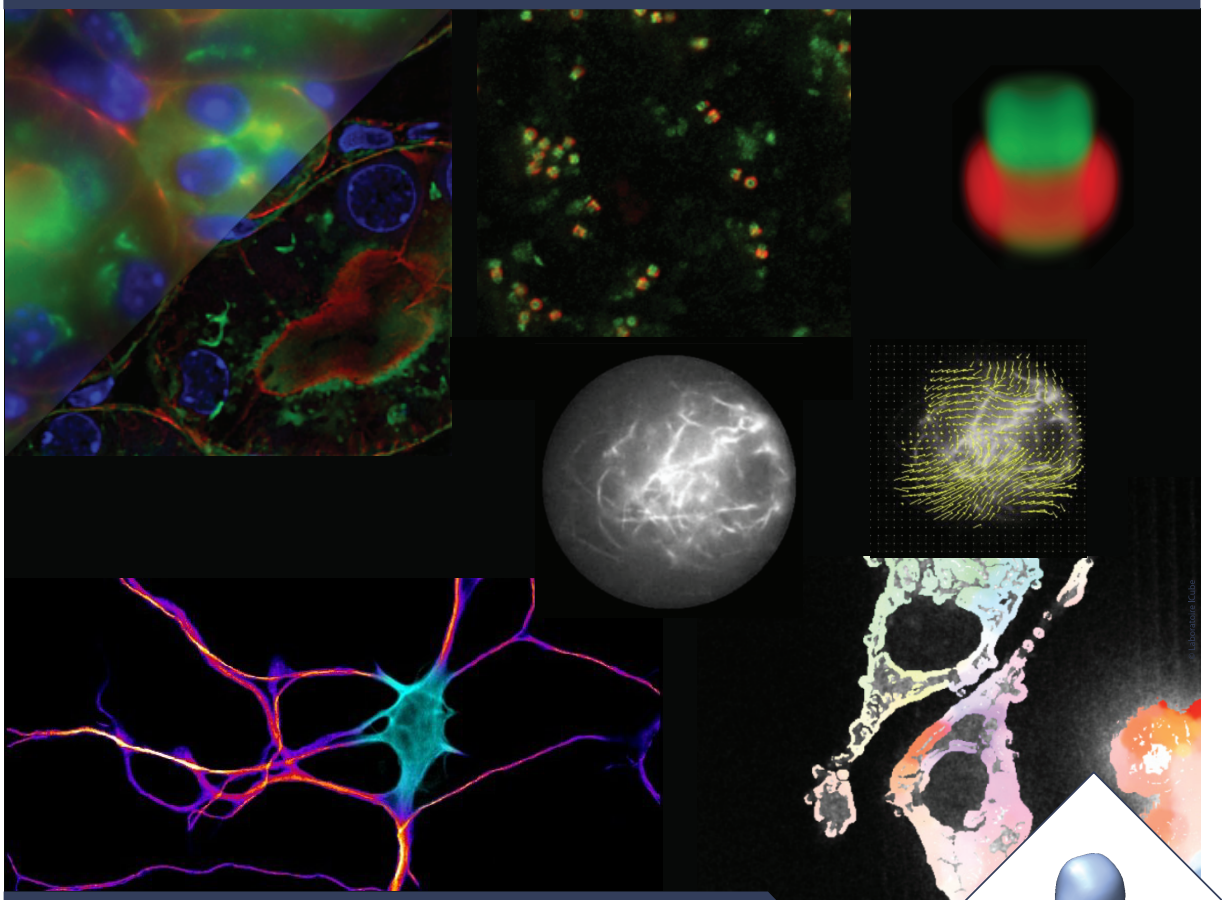
Journées communes des GdR GdR MIA et ImaBio

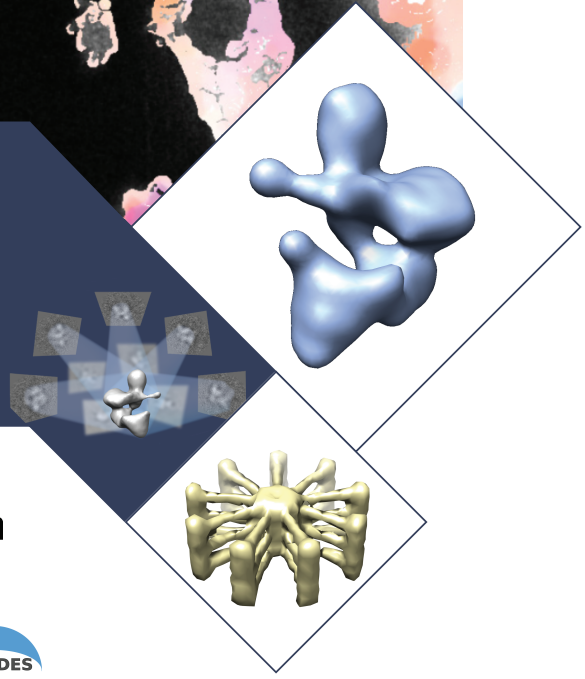
Microscopie et traitement d'image



6 au 8 Novembre 2018

Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC)

Illkirch Graffenstaden



iCUBE · cnrs · Université de Strasbourg · Inserm

igbmc INRT · igbmc · GdR ISIS · ANR · RHODES

Programme

Mardi 6 Novembre

9h-9h50	**Michael Unser** *Steerable detectors for bioimage analysis*
9h50-10h10	**Jérôme Mutterer** *Setting up an open, web-based platform for reproducible image processing*
10h10-10h30	**Charles Kervrann** *A Monte Carlo Framework for Denoising and Missing Wedge Reconstruction in Cryo-Electron Tomography*
10h30-11h	*Pause*
11h-11h50	**Perrine Paul-Gilloteaux** *Computer vision methods for multidimensional correlative microscopies*
11h50-12h10	**Sandeep Manandhar** *Census Signature based 3D Optical flow for 3D Light Microscopy Image Sequences: Application to Cell Migration*
12h10-12h30	**Nacho Molina** *Stochastic modeling of nuclear diffusion and transcription dynamics using live imaging data*
12h30-14h	*Repas*
14h-14h50	**Michael Liebling** *Cardiac Microscopy: Expanding Imaging Dimensionality without Compromising Temporal Resolution*
14h50-15h10	**Gilles Charvin** *High-throughput tracking of cell cycle progression in single dividing cells*
15h10-15h30	**Quentin Garçon** *Image processing for detection and characterization of label free biotic and non-biotic nanoparticles with a nondestructive, full field, single-arm interferometric technique*
15h30-16h	*Pause*
16h-16h50	**Rémi Galland** *Monitoring protein organization, dynamics and interactions at high spatial and temporal resolution*
16h50-17h10	**Maxime Woringer** *Complex diffusion analysis challenge*

