology

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A042-Remember your wavefront: adaptive optics and memory effect in different regimes

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Abstract : Wavefront shaping is opening new frontiers on optical imaging. Out of many concepts, the idea of memory effect (ME), or isoplanatic patch, is crucial to understand applicability and limits of the techniques which take advantage of this branch of optics, as Adaptive Optics (AO). We will take an overview on the actual techniques and approaches to recover a focus after medium across different regimes, from low aberrations to highly scattering medium. We will give a clear and physical view on the of the key parameters which affect the quality of the obtained focus, and image retrieval capabilities. With a liquid crystal phase Spatial Light Modular (SLM), and few lines of code, we will see how to perform a basic optimization procedure in the different regimes, and with different balance of thickness, mean free path, and transport length, and we will show which are the limit on

the aforementioned techniques. At the end of this workshop participants will be able to have a solid vision of the memory effect and its role in adaptive optics, gain knowledge about basic control of a SLM, and how to perform simple optimization scheme for adaptive optics in highly scattering samples.
Prior knowledge of optics and programming is a plus, but not mandatory.
And remember: bring your sample!
Keywords : memory effect, wavefront shaping, adaptive optics, optimization

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A044-étude de l'organisation des composants du cytosquelette et de l'autophagie dans un cadre infectieux sur des cellules à géométrie contrôlée
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Elisabeth Werkmeister (elisabeth.werkmeister@ibl.cnrs.fr)
Abstract : Ces dernières années mettent en évidence un intérêt de plus en plus fort pour l'étude des propriétés mécaniques cellulaires. Ces dernières jouent un rôle majeur dans de nombreuses fonctions biologiques essentielles, notamment la migration, l'adhésion, la différenciation et le vieillissement. Ainsi, la façon dont les cellules répondent à leur environnement (forces adhésives, déformations exercées par les cellules adjacentes et influence de la matrice extracellulaire) et répondent à des sollicitations mécaniques constituent une problématique fondamentale mais qui reste encore à l'heure actuelle peu comprise.
Nous nous sommes particulièrement intéressés à la manière dont l'autophagie pouvait moduler les propriétés mécaniques, en induisant ou bloquant ce phénomène cellulaire. Il est en effet établi que lors du vieillissement, les propriétés mécaniques des cellules se trouvent modifiées et l'autophagie est altérée. Nous recherchons donc à déterminer quelle pourrait être l'influence de modifications dans la réponse cellulaire à des changements mécaniques sur l'autophagie et, en tant que voie de dégradation des pathogènes intracellulaires, sur l'infection. Y-a-t-il corrélation ?
Durant ce TP nous nous intéresserons à l'organisation des composants du cytosquelette (actine, microtubules, filaments intermédiaires) dans le cadre de l'autophagie pour des cellules dont l'environnement mécanique est contrôlé grâce à l'utilisation de surfaces contrôlées.
Keywords : micro patterns, cytosquelette, autophagie, imagerie de haute résolution

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A045-Ultrastructure cellulaire par microscopie d'expansion
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Abstract : L'ultrastructure d'une cellule est très souvent étudiée par microscopie électronique, la microscopie photonique étant trop limitée en résolution spatiale. Depuis quelques années, les approches de microscopie d'expansion qui consistent à agrandir physiquement son échantillon offrent une nouvelle perspective d'étude d'ultrastructure cellulaire par la microscopie photonique. Il est alors aussi possible de localiser sa protéine favorite à l'échelle sub-cellulaire. Il n'est donc plus absolument nécessaire d'utiliser la microscopie électronique qui peut être lourde à mettre en place. Toutefois, les résolutions atteintes ne seront jamais celles de la microscopie électronique.
L'atelier proposé permettra de découvrir en pratique la microscopie d'expansion appliquée à une mono-couche cellulaire et d'en évaluer les limites en terme de résolution spatiale. Les échantillons

seront observés après expansion en microscopie confocale à balayage laser. Certains échantillons seront également imagés en STED afin d'évaluer le gain de résolution en combinant la microscopie d'expansion et le STED. Il sera ainsi possible d'observer en direct un centrosome, microtubule et un cil primaire. Les pores nucléaires seront imagés en mode STED avant ou après expansion..... Toutes ces structures caractéristiques pourront être couplées à la détection de sa ou ses protéines favorites par immunofluorescence.
A la fin de l'atelier, les participants de l'atelier sauront comment réaliser une expérience de microscopie d'expansion et observer leur échantillon en microscopie confocale ou STED.

Keywords : microscopie d'expansion, ultrastructure cellulaire, super-résolution

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A046-Comment adapter son microscope TIRF pour faire du STORM!

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Abstract : Pour répondre aux besoins des chercheurs en matière de super résolution, et à moindre coût, il est possible d'apporter des modifications à un microscope conventionnel TIRF, pour accéder à la technique de super résolution STORM. Dans cet atelier, nous verrons toutes les étapes de la réflexion du projet à sa réalisation technique et sa validation. Nous identifierons les points sensibles et verrons comment lever les verrous techniques.
Cet atelier s'inscrit dans un parcours d'ateliers autour du STORM. Il s'adresse à un public de chercheurs ou d'ingénieurs biologistes, ayant des connaissances de base en microscopie de molécule unique.
Déroulement de l'atelier avec l'implication active des participants :
1 _ Analyser les étapes, les besoins pour cette transformation, quels lasers pour quelles techniques ?
2 _ Atelier pratique manipulation de lasers : installation et alignement du laser dans la fibre à l'aide d'un puissance mètre.
Discussion : Pourquoi partir d'un microscope TIRF ? Intérêt du bras TIRF, des éléments d'optique (filtres, objectif, caméra)
3 _ Atelier pratique acquisition d'images : réalisation d'acquisitions d'images par les participants, en jouant sur différents paramètres des lasers.
4 _ Atelier pratique reconstruction d'images : utilisation d'un logiciel libre (UNLOC) pour reconstruire les images super-résolues.
Discussion : Impact de l'alignement et de la puissance des lasers sur le clignotement des fluorophores. Évaluation de la fiabilité d'un système « maison » en utilisant les informations données par le logiciel UNLOC. Avantages et limites d'un tel système.
A l'issue de cet atelier, les participants auront acquis des connaissances théoriques et pratiques, leur permettant d'upgrader un microscope TIRF pour faire du STORM. Ils auront pu se familiariser avec certains gestes expérimentaux et seront sensibilisés aux éléments importants d'un microscope STORM, afin d'avoir un regard critique lors de l'achat d'un système commercial.
Keywords : Microscopie TIRF, STORM, home made

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A048-Mécanobiologie de cellules tumorales circulantes

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Abstract : L'objectif de l'atelier est d'observer la déformation et relaxation de cellules tumorales circulantes (CTCs) sous flux imposé, dans des constrictions microfluidiques reproduisant la topologie de la microvasculature. Ce dispositif microfluidique permet de reproduire les stress et déformations subies par les CTCs dans la circulation sanguine, afin de comprendre les mécanismes mis en jeu dans la survie des CTCs dans le contexte du développement de métastases.
La cellule sera imagée le long de son parcours dans la constriction jusque dans le piège prévu pour la capturer et l'observer lors de sa récupération de la déformation. Des marquages du noyau et de la membrane permettront de suivre la forme de la cellule et de son noyau au cours de la déformation. La lignée cellulaire a été transfectée stablement pour exprimer des marqueurs fluorescents pour les ruptures de l'ADN double-brin (53BP) et ruptures de la membrane nucléaire (cGAS). L'évolution des dommages post-constriction sera suivie en imageant la cellule dans le piège.

A la fin de l'atelier, les participants seront sensibilisés au travail sur puce microfluidique et aux différents défis que cela suppose : marquage, acquisition de phénomènes dynamiques, objets en mouvements dans le champ de vue. Ils auront découvert la gestion et l'analyse de fichiers volumineux, issus de l'acquisition d'images de haute qualité à haute fréquence.
Keywords : Cellules circulantes, Microfluidics, Cell Mechanics, 3D

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A050-Multiplexed FRET biosensor imaging to visualize (part I) and quantify (part II) the dynamic coordination between cell signaling and mechanics during collective cell migration.

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Abstract : Biological images are a very rich source of information. Especially with fluorescent reporters and live cell imaging, researchers can gain a tremendous amount of data. With the help of automated image analysis algorithms, the information can be quantified. Automated image analysis reduces subjectivity and increases throughput but can also detect changes too subtle for the experimenter to assess. Rich multiparametric experimental raw data generated in the complementary workshop (Part I) will be analyze here. We will address this challenge by using our home-made image analysis pipeline developed on ImageJ/MatLab for analyzing hundreds to thousands of images. First the software tools allow images pre-processing to correct illumination and anomalies, then images segmentation and cells tracking to identify and follow individual cells within the collective using automated thresholding and configured segmentation algorithms. Lastly, particular features of interest will be extracted from every cell in every image, including categories such as morphology and displacements parameters (size, velocity, etc.), fluorescence intensities for FRET calculation, and the correlation of these metrics in space and time, to uncover relationships between the dynamics of kinase activities and molecular mechanics that are involved in collective cell migration. At the end of this workshop (Part II), participants will be able to process their data (obtained in Part I) using this integrated and user-friendly image analysis workflow for automatically delivering robust and quantitative data.
Keywords : FRET biosensor, Multiplexed FRET, image analysis workflow, cell tracking, cell tracking, correlation

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A052-Quantitative 3D Spatial Analysis of multicellular specimens (Organoids w/o clearing)

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Abstract : Background: The large size and optical opacity of new promising mammalian models such as organoids and cleared tissues has turn up its own set of challenges related to data acquisition and storage, and subsequently image visualization and analysis. Experiments can generate gigabyte-sized data sets. The investigation of the architectural organisation of organoids at the cellular level will answer the relevant questions of cellular differentiation, apoptosis, and cellular interactions. This can only be achieved through detailed visualization of different cell types, their quantification, and their analysis through spatial statistics. Thus, image-based spatial characterization of these different cells and structures is crucial to define the functionality of organoids.

Goal of the Workshop: The goal of this experimental workshop is to make attendants to visualize and spatially analyze colon and bladder human organoids. They will create an analysis pipeline that uses both Spots (cells), and Surfaces (either cells or other extended structures) to study their distribution in term of cellular differentiation. This workshop will be a hands-on-session with a computer for each participant, working with the same datasets (light sheet and confocal image multicolor stacks). They will follow a step by step guiding workflow.

At the end of this workshop, participants will be able i/ to correct the 3D photobleaching which can negatively impact image data quality and downstream reconstruction, ii/ to use a toolset to quantify number of cells, % of differentiating cells and % of apoptotic cells, and at the end, iii/ create a 3D movie rendering for presentation.

Keywords : organoids, 3D multicellular specimens, quantitative analysis, 3D rendering, segmentation, cell counting, morphometry, analysis pipeline, data representation

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A056-Microscopy on thin resin sections: multimodal and correlative approaches using scanning electron microscopy.

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Abstract : Biological samples are conventionally imaged through thin resin sections using transmission electron microscopy (TEM). This technique allows for the visualization and direct identification of tissular and cellular components (with an almost global contrast), as well as the localization of events ranging from tissue to macromolecular scales (with a nanometric lateral resolution). These approaches are now feasible by scanning electron microscopy (SEM), conventionally used in biology for topological imaging of bulk specimens. These microscopes with new generation optics and detectors are now capable of producing “TEM-like imaging”, assisted by powerful automation software. These great technical advancements offer the possibility to:

-Use larger and various supports enabling multimodal approaches (without the limitations of conventional TEM grids).

-Take advantage of autonomous operation to acquire a large FOV and/or large volume of sliced-samples. By this way, the cutting thickness (around 50 nm) offers a high level of axial resolution.

In this workshop, we propose to:

-Train the participants in the basics of SEM, with an active comparison with standard confocal laser scanning microscopy (CLSM) in terms of logic of settings (CLSM skills are a prerequisite).

-Present examples of samples preparations protocols to obtain thin sections, focusing on potential multimodal analysis.

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

-Use and adapt the main settings of a SEM.
-Acquire a general overview of strategies to screen and target events between complementary techniques as well as bring the know-how to their laboratories.
-Understand the advantages, disadvantages, limitations, and complementarities of presented techniques as well as the ability to deal with different scales of observations and renderings.
Keywords : Scanning Electron Microscopy, Thin Sections, Screening and Targeting Events, Multimodal approaches

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A057-Introduction to single molecule localization super-resolution microscopy (SMLM)
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Abstract : Single molecule localization microscopy techniques (SMLM) are more and more used and accessible. Still these techniques represent a challenge in terms of choice of the techniques, sample preparation and data extraction, analysis and interpretation.
The aim of this introduction workshop is to present the different SMLM techniques (PALM, STORM, PAINT...), their specificities (sample preparation, dyes used...), the resolution reached and what are the biological applications.
After a theoretical presentation of the techniques, we will also show an example of STORM acquisition on a biological sample. It will allow to demonstrate the practical implementation of this kind of technique and discuss the important parameters to control for a proper acquisition with participants.
At the end of this workshop the participants will be able to know what kind of SMLM technique will be more adapted for their biological question.