Mercredi 7 Novembre

9h-9h50	**Pierre Bon** *Quantitative Phase Imaging, from white light imaging to fluorescence super-resolution*
9h50-10h10	**Julien Vermot** *Mapping and quantification of nuclear positions in tissues with approximate cylindrical geometry to study vascular morphogenesis*
10h10-10h30	**Philippe Carl** *Traction Force Microscopy: Data acquisition and processing*
10h30-11h	*Pause*
11h-11h50	**Quentin Denoyelle** *Super-Resolution Microscopy Using the BLASSO*
11h50-12h10	**Leonid Andronov** *Analysis and 3D visualization of protein complexes by Voronoi tessellation in super-resolution microscopy*
12h10-12h30	**Bertrand Cinquin** *Quantification of Patchiness in the fly eye*
12h30-14h	*Repas*
14h-14h50	**Graham Knott** *Focused ion beam scanning electron microscopy to explore brain connections*
14h50-15h10	**Laurent Thomas** *Automatic regions of interest detection for smart microscopy applications in whole organism screening*
15h10-15h30	**Ryshtee Mary Tannoo** *Characterization of the interaction of coreceptor CD44v6 and MET after ligand-induced activation*
15h30-16h	*Pause*
16h-16h50	**Bruno Klaholz** *Common concepts in cryo-EM and super-resolution fluorescence microscopy - the way towards integrated structural biology*
16h50-17h10	**Gabor Papai** *Single particle cryo electron microscopy of the promoter-bound transcription factor TFIID*

Jeudi 8 Novembre

9h-9h50	**Jérôme Idier** *On the super-resolution capacity of fluorescence microscopy using random speckle illuminations*
9h50-10h10	**Pierre Weiss** *Défloutage aveugle et calibrage de microscope*
10h10-10h30	**Yannick Spill** *Binless: bayesian interaction and difference detection in Hi-C data*
10h30-11h	*Pause*
11h-11h50	**Guillaume Maire** *Computational diffraction microscopy for high resolution quantitative imaging*
11h50-12h10	**Anastasiia Bozhok** *Microscopie ptychographique de Fourier: théorie, instrumentation et études de bruit*
12h10-12h30	**Stéphane Perrin** *Microsphere-assisted microscopy for biological imaging*
12h30-14h	*Repas*

Résumés des présentations

Mardi 6 Novembre

9h – 9h50

Steerable detectors for bioimage analysis

Michael Unser, Virginie Ulhmann, Julien Fageot, Zsuzsanna Püspöki , Daniel Sage and Adrien Depeursinge

Ecole Polytechnique Fédérale de Lausanne

Steerable filters are a powerful framework for detecting patterns that can appear at arbitrary orientations in images. The underlying idea, which goes back to Freeman and Adelson in 1991, is the ability to express any rotated version of a given template as a suitable linear combination of a fixed number of basis filters (or filterbank). One of the better known incarnation of the concept is the steerable pyramid, which provides a reversible decomposition of images in terms of steerable wavelets. We shall recall how the latter can be used effectively for image denoising, feature extraction, and morphological component analysis. We then introduce a mathematical framework for the optimal design of steerable detectors that explicitly takes into account the spectral properties of the underlying background signal. We also provide estimates on the angular accuracy of such detectors. We then describe a dedicated ImageJ plugin that facilitates the design and deployment of application-specific steerable detectors. We illustrate the usefulness of this tool for bio-image analysis; specific examples include the detection of DNA filaments in cryoelectron micrographs, the angular localization of double-helix PSF in super-resolution microscopy, and the detection/localization of bacteria in high-throughput microscopy.

***

9h50 – 10h10

Setting up an open, web-based platform for reproducible image processing

Jérôme Mutterer[1], Erwan Grandgirard[2]

[1]*Institut de biologie moléculaire des plantes, Strasbourg*
[1] *Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch 67404, France.*

In this presentation, we'll introduce a web tool that allows setting up and running remote image processing workflows. The solution was developed using Galaxy project, "an open, web-based platform for accessible, reproducible, and transparent computational biomedical research". In short, we developed a Galaxy tool that invokes ImageJ remotely. Both Galaxy and ImageJ are stable and standard tools readily used in the biomedical community, which allows to build on existing expertise for the generation of potential new tools or workflows. From a user point of view, applying Galaxy tools that can wrap complex base operations is time saving, whilst the easy graphical workflow creation in Galaxy enables creating original programs. We'll present the general concepts and implementation details based on a simple example, and the suggested approach for developing similar tools.

***

10h10 – 10h30

A Monte Carlo Framework for Denoising and Missing Wedge Reconstruction in Cryo-Electron Tomography

Charles Kervrann

Inria Rennes-Bretagne Atlantique

We propose a statistical method to address an important issue in cryo electron tomography image analysis: reduction of a high amount of noise and artifacts due to the presence of a missing wedge (MW) in the spectral domain. The method takes as an input a 3D tomogram derived from limited-angle tomography, and gives as an output a 3D denoised and artifact compensated tomogram. The artifact compensation is achieved by filling up the MW with meaningful information obtained with Monte-Carlo samples generated by a dedicated Metroplolis-Hastings algorithm. The method can be used to enhance visualization or as a pre-processing step for image analysis, including segmentation and classification. Results are presented for both synthetic and experimental data in 3D cryo-tomography.

***

11h – 11h50

Computer vision methods for multidimensional correlative microscopies

Perrine Paul-Gilloteaux

SFR santé CNRS INSERM Univ. Nantes UMS3556, Nantes
Inbs France Bioimaging

Correlative microscopy allows combining different scales of observations and different contents, functional and morphological, based on the large panel of microscopy technologies available for life or material sciences. Data fusion methods have to be considered when developing an imaging workflow, from the sample preparation to imaging acquisition. The purpose is to accurately follow a region of interest across modalities and scales of imaging, for guiding and targeting the specimen preparation procedure needed for another modality, to guide the acquisition at the second microscope and to adjust the field of view of the microscope to this feature. In a last, and most accurate registration step, the acquired data from both modalities are mapped.

The challenges in registration for correlative microscopies are due to:

a) The variations of densities in the points extracted, due partly to the difference in resolution: several elements of one high-resolution modality, would appear as a single element in a larger field of view modality due to the diffraction limit;

b) Missing data: some modalities are not specific and show the whole content of the area imaged, meaning that many structures will not appear in a functional imaging modality

c) The large variations in image scale, rotation, and translation, and deformation of the sample due to the sample preparation or to the sample itself;

d) The large diversity of imaging content and scale at the different steps of a correlative microscopy workflow;

e) The lack of methods for computing the accuracy of the registration.

In this talk, we will also present a point-based registration paradigm, which presents several advantages. The only step of our registration workflow which differs between workflows, whatever the dimensionality of data, is the feature extraction step, which can be learned on uncorrelated images. It includes a method for the selection model of non-rigid transformation, for which the transformation basis is selected according to its physical significance. We also present a statistical framework of error estimation on the registration error allowing to answer the question of the confidence of the matching of structures.