Keywords : Single molecule localization microscopy (SMLM), super-resolution, sample preparation, STORM, PALM, PAINT

A058-Probabilistic pipeline to extract reliable information from single molecule microscopy data.
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Abstract : Tumor necrosis factor receptors (TNFR) and their interacting ligands are crucial for immune regulation, cell proliferation, survival and death. They have demonstrated ability for treating infectious disease, autoimmunity and cancer. TNFR has been shown to be capable of enhancing TCR-dependent activation of both CD4 and CD8 T cells, inducing increase of IL-2 receptors levels, enhancing T cell proliferation and cytokine production. TNF superfamily (TNFSF) ligands and their receptors have several functional and structural characteristics implicated in cell signal transduction and protein recruitment. To trigger signaling, the binding of trimeric ligand to three monomers receptors is necessary, creating hexagonal network of TNF-TNFR.
Single molecule microscopy can achieve 10nm of resolution, allowing the visualization and a precise analysis of checkpoint receptors dynamic and interaction. Since little is known about how really the checkpoint receptors interact each other in single or double activation. Cambulac will help us to elucidate those interaction and determine which multispecifics mAbs structure gave the best T cells immune response. Both T cells CD4, CD8 and T regulatory will be screened using Bayesian inference and deep learning approach.
Cambulac will also help in others projects where complex molecules binding and interactions are involved. These unique statistical tools to compare complex experiment at single molecule scale will be an added value to accelerate and unravel unsuspected molecular interactions on cell surface

membrane. Many applications in dissecting immune-oncology and immune-inflammatory in vitro and in vivo mechanism of action of immune checkpoint mAbs will be then possible.
Keywords : Single molecule microscopy, receptors dynamics,bayessian analysis,artificial intelligence

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A059-Coordinate-based quantification of multidimensional and multicolor single-molecule localization microscopy data.
Florian Levet (florian.levet@inserm.fr)
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. As a support, we will use simulation and experimental data.
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.
Keywords : Single-molecule localization microscopy, coordinate-based quantification, segmentation, 2D and 3D analysis, colocalization analysis

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A061-Acquisitions intelligentes sous micromanager
Jerome Mutterer (jerome.mutterer@cnrs.fr) Christian Rouviere (rouviere@obs-vlfr.fr)
Abstract : Une acquisition “intelligente” est un protocole d’acquisition d’images dont certaines étapes peuvent évoluer en cours d'exécution sur la base de critères propres au développement du spécimen ou de son environnement. Cette flexibilité n’est pas possible dans le cadre simple d’une acquisition de microscopie de fluorescence multidimensionnelle, mais nécessite de pouvoir évaluer des paramètres de l’échantillon par analyse d’image, ou des paramètres environnementaux (mesures externes issues de capteurs).
Nous utiliserons une extension du langage macro d’ImageJ qui permet un pilotage facile du logiciel d’acquisition Micro-Manager. (https://github.com/mutterer/MM2MacroExtensions)
Cette solution combine les avantages de Micro-Manager (richesse des pilotes de statifs et de périphériques, solution de choix pour le développement de périphériques home-made) et d’ImageJ pour le traitement et l’analyse des images acquises. Elle comble un espace en rendant accessible le pilotage de périphérique dans le langage macro d’ImageJ, sans doute le langage de programmation le plus accessible et le plus largement adopté dans la communauté des biologistes investis dans l’imagerie du vivant.
A l’issue de cet atelier les participants seront capables de programmer en langage macro d’ImageJ des scripts de pilotage de stations de microscopie et de les intégrer avec des éléments d’analyse d’image pour réaliser des boucles de rétroaction en fonction de paramètres extraits des images acquises.
Keywords : smart microscopy, pilotage, analyse d'image, imagej, micromanager

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A062-Contrôle qualité des données brutes de SMLM en temps réel

Sébastien Mailfert (mailfert@ciml.univ-mrs.fr) Nicolas Bertaux (nicolas.bertaux@fresnel.fr)

Abstract : Derrière les belles images de SMLM, il y a des données brutes. Tout au long du processus d'enregistrement et d'analyse des données SMLM, PALM, SPT, DNA-PAINT, vous êtes confrontés à deux contraintes majeures : (1) minimiser le délai entre une expérience et le résultat final d'une expérience qui nécessite un long workflow (acquisition et analyse), (2) réaliser une évaluation objective de la qualité des données enregistrées qui conditionnent la qualité de l'image reconstruite et la quantification qui en résulte.

La SMLM est aujourd'hui une technique bien connue mais qui nécessite la prise en compte de nombreux facteurs expérimentaux et analytiques tels que la préparation de l'échantillon, l'acquisition/analyse des données et la quantification. Chacune de ces étapes, si elle n'est pas réalisée avec soin, empêche finalement d'obtenir la résolution nanométrique attendue.

Dans cet atelier, vous apprendrez par une approche pratique de test/erreur comment régler les paramètres d'acquisition (puissance laser, temps d'exposition, etc.) sur un système commercial. En pratique, vous évaluerez ces effets sur la qualité des données brutes en termes de densité, de SNR, de fond, de nombre et de précision de localisation, et d'image reconstruite.

Grâce à un tout nouveau logiciel, cela se fera en mesurant en temps réel des indicateurs tangibles sur la qualité des données acquises à des fréquences proches de 100 fps pour des images de 1024x1024 pixels. Nous discuterons également de nos connaissances pratiques et théoriques du point de vue de la biologie et du traitement du signal.

A la fin de l'atelier, les participants seront capables de porter un regard critique sur leurs acquisitions SMLM et repartiront avec un document où ils pourront retrouver toutes les discussions pratiques et théoriques.

Public: biologiste ou non (débutant, confirmé ou expert) pratiquant la microscopie SMLM au quotidien, francophone.

Avoir lu absolument Mailfert et al., BiophysJ, 2018, (UNLOC)

Keywords : SMLM, real-time analysis, quality control

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A063a-Transparisation, acquisition au microscope à feuillet de lumière et post traitement de sphéroïdes

Chloé Dominici (chloe.dominici@univ-cotedazur.fr) Cédric Gaggioli (Cedric.GAGGIOLI@unice.fr)

Abstract : La culture tri-dimensionnelle de sphéroïdes représente aujourd'hui un outil puissant afin d'observer in vitro l'interaction de différents types cellulaires. Toutefois, l'évolution du sphéroïde en un amas opaque de cellules rend impossible une imagerie au cœur même de l'échantillon et ainsi visualiser la totalité des interactions présentes. Les techniques de transparisation mises au point ces dernières années permet de contrer ce problème.

Nous proposons dans cet atelier de former les participants au protocole d'immunomarquage et de transparisation mis au point dans notre laboratoire. Ce protocole à base de solutions aqueuses est aussi adapté aux sphéroïdes avec un marquage endogène. Les participants pourront apporter leurs propre échantillons avec un marquage endogène ou nous pourrons envoyer le protocole d'immunomarquage des sphéroïdes en amont eux participants qui le souhaite.

Par la suite, nous proposons d'acquérir au microscope à feuillet de lumière ou confocal les sphéroïdes préalablement transparisés. Nous détaillerons ainsi le montage de l'échantillon dans le microscope ainsi que les réglages à effectuer.
Pour finir, nous verrons avec le logiciel Imaris le post traitement des acquisitions effectuées.
A la fin de cet atelier, les participants auront acquis des bases solides sur tout le processus de transparisation des sphéroïdes jusqu'aux post traitements des images et pourront ainsi être autonomes afin de traiter leurs propres échantillons.
Keywords : spheroïde, microscope à feuillet de lumière, transparisation, Imaris

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A063b-Transparisation, acquisition au microscope à feuillet de lumière et post traitement de sphéroïdes

Chloé Dominici (chloe.dominici@univ-cotedazur.fr) Cédric Gaggioli (Cedric.GAGGIOLI@unice.fr)

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Keywords : spheroïde, microscope à feuillet de lumière, transparisation, Imaris

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A065-Getting the most out of 3D pheroids by combining microfabricated wells, clarification techniques, standard confocal imaging and deep learning image processing

Charlotte Riviere (charlotte.riviere@univ-lyon1.fr) Ali Ahmad (ali.ahmad@insa-lyon.fr)

Abstract :
Context : Studies are now directed to 3D in-vitro models to try to better replicate in-vivo complexity of tumor microenvironment. Among them, multicellular tumor spheroids (MCTS) recapitulate many tumor features. Immunostaining, image acquisition and analysis of such 3D biological samples remains time consuming and challenging.
We will present a method based on micro-fabrication to handle efficiently such spheroids that is compatible with all-mount immunostaining and confocal analysis. To that aim, we will present different efficient clarification techniques for such biological sample.
We will then show how machine learning strategies could be used even for non-specialist to extract interesting features from the stacks of images. We will focus on signal to noise ratio/depth

penetration issues on clarified and non-clarified samples and propose some annotation strategies to deal with variation of signal to noise ratio from one sample to the other.
Objective: At the end of this workshop the participants will have an overview of a complete workflow for efficient cell phenotypic characterization within 3D spheroids (including sample preparation, multi-points automatic acquisition, image analysis and readouts quantification using machine learning strategies).

Keywords : Spheroids, confocal imaging, clarification, image analysis, machine learning

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A071-Microscopie d'expansion : stratégies et astuces pour l'analyse des cellules de mammifères en culture, de la levure S. cerevisiae et pour la visualisation de l'organisation mitochondriale

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Abstract : Contrairement à d'autres techniques d'imagerie optique de super-résolution, qui augmentent la résolution par des améliorations très couteuses et complexes des microscopes, 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.
Nous avons appliqué cette technique de super-résolution à faible coût pour étudier la nanostructure et l'organisation des membranes mitochondriales et le réseau de microtubules et nous avons développé un protocole permettant l'ExM sur la levure S. cerevisiae.
Au cours de cet atelier, nous décrirons les trucs et astuces que nous avons développés pour adapter l'ExM à nos problématiques, simplifier la technique au niveau pratique et préserver et/ou amplifier le signal des protéines fluorescentes. 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 le protocole et de l'adapter à leur propre thématique.

Keywords : super-resolution, Expansion Microscopy, mitochondrie, GFP, mCherry, levure S. Cerevisiae, microtubule

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A072-Evolution of brain morphology from invertebrates to mammals. Everything we can learn from in toto 3D imaging of autofluorescence signals ?

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Abstract : Over last years, advanced tissue-clearing methods combined to imaging technologies led to 3D imaging of whole tissues at cellular resolution. Challenges of thick tissues observations are now limited by improvement of immunostaining procedures and development of imaging approaches dedicated to big tissues. Extensive studies have been conducted to compare efficacy of various clearing protocols preferentially on mammalian brains, thus extended to other mammalian tissues or other vertebrates such as zebrafish. Image analyses of cleared mammalian brain allowed among other visualization of neuronal network, distribution of neuronal cell populations, and establishment of brain atlas taking into account the brain distortion induced by clearing treatments.

Objectives
We propose to conducted comparative analysis of brain anatomy based on 3D acquisitions of various organisms post clearing.
For this aim, the organizers will provide various samples: fish species (zebrafish, trout and carp), mouse brain but also fly and lizard, which would have been clarified previously.
During the workshop, we will address the following points:
- selection of clearing protocol based on the biological sample (pigmentation, nature, size,…),
- training of the participants to mount the sample they will have selected for light sheet acquisition
- use of the light sheet microscope for imaging of brain autofluorescence signals
- image processing will be proposed at the end for whole brain visualisation in 3D and exemple of segmentation of selected brain areas to gain insight into evolution of brain morphology.
Keywords : Tissue clearing, lightsheet microscopy, autofluorescence, 3D image analysis, comparative morphology

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A073-Microscopie plein champ "haute résolution" et traitement numérique sur échantillon épais
Vicky Diakou-verdin (vicky.diakou@univ-montp2.fr)
Abstract : En microscopie plein champ, de plus en plus d'industriels intègrent des algorithmes de traitements d'images à leur logiciel d'acquisition dans le but d'améliorer la qualité des images produites (amélioration de la résolution et du rapport signal sur bruit, débruitage, défloutage). Ces traitements post-acquisition utilisent des approches algorithmiques souvent complexes comme la déconvolution, les filtres de Wiener, les approches par ondelettes, etc.
L'objectif de cet atelier est de tester si ces techniques alliant la microscopie plein champ et le traitement des images peuvent être utilisées pour tous type d'échantillons ? Quelles sont leurs avantages et leurs limites ? Les images sont-elles ensuite quantifiables ? Ces techniques peuvent-elles concurrencer des approches plus classiques d'amélioration du signal (imagerie confocale, déconvolution par exemple). Peut-on revenir à de la microscopie « basique » ?
Nous observerons à différentes échelles plusieurs types d'échantillons fixés (cellules de mammifères, embryon de drosophiles, racine de plantes) qui nous amènerons à trouver les avantages et les limites de ces techniques. Nous verrons comment paramétrer et optimiser les traitements post-acquisition et nous pourrons comparer différentes techniques. Nous utiliserons aussi des méthodes et des outils pour s'assurer que les techniques utilisées sont bien quantitatives.
A la fin de l'atelier, les participants pourront déterminer :
- Si la microscopie plein champ associée à une méthode de traitement d'images peut être une alternative acceptable aux microscopies plus conventionnelles comme l'imagerie confocale.
- Quels types d'échantillons sont compatibles avec ces approches
Keywords : Widefield, deconvolution, debluring

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A074-3D High Resolution imaging by PSF engineering using ZOLA-3D
Proposer
Mickaël Lelek (MICKAEL.LELEK@PASTEUR.FR)
Abstract
The goal of this workshop is to present:
- A semi homemade 3D high resolution imaging system (PALM/STORM) using PSF shaping of single molecules

- ZOLA-3D: A user friendly algorithm implemented as a FIJI plugin to localize any kind of PSF shape and to reconstruct super resolution images in 3D

PALM/STORM super-resolution imaging systems allow to improve the spatial resolution of common fluorescence microscope by a factor of ~10. Their principle is based on the localisation of isolated fluorescent dye. Making use of the shape of PSF and its modulation by a spatial phase mask placed in the emission path of the microscope, it's possible to reconstruct a 3D high resolution image without mechanical scanning. We developed ZOLA-3D, a user friendly FIJI plugin in order to reconstruct 3D high resolution images from any kind of PSF shape (astigmatism, double helix, tetrapod), while taking into account the spherical aberration due to refractive index mismatch (Aristov, Lelandais et al. Nat Comm 2018 et résultats non publiés).

After a short presentation of 2D-3D single molecule localization microscopy methods, the participants will discover theoretically and experimentally the influence of 3 different spatial phase masks on the PSF shape. The participants will understand how to determine the PSF model using ZOLA-3D and images of fluorescent beads. They will also learn how to acquire and reconstruct high resolution images of nuclear pore complexes.

At the end of this workshop the participants will know how to obtain 3D high resolution images using any kind of PSFs. They will understand the advantages and the limits of each studied PSF shape. The participants will be able to model the PSF and to reconstruct 3D high resolution images with ZOLA-3D

Keywords : 3D Single Molecule Localization Microscopy, PALM/STORM, PSF shaping, PSF engineering

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A075-Adaptive optics fluorescence microscopy for biological imaging

Alexandra Fragola (alexandra.fragola@espci.fr), Sophia Imperato, 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 light sheet microscopy based on a novel wavefront measurement approach, for embryo or drosophila brain imaging. 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.

Keywords : adaptive optics, aberration, in depth imaging, light sheet microscopy

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A076-Alternative strategies to image multiple proteins in single molecule localization microscopy

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Abstract : Imaging multiple proteins is still a challenge in Single Molecule Localization Microscopy (SMLM). In DNA-PAINT so far only two complementary dyes(atto 655 and CY3B) are commercially available. In dstorm, the photophysics of all the fluorophore should be compatible with the same buffer. Combining multiple laser (532 and 640) not only induce chromatic aberrations but as so far been difficult to obtain. More recently, strategy based on a single excitation at 640 nm for dyes with slightly shifted spectra have been proposed (AF647 or CF647/CF660C/CF680) which can also be demonstrated in DNA PAINT (attot647N/ atto680).

In this workshop, we will overview these current strategies mainly based on spectral properties of the fluorescent dyes. We will introduce ratiometric spectral demixing to image 2/3 dyes simulatneously with a single excitation laser. We will discuss how to choose the protein/dye combination for an optimal spectral demixing. Different acquisition with 2 or 3 dyes will be performed to demonstrate these different points and evaluate the spectral demixing. We will image COS7 cells with tubulin/clathrin labelling(cf647/CF680 CF647/CF660, DNA PAINT with atto647N/atto680). We will also present new approaches based on alternative information intrinsically present within the sample which can be used to support/replace spectral demixing and illustrates on datas. At the end of the workshop participants will be able to identify which dyes should be used, what are the performances of spectral demixing for dSTORM/DNA-PAINT , and become familiar with the use of alternative fluorescent properties to enhance multiple proteins imaging

Keywords : single molecule localization microscopy, spectral dmeixing, DNA-PAINT, dSTORM

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A077-BIAFlows

Volker Bäcker (volker.baecker@mri.cnrs.fr)

Abstract : Image analysis is key to extract quantitative information from scientific microscopy images. In this workshop we explore the question of how image analysis workflows can be deployed in a reproducible way. Reproducibility is at the core of the scientific method itself. If a research result can not be reproduced the conclusions implied are not considered valid. This means that the image analysis that is part of a scientific project must also be reproducible.

It might seem that in the deterministic world of computation reproducibilty could be easily achieved. However in practice it turns out that the reproducible sharing of image analysis workflows is a complex task. Some keypoints for achieving repoducibility in this context are:

- the availability of the original data
- the availability of the original metadata, including parameters of the workflow at hand
- the availability of the versioned source code
- a clear path of how to get an executable from the source code
- the availability of the versioned computational environment, including the operating system, the software platform, libraries and plugins
- documentation of how to use the workflow
- access to the computational resources needed to execute the workflow

In the context of the COST Action (CA15124) NEUBIAS (Network of EUropean BioImage AnalystS), we developped a cloud based and user friendly open source tool BIAFlows, that helps to make image analysis reproducible.
In this workshop participants will learn how to use BIAFlows to run and compare existing workflows and how to integrate their own workflows and data into BIAFlows.
Keywords : reproducibility, image analysis workflow, cloud computing, image analysis, image database, docker, git, biaflows

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A078-BioImage-IT: Implement image processing workflows with tools from multiple software
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Abstract : New microscopes are high resolution and/or high content. They then generate a large quantity of data that cannot be analyzed manually anymore. Many software have been developed to deal with image analysis. We can cite Fiji, Icy, CellProfiler for software dedicated to biological imaging and more generic software like Matlab, Keras that are frequently used in data science. Each software have it pros and cons and the variability of existing software is justified by the variety of technologies for data science going from small data to big data, and from simple image processing to deep learning.
As a consequence, when a scientist wants to create an image processing workflow to analyze images and answer a biological question, he or she has to install and use multiple software and write scripts in multiple languages. This is a tedious task.
In this workshop, we will teach the participant to build image processing workflow connecting multiple software with a high level application called BioImage-IT.
BioImage-IT is developed by France-BioImaging. It is a “bandmaster” application that allow any scientist to annotate data, process data and analyze data using only one single high level application build on top of many existing software.
During this workshop, we will demonstrate the use of BioImage-IT to analyze a sample dataset to analyze recycling endosomes localizations . Participant will annotate images and design an analysis workflow. At the end of this workshop the participants will be able to create an image processing workflow either graphically or by writing a python script.
Keywords : image analysis, data indexation, cloud computing, python

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A079-Bioprinting as a solution for recreating a physiological environment on a slide
Alessandro Furlan (alessandro.furlan@univ-lille.fr) Lisa Terrassoux (lisa.terrassoux.etu@univ-lille.fr)
Abstract : Bioprinting has emerged in the last years as a powerful tool to produce scaffolds useful for both fundamental and applied research.
During this workshop participants will use a bioprinter to deposit cell solutions according to various geometric settings and realize their multicellular structures.
Participants will adjust several parameters to optimize the proper deposition of biocompatible inks, including cells or not.
They will then observe on the microscope the layout of their scaffold.

With a confocal microscope, it would be interesting to measure the signal-to-noise ratio of fluorescently-labelled cells, as a function of the thickness of the bioprinted sample, of the matrix/ink used, and of the distance to the coverslip.
Structures will be also prepared before the workshop, in which different cell types will be printed in different matrix compartments. This will help us to quantify migration of cells and possible chemoattraction between cells.
The aim of this workshop is to provide participants with the basics of bioprinting and imaging of bioprinted cells.
Keywords : bioprinting, cell interactions, imaging, signal-to-noise ratio

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A080-Conception de prototypes connectés sous rapsberry et arduino Surveillance d'une expérimentation a distance (relevé de paramètres "connecté") pour moins de 100€.
Brice Ronsin (brice.ronsin@univ-tlse3.fr) Thierry Legou (thierry.legou@lpl-aix.fr)
Abstract : Certains microprocesseurs (Rapsberry, Arduino...) permettent aujourd'hui de développer des prototypes peu coûteux. Cependant leur mise en place demande l'apprentissage du langage de programmation et la compréhension de leur mise en œuvre. Nous proposons donc de lever cette barrière lors d'un atelier en trois modules (débutant à avancés). Nous assisterons les participants au cours de trois modules pour la fabrication d'un prototype fonctionnel et connecté, permettant d'envoyer sur un site web, les paramètres environnementaux et la distance entre deux capteurs en temps réel. Ces modules peuvent être réalisés comme un parcours ou se faire de manière indépendante selon trois axes :
module 1 (débutant): connexion et programmation de deux capteurs (température et capteur de proximité) pilotés par un"Arduino".
module 2 (intermédiaire) pré requis: avoir déjà programmé afin de s'adapter à la compréhension du code que nous écrirons en Python) : génération d'une série de chiffres aléatoires et envois de ceux-ci sur un site web (Rapsberry PI)
module 3 (avancé) : pré requis: avoir déjà programmé et connaitre un microcontrôleur "Arduino" ou avoir assisté au premier atelier. Mis en œuvre de la communication entre deux microcontrôleur (Arduino: plateforme de réception des mesures) et un Rapsberry PI par communication I2C ou série envoyant les informations recueillies vers le site web.
A l'issue de ces modules les participants seront capables selon l'atelier suivi :
module 1: Mise en place d'un environnement de programmation Arduino : brancher et piloter deux capteurs (acquis général pour la mise en œuvre d'un autre type de capteur).
module 2 : Mise en œuvre un mini ordinateur Rapsberry : installer un système d'exploitation de type Linux, programmer un script sous python pour générer un chiffre aléatoire et l'envoyer sur un site web.
module 3: Programmer en python un script de communication entre Arduino et Rapsberry pour le transfert de données et l'envoie sur un site web.
Keywords : Rapsberry PI, Arduino , capteurs , objets connectés, communication I2C, communication série, suivi de mesure, accessibilité, connexion web, transfert de données, thingspeak, fun

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A084-Dessiner, paramétrez et Imprimer en 3D

Brice Detailleur (brice.detailleur@univ-amu.fr) Ludovic Ludovic.leconte (ludovic.leconte@curie.fr)

Abstract : La communauté scientifique fait de plus en plus usage de l'impression 3D pour répondre à des besoins atypiques. Ces technologies ne sont, hélas, pas intuitives et demandent, entre autres, des compétences en CAO mais aussi en impression 3D.

Cet atelier a pour objectif d'aider la communauté à cibler et à utiliser des outils de CAO permettant la conception d'objets, autour de la microscopie et de la biologie, (insert de microscope, moule pour empreinte de culture cellulaire, porte échantillons pour organoïdes…) pouvant être imprimé en 3D.

•Deux logiciels de CAO pourront être abordés, SolidWorks (logiciel professionnel) et Freecad (logiciel Open Source). Une approche pratique de ces logiciels sera proposée afin de permettre aux utilisateurs d'appréhender leur utilisation mais aussi de comparer ces deux logiciels. Nos compétences en CAO nous permettent aussi de proposer, à la demande, d'autres logiciels tel que Inventor ou Fusion 360…

•Au travers de deux technologies d'impression 3D, FFF (dépôt de fil fondu) et SLA (polymérisation de résines) nous initierons la communauté à l'utilisation de ces procédés de fabrication additive en ayant préalablement listé les différentes technologies utilisées dans ce domaine de fabrication. Des contacts avec des sociétés ont déjà été établi afin d'obtenir le prêt de ces technologies pendant la durée de l'école.

A l'issue de cet atelier les participants seront capables de créer une pièce physique à partir d'un objet numérique 3D qu'ils auront modélisé, graver ou découper.

Keywords : Impression 3D, Conception Assisté par Ordinateur, Microscopie, Biologie, Partage de connaissances et compétences

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A090-Label-free virus detection and sorting with full-field interferometric microscopy

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Abstract : In this workshop, we will introduce a new, sensitive, interferometric, and non-destructive optical approach to detect, count and sort different types of label-free, biotic and non-biotic single nanoparticles (NPs) (Boccara et al, Biomed. Opt. Exp. 2016). In short, we measure the light scattered by the nanoparticles and obtain an interferometric signal related to their size and refractive index; this measurement is complemented with single particle tracking of their Brownian motion.

With this technique, one can study the diffusion properties of different biological samples such as bacteriophages in different aquatic environments, membrane vesicles, etc. The ability of classifying virus in a given environment according to their size and structure is an essential tool in applications ranging from environmental to medical studies.

At the end of this workshop, the participants will have learned how to build such a set-up, where the signal comes from in an interferometric microscope, and understand the differences with fluorescence microscopy. They will have also learned about single-molecule detection in a non-fluorescence context, as well as the basics of Brownian diffusion and its application for analyzing a data set of single-particle traces.

Keywords : Virus detection; Interferometry; Single Particle Tracking; Brownian diffusion;

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A093-Measuring protein's orientation and organization by polarized fluorescence and polarized super resolution imaging

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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 however challenging. We will describe and demonstrate different approaches that we have developed to solve this problem, based on polarized fluorescence imaging and splitting of polarized detection channels [1,2] or PSF engineering [3]. 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. At last, we will apply polarization splitting to structural imaging in dense samples, using pure polarized fluorescence imaging.

[1] C.A. Valades Cruz, et al. Quantitative nanoscale imaging of orientational order in biological filaments by polarized super-resolution microscopy, Proc. Natl. Acad. Sci. 113 (7) E820-E828 (2016)
[2] C. Rimoli, et al. 4polar-STORM polarized super-resolution imaging of actin filament organization in cells. Submitted, bioRxiv 2021.03.17.435879 (2021)
[3] 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)

Keywords : light polarization, molecular orientation, polarized fluorescence, proteins organization, cytoskeleton filaments alignment

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A094-Modulated excitation for enhanced localization : ModLoc

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Abstract : Single Molecule Localization Microscopy (SMLM) is widely used in bioimaging, both for structural Imaging and tracking. The molecular localization usually relies on a centroid technique that strongly relies on the point spread function shape. Axial localization is usually obtained by introducing a PSF engineering method. This conventional localization strategy leads to lateral/axial precision that varies along the depth of field but also induced a reduced depth of observation
In this workshop, we propose an alternative strategy, named ModLoc, where the introduction of a modulated excitation can be used to retrieve the single molecule position. We will discuss the various potential implementation and benefits. This new principle will be demonstrated for an enhancement along the axial direction during the workshop. Performances of this new approach will be demonstrated on calibration samples and on biological samples.
At the end of the workshop, the participants will be reminded the limitations of current localization strategy, be familiar with the concept of a modulated excitation for localization, known the key parameters for an implementation on a SIM microscope, have seen an experiment with this concept

Keywords : SMLM, modulated excitation, SIM, dSTORM, in depth imaging

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A096-Multimode fiber based-endoscope for fluorescence imaging using wavefront shaping

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Abstract : Wavefront shaping has become a ubiquitous technique to control the light field through scattering layers or multimode fibers and opens interesting alternatives for imaging deep into biological tissue. For example, by controlling the output of multimode fibers (MMF), ultrathin endoscopes (125 µm) have been demonstrated: made of a single MMF, they can be inserted in the brain of small animals while causing minimal damage and used to acquire fluorescence images with micrometer resolution.

When light propagates in an MMF, incident light is projected into a large number of components (or modes) that undergo unpredictable phase delays, so that the output pattern is usually speckle-like. However, as long as the relation between the input and output fields is linear and deterministic, the response of the MMF can be precalibrated by measuring a 'transmission matrix'. Then this information can be used to rephase all the modes by means of a phase modulator and obtain the desired output pattern. Phase modulators include liquid-crystal spatial light modulators (SLM) and, more recently, digital-micromirror devices (DMDs). The latter has the advantage of much improved switching rates (up to 20 kHz).

The goal of this workshop is to introduce the concepts of wavefront shaping and build a point-scanning ultrathin endoscope for fluorescence microscopy using a single multimode fiber and a DMD.

At the end of this workshop, the participants will know how to use a DMD for phase modulation, how to measure the transmission matrix of a complex medium (scattering layer or MMF) using off-axis holography and how to implement wavefront shaping to focus through such a medium.

Keywords : endoscopy, wavefront shaping, digital micromirror device (DMD), multimode fiber

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A097-Nuclear Pores Complexe : a tool for metrology in Single Molecule Localization Microscopy

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Abstract : Single Molecule Localization Microscopy (SMLM) allows to overcome the diffraction limitation and typically offers a localization precision lower than 20nm. Evaluation of the resolution performances of the microscope is an important step but is however not straightforward. Indeed, final image quality in SMLM can vary from one microscope to another, but as it includes multiple parameters such as the sample preparation and the analysis on top of the microscope performances, it is not easy to disentangle their various influences. Calibrated beads or spheres can be used but the possibility to image a known biological structure in cells appears as a promising solution.