***

11h50 – 12h10

Census Signature based 3D Optical flow for 3D Light Microscopy Image Sequences: Application to Cell Migration

Sandeep Manandhar[1], Patrick Bouthemy[1], Philippe Roudot[2], Erik Welf[2], Charles Kervrann[1]

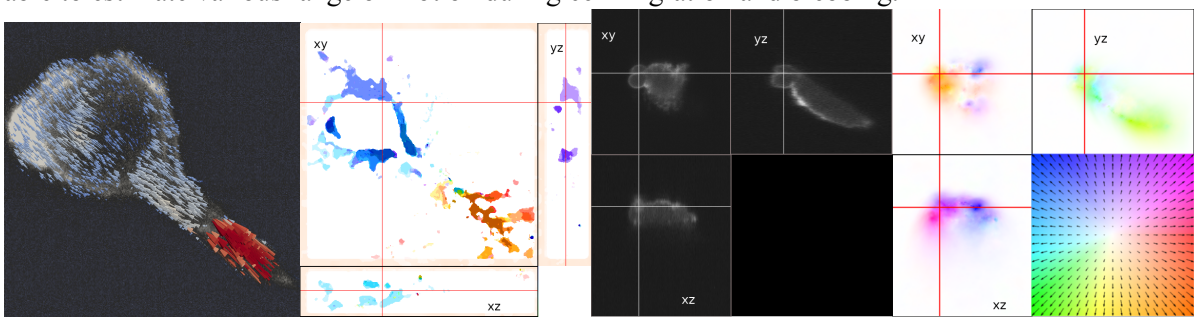
[1]*Inria, Centre de Rennes – Bretagne Atlantique, France*

[2]*Department of Cell Biology, University of Texas Southwestern Medical Center, Dallas, USA*

Keywords: Optical flow, Census Transform, Patch Match, Fluorescence Microscopy, Cell Migration

Three dimensional (3D) motion estimation for light-sheet microscopy is challenged by the heterogeneous scales and nature of intracellular dynamics [2, 3, 4]. As typical examples in cell imaging, blebbing of a cell has smaller motion magnitude while cell migration may show large displacement between frames. To tackle this problem, we present a two-stage 3D optical flow method. The first stage involves an extension of two-dimensional PatchMatch [1, 2] paradigm to 3D data that operates in a coarse-to-fine manner. We exploit multiple spatial scales to explore the possible range of intracellular motions. Our findings show that the metric based on Census transform [6] is more robust to noise present in the images and to intensity variation between time steps. Only discrete displacements are estimated in this stage. We then devise a 3D variational method in next stage to obtain a sub-voxelic flow map. The variational approach still involves a data fidelity term based on the Census transform. The combination of the PatchMatch and the variational stage is able to capture both large and small displacement.

We assessed the performance of our method on data acquired with two different light sheet microscopes [7, 8] and compared it with a couple of other methods. The dataset depicts blebbing and migration of MV3 melanoma cells, and collagen network displacement induced by cell motility. As seen in Fig. 1, our method is able to estimate various range of motion during cell migration and blebbing.



a.1.1 a.1.2 b.1.1 b.1.2

Figure 1: (a) Migration of MV3 melanoma cell in collagen; (a.1.1) Depicting 3D flow field in a slice of cell data with glyphs. Larger motion magnitude is coded in warm colors and smaller motion in cold colors. (a.1.2) Depicting motion map of collagen channel in 3 orthogonal planes (see b.1.2 for color code). (b) Blebbing of MV3 cell in a cover-slip; (b.1.1) Depicting 3 orthogonal planes of cell data. (b.1.2) Depicting motion map of the cell data in 3 orthogonal planes. The motion map is color coded as shown in lower-right corner of b.1.2.

References :

[1] C. Barnes,E. Shechtman, A. Finkelstein, and D.B. Goldman, SIGGRAPH, 2009.
[2] S. Manandhar, P. Bouthemy, E. Welf, P. Roudot, and C. Kervrann, ISBI, 2018.
[3] F. Amat , E.W. Myers and P.J. Keller, Bioinformatics, vol. 3, pp 373-380, 2013.
[4] A.B. Pujadas, M. Manich, N. Guillén, J.-C. Olivo-Marín, and A. C. Dufour, Scientific Reports, 2017.
[5] D. Fortun, P. Bouthemy, P. Paul-Gilloteaux, and C. Kervrann, ISBI, 2013.
[6] D. Hafner, O. Demetz, and J. Weickert, SSVM, 2013.
[7] K.M. Dean, P. Roudot, E. Welf, G. Danuser, and R. Fiolka, Biophysical Journal, vol.108, 2807-2815, 2015.
[8] K.M. Dean, P. Roudot, C. Reis, E. Welf, M. Mettlen, and R. Fiolka, Biophysical Journal, vol. 110, pp 1456-1465, 2016.

***

12h10 – 12h30

Stochastic modeling of nuclear diffusion and transcription dynamics using live imaging data

Nacho Molina

[1] *Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch 67404, France.*

In my team we develop stochastic and biophysical models as well as deep learning methods to characterize the process of gene regulation. Our work lays at the interface between computational biology and theoretical biophysics combining tools and methods from both fields to investigate gene regulation processes based on both large-scale genome-wide data and single-cell imaging data. In this talk I will briefly present three projects where we study the nuclear diffusion dynamics of transcription factors, the movement of chromatin fibers and the transcription kinetics in mammalian single cells by analyzing live-cell imaging data.

***

14h – 14h50

Cardiac Microscopy: Expanding Imaging Dimensionality without Compromising Temporal Resolution

Michael Liebling

Idiap Research Institute
University of California Santa Barbara

Imaging is an essential tool for characterizing the mechanisms underlying many dynamic biological systems. Despite tremendous advances in microscopy hardware, many live samples remain difficult to observe with off-the-shelf instruments. The developing heart, which beats already before it is fully formed, is particularly challenging to image as the signal is often dim or because the cells move rapidly. Even when direct optical access to the heart is possible, imaging multiple channels or multiple samples, imaging from multiple view-points or in multiple colors—without giving up high frame rate—is difficult to achieve. Computational imaging methods in microscopy, which capitalize on the joint interplay between custom acquisition hardware or protocols and digital image processing, have proven to be highly promising to break physical limitations imposed by the sample or the instrument.

In this talk, I will present different computational imaging strategies that we developed to build multi-dimensional volumes, acquire time-lapses, achieve temporal super-resolution or multi-spectral images of the heart, all without compromising the frame-rate. These techniques gather images over multiple cardiac cycles, requiring no additional gating signals than the images themselves. We found that fairly weak assumptions on the cardiac dynamics (such as motion repeatability and asymmetry) are sufficient to guarantee unique dynamic reconstructions. In particular, we devised imaging arrangements and reconstruction methods that self-validate the reconstructions to rule out implausible reconstructions. This allows building multi-dimensional images of the beating heart with temporal resolution equal to or exceeding that of the hardware. I will also describe our ongoing effort to set up an imaging platform to collect dynamic microscopy images that allow characterizing our methods within a dynamic (but controlled) environment.

***

14h50 – 15h10

High-throughput tracking of cell cycle progression in single dividing cells

Gilles Charvin

Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch 67404, France.

Live imaging of dividing cells can yield a large amount of information, yet it is difficult to precisely determine the timings of cell cycle phases with single cell resolution. We have developed an image analysis pipeline that allows us to accurately track the progression through the cell cycle in single cells growing in a microfluidic device. This methodology, has allowed to collect more than ~10000 cell cycles timings in more than 20 yeast mutant backgrounds and has revealed new insights regarding the mechanisms that coordinate growth and division during cell cycle progression.

***

15h10 – 15h30

Image processing for detection and characterization of label free biotic and non-biotic nanoparticles with a nondestructive, full field, single-arm interferometric technique

Quentin Garçon[1], Matthieu Greffet[1,*], and Yasmina Fedala[2]

[1]*Myriade, 68 boulevard de Port-Royal, 75005, Paris*
[2]*Institut Langevin, ESPCI Paris, PSL Research University, CNRS UMR 7587, 1 rue Jussieu, 75005, Paris*
[*]*matthieu.greffet@myriadelab.com*

Background: Enumerating and distinguishing nanoparticles such as viruses, extracellular vesicles (ECV) or phages are major importance for many applications such as bioproduction of viral vectors for cell therapy, phages batches manufacturing or ECV for drug delivery. Detection methods such as PCR or epifuorescence microscopy for these applications are quite time-consuming and not easy to implement without labelling or damaging the sample. Moreover, there are currently no existing optical methods which are able to discriminate vesicles in real time. Thus, we develop a new technique based on full-field interferometry which makes possible the counting and characterization of nanoparticles in the range 30 - 200nm within seconds and with a 5µL droplet (Boccara et al, Biomed. Opt. Exp. 2016).

Image Processing: The raw interferometry signal is recorded and has to be processed. After enhancing the image, we convolve it with a Bessel function. The spots are detected by thresholding and then the coordinates can be used to track particles with a Nearest Neighbour algorithm. Thanks to the Mean Square Displacement method and the Einstein formula, we finally have access to the diffusion coefficient and so the particles diameter.

Perspectives: To get more accurate results, more particles per image are required. However, the tracking algorithm is not suitable for a high density of spots. To cope with this tracking issue and the specificities of our patented technique, we will create a global approach based on minimizing a cost function. Furthermore, a machine learning-based method could be an effective alternative to detect and segment the particles at the same time.

Keywords: Viruses Detection, One-arm Interferometry, Single Particle Tracking, Brownian Motion, Nanoparticles Segmentation, Vesicles Characterization, Machine Learning detection, Viral Bioproduction

***

16h – 16h50

Monitoring protein organization, dynamics and interactions at high spatial and temporal resolution

Rémi Galland[1], Anand P. Singh[2], Corey Butler[1], Virgile Viasnoff[3], Timothy E. Saunders[2], Vincent. Studer[1], Jean-Baptiste Sibarita[1]

[1]*Interdisciplinary Institut for Neurosciences, University of Bordeaux, CNRS UMR5297*
[2]*Mechanobiology Institute, NUS, Singapore*
[3]*Biomechanics of Cell-Cell Contact, NUS/CNRS, UMI3639, Singapore*

Protein organization, dynamics and interactions play a crucial role in all living systems. However, probing such spatio-temporal organization in intact systems with sufficient spatial and temporal resolution is challenging. Fluorescence-based microscopy provides several tools to reach this goal. For example, Fluorescence Correlation Spectroscopy (FCS) enables to quantify fast proteins dynamics and interactions in different environment. However, FCS measurements are usually restricted in their throughput and/or penetration depth. On another side, Single Molecule Localization Microscopy (SMLM) techniques have proven to be essential to quantitatively monitoring protein organization and dynamics with high spatial and temporal resolutions. However, they also face important limitations with respect to their penetration depth capabilities and in their ability to measure protein interactions. To overcome some of these limitations, we have developed two complementary approaches in order i) to improve the penetrations depth of FCS and SMLM techniques and ii) to increase the spectral content of SMLM:

- A new light-sheet microscope architecture, called soSPIM, which relies on the use of a single high NA objective in combination with micro-fabricated chips featuring 45° mirror alongside micro-wells [1]. This architecture provides both good optical sectioning and high sensitivity in depth, which we demonstrated to allow image-FCS [2] and multicolour SMLM at up to a few tens of microns above the coverslip.
- A SMLM with 4π geometry which combines two microscope objectives in order to increase the spectral content of SMLM without compromising neither the spatial nor the temporal resolution. It allows monitoring simultaneously the dynamics of several protein's population, providing insights in their respective interactions.

References

[1] Galland, R. R. et al. 3D high- and super-resolution imaging using single-objective SPIM. Nat. Methods 12, 641–644 (2015).
[2] Singh, A. P. et al. 3D protein dynamics in the cell nucleus. Biophys. J. 112, 133–142 (2017).

***

16h50 – 17h10

Complex diffusion analysis challenge

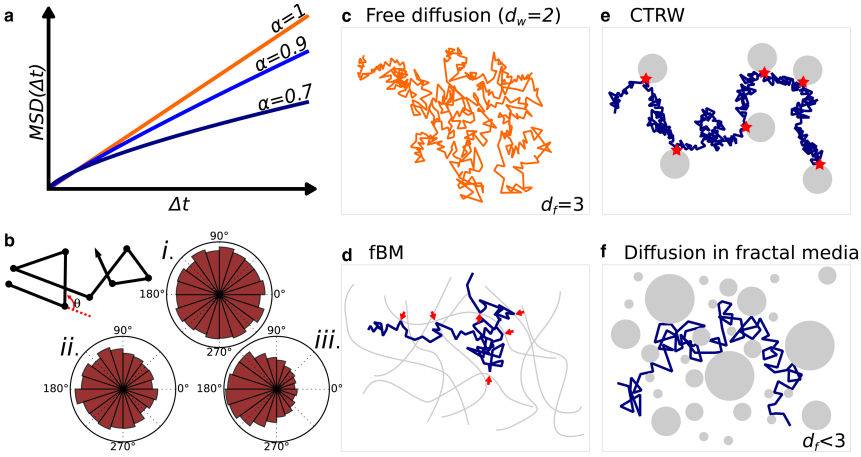
Maxime Woringer[1,2,&], Hugues Berry[3,4,&], Dominique Bourgeois[5,&], Cyril Favard[6,&] and Ignacio Izeddin[7,&]