Nuclear Pores complexes (NPC) are well known 3D structures, which have been recently proposed to evaluate within true biological conditions the microscope resolution.

During this workshop we will :

- remind the alternative strategies to evaluate precision/resolution of a SMLM microscope
- present the NPC structure
- acquire 3D images of NPC in U2OS stable cell line, with a description of the 3D strategy
- analyse the images with an open source software developed by Jonas Ries

At the end of the workshop, the participants will be able to evaluate the resolution of their microscope with NPC cell lines, including how to optimize the acquisition. They will be more familiar with a 3D imaging setup, and how to process these data.

Keywords : Nuclear Pores, SMLM, Precision metrology tool

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A099-Practical considerations for reporting change in cell membrane tension using FLIM

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Abstract : The recent development of fluorescent probes combined to the accessibility to FLIM approach have paved the way to ease the reporting of sub- and cellular environment changes. Here we focus on changes in membrane tension using FliptR (Colom et al., 2018. Nat. Chem.). To illustrate the practical considerations while using FLIM approach, we propose to image and hence report changes in cell membrane tension during mitotic process. Results will be heavily discussed to better highlight the key parameters while carrying out FLIM imaging to report change in membrane tension. At the end of this workshop people the participants will be able to understand the basic principles of FLIM approach illustrated with the imaging of membrane tension reporters. The significance of the retrieved data will also be discussed.

Keywords : FLIM, tension probe, cells, microscopy, imaging

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A100-Préparation d'échantillon, acquisition et analyse d'image pour les applications de culture cellulaire 3D

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Abstract : Les modèles cellulaires de culture 3D in vitro (organoïdes, spheroïdes) ont révolutionné la modélisation du développement d'organes, et celle des maladies comme le glioblatome.
Durant cet atelier, nous allons transpariser, acquérir et reconstruire un organoïde. Nous nous attacherons à décrire chaque étape en les mettant en perspective. Ce sera notamment l'occasion de discuter des différentes techniques de transparisation, des différents types de système qui peuvent être utilisés pour l'acquisition (feuillet de lumière / confocal / 2-Photon) ou encore des différents moyens de reconstruire et analyser les structures imagées. Ces mises en perspectives permettront d'établir le dialogue avec les participants. Cet atelier s'adresse aux personnes qui débutent ou veulent se lancer dans l'acquisition d'organoïdes mais vous êtes tous bienvenus.

Keywords : Organoïde, sphéroïde, confocal, transparisation (FocusClear t RapidClear et fructose-glycérol), imagerie 3D haute resolution, scanner résonnant

A102-Publishing FAIR-ly with OMERO

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Abstract : The digital image has revolutionized the world in a very short period. Practically, all current microscopes provide images in a digital format. Digitalized images provide a great number of key advantages for scientific imaging, mainly the integrity of data over time and the ability to store, not only the raw data, but all the metadata in close and unequivocal association. These features are key for scientific reproducibility. On top of the digital image format, the advent of the web has, yet again, changed the game of how data is stored and retrieved.
While there is a great potential for these technologies to change the way that we manage our image data, the daily practice of biology researchers is, in general, very poor. Image files are saved in infinite hierarchies of folders in memory sticks, hard disks or, in the best case, network drives. The results of the analysis are too often managed in a similar way.
- This problem has been recognized by the community and the authorities

- This recognition has led to the declaration of the FAIR principles (Findable, Accessible, Interoperable, Reusable)
OME Remote Objects (OMERO, www.openmicroscopy.org) is an integrated solution to manage image data during all its lifetime, from the acquisition to the publication. In this workshop we are going to illustrate the potential of OMERO to publish image and associated data as well as the analysis workflows. Through demonstrations and practical exercises, we will show how to use OMERO to create figures for publication, publish data and metadata, either for visualization on a website or making them accessible following the FAIR principles.
At the end of the workshop the attendees will be able to:
-Have a better understanding of what it means to be "FAIR" in practice.
-Create a figure for publication using OMERO.figure.
-Understand the interest of sharing data in a structured and persistent way.
This workshop has no pre requirements.
Keywords : Image publication, FAIR principles, data management

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A106-Serial Block Face Imaging: imagerie 3D sans marquage de gros échantillons
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Abstract : La technique de Serial Block Face Imaging (SBFI) permet une analyse tridimensionnelle d'un échantillon à partir d'une imagerie sans marquage. Le principe de cette méthode est de prendre une image de la surface du bloc d'un échantillon inclus en paraffine au fur et à mesure de sa coupe par un microtome. La série d'images de contraste obtenues est alors déjà alignée et permet la visualisation volumique de l'échantillon. La récupération des coupes histologique associées permettra par la suite colorations ou marquages spécifiques afin d'obtenir des données morphologiques pour des zones d'intérêt.
Contrairement aux imageries confocale, multiphotonique ou encore à feuille de lumière, qui passent par des étapes longues et parfois onéreuses de transparisation pour la visualisation d'échantillons épais, le SBFI peut s'avérer être une solution peu onéreuse, rapide et relativement simple à mettre en œuvre. Elle offre des informations sur la morphologie générale de l'échantillon à une résolution cependant relativement faible (jusqu'à 10 µm). Il est donc intéressant d'associer cette technique à une imagerie multimodale/multi-échelle, le but étant par une approche corrélative, d'associer les coupes histologiques obtenues avec le rendu volumique de l'échantillon entier. On peut alors placer dans un contexte tissulaire des images de microscopie de fluorescence ou d'histologie dans un jeu de données donnant accès à des informations sur la morphologie générale de l'échantillon d'intérêt.

A la fin de cet atelier, les participants seront capables de comprendre dans quel contexte mettre en place cette technique et de connaître les étapes ainsi que les contraintes et les améliorations pouvant être apportées lors d'un développement home-made cette technique.

Keywords : Serial Block Face Imaging, imagerie 3D sans marquage, analyse macroscopique, histologie, imagerie multimodale

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A107-Standardization of organoids culture allowing high throughput 3D live imaging using sospim technology

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Abstract : Organoids are emerging as a very promising technology for fundamental research and health sciences. These self-organized 3D cellular structures grown in vitro mimic all or part of the morphological aspects and functions of real organs. However, the adoption of organoids for routine use is nowadays hindered by the lack of standardized and parallelized culture devices and appropriate 3D quantitative imaging methods. Indeed, conventional cell culture devices lead to a huge variability in the organoids generated, and while specific 3D cell culture devices can homogenize organoid culture, they are often incompatible with standard imaging platforms. Furthermore, 3D imaging methods such as confocal or spinning disk microscopy are not suitable for long-term 3D live imaging due to high photo-toxic effects. Conventional light-sheet microscopy approaches (or SPIM technologies) are live compatible, but their limited throughput due to complex sample mounting hinder their use in routine biology workflows.

In this workshop, we will present a solution that aims to provide a complete workflow for parallelized organoid culture and 3D live imaging.

This approach relies on (1) a single-objective SPIM (soSPIM) technology and (2) a newly developed versatile 3D cell culture device. The soSPIM technology allows to perform SPIM on a conventional inverted microscope thanks to the use of dedicated microfabricated devices embedding 45° mirrors and a beam-steering module plugged onto the microscope-body. Our new soSPIM devices are designed for the parallelized and standardized 3D culture of organoids in a well-defined array, allowing high-throughput monitoring of organoid morphogenesis.

At the end of the workshop the participants will have learned (1) the technical challenges inherent to the organoid culture and imaging, (2) a way to culture their organoids in a more reproducible and robust manner, and (3) expertise and skills to conduct 3D live imaging on 3D cell culture.

Keywords : 3D cell culture, Organoids, Light-Sheet Fluorescence Microscopy, High Content Screening

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A111-Optogenetic control and measurement of cell contraction 1/2 (Artur Ruppel/Martial Balland)

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Artur Ruppel (artur.ruppel@univ-grenoble-alpes.fr)

Abstract : Mechanical forces are implied in most of biological movements ranging from single cell migration to embryo morphogenesis. Understanding how this forces are generated and transmitted within supra cellular assemblies is a key point to the understanding of those phenomenons. This workshop aims at studying how these forces are generated by single cells. Especially we start to ask ourselves questions about the relationship between the actiovation of a signalling pathway and the amount of physical forces that can be generated. To this end, an optogenetic microscopy set up provided by the compagny will be used to trigger the contraction of single "optogenetic" cells. Practically the optogenetic stimuli will target ArhGEF11, a major regulator of cellular contractility. The bio-mechanical response of each biological sample will be quantified in a coupled practical where the participants will work on both home made software solutions and open source codes for force analysis. At the end of this workshop the participants will be autonomous on the use of the optogenetic setup, will be able to design a typical sequence of optogenetic stimulation coupled to image acquisition and finally will be trained on the software solutions for force imaging of single cells.

Keywords : optogenetic , force, mechanotransduction, imaging, microfabrication

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A112-Virtual reality for multidimensional data visualization and analysis

Thomas Blanc (thomas.blanc@curie.fr)

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.

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 generated in single-molecule localization experiments.

At the end of this workshop, the participants will be able to grasp the concepts behind virtual reality and judge the utility of such technology in several contexts.

co-participants: Thomas Blanc, Charlotte Godard, Mohamed El-Beheiry, Jean-Baptiste Masson, Bassam HAJJ

Keywords : Virtual Reality, Quantitative Multidimensional Microscopy Data, Super Resolution Microscopy, Visualization Software

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A113-An example of feedback microscopy: Developing a High Content Screening (HCS) Optogenetics experiments.

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Abstract : The objective of this workshop is to introduce the participants to adaptive feedback microscopy through the development of a high content screening optogenetic experiment. Optogenetics allow acute manipulation of signaling pathways in living cells using light. However, experiments are often performed at the single cell scale. Thus, these experiments are time consuming and required multiples replicates in order to obtain statistically manageable data. With this in mind, we propose to develop an automated method of high content screening optogenetics experiments allowing multiple optic manipulation of living cells. The experiment will be developed with the Open Application Development (OAD) module, which use Python scripts to customize and automate workflows one the ZEN software.

We will guide the participant through a workflow which allow us to automatically detect cells of interest, perform an optogenetic experiment on them and measure and display the results.

Keywords : Optogenetic, adaptive feedback microscopy, python, image segmentation and analysis

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A114-Deep learning made easy for microscopy: an introduction to ZeroCostDL4Mic and DeepImageJ

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Abstract : Deep learning methods are the current the state-of-the-art for many image analysis problems. They can notably be used for image classification, object segmentation, pixel classification (also called segmentation), image denoising or image-to-image translation. Unfortunately, these tools are quite difficult to understand and to use in their post-publication format. The goal of ZeroCostDL4Mic and DeepImageJ is to leverage this difficulty. ZeroCostDL4Mic is an online tool for deep learning. It runs on Google Cloud computing platform without the need of any setup on a local machine, a usually tedious step, even for developers. Data are first uploaded on a Google Drive and then a deep learning model can be trained on it through a coding-free graphical interface. This trained model can be then used to process novel unseen sets of data. It can also be exported and used offline directly from the ImageJ software thanks to the DeepImageJ plugin. This plugin let a user unfamiliar with coding using deep learning methods. These can either be models exported from ZeroCostDL4Mic or directly downloaded from DeepImageJ's website. At the end of this workshop the participants will be able to understand the basic underlying concepts behind both tools and to use them to segment a set of 2D and 3D microscopy images.

Keywords : Deep Learning, Bioimage analysis, DeepImageJ, ZeroCostDL4Mic

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A116-Bioimage Analysis: Practice Deep Learning Without Coding

Anaïs Badoual (anais.badoual@inria.fr) Daniel Sage (daniel.sage@epfl.ch)

Abstract : The emergence of deep learning techniques (a.k.a. neural networks) has drastically transformed the field of bioimage analysis and its quest for the understanding of biological processes. Successful use of these learning-based techniques in various biological imaging problems are found on a regular basis and the trend is likely to continue. Unfortunately, the deployment of deep learning models is often riddled with technical challenges for non-expert users, and their appropriate use requires deep learning knowledge and good programming skills. Since 2020, efforts have been made to democratize the use of deep learning with the deployment of notebooks, zoos of pre-trained models and plugins such as DeepImageJ. However, these user-friendly and code-free tools deserve to be better known and disseminated in the biology community.

The goal of this workshop is to contribute to the spread and assessment of deep learning models in life-sciences applications and bioimage informatics. First, we will get an intuitive understanding of deep learning concepts. Then, we will use pre-trained models with DeepImageJ, and finally, we will train neural networks without progamming for image segmentation. For this purpose, we will exploit with DeepImageJ the pre-trained models gathered on the Bioimage Model Zoo. We will also use the notebooks developed by ZeroCostDL4Mic to train neural networks on google Colab. We rely on our long experience in teaching image processing on ImageJ for Master students to guide the biologists throughout the workshop. At the end of this workshop the participants will know how to choose the appropriate pre-trained model according to their application and how to test it on their own images. They will also leave the workshop with basic knowledge on how to train a model from scratch if needed.

This workshop is designed for participants without any skills in programmation and without machine-learning knowledge.

Keywords : Deep learning, bioimage analysis, friendly tools, DeepImageJ, ImageJ, cell segmentation;

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A117-Fluorescent imaging and techniques to study lignocellulosic biomass at the nanoscale

Anouck Habrant (anouck.habrant@inrae.fr)

Abstract : There is an increasing interest in the use of plant biomass for the production of sustainable liquid fuels and chemicals to replace fossil carbon to mitigate increasing atmospheric CO2. Plant cell wall biomass consists of a framework of cellulose surrounded by a matrix of complex polymers including hemicellulose and lignin. Non-covalent and covalent linkages interconnecting these polymers result in a complex structural and chemical network leading to recalcitrance to biological deconstruction. Physical and chemical barriers limit enzymatic penetration and progression, leading to limited enzymatic conversion of lignocelluloses thus to high cost of conversion into bioproducts.
In this workshop, we propose to show fluorescence microscopy techniques applied to wood and to demonstrate their interest for the study of the structures and properties of the plant cell wall. We will show methods of preparation of the samples and the interest of chemical and/or physical pretreatments to reduce the recalcitrance of cell walls and facilitate their biological deconstruction. We will show the advantages of combining fluorescence imaging and F-techniques using fluorescent probes to study the porosity of the samples and the accessibility of the polymers constituting the plant cell walls.
At the end of this workshop the participants will be able to prepare wood samples for direct fluorescence microscopy imaging and run experiments using fluorescent probes to characterize the samples at the structural level.