[1] *Unité Imagerie et Modélisation, Institut Pasteur, 25 rue du Docteur Roux, 75015 Paris, France;*
[2] *Department of Molecular and Cell Biology, Li Ka Shing Center for Biomedical and Health Sciences, CIRM Center of Excellence, University of California, Berkeley, CA 94720, U.S.A.;*
[3] *INRIA, Villeurbanne 69603, France;*
[4] *Université de Lyon, LIRIS UMR 5205, Villeurbanne 69621, France;*
[5] *Institut de Biologie Structurale, Université Grenoble Alpes, CEA, CNRS, F-38044 Grenoble, France;*
[6] *Membrane Domains and Viral Assembly, IRIM, UMR9004 CNRS / Université de Montpellier, 1919, route de Mende, 34293 Montpellier Cedex;*
[7] *Institut Langevin, ESPCI Paris, CNRS, PSL Research University;*
[&] *CNRS GdR ImaBio*

The life of a cell is governed by highly dynamical nanoscopic processes. Two notable examples are the diffusion of membrane receptors and the kinetics of transcription factors governing the rates of gene expression. Different fluorescence imaging techniques have emerged to study (macro)molecular dynamics in

the living cell. Among them, fluorescence correlation spectroscopy (FCS) and single-particle tracking (SPT) have proven to be instrumental to our understanding of cell dynamics and function down to the molecular level.

Beyond insights on the biochemistry of the cell, these techniques have also unraveled an unforeseen complexity and diversity of mechanisms of protein diffusion. Many efforts have been devoted to analyze datasets generated by FCS or SPT, ranging from diffusion coefficient estimations to inference approaches. Among this array of algorithms, choosing the appropriate software for a given biological question can be challenging.



Models of anomalous diffusion and plausible underlying physical structures. From Wroinger and Darzacq, Biochemical Society Transactions 46 (2018)

Indeed, the richness of experimental data often makes it difficult to determine which are the models to be considered and the relevant biophysical parameters to be estimated.

Within this context, the CNRS GdR ImaBio proposes the **Complex diffusion analysis challenge 2019**. Our goal is to foster the development of new state-of-the-art analysis algorithms. We aim at providing a unified data benchmark based on a set of biologically and physically relevant metrics in order to compare the diffusion analysis software available for the community.

Following an international call, we will provide researchers from the fields of image analysis, statistics and machine learning with a series of computer-generated datasets emulating realistic acquisitions produced by the single-molecule imaging and correlation spectroscopy communities. Part of this reference dataset will disclose the generating model and parameters used to generate the trajectory, to be used as training set. The performance of trajectory analysis algorithms will be quantified based on their ability to correctly infer the model used to generate a set of unlabeled trajectories (test set).

With this challenge, we hope to provide the molecular imaging community with a comprehensive set of data and metrics allowing to objectively evaluate existing and new analysis tools, as well as instigating an open discussion about the limitations and challenges of analyzing and modeling diffusion of molecules in the complex environment of the cell.

Mercredi 7 Novembre

9h – 9h50

Quantitative Phase Imaging, from white light imaging to fluorescence super-resolution

Pierre Bon

CNRS (LP2N), Université de Bordeaux & Institut d'Optique, Talence France

Email: pierre.bon@institutoptique.fr

Quantitative Phase Imaging has been developed to harvest information from biological samples. We have developed an approach based on generating self-interferences from the light emerging from an optical microscope[1]. More than a simple contrast enhancement technique, the quantitative information provided allows many possible applications, both in the coherent (transmission white-light) and the incoherent (fluorescence) world.

Retrieving and deciphering the biophysical information from a raw acquisition requires algorithms that are specific for each imaging modality and application. We will see that in transmission white-light imaging makes the approach applicable to multiple investigations such as label-free cytoskeleton imaging[2], identification of sub-cellular compounds[3] or dry mass measurement[4]. In the fluorescence world it can lead to 3D super-resolution imaging even deep inside biological samples[5].

References

1. Bon, P., Maucort, G., Wattellier, B. & Monneret, S. Quadriwave lateral shearing interferometry for quantitative phase microscopy of living cells. *Opt. Express* **17,** 13080–13094 (2009).
2. Bon, P., Lécart, S., Fort, E. & Lévêque-Fort, S. Fast Label-Free Cytoskeletal Network Imaging in Living Mammalian Cells. *Biophys. J.* **106,** 1588–1595 (2014).
3. Bon, P., Savatier, J., Merlin, M., Wattellier, B. & Monneret, S. Optical detection and measurement of living cell morphometric features with single-shot quantitative phase microscopy. *J. Biomed. Opt.* **17,** 76004 (2012).
4. Aknoun, S. *et al.* Living cell dry mass measurement using quantitative phase imaging with quadriwave lateral shearing interferometry: an accuracy and sensitivity discussion. *J. Biomed. Opt.* **20,** 126009 (2015).
5. Bon, P. *et al.* Self-interference 3D super-resolution microscopy for deep tissue investigations. *Nat. Methods* **15,** 449–454 (2018).

***

9h50 – 10h10

Mapping and quantification of nuclear positions in tissues with approximate cylindrical geometry to study vascular morphogenesis

P. Campinho[1,2,3,4], P. Lamperti[1,2,3,4], F. Boselli[1,2,3,4], J. Vermot[1,2,3,4]

[1] *Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch 67404, France.*
[2] *Centre National de la Recherche Scientifique, UMR7104, Illkirch 67404, France.*
[3] *Institut National de la Santé et de la Recherche Médicale, U964, Illkirch 67404, France.*
[4] *Université de Strasbourg, Illkirch 67404, France.*

Organogenesis is associated with multiple morphogenetic events that require tissue plasticity and creat a large variety of transient three-dimensional geometries. These global responses are driven by tight spatio-temporal regulation of the behaviours of individual cells composing tissues. Developing image analysis tools that allow for extraction of quantitative data focusing on individual cell behaviours is essential to understand tissue morphogenesis. There are many image analysis tools available that permit extraction of cell parameters. Often,

they are optimized for tissues with relatively simple geometries such as flat epithelia. Things can become difficult when the tissue of interest follows more complex three-dimensional geometries. Here, we present an approach focusing on tissue with cylindrical geometry and describe the image analysis routines developed to extract quantitative data on individual cells in such tissues, as well as the image acquisition and sample preparation methodology.

***

10h10 – 10h30

Traction Force Microscopy: Data acquisition and processing

Philippe Carl

Laboratoire de Bioimagerie et Pathologies, UMR CNRS 7213, Illkirch

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. Thus, characterization of cellular forces is important for a greater understanding of the cellular mechanosensing process. 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.