Keywords : Biomass, Cell wall, Imaging, autofluorescence, Fluorescence microscopy, Fluorescence techniques, Fluorescent probes, accessibility, porosity

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A118-Relative localization of dendritic spine proteins in mouse brain tissue using 3D-STED microscopy and deconvolution.

David Mazaud (david.mazaud@curie.fr)

Abstract : Study of biological samples such as brain tissue by microscopy has long been essentially done using confocal/2-photon microscopy. While it gives a rough estimate of protein localization in the first tens or hundreds micrometers of the sample, the limit of the resolution obtained by these techniques does not allow precise measurements between two proteins in very close proximity .
The workshop will be split in two parts: Data acquisition and then image processing (deconvolution). It will be accessible from Beginners to experts.
The aim is to demonstrate the advantage of super resolution microscopy in thick tissue to answer biological questions that cannot be addressed by conventional microscopy. Also, image processing and analysis will be discussed, especially in the case of multicolor acquisition (up to 3 colors), where a careful attention is needed to reconstruct Z-stack images.
Using fixed 40µm thick brain tissue immunostained with red and far red antibodies to localize dendritic proteins and the green channel to identify dendritic spines, we will observe how 3D-STED microscopy can discriminate two proteins in close proximity within spine heads. Indeed, the fact that two proteins are “above” or “below” one another is key to generate models for the speed of Calcium entry and propagation in the spine. 2 or 3 color STED will be done, especially to discuss the limits of multicolor (green and reds) STED imaging on Z-stacks acquisitions.
Image postprocessing by reconstruction and deconvolution using ImageJ and Huygens will be shown and discussed. The setup of precise parameters for deconvolution is necessary to avoid overinterpretation of the signal.

Participants will be invited to modulate different parameters to observe the impact and importance of each (laser power, gating, dwell time, averaging, pixel size, Rescue mode (if available)). Comparison between 2D and 3D mode will also be evaluated.
At the end of the workshop, participants are expected to have understood the advantage of 3D-STED microscopy for thick biological samples. They will know the most important parameters to play with to get the best image quality of their samples. Finally, they will understand the pros and cons of 2D vs 3D-STED, on sample preparation (secondary antibodies and antibody concentration and incubation) and the basic parameters for faithful deconvolution.
Keywords : 3D-STED, brain tissue, multi-color STED, deconvolution

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A119-L'autofluorescence chez les plantes: adversaire ou alliée?
Geneviève Conéjéro (genevieve.conejero@inra.fr) Elodie Jublanc (elodie.jublanc@inrae.fr)
Abstract : Les plantes contiennent beaucoup de molécules fluorescentes, notamment des métabolites secondaires jouant un rôle crucial pour les défenses de la plante et son adaptation aux variations de l'environnement (température, pollution, nutrition minérale, pathogènes…)
Cette autofluorescence est souvent problématique et peut gêner l'observation de fluorochromes exogènes, colorants/ protéines fluorescentes lors des expériences. Mais elle est aussi un atout important pour comprendre le rôle de ces molécules endogènes fluorescentes qui représentent un enjeu sur le plan agronomique en cette période de changement climatique et de stress environnementaux (par exemple des molécules aux propriétés d'insecticide naturel). L'imagerie peut contribuer à une meilleure connaissance de ces molécules d'intérêt à condition de pouvoir exploiter correctement leur autofluorescence.
Dans cet atelier, nous proposons de réaliser dans un premier temps des acquisitions spectrales d'échantillons végétaux vivants ou fixés en microscopie multiphotonique équipé d'un détecteur spectral, le laser pulsé IR permettant d'atteindre des couches cellulaires plus profondes et d'obtenir une cartographie tissulaire de la répartition de ces molécules. Des spectres auront été au préalable acquis sur une liste de métabolites purs connus et nous réaliserons des déconvolutions spectrales.
Puis dans un second temps, une fois connus les spectres d'émission de ces molécules, nous pourrons faire des images avec des systèmes confocaux qui permettent d'augmenter notablement la résolution par rapport aux systèmes classiques. Une meilleure résolution permettra d'affiner la localisation subcellulaire, notamment dans les plastes, le noyau ou des vésicules de taille variable. Enfin nous réaliserons si besoin des images en 3D afin de visualiser la répartition de ces molécules dans le volume cellulaire.
Keywords : autofluorescence, plantes, imagerie spectrale, microscopie multiphotonique

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A120-Imagerie sur petit organoides 3D, Troubleshooting
Adeline Boyreau (adeline.boyreau@u-bordeaux.fr)
Laetitia Andrique (laetitiaandrique@hotmail.com)
Abstract : Objectif : Proposer une méthodologie par maximiser ses chances d'obtenir des résultats interprétables sur des échantillons 3D de taille moyenne.
Depuis une dizaine d'années, les organoïdes, sphéroïdes et autres objets/tissus en 3D sont devenus des modèles de choix en biologie à la fois car ils portent en eux plus de complexité que les modèles 2D et sont une alternative possible aux modèles animaux (3R). Ces nouveaux échantillons

nécessitent donc de repenser le pipeline expérimental qui comprend l'échantillon, sa coloration et le mode d'imagerie. Après une présentation non exhaustive de quelques techniques de production d'organoïdes, nous proposerons de répondre à un exemple de problématique courant en imagerie 3D : « Je n'ai pas de signal au centre de mon échantillon ». Dans cet exemple, nous imagerons des cibles biologiques simples, comme un marquage nucléaire 1/ par intégration d'une sonde fluorescente, 2/ par expression cellulaire d'un vecteur et 3/ par immuno-marquage. Au cours de cet atelier, nous développerons ensemble une méthodologie basée sur une série de questions simples et de réponses pratiques pour obtenir des résultats interprétables en imageant ces organoïdes.
Ce TP ne prendra pas en compte les plus gros échantillons (cerveaux, organes, spécimens entiers), souvent associés à des méthodes de clearing complexes et montages/microscopes spécifiques.
Nous souhaitons, en conclusion de l'atelier, présenter un listing de sondes testées par nos soins en imagerie 3D, en donnant leurs caractéristiques (concentration, temps d'incubation, distance de pénétration de la sonde, type de tissu imagé, type de microscope/objectif utilisés), que nous complèterons en direct avec les participants en fonction des expériences de chacun.
A l'issue de cet atelier les participants seront capables d'obtenir des résultats interprétables de leur échantillon 3D en adaptant leur protocole expérimental et en conduisant un raisonnement méthodique.
Keywords : Organoides, 3D, méthodologie, protocole optimisé

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A121-CentrO2 : Influence de la concentration en oxygène pour l'imagerie dSTORM multi-couleur du cil primaire

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Abstract : Nous avons développé un nouveau tampon (Eternity/Everspark) pour l'imagerie de super-résolution dSTORM (direct Stochastic Optical Reconstruction Microscopy), sans dégazage enzymatique (Provost and Rousset, Sci. Rep., 2019) qui conserve le scintillement des fluorochromes pendant plusieurs semaines (versus plusieurs heures pour les tampons enzymatiques, classiquement utilisés). Lors de cet atelier, nous allons démontrer que le scintillement effectif des échantillons, observable sur un système d'imagerie de molécules uniques (ELYRA de Zeiss ou Vutara de Brucker) est directement corrélé à l'absence d'Oxygène dans le milieu en faisant une mesure effective de la concentration en oxygène dans le milieu par une fibre optique sur un système déporté avec des optodes inclues dans le milieu.
Les échantillons observés seront des immuno-marquages double ou triple couleur de protéines du corps basal (centrosome) et du cil primaire dans des cellules de la rétine. L'objectif de notre atelier est de démontrer qu'un contrôle qualité des échantillons scellés peut être réalisé en faisant une simple mesure d'oxygène dissout dans le tampon grâce à une fibre optique et un capteur déporté dans l'échantillon scellé. Les images de reconstruction multi-couleurs devraient donc permettre d'observer la structure de la base du cil et du cil primaire avec une précision de l'ordre de 10 à 20 nm.
Keywords : dSTORM, mesure oxygène par fibre optique, super-résolution, centrosome, corps basal, cil primaire, qualité tampon dSTORM, multi-couleur, Everspark

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A122-Mapping elasticity of micro-patterned living cells by AFM

Sébastien Janel (sebastien.janel@cnrs.fr)

Abstract : Atomic force microscopy has gained popularity out of its original physics field and is now applied in many diverse studies in biology, ranging from the imaging of molecules on lipid membrane to mechanobiology on cell and tissues. Among the many possibilities offered by AFM, one of its most remarkable feature for biologists is to be able to probe cell mechanics in a physiological environment, at the nanoscale, sometimes even in 3D1. This gives crucial information concerning the state of the cell upon genetic modification2, drug treatment, intoxication3 etc.

Micropatterning is a technique that allows constraining cells in a given shape by structuring the cell substratum. Applications are manyfold and thriving nowadays. One of the key point of the technique is to keep the cell in a more native environment, and not to spread as much as it does on a glass coverslip. Such patterning can also be performed in 3D5 to resemble the situation of cells in organs. Another major point for mechanobiology is the possibility to get many data with the same cell geometry, increasing the statistical significance of such studies.

We propose here to teach how to probe cell mechanics on such 2D micropatterned cells4 with the following key aspects:

- Calibration of the microscope6 (invOLS, spring constant)
- Performing force maps with suitable parameters
- Data analysis
- Data averaging

Keywords : mechanobiology, Elasticity, AFM, Young's modulus, micro-patterning

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A123-Atomic Force Microscopy analysis of SARS-CoV-2 virus-like particles and producing cells: nanoscale imaging to mechanical characterization

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Abstract : Whereas fluorescence microscopy has become essential for cell biology studies, the diffraction barrier has for a long time set a limit for observation of structural dynamics of biological assemblies, especially viruses. AFM partly bridges that spatial resolution gap and also brings force application/measurement capabilities for investigation of the mechanical properties of viruses, virus-like-particles (VLP), infected or VLP-producing cells. This has been recently used to analyze SARS-CoV-2 morphology and stiffness at the nanoscale (1, 2). We will focus here on the application of AFM in the analysis of non-infectious virus-like particles mimicking SARS-CoV-2 assembled from the structural viral proteins M, N, E and S (3) and produced from transfected cell cultures. On the technical side, the workshop will be focused in how to set up AFM imaging and stiffness measurements on (i) SARS-CoV-2 VLP samples adsorbed on glass or mica surface or (ii) cells (live of fixed) producing these VLPs. On the biological side, we will explore the differences (if any) in VLP morphology and stiffness, or VLP producing cells mechanical properties, depending on VLPs with or without Spike. Part of the workshop will deal with the calibration procedure, choice of cantilevers, imaging modes and acquisition speeds for obtaining robust and high resolution images. At the end of this workshop, the participants will be able to understand the basic functioning of a modern bio-AFM, the formation of an AFM image according to the different acquisition channels and the integration of AFM imaging of viruses with optical microscopy.

(1) Kiss, B. et al., Topography, spike dynamics, and nanomechanics of individual native SARS-CoV-2 virions. Nano Lett. (2021).

(2) Lyonnais, S. et al. Atomic force microscopy analysis of native infectious and inactivated SARS-CoV-2 virions. Sci Rep (2021).
(3) Swann, H. et al. Minimal system for assembly of SARS-CoV-2 virus like particles. Sci Rep (2020)

Keywords : AFM, virus, cells, mechanical properties, quantitative imaging, young's modulus

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A124-QuPath: pyramid image analysis for everyone. Case study: Deep-learning cell counting and quantification of histological slides.

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Philippe Mailly (philippe.mailly@college-de-france.fr)

Abstract : During this workshop, you will image serial sections of histological slides on a slide scanner to analyze fibrosis and cell death within the liver tissue.
Using QuPath, an open software platform for whole slide image analysis, you will perform deep-learning apoptotic cell counting and quantification of tissue fibrosis.
At the end of this workshop, participants will have acquired new knowledge in terms of microscopy technique and also in image processing and analysis. You will be able to test the slide scanner and the QuPath software.

Keywords : Slide scanner, Histological staining, QuPath, Deep-learning, Counting, Quantification

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A125-Speckle-based computational microscopy : harnessing scattering for enhanced imaging of tissues

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Abstract : Scattering media, ubiquitous in biological systems, has been seen as a nuisance: because of scattering, an image can not be created with fine contrasts especially when deep in a specimen. In the last decades, adaptive optics and wavefront shaping has been proposed to overcome these challenges, enabling sharp imaging by controlling the advert effects due to scattering. These ideas will be covered by the introduction atelier *Remember your wavefront: adaptive optics and memory in different regimes*). Conversely, the emerging concept of computational microscopy with speckle patterns exploits advanced algorithms to image behind a scattering medium or to use scattering itself as an advanced and low-cost tool. In this atelier, we will perform two well-established experiments that show the advantage of using speckles with computational methods, on the same experimental setup as the one used in the atelier organized by our group, previously aforementioned.
Prerequisites
• have a basic knowledge of optics
• have some knowledge of code (Matlab)
• have some knowledge of biology
However, we will be as educational as possible and will redefineall the notions.
At the end of the workshop, participants will be able to use an optical setup to focus and image through scattering media with different computational methods.

Keywords : fluorescence ; microscopy ; NMF ; SIM ; Blind SIM ; scattering medium ; transmission

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A126-Microscopie quantitative : dynamique moléculaire par Spectroscopie de Corrélation de Fluorescence - Mise en oeuvre, calibration et analyse

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Abstract : Fluorescence correlation spectroscopy (FCS) is a technique for measuring the diffusion coefficients and concentrations of fluorescent constructs. FCS is usually performed with a confocal microscope and can be applied to any fluorescently labeled molecule in solution (usually water or a buffer solution), on membranes and in living cells. To observe proteins it is quite easy today to create fusion proteins with autofluorescent molecules (GFP, mRFP, flag,...). From an instrumental point of view, FCS is a fairly easy technique to implement. However, the quality and relevance of the measurements depend strongly on the quality of the optical device and the reliability of the measurement methodology. During this workshop we will deal with simple cases allowing to implement this technique in order to realize precise and reproducisble quantification of molecular concentration and diffussion in living cell.

Keywords : FCS, quatification, diffusion.

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A127-Microscopie large champ sur mésoscope homemade

Benoit Rogez (benoit.rogez@fresnel.fr)

Abstract : L'étude d'une population cellulaire constitue un défi du fait de son hétérogénéité intrinsèque. Chaque cellule présente des caractéristiques qui lui sont propres, et remonter à des propriétés moyennes nécessite une étude statistique sur un grand nombre de cellules. Ces études sont toutefois contraintes par les systèmes d'imagerie disponibles. D'un côté, les objectifs de microscopie présentent un champ de vue limité à quelques mm2, ce qui limite le nombre de cellules observables. De l'autre, la cytométrie de flux ignore les événements rares et est peu adaptée à l'étude de cellules adhérentes. Afin de dépasser ces limitations, une collaboration entre l'Institut Fresnel, Horiba Medical et le CEA LETI a permis le développement d'un microscope très large champ (7 x 5 mm2) disposant d'une résolution à l'échelle de la cellule unique (3 µm).
Deux aspects seront abordés dans cet atelier :
Le Mésoscope sera présenté afin d'illustrer ses capacités d'imagerie sur des échantillons biologiques (amenés par les participants). A l'issue de l'atelier, les participants seront capable de reproduire et d'utiliser le Mésoscope de manière autonome dans leur laboratoire.
Puis, on discutera des aspects « DIY » du Mésoscope qui a été conçu dans l'optique d'être le moins cher possible

Keywords : Imagerie de champ millimétrique, DIY

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A128-Mechanical characterisation by AFM of murine oocytes to predict their fitness

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Abstract : Oocyte production during meiosis in human females is essential for sexual reproduction. However, this process is error-prone and generates a basal rate of bad quality oocytes, having deleterious consequences for fertility and offspring development. It has been shown that aberrant oocyte stiffness alters the developmental capacity of the oocyte (Chaigne et al., 2013, 2015; Yanez et al. 2016; Bennabi et al. 2020). Thus, mechanical properties could be used to predict developmental potential of oocytes and guide their selection for assisted reproductive technologies.

The mechanical properties of such big cells have been studied using the micropipette aspiration technique (Larson et al. 2010,Chaigne et al., 2013, 2015, 2016;Yanez et al., 2016;Bennabi et al. 2020). However, this method has a low yield and not fully adapted to follow oocyte development.
Atomic Force Microscopy (AFM) has a much higher throughput and provides robust measurement of mechanical parameters, and is therefore a good alternative to micropipette aspiration. We will present the protocol and the analysis work-flow we have designed to measure the evolution of murine oocytes mechanical properties during their development. The experimental setup is adapted to the specific characteristics of oocytes which are large (80µm diameter) non-adherent cell. The force-distance curves obtained will be analysed using a Sneddon model combined with a linear model to extract the elasticity and cortical tension values. Our preliminary results have shown that both these parameters decrease during oocyte development. They confirm our previous micropipette measurements and indicate that we can use this technique in a high throughput manner to score oocytes and set cortical tension threshold values correlating with a good developmental potential. Finally, this workshop will present an AFM indentation experiment on oocytes that may be of interest to scientists wishing to study the mechanics of other non-adherent cell types.
Keywords : AFM, Oocytes, Cortical Tension, Elasticity

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A129-AFM on microbial surfaces: basics of force spectroscopy measurements
Audrey Audrey beaussart (audrey.beaussart@univ-lorraine.fr)
Sofiane El-kirat-chatel (elkirat1@univ-lorraine.fr)
Abstract : More than an imaging tool, AFM has been instrumental in measuring the mechanical response of living cells and to understand the distribution and unfolding properties of their surfaces biomolecules. In this practical, we will demonstrate how to measure molecular and cellular interactions with a piconewton sensitivity.
For that, AFM tips need to be functionalized i) with ligands to map the distribution of the corresponding receptors at the cell surface,
ii) with chemical functions to decipher specific physico-chemical properties or
iii) with the cell itself to measure its adhesion towards (a)biotic substrates.
i and iii will be explained theoretically and demonstration of ii will be done during the workshop. For that, gold AFM probe will be functionalized with hydrophobic thiols and participants will be invited to measure forces toward abiotic hydrophobic and hydrophilic samples to sense how to measure hydrophobic properties at the nanoscale.
Data treatment will be explained (and compared to previous data obtained in the lab using other types of functionalized tips, eg. Abs).
At the end of the practical, participants should be able to place the tip in the AFM set-up, align the laser, and acquire and interpret simple force-distance curves.
This workshop is open to biologists, chemists and physicists and competences in AFM or biophysics are not required.
Keywords : AFM, hydrophobicity, force spectroscopy, adhesion

A130-Optogenetic control of 3D micro- tissue 2/2
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Adrien Méry (adrien.mery@univ-grenoble-alpes.fr)

Abstract : Mechanical forces are implied in most of biological movements ranging from single cell migration to embryo morphogenesis. Understanding how this forces are generated and transmitted within supra cellular assemblies is a key point to the understanding of those phenomenons. This workshop aims at studying how these signals are spatially propagated in biological micro-tissues. To this end, an optogenetic microscopy set up provided by the compagny will be used to trigger the contraction of 3D engineered microtissues. Practically the optogenetic stimuli will target ArhGEF11, a major regulator of cellular contractility. The bio-mechanical response of each biological sample will be quantified in a coupled practical where the participants will work on both home made software solutions and open source codes for force analysis. At the end of this workshop the participants will be autonomous on the use of the optogenetic setup, will be able to design a typical sequence of optogenetic stimulation coupled to image acquisition and finally will be trained on the software solutions for force imaging of both single cells and and 3D microtissues.
Keywords : optogenetic , 3D microtissue, mechanobiology

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A131-Imagerie 3D d'organes transparisés par microscopie à feuille de lumière
Sébastien Dupichaud (sebastien.dupichaud@inserm.fr)
Louison Lallemant (louison.lallemant@gmail.com)
Abstract : La capacité d'imager un échantillon biologique dans son intégralité est un enjeu majeur dans beaucoup de questions biologiques. Plutôt que de réaliser des coupes en séries d'un organe, il existe maintenant des techniques de transparisation qui permettent d'imager intégralement l'échantillon. Couplées à la microscopie en feuille de lumière, on peut acquérir rapidement des volumes importants de données.
Pour montrer l'intérêt de la microscopie à feuille de lumière et de la transparisation, des modèles d'organes murins seront utilisés durant cet atelier.
A la fin de l'atelier, les participants seront familiarisés avec l'intérêt de la transparisation, les différents types de techniques et comment choisir la technique de transparisation à utiliser. La prise en main de la microscopie à feuille de lumière leur permettra de voir les points forts (vitesse d'acquisition, faible photo blanchiment) et les limites (taille de l'image, résolution) de cet outil.

A132-Structured Illumination Microscopy : SIM on cells !
Aurélien Dauphin (aurelien.dauphin@curie.fr) Audrey Salles (audrey.salles@pasteur.fr)
Abstract : The ultrastructure of the cell is often studied by electron microscopy since the resolution of photon microscopy is limited to the diffraction of the light in photon microscopes. However, since many years, super-resolution technics have overcome this limitation with different strategies and results. Among them, one is often seen has the less phototoxic, while doubling the resolution in 2 or 3D by a factor of 2 : the Structured Illumination Microscopy (SIM).
In this workshop, you'll discover in practice the SIM and the entire workflow required to achieve a super-resolution image from sample preparation to artefact analysis :
We will see the pattern of illumination, the gain of resolution, we will image different nuclear and cytosolic structures and reconstruct the images. Depending on the availability of samples, we will also be able to carry out super-resolution imaging on living samples and see together how to adapt the acquisition parameters. We will check the quality of the reconstruction process, and the possible artefacts. At the end of the workshop, the participants will be able to make a SIM acquisition and reconstruction and check for the presence of artefacts. We will then discuss the pros and the cons of SIM among the Super-resolution methods.

Keywords : SIM, Structured Illumination, ultrastructure, super-résolution

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A133-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

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Alessandro Furlan (alessandro.furlan@univ-lille.fr)

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., the immortalised intestinal cell line Caco-2.
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, both by orthodox balneation and by intra-cystic micro-injection.
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.

Keywords : Organoids, spheroids, aggregating methods, microfluidics, live labelling, mounting

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A134-Measuring turgor pressure of living plant cells with an Atomic Force Microscope

Simone Bovio (simone.bovio@ens-lyon.fr)

Abstract : Atomic force microscopy (AFM) belongs to the scanning probe microscopy (SPM) family, where a tip, often with a nanometric radius, scans the surface of a sample. In this kind of microscopies, the surface is detected via the interaction forces between the tip and the sample. If the sample is scanned by maintaining constant the tip-surface force, the tip would consequently have to move up and down to follow the surface profile: those displacements may be used to reconstruct the 3D topography of the sample. Beyond the topographic information, this technique allows the measurement of any type of interaction forces such as electrostatic, van der Waals or contact forces. Furthermore, the tip can be used to apply forces to the surface of a sample and to measure the resulting deformation, the so-called "indentation", in order to determine its mechanical properties (e.g., Young's modulus, viscoelastic properties).
Plants cells can be seen as balloons under pressure. Indeed, walled cells as in plants, fungi or bacteria, contain a high hydrostatic pressure called turgor pressure. Turgor pressure (that can be up to several MPa) is a driving force in cell growth and participates to the overall mechanical stability of the plant. Different methods exist for measuring turgor pressure, like the psychrometer, the pressure bomb or (for a direct measurement) the pressure probe1. Anyway, the turgor pressure can also be calculated from indentation measurements2, if the cell wall thickness is known, which is the scope of this workshop.

Keywords : AFM, Atomic Force Microscopy, force spectroscopy, plant tissue, turgor pressure

A135-Imaging multiprotein complexes in the cytosol by super-resolution fluorescence. Introduction to lattice and dual iterative SIM

Stanimira Valeva (stanimira.valeva@inserm.fr) Elodie Chatre (elodie.chatre@ens-lyon.fr)

Abstract Structured illumination microscropy (SIM) is a technique that doubles the resolution typically obtained in confocal microscopy as SIM imaging allows to reach lateral resolution of 100-130 nm (1,2) and axial resolution of 280-350 nm (3,4). Multicolor 3D image acquisition in SIM is fast, can be done on living on fixed samples and is compatible with most chemical or biological stains. Thus SIM can be particularly useful for studying cellular structures, dynamics and/or interactions on a cellular or tissular level.

The purpose of this workshop is to present the SIM methodology and the functionalities of the lattice SIM system of the Elyra 7 microscope from Zeiss. We will also introduce the new processing method developed by Zeiss – deconvolution applied to SIM, called diSIM (dual iterative SIM) or SIM2. Finally, this workshop will give to the participants the opportunity to evaluate the usefulness of SIM for the needs of their projects.

This workshop will be interactive and adapted as best possible to the interests of the participants. It can be conducted in French or in English as needed.

Keywords : Structured illumination microscopy (SIM), dual iterative SIM, super-resolution, cellular structures, multiprotein complexes

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A136-Biosensing the cell: FRET by FLIM using AurkA kinase activation biosensor

Marc Tramier (marc.tramier@univ-rennes1.fr)

Abstract : In this workshop, we will present user-friendly approaches of FLIM to monitor FRET biosensors. As an example, we will determine activation of AurkA kinase in living cells. 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. Moreover, we will present that FLIM allows us to measure single color FRET biosensor by using dark acceptors, opening the way to multiplex.

Keywords : FRET – FLIM – genetically encoded FRET biosensors – TauSense – dark acceptor - AurkA

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A137a-Imagerie de fluorescence par microscopie confocale spinning-disk : une exploration des avantages et limitations techniques sur 4 systèmes (en 2 parties)

Thomas Guilbert (thomas.guilbert@inserm.fr) Baptiste Monterroso (baptiste.monterroso@unice.fr)

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. D'autres techniques exploitent également cette pratique du confocal multipoint en utilisant un système de fentes. 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.
Cet atelier se décomposera en deux parties dans le but d'explorer les possibilités, avantages et limitations des 4 différents systèmes SDCM présents cette année à MiFoBio. Les participants à cet atelier découvrirons ou approfondirons leurs connaissances sur cette technique. Les notions de résolutions, d'échantillonnage, d'homogénéité de champs et de diaphonie en milieux diffusant seront abordées, parmi d'autre. L'aspect de suivis dans le temps des performances d'un tel système sera également évoqué. Toutes les questions seront les bienvenues dans le but de repousser les limites de cette technique devenue courantes dans nos laboratoires.
Keywords : Microscopie de fluorescence à disque rotatif, Spinning-disk, Résolution, Métrologie

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A137b-Imagerie de fluorescence par microscopie confocale spinning-disk : une exploration des avantages et limitations techniques sur 4 systèmes (en 2 parties)

Thomas Guilbert (thomas.guilbert@inserm.fr) Baptiste Monterroso (baptiste.monterroso@unice.fr)

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.
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Cet atelier se décomposera en deux parties dans le but d'explorer les possibilités, avantages et limitations des 4 différents systèmes SDCM présents cette année à MiFoBio. Les participants à cet atelier découvrirons ou approfondirons leurs connaissances sur cette technique. Les notions de résolutions, d'échantillonnage, d'homogénéité de champs et de diaphonie en milieux diffusant seront abordées, parmi d'autre. L'aspect de suivis dans le temps des performances d'un tel système sera également évoqué. Toutes les questions seront les bienvenues dans le but de repousser les limites de cette technique devenue courantes dans nos laboratoires.
Keywords : Microscopie de fluorescence à disque rotatif, Spinning-disk, Résolution, Métrologie

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A138-Colocalisation de complexes macromoléculaires en super-résolution PALM/STORM

Xavier Marques (xavier.marques@sorbonne-universite.fr)

Abstract : L'avènement de la microscopie à super-résolution est une avancée majeure pour la microscopie optique. Elle améliore considérablement notre capacité à décrire et expliquer l'organisation biologique à l'échelle nanométrique.

Parmi les méthodes proposées, la microscopie de localisation de molécules uniques (Single Molecule Localization Microscopy, SMLM) est basée sur l'observation des signaux produits par des fluorophores individuels. De cette manière, il est possible de construire une image par pointillisme dont la résolution dépend essentiellement du rapport signal/bruit. Typiquement, la résolution atteinte est de l'ordre du dixième de nanomètre (~10 fois plus que la résolution d'un microscope optique).