Description: The TFM (Traction Force Microscopy) technique is used to extract the adhesion forces that cells exert on a substrate. For this purpose, cells are plated on polyacrylamide (PA) gels containing fluorescent microbeads. The chemical and mechanical properties of PA gels are ideal for the study of traction forces. Indeed, PA gels are optically transparent, allowing cells to be easily imaged. Furthermore, the use of fluorescent markers embedded into the gel allows the user to measure deformations caused by cell adhesion/migration processes using standard fluorescent microscopy. More importantly, PA gels are elastic and thus will be deformed in proportion to a broad range of applied forces. Once these forces are removed, PA gels reproducibly recover the initial unstressed conformation. The mechanical properties of the PA gels expressed as the Young's modulus is also easily tunable to suit the in-vivo conditions simply by altering the ratio of acrylamide to bis-acrylamide. Thus a typical TFM experiment include (1) recording the position of the beads by acquiring images of the gel with a classical EPI-fluorescence microscope in stressed and unstressed conditions and (2) simultaneously acquiring phase-contrast or epifluorescence images of the cells. For the unstressed condition we use trypsin to wash out the cells from the PA gel and thus relax the gel back to its initial position. Then, from the beads displacement between the support at rest and with the presence of cells, are calculated the adhesion forces of these cells at a given time. Moreover, particularly in the vicinity of focal adhesions, the need for a finer evaluation of the force distribution involves the use of high resolution tensile force microscopy. This can be done by using a mixture of smaller beads and emitting at different wavelengths so that we can combine their displacements. The analysis of the acquired pictures is performed automatically using a graphical user interface ImageJ plugin which with will be launching a mix of plugins available online and / or developed in the laboratory. First the algorithm will extract the best focused slice over all the marked positions and times as well as for the reference pictures obtained after cells detachment. Then, taking the focused beads pictures after cells detachment for all the marked positions as reference, the x-y drift error is corrected using a template matching algorithm from the focused beads pictures acquired over all the marked positions and times. The same correction is then applied to the bright field pictures. Once the drifting has been corrected, the beads displacements induced by the cells over all the marked positions and times are calculated using a PIV (Particle Image Velocimetry) algorithm. Finally from the last result, the forces applied by the cells

on the substrate are finally deduced using a FTTC (Fourier Transform Traction Cytometry) method. Once the forces calculated and in order to compare different cells conditions or environments, different values or parameters can be measured or calculated like the total force over a cell, its resulting force vector (length, orientation, direction) its zero force point,…, and this combined with geometrical (area, perimeter, aspect ratio, circularity,…) or kinetic (displacement vector, speed,…) parameters.

Keywords: Mecanobiology, Traction Force, Adhesion, Migration, ImageJ, Image processing

***

11h – 11h50

Super-Resolution Microscopy Using the BLASSO

Quentin Denoyelle[1], Vincent Duval[2], Gabriel Peyré[3], Emmanuel Soubiès[1]

[1] Biomedical Imaging Group, EPFL
[2] INRIA Paris, MOKAPLAN
[3] CNRS & ENS

We propose to review the BLASSO, an off-the-grid or grid-less counterpart to the well-known LASSO, for the deconvolution of measures composed of Dirac masses and present theoretical guarantees for the super-resolution problem i.e. the recovery of possibly infinitely close Dirac masses with noise contaminating the data. Then we present a new solver for the BLASSO, called the Sliding Frank-Wolfe (SFW), which is an adaptation of the Frank-Wolfe (or conditional gradient) algorithm. It follows a recent trend of interleaving convex optimization updates (corresponding to adding new spikes) with non-convex optimization steps (corresponding to moving the positions of the spikes). Our main theoretical finding for this algorithm is that it terminates in a finite number of steps under a mild non-degeneracy hypothesis. Finally, we use the SFW algorithm for the comparison of three PSF models for Single-Molecule Localization Microscopy (SMLM) in 3D. These modalities based on photoactivated localization microscopy (PALM) or stochastic reconstruction microscopy (STORM) have PSFs that vary in the axial direction. The multi-focal astigmatism and double-helix models are considered but also Multi-Angle Total Internal Reflection Fluorescence (MA-TIRF) acquisitions. The goal is to show the practical performance of the SFW, but also to study the potential of each method of acquisition in an ideal case and serve as a proof of concept for further developments.

***

11h50 – 12h10

Analysis and 3D visualization of protein complexes by Voronoi tessellation in super-resolution microscopy

Leonid Andronov, Jonathan Michalon, Khalid Ouararhni, Yves Lutz, Igor Orlov, Ali Hamiche, Jean-Luc Vonesch, Bruno Klaholz

Centre for Integrative Biology (CBI)
Institute of Genetics, Molecular and Cellular Biology (IGBMC)
Centre National de la Recherche Scientifique (CNRS)
University of Strasbourg
1 rue Laurent Fries, BP 10142, 67404 Illkirch CEDEX, France
andronov@igbmc.fr

While many tools exist for the 3D analysis and visualization of crystal or cryo-EM structures little exists for 3D single-molecule localization microscopy (SMLM) data. SMLM can play an important role in integrated structural biology approaches to identify, localize and determine the 3D structure of cellular structures [1]. These can provide unique insights but are particularly challenging to analyze in three dimensions especially in a dense cellular context.

We have developed visualization software and a segmentation method that will facilitate cellular structural biology in the future.

We first developed ClusterViSu, a robust pipeline for processing of 2D localization data based on Voronoi tessellations [2, 3]. ClusterViSu is suitable for the convenient visualization and quantification of the localization and distribution of fluorescently labeled complexes, which allows segmentation, cluster analysis and estimation of the amount of co-localization. Voronoi diagrams allow performing a statistical analysis of the clusters, their occurrence and inter-cluster distance distribution, and work well including for the analysis of weak signals. ClusterViSu is available under https://github.com/andronovl/SharpViSu

We now demonstrate an extension of this method to address 3D single-molecule localization microscopy data [4]. We show that 3D Voronoi diagrams can be useful for precise local density estimation, noise reduction, rendering and cluster analysis of 3D data. One of the advantages of the Voronoi tessellation method is that it does not require any a priori knowledge for the clustering. Because the clustering uses an internal reference generated with Monte-Carlo simulations of randomized data to automatically determine the threshold value for forming clusters between neighboring Voronoi zones, it is fully automated for a given region of interest.

The general applicability of the method is illustrated on microtubules, histones H2B and CENP-A chromatin data. 3DClusterViSu, a standalone software for data processing and 3D visualization is available under http://cbi-dev.igbmc.fr/cbi/voronoi3D

References:

1. I. Orlov, A.G. Myasnikov, L. Andronov, S.K. Natchiar, H. Khatter, B. Beinsteiner, J.-F. Ménétret, et al. Biol Cell. 2017, 109, 81-93.
2. L. Andronov, Y. Lutz, J.-L. Vonesch, B.P. Klaholz. Bioinformatics, 2016, 32, 2239-2241.
3. L. Andronov, I. Orlov, Y. Lutz, J.-L. Vonesch, B.P. Klaholz, Sci. Rep. 2016, 6, 24084.
4. L. Andronov, J. Michalon, K. Ouararhni, I. Orlov, A. Hamiche, J.-L. Vonesch, B.P. Klaholz, Bioinformatics, 2018, bty200.