Différentes techniques SMLM sont disponibles, en fonction de l'observation des fluorophores individuels imagés. Les exemples les plus populaires sont la stochastic optical reconstruction microscopy (STORM) et la photo-activated localization microscopy (PALM), qui diffèrent simplement par la manière dont les molécules individuelles sont marquées et imagées. Des images super-résolues à deux couleurs peuvent être obtenues soit en combinant deux fluorophores différents qui conviennent à STORM, soit en combinant STORM et PALM.

Keywords : Super-resolution, Single Molecule Localization Microscopy, PALM, STORM

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A139-Confronting Lattice SIM imaging to various scattering samples of different thickness

Philippe Bun (philippe.bun@inserm.fr) Lydia Danglot (lydia.danglot@inserm.fr)

Abstract : Structured Illumination Microscopy (SIM) is a fluorescence microscopy technique that can surpass the diffraction limit by exploiting interference patterns (Moiré fringes). One of the main pros of using SIM is that such approach does not require any sophisticated sample preparation protocols, and can be readily applied to samples prepared for "conventional" fluorescence microscopy. On top of its capacities of multicolor imaging and optical sectioning, SIM is the method of choice when conducting live cell imaging experiment. However, SIM approach is sensitive to out-of-focus light and suffers when imaging thick or densely labelled samples. Using the latest and commercially-available SIM system, we propose to conduct SIM imaging on both thin (cultured cells) and thick scattering (organoids or tissue) samples. We aim to find the adequate set of imaging and 3D reconstruction parameters for the different type of biological samples.

Keywords : Super-resolution fluorescence imaging, Structured Illumination Microscopy, Neurons, Brain section.

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A140-Du plus petit échantillon au plus gros, la microscopie à feuille de lumière face à l'enjeu du multi-échelle

Julien Dumont (julien.dumont@college-de-france.fr) Astou Tangara (tangara@biologie.ens.fr)

Abstract : De par ses qualités intrinsèques, la microscopie à feuille de lumière a connu un succès retentissant depuis sa remise au goût du jour par l'équipe d'E. Stelzer (2004). Cette technique limite le photoblanchiment et la phototoxicité, permet une acquisition totale grâce à une rotation de l'échantillon, tout en assurant vitesse et résolution à l'acquisition sur des échantillons au volume parfois important. L'une des difficultés majeures de cette technique réside dans la mise en place de cet échantillon. En effet, la nécessité d'obtenir un montage permettant la rotation de l'échantillon

représente très souvent un casse-tête technique, casse-tête qui évolue en fonction de l'échelle de l'échantillon.
Au cours de cet atelier, nous allons nous confronter aux différentes échelles d'échantillons pour leur mise en place sur un microscope à feuille de lumière. Le but de cet atelier sera de réaliser des acquisitions de microscopie à feuille de lumière sur des échantillons aux volumes très différents, et d'adapter les conditions de montage en accord avec les besoins des échantillons. Nous traiterons également des différents aspects de l'acquisition d'image et de l'optimisation des paramètres d'acquisitions propre à chaque condition.
A l'issue de l'atelier, les participants auront développé un point de vue élargi sur les enjeux et les limites de la microscopie à feuille de lumière. Ils auront appris à réaliser des montages d'échantillons pour les différentes échelles rencontrées, ainsi qu'à réaliser l'acquisition d'images correspondantes.
Keywords : microscopie à feuille de lumière, lightsheet, zebrafish, drosphile

A141-Medium throughput imaging of thick samples: a practical comparison of different samples (Drosophila tissues and encapsulated spheroids) in native opaque state and after light-penetration facilitation

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Abstract : This workshop is dedicated to illustrate how light-sheet microscopy could be used as a medium-throughput 3D microscopy method for extended imaging of a diversity of thick samples.
Light-sheet microscopy is now well identified as a state-of-the-art technique for 1/ high resolution, 2/ low photodamage, 3/ its aims to isotropy and 4/ fast-scanning imaging of 3D living samples, including zebrafish larva, drosophila embryo, mouse egg-cylinder etc…
Although there are now several designs available in the market as commercially available (turn-key) systems, most of them rely on a design that requires the sample to be either embedded on an agarose cylinder (that is an issue for constrain-free morphogenesis) when using a vertical sample holder (the original design) or inserted in a small and tight cavity on a stage when using the upright design (similar to the one implemented for the lattice-light-sheet microscope).
Here we will take advantage of the original design of an inverted light-sheet microscope where the samples are deposited on a transparent and flexible groove (a FEP foil).
During this workshop, we will mount and image samples which are chosen to illustrate different challenges: 1/ a Drosophila imaginal disc (or embryo) and its counterpart, 2/ a cleared and expanded disc (or embryo), 3/ encapsulated plain spheroids (thick balls of cells), 4/ encapsulated hollow spheroids (cystic shape) and 5/ the later after a soft and safe transparisation protocol. The encapsulated cells are Caco2 cells, that arrange within the capsules in either a solid and compact spheroid in the absence of Matrigel® or in hollow pseudo-monolayers cyst.
We will take the attendees in a journey about questioning their samples, how they are amenable to in-depth imaging and how to improve their transparency to light. This specific microscope and its geometric features would be as well an occasion to discuss the necessity of imaging a reasonable number of samples at the same time, especially when sa
Keywords : Encapsulated spheroids, drosophila tissues, expanded tissues, light sheet imaging, soft transparisation

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A142-Microscopie à feuillet de lumière pour l'imagerie volumique

Basile Gurchenkov (basile.gurchenkov@inserm.fr)

Abstract : Precise spacio-temporal description of morphology and developmental dynamics is essential for understanding of functioning of living systems. This concerns both "natural" objects such as developing embryos and also the artificial multicellular systems aimed to recapitulate structure and physiology of a tissue or an entire organ. The multidimensional microscopy plays major role in addressing this challenge.

Light-sheet microscopy is a classical technique applied to the imaging of three-dimensional biological samples. During this workshop we will give a brief historical overview of lightsheet microscopy and its application. We will discuss the advantages and the drawbacks of the technique when applied to biology.

During the hands-on part we will introduce the participants to the mounting strategies and establishing of imaging protocols.

Keywords : SPIM, Light-sheet , 3D+T

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A143-Imagerie à feuillet de lumière des échantillons 3D montées d'une manière peu contraignante

Basile Gurchenkov (basile.gurchenkov@inserm.fr)

Abstract : L'imagerie longitudinale des échantillons vivants nécessite de les immobiliser : il faut généralement leur imposer des contraintes mécaniques afin de les immerger dans un milieu de montage solide (tel qu'un gel d'agarose). Or ces contraintes mécaniques sont souvent une source de modification de leur morphologie et/ou de leur comportement cellulaire, en particulier dans le cas des immersions de longue durée (au-delà de quelques heures). Le montage de l'échantillon ne peut donc pas être préparé à l'avance. De plus, le temps nécessaire pour la solidification d'un gel de montage peut empêcher l'observation de certains évènements biologiques, par exemple les premières divisions cellulaires au cours de la segmentation d'un embryon de poisson zèbre.

Lors de cet atelier, nous discuterons de l'intérêt d'étudier des systèmes modèles 3D biologiques en imagerie optique et des approches méthodologiques permettant de minimiser les contraintes mécaniques lors de leur immobilisation. Nous illustrerons ces approches à l'aide d'un microscope permettant le montage d'échantillons 3D sur une lamelle de verre incurvée.

Keywords : SPIM, light-sheet, spheroids, sample mounting

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A144-Use of detrended Fluorescence Lifetime Correlation Spectroscopy (dFLCS) to assess protein dynamics in the cell nucleus

Aymeric Leray (aymeric.leray@u-bourgogne.fr) Sébastien Huet (sebastien.huet@univ-rennes1.fr)

Abstract : Fluorescence Correlation Spectroscopy (FCS) is used for investigating the intracellular dynamics of labeled proteins at single molecule sensitivity. By parking the beam at a specific cellular location, it is possible to assess the local dynamics of a tagged protein based on the auto-correlation of fluctuations arising from fluorophores crossing the confocal volume. Nevertheless, these fluctuations do not only arise from protein movements but also from photobleaching or cell movement, leading to artifacts. Because these sources of fluctuations display different time characteristics, it is possible to detrend the raw traces to suppress slow fluctuations unrelated to protein dynamics.

Another artefact that could occur in FCS measurement is due to the detectors afterpulsing, meaning that each genuine photon detection events can be followed by a spurious photon detection at a later time. Such afterpulsing can generate a deviation of the autocorrelation function on a short time

scale. However, this afterpulsing can be easily suppressed by exploiting the fluorescence lifetime for separating the true fluorescence signal from afterpulsing events. This approach is known as FLCS (Fluorescence Lifetime Correlation Spectroscopy). Nevertheless, similar to FCS, FLCS is also sensitive to slow fluctuations. We have developed an analysis tool to perform detrended FLCS (dFLCS) to suppress these slow fluctuations, thus making the FLCS acquisitions more robust in living cells and usable for a larger spectrum of proteins.

The aim of this workshop is to introduce theoretically the principle of dFLCS then to perform experimental one color FLCS acquisitions in living cells expressing tagged proteins, and finally to analyze these data with a home-made software and discuss some strategies for improving the results.

Keywords : Fluorescence Lifetime Correlation Spectroscopy, nuclear protein

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MINI SYMPOSIUM

Round tables Lectures

New in 2021, two mini symposiums are proposed in order to prolong the exchanges and also to give the floor to the participants, in particular to the young researchers.

Symposium : SMLMS et intelligence artificielle

Organisation : **Juliette Griffie et Hippolyte Verdier**

Monday 14h-18h

Salle Méditerranée

«Molecular organisation and dynamics: is deep learning bringing something to the table? »

This mini symposium focuses on cluster/structure analysis methods of fixed single molecule localisation microscopy data sets, as well as analysis tools dedicated to quantifying live cell molecular diffusion. The analysis tools will be presented by their developers and the track will be concluded with a round table "

Program :

Florian Levet: A tale of tiles: multidimensional analysis of SMLM data with tessellations
Hippolyte Verdier: Model-free analysis of biomolecule dynamics with simulation-based inference
Cyril Favard: HIV-1 assembly at T-cell membranes: a crossroad between physics, molecular modeling and single molecule dynamic microscopy
David Williamson: Cluster analysis of localization microscopy data with machine learning
Arnauld Sergé: Analyzing and disrupting leukemic stem cell adhesion to bone-marrow stromal cells by single molecule tracking nanoscopy

symposium : Cell signalling, mecanobiology, mecanotransduction

Organization : ***Marie-Emilie Terret and Laëtitia Kurzawa***

Thursday November 11th 13h45-17h

Salle Méditerranée

The mechano-biology session of Mifobio 2021 will be held on the 10th and 11th November, with **Peter Lenart**, MPI for biophysical chemistry, Gottingen, **Nathalie Sauvonnet**, Institut Pasteur, **Guillaume Charras**, Cambridge University, **Stéphanie Miserey-Lenkei**, Institut Curie, **Stéphane Vassilopoulos**, Institut de Myologie, **Matteo Rauzi**, IBV, Nice. We are extending it by organizing a mini-symposium with **Laurent Blanchoin** (CEA, Grenoble) and **Hervé Turlier** (CIRB, Collège de France) as invited speakers. The rest of the symposium will be led by young researchers, who will present their research.

Thursday November 11th 13h45

Introduction

Invited speakers :

Directed Actin Cytoskeleton Self Organization and Contractility.
Laurent Blanchoin,
Cytomorpholab, IRIG, CEA, Grenoble, France

Mini symposia :

Magnetic muscular multicellular aggregates: focusing on fluid-like properties of tissue models and driving macroscopic organization

Irène Nagle[1], Florence Delort[2], Sylvie Hénon[1], Sabrina Batonnet-Pichon[2], Claire Wilhelm[1,3], Myriam Reffay[1]

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Mechanics of tissues drives growing interest in mechanobiology but it implies to develop new approaches to control and deform them. We address these two challenges on muscle tissue models by using magnetic muscle cells via the incorporation of biocompatible superparamagnetic nanoparticles (γ-Fe2O3). This magnetic labelling enables both the manipulation of cells at distance to create purely cellular aggregates of controlled shapes and the application of forces to measure their mechanical properties. Using mouse muscle precursor cells C2C12, we obtained multicellular aggregates without support matrix of unprecedented size (1 mm). The aggregate deformations with a magnetic field enable to measure its macroscopic mechanical properties (surface tension, Young's modulus). We looked at the interplay between the individual cell

properties (cell-cell adhesions, actin structure and tension) and the mechanical properties at the tissue scale revealing the importance of desmin disorganization in macroscopic rigidity and surface tension. By studying desmin-mutated muscle precursor cells (point mutations involved in desminopathies), we enhanced the fundamental role of the intermediate filament network architecture in this 3D tissue model. Moreover, magnetic forces can be used to drive muscle cell differentiation by first reproducing their alignment and secondly stimulating them. We develop a magnetic stretcher to stretch multicellular aggregates of muscle precursor cells trapped between two mobile magnets and induce their differentiation into fused aligned muscular cells. This approach will enable to study force generation in purely cellular 3D tissue models.

Integrated microfluidic and microscopy set-up to measure oocytes mechanical properties and predict their fitness

Lucie Barbier[1], Rose Bulteau[2], Enzo Lescure[1], Marie-Hélène Verlhac[1], Clément Campillo[2], Marie-Emilie Terret[1]

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Oocyte production during meiosis in human females is error-prone and generates a basal rate of bad quality oocytes. They are associated with miscarriages and congenital diseases such as Down syndrome. Moreover, their rate increases with maternal age and leads to the need for medically assisted reproduction procedures such as in vitro fertilization (IVF). Therefore, it is crucial to move from qualitative to quantitative approaches and find new technologies to assess oocyte quality and increase the rate of successful IVF.

Recent works have shown that oocytes mechanical properties could be used as a biomarker of their development potential[1,2,3]. Indeed, oocytes with too high or too low cortical tension are not able to develop after fertilization[1]. Moreover, low cortical tension leads to chromosomes miss-segregation and aneuploidy[3]. However, current methods used in research laboratories to measure oocyte cortical tension are low throughput, require expensive equipment and manipulation skills, limiting their application in medical environment. Thus, we aim to develop a simple minimally invasive microfluidic device able to sort oocytes depending on their cortical tension to implement its use as a predictive biomarker in clinical practice.