***

12h10 – 12h30

Quantification of Patchiness in the fly eye

Joyce Kao[1], Bertrand Cinquin[2]

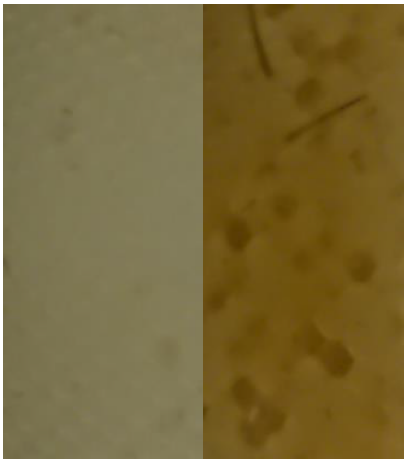
[1] *Center for Genomics and Systems Biology, New York University, New York, NY, USA*
[2] *LBPA, ENS Saclay, Cachan France.*

Color is a phenotype that has been used throughout the history of genetics research. Almost every organism ever formally studied has a color-based phenotype including the fruit fly, *Drosophila melanogaster*. And when the samples had no particular color, they were painted/stained/constrasted. One of the first phenotypes described in *D. melanogaster* was an eye color mutant of the sex-linked gene, *white*, where mutant flies had white eyes as opposed to the wild-type red (Morgan, 1910). While these mutants are usually recognized without problems by experienced (and non-colorblind) Drosophilists, more complicated eye pigmentation mutants arose as more and more genetic tools were being developed in the fruit fly model. One mutant of note is the *wm4* mutant (Muller, 1930). The *wm4* mutant is a classical example of position effect variegation (PEV). An inversion on the X-chromosome relocates the *white* gene next to pericentric heterochromatin so that the neighboring chromatin state determines whether or not the *white* gene is expressed. When the neighboring chromatin is in the euchromatic state, *white* is expressed whereas in the heterochromatic state, *white* is silenced. In the age of digital images, we can bypass the biases produced by the subjectivity and variability of human perceptions and obtain objective and consistent measurements. We therefore describe an automated method which can quantify eye color from images of fly heads using a commercially available imaging setup and

open-source analysis software. In addition, we extend this method to quantify position effect variegation with an additional metric to assess the "patchiness" of variegation.

The "patchiness" of the fly eye can easily estimated using a Likert scale but it will fail to render the more complex reality of the different observed phenotypes. We realize that if such a criteria has a practical definition for the human eye, it is not for our standard analytic tool. Patchiness is more a convolution of different quantitative factors such as : the number of patches, their size, their distance relative to each other (cluster). Observing a defined area means that for a certain size and a certain number, the repartition of the patches has a limit that can be computed and a measure of level of organization can be reached.

We create a workflow that tends to do all that with additional cleaning and sorting steps. We will present our successes and remaining challenges to escape the practical but unprecise Likert scale.



Example of two fly eyes (not patchy and very patchy)

***

14h – 14h50

Focused ion beam scanning electron microscopy to explore brain connections

Graham Knott

Ecole Polytechnique Fédérale de Lausanne

The fine structure and connectivity of the nervous system can only be seen with electron microscopy. The focused ion beam scanning electron microscope has the capability of imaging significant volumes of brain tissue with isotropic voxels that enable detailed morphological analysis of its neurons and their synapses. This presentation will show how combining light microscopy imaging with FIBSEM is able to provide answer important questions about the brain's structure; from the plasticity of its connections to the structural changes that occur during aging. I will also show recent work with a correlative light and electron microscopy method that characterizes the morphology of the neuromodulatory axons in the adult mammalian brain.

***

14h50 – 15h10

Automatic regions of interest detection for smart microscopy applications in whole organism screening

L. Thomas[1,2], J. Gehrig[1]

[1]*ACQUIFER is a division of DITABIS, Digital Biomedical Imaging Systems AG, Pforzheim, Germany*
[2]*Centre of Pediatrics and Adolescent Medicine, University Hospital Heidelberg, Germany*
Email : l.thomas@acquifer.de, j.gehrig@acquifer.de

In whole organism screening scenarios, the targeted higher resolution acquisition of regions of interest and automated analysis of complex multidimensional datasets remains a major challenge. While common image

processing techniques can be applied, their usage often results in highly application-specific solutions and algorithm developments that lack broad usability. Alternatively, object-detection and pattern-recognition algorithms can be applied to detect region of interest; however their usage is often limited to expert users due to lack of easy-to-use implementations in common bio-image analysis tools. To overcome this limitation, a toolbox for region of interest detection based on various pattern recognition algorithm from different open source repositories is being developed. Algorithms are benchmarked on zebrafish screening datasets and protocols for their usage are established. Moreover, they are combined with smart microscopy applications for automated imaging of regions of interest, rare event detection, zooming-in on structures or decision making based on presence of relevant features. The toolset will be implemented in Fiji-Plugins and KNIME workflows thus providing wider access including non-expert users. Additionally, a Fiji update site and GitHub repository for distribution of updates and further developments of the pattern recognition toolbox will be set up. The Fiji toolbox developed in python/jython will be macro-recordable to ensure that it can be readily re-used to write custom macro.

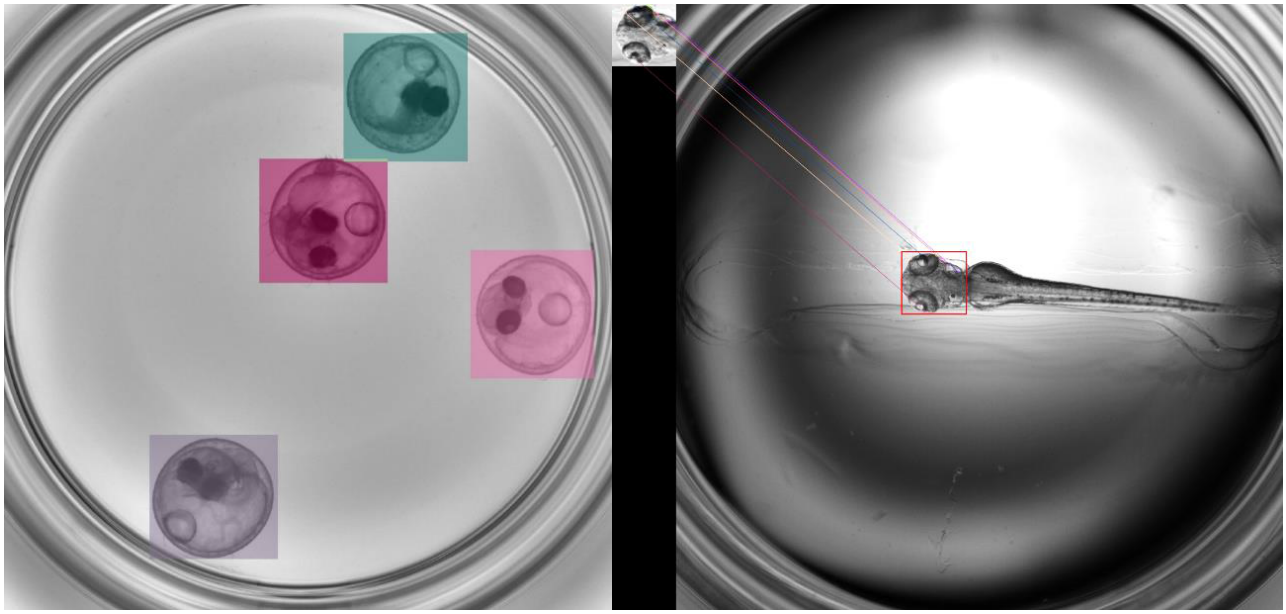


Fig. 1: Examples of automatic regions of interest detection.
Left: Template matching, Right: Keypoints matching