We based our approach on micro-metric constriction as cells mechanical properties can be linked to different transit score parameters[4]. We are implementing an integrated environment to control both the flow in the microfluidic device and image recording of oocyte deformation to allow their measurement by non-trained users on a standard video microscope. By recovering oocyte after measurement, we have shown that oocyte transit in the microfluidic device did not alter their development. Moreover, we found significant variability in passage time within a wild-type oocyte population reflecting the diversity in cortical tension in murine oocytes. These values are currently being coupled with tension measurements to confirm that the microfluidic device can discriminate oocytes by their mechanical properties. These preliminary experiments show the potential use of our comprehensive set-up to assess oocytes mechanical properties in a user friendly and non-invasive manner.

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Mechanisms of IRSp53 driven filopodia initiation

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Filopodia are actin-rich membrane protrusions essential for cell morphogenesis, motility, and cancer invasion. It remains elusive how cells control filopodia initiation on the plasma membrane. To address this fundamental question in cell biology, we performed experiments *in cellulo*, *in vitro* and *in silico*. We showed that IRSp53, a membrane curvature sensor critical for filopodia generation, self-assembles into clusters on PIP2-containing membranes in the absence of signalling regulators as those found in cells. Our simulation work revealed that PIP2 is key for IRSp53 clustering. We further showed that IRSp53 is required for the recruitment of actin polymerases such as VASP to assemble actin filaments locally, leading to the generation of membrane protrusions filled with fascin-mediated actin bundles. By following filopodia growth in live cells, we revealed that fascin enhances filopodia elongation rate and stability. Overall, our novel *in vitro* reconstitution system was able to faithfully mimic the generation of actin-rich filopodia. Moreover, our results indicate that once IRSp53 is active and able to bind to membranes and recruit downstream partners, it readily induces protrusion formation. However, by pulling membrane tethers from live cells, we observed, for the first time, that IRSp53 can only be enriched and trigger actin assembly in tethers at highly dynamic membrane regions where it is membrane-bound and presumed active. Our work thus supports a cellular regulation mechanism of IRSp53 in its attributes of curvature sensation and partner recruitment to ensure a precise spatio-temporal control of filopodia initiation.

Discussion

With speakers of Module 7 and allspeakers of Symposium cession

Caffee Break

Invited speaker :

Unifying microscopy data and physical models.

Hervé Turlier,
CIRB, Collège de France, Paris, France

Mini-symposia:

Long-term nuclear regulation of cancer cells under confinement

Malèke Mouelhi[1], Charlotte Rivière[1] and Sylvain Monnier[1]
(1) Institute of Light and Matter (ILM), UMR5306 Univ. Lyon, Univ. Claude Bernard Lyon 1, France

The physical properties of the tumor microenvironment are strongly modified during tumor growth and participate in the development and invasion of cancer cells [1], including not only stiffness, but also compression [2].

In particular, the nucleus is critically affected during compression [3] and is appearing as an important mechanosensor of deformations [4,5]. Nevertheless, most studies focus nowadays on short-term cell response (from minutes to few hours). New questions are open on the long-term adaptation to deformations and the mechano-sensing mechanism involved. We have recently developed a new agarose-based microsystem copping with media renewal impediment to investigate cell response to prolonged confinement [6].

We used this device to apply a tunable and controlled 1D confinement on the colorectal cancer cell line HT-29 up to several days. We evidenced a decrease of the nuclear volume after 24 hours under confinement. The overall nuclear shape is also dynamically regulated with the apparition of transient nuclear blebs. We are currently analyzing the mechanisms and consequences of such adaptation on cell division, transcription activity and protein expression. Such long-termed adaptation to mechanical constrains may be of importance for cancer cell plasticity and play a role in their resistance to treatments.

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Focal adhesion assembly and force sensing in artificial cells

Marcelina Cardoso Dos Santos [1], Audrey Ntadambanya [1], Julien Pernier [1], Kimihiro Susumu [2], Igor L. Medintz [2], Niko Hildebrandt [3,4], Christophe Le Clainche [1]
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The formation of focal adhesions (FAs) involves the recruitment of various types of proteins and it is extremely challenging to reconstitute the dynamics of this process. Although several studies have partially revealed the architecture of FAs 1,2, the cartography and chronology of protein interactions leading to the assembly of FAs, and actomyosin cytoskeleton mecanosensing are poorly understood.

In this work we try to unravel the spatio-temporal dynamics of adhesion assembly in an artificial cell model. To this end, we have developed an unprecedented strategy of microinjecting proteins of the cell adhesion-contraction machinery (talin, vinculin, actin, myosin) into Giant Unilamellar Vesicles (GUVs) containing transmembrane integrins, mimicking the cell membrane, Fig 1 A-E. The microinjection of purified proteins one by one in this simplified system allows to measure the impact of each of them while avoiding undesired interactions with various other signaling pathways, a recurrent problem when working with living cells. To date, there appears to be no model that can accurately reconstruct full cell adhesion while specifically controlling its composition from inside as well as outside. Nanoscale interactions will be solved using FRET (Förster Resonance Energy Transfer) nanosensors. These versatile probes allow multiplexing (detection of several events simultaneously) and their role as inter- and intramolecular biosensors arise a lot of interest. Previously, we have shown the potential of FRET nanosensors for a multiplexing imaging in live cells (Fig 1F) and long-lived fluorescence in zebrafish3.

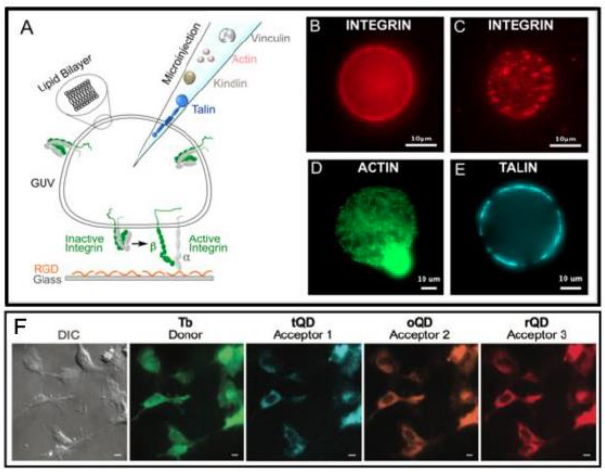

Figure 1. (A) Principle of setting up the synthetic cell: microinjection to adhered GUV. (B) GUV containing integrins IIb3 -Alexa546 imaged in epifluorescence at its maximum radius and (C) at the adhesion surface coated with RGD peptides. (D) GUV microinjected with G-actin-Alexa488 or (E) talin-QD recruited on the membrane. (F) Example of multiplexed detection that we plan to implement in the invitro assay presented in (A). Terbium (Tb)-Quantum Dot nanosensors composed of turquoise (tQD), orange (oQD), red (rQD) QDs as acceptors and Tb as donor are microinjected into live COS-7 cells.

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Inverse blebs in blastocoel formation

Markus Frederik Schliffka[1,2], Julien Dumortier[1], Jean-Léon Maître[1]
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Mammalian preimplantation development culminates in the formation of the blastocyst with the appearance of the first mammalian lumen. Pressurized fluid fractures cell-cell contacts and accumulates into a multitude of microlumens, which eventually coarsen by emptying their content into a single lumen. While the mechanics underlying hydraulic fracturing and microlumen coarsening were investigated, the cellular processes controlling these phenomena are unknown. Using

multiscale spinning disk and light sheet microscopy, we uncover shortlived membrane protrusions bulging into cells at their contacts. These protrusions are characterised by extension of the cell membrane into the cytosol, followed by rapid recruitment of actin and myosin and subsequent retraction of the protrusion within 1 minute. Due to their similarity with well-characterised membrane blebs, we call these protrusions « inverse blebs ». Like forward blebs, inhibition of actomyosin contractility results in impaired inverse bleb retraction. As for their nucleation, fluid accumulation is required, suggesting that fluid pumping would generate the hydrostatic pressure driving bleb expansion into the cells. To prevent pressure from building up locally while leaving the fluid pumping machinery intact, we use Cdh1 mutants, which show weaker cell-cell adhesion and fracture faster than wild-type embryos. We observe that adhesion deficient embryos form a lumen without inverse blebs. Therefore, the confinement provided by strong cell-cell adhesion seems necessary to build up the local pressure nucleating inverse blebs. Since inverse blebs will always extend into only one of two contacting cells, future research will address whether differences in cell contractility, cell pressure or local cortical weaknesses can determine the directionality of inverse blebs. Altogether, inverse blebs provide new insights into the interplay between cellular contractility, adhesion and extracellular fluid pressure during the formation of the first mammalian lumen.

Dynamics of early stages of cell adhesion on fluid substrate

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Adhesion is a ubiquitous process for virtually all cells from those that form together tissues and organs of multicellular organisms to single immune cells that migrate adhering to the extracellular matrix in search of pathogenic cells. Cells adhere to substrates or other cells not only to maintain physical cohesion, but also to receive information on their microenvironment. Adhesion is thus a platform for mechano-chemical communication between a cell and a substrate or another cell.
In this work, we focus on integrin-mediated adhesion. Integrins are the main players of this mechano-chemical communication that assemble in clusters in a mechanosensitive manner. Mechanical stimuli that act on these clusters are converted into biochemical signals and transmitted inside the cell. Although cell adhesion on rigid substrates is very well studied, much about adhesion on soft fluid substrates like membranes remains unknown.
Here we study the evolution of integrin clusters in cells on fluid supported lipid bilayers (SLBs) functionalized with cell ligands of different affinities (RGD peptide, bacterial protein *Invasin*). Ligand affinity to integrins is very important in integrin activation and consequent formation of adhesion clusters. We have used advanced confocal microscopy, fluorescence calibration and custom-made image analysis routines to quantify integrin densities in adhesion clusters. We have found that integrin densities in clusters on *Invasin* are significantly higher than the ones on RGD reaching the densities reported for focal adhesions on glass. Additionally, we have observed the recruitment of the proteins that are associated with mature adhesions to integrin clusters like zyxin and VASP. Finally, we have shown cells are able to deform SLBs by pulling membrane tethers from them. These results suggest the presence of mechanotransduction in integrin clusters on fluid substrates and that ligand affinity is a critical parameter in integrin cluster growth.

Final Discussion

With speakers of Module 7 and allspeakers of Symposium

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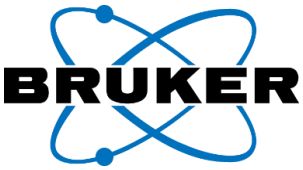
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	Friday 5th Nov	Saturday 6th Nov	Sunday 7th Nov	Monday 8th Nov	Tuesday 9th Nov	Wednesday 10th Nov	Thursday 11th Nov
8h30	Pre-module (optionnel)	Module1 P Sondes fluo M. Erard K. Podgorsky Module3 M IA D. Sage P. Paul-Gilloteaux	Module1 M Sondes fluo A. Klymchenko U. Edensfelder Module2 P Nanoscopie S. Cox Y. Shechtman	Module6 P Dynamique J. Gebhardt M. Lagomarsino (Visi Module 5 M Ondes P. Berto Chamard	Free Sport	Module7 P Mecanobiologie S. Miserey S. Vassipopoulos Module4 M Multicellulaire A. York (Visio) L. Malaquin (Visio)	Module4 P Multicellulaire C.Riviere F. Pampaloni (Visio) Module7 M Mecanobiologie N. Sauvonnet M. Rauzi
10h10		Coffee sponsored by Treefrog	Coffee sponsored by Bioaxial -Telight	Coffee sponsored by Optonlaser		Coffee sponsored by Coherent	Coffee sponsored by Treefrog
10h40		Module 3 M P Intelligence artificielle L. Royer M. Weigert	Module 1 M P Sondes fluorescentes M. Bates	Module 5 M P Ondes N. Ji J. Mertz		Module 4 M P Multicellulaire S. Descroix	Module 7 M P Mecanobiologie P. Lenart G. Charras
12h15			S3 N. Borghi			S8 F. Meunier (visio)	
13h30				Lunch	Flash tutorial 5		
		Flash tutorial 1	Flash tutorial 3	Flash tutorial 4	Presentation YISN / Prix Poster	Flash tutorial 6	Flash tutorial 7
14h00	M P	Workshops Round-tables Advanced modules	Workshops Round-tables Advanced modules	Workshops Round-tables Advanced modules **SYMPOSIUM IA for SMLM**	Module6 M Dynamique J. Mine-Hattab S.Huet Module5 P Ondes I. Izeddin S. Brasselet	Workshops Round-tables Advanced modules	Workshops Round-tables Advanced modules **SYMPOSIUM Mécanobio/transduction**
15h45	15h Introduction Cours fondamentaux			coffee break (25')			
16h10		Module2 M Nanoscopie A. Fragola S. Lévêque-Fort Module3 P IA D. Mateus A. Zaritzky	Workshops Round-tables Advanced modules	Workshops Round-tables Advanced modules **SYMPOSIUM IA for SMLM**	Module 6 M P Dynamique F. Robin J. Bothma	Workshops Round-tables Advanced modules	Workshops Round-tables Advanced modules **SYMPOSIUM Mécanobio/transduction**
18h00	M P			coffee break (15')			
18h15	S1 M.H. Verlhac S2 L. Lavis	Module 2 M P Nanoscopie J. Ries E. Katrukah	S4 S. Abrahamsson M P Module 1 Sondes Fluo M P N. Shaner (visio)	S5 C. Eggeling M P Apéritif Champagne Party offert par Hamamatsu Photonics	S6 J. Enderlein M P S7 L. Collinson M P	Module 4 Multicellulaire M P E. Hillman (visio) S9 N. Minc M P	S10 C. Paraponaris M P Bilan/AG GDR M P
20h15		Flash tutorial 2					Aperitif
21h30	Poster session organisée par YISN	Workshops Round-tables Advanced modules	Workshops Round-tables Advanced modules	Biophotonic party sponsored by Life Imaging Service	Workshops Round-tables Advanced modules	Workshops Round-tables Advanced modules	soirée YISN
0h							

M Salle Méditerranée (salle de conférence n°1)
P Salle Porquerolles (salle de conférence n°2)
M P Salle Méditerranée + retransmission visio en Salle Porquerolles