***

15h10 – 15h30

Characterization of the interaction of coreceptor CD44v6 and MET after ligand-induced activation

Ryshtee Mary Tannoo[1,2], Ludovic Richert[1], David Koschut[2], Véronique Orian-Rousseau[2] and Yves Mely[1]

[1] *Laboratoire de Bioimagerie et Pathologies, UMR 7021 CNRS, Faculté de Pharmacie, Université de Strasbourg, Illkirch Cedex, France,*

[2] *Institute of Toxicology and Genetics, Karlsruhe Institute of Technology, 76131 Karlsruhe, Germany*

The receptor tyrosine kinase (RTK) MET and its ligand Hepatocyte Growth Factor (HGF) are key regulators of cell signaling (Fig. 1). They control cell survival, proliferation, differentiation and mobility [1], [2]. An uncontrolled upregulation of MET/HGF pathway may promote tumoral progression and metastasis [3], [4]. We have shown that HGF binding to MET causes its dimerisation by molecular biology and quantitative microscopy (FRET-FLIM and FCS) [5].

For MET/HGF pathway, the team of Prof Orian-Rousseau (KIT, Karlsruhe) has shown that the transmembrane glycoprotein CD44v6, isoform of CD44 family, acts as a coreceptor for signaling. It also promotes the formation of a ternary complex with MET and HGF (Fig. 1) [6]. The coreceptor functions are two-fold : the extracellular part of CD44v6 is involved in MET phosphorylation, while the cytoplasmic tail of CD44v6 connects to the cytoskeleton through ERM proteins (ezrin, radixin, moesin), thereby inducing MET-dependent signaling [7].

To characterize the mechanisms of interaction of CD44v6 with MET promoted by HGF, quantitative microscopy techniques are carried out in live HEK-293T cells. With FRET followed by FLIM, we showed how HGF can modify the oligomerization of MET (Fig. 2) and the formation of CD44v6/MET complexes. In parallel, analysis by different approaches of Fluorescence Fluctuation Spectroscopy (FFS) are used to determine the behavior of CD44v6 and MET in non-induced cells and in the presence of HGF. These results allow us to identify the diffusion range of MET and its coreceptor CD44v6 in the plasma membrane before and after binding with HGF. Hence, we can track a possible relocalisation of activated proteins in the membrane, allowing a better understanding of MET pathways.

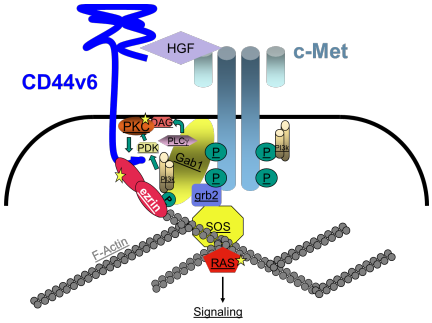


Fig. 1. Met signaling pathway

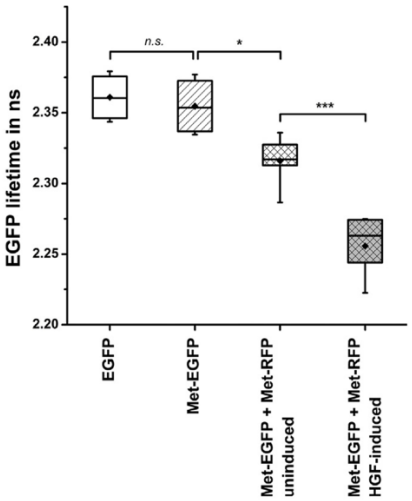


Fig. 2. FRET-FLIM shows HGF-induced Met dimerization in living cells. HEK-293T cells were transfected with the coumermycin A1-regulated expression vectors Met–EGFP, Met–RFP and pReg (1:2:1 ratio), or Met–EGFP and pReg (1:1 ratio), or EGFP [5]

.

References

1. Blume-Jensen, P., Hunter, T., 2001. Oncogenic kinase signalling. Nature 411, 355–65.
2. Schlessinger, J., 2000. Cell signaling by receptor tyrosine kinases. Cell 103, 211–25.
3. Gherardi, E., Birchmeier, W., Birchmeier, C., Woude, G.V., 2012. Targeting MET in cancer: rationale and progress. Nat. Rev. Cancer 12, 89–103.
4. Orian-Rousseau, V., 2010. CD44, a therapeutic target for metastising tumours. Eur J Cancer 46, 1271–7.
5. Koschut D., Richert L., Guiseppina P., Niemann H., Mély Y., Orian-rousseau V., 2016. Live cell imaging shows hepatocyte growth factor-induced Met dimerization. Biochimica et Biophysica Acta 1863,1552–1558.
6. Orian-Rousseau, V., Chen L., Sleeman, J., Herrlich, P., Ponta, H. CD44 is required for two consecutive steps in HGF/c-Met signaling. Genes & Development 2002, 16, 3074-3086.
7. Hasenauer, S., Malinger, D., Koschut, D., Pace, G., Matzke, A., von Au, A., Orian-Rousseau, V., 2013. Internalization of Met Requires the Co-Receptor CD44v6 and Its Link to ERM Proteins. PLoS ONE 8, e62357.

***

16h – 16h50

Common concepts in cryo-EM and super-resolution fluorescence microscopy - the way towards integrated structural biology

Bruno Klaholz

Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch 67404, France

The analysis of structure-function relationships in molecular and cellular biology is moving towards the integration across various scales and requires the combination of complementary approaches in imaging, structural biology and methods developments at the interfaces, including innovative tools for image processing and data analysis. This includes in particular the combination of X-ray crystallography, high-resolution cryo-EM, cryo electron tomography, FIB/SEM and single-molecule localization microscopy. Common concepts for image processing and examples of data integration on different biological topics will be given during the presentation.

References:

L. Andronov, J.-L. Vonesch & B. P. Klaholz. Practical aspects of super-resolution imaging and segmentation of macromolecular complexes by dSTORM. Invited Book chapter, submitted.

P. V. Afonine, B. P. Klaholz, N. W. Moriarty, B. K. Poon, O. V. Sobolev, T. C. Terwilliger, P. D. Adams & A. Urzhumtsev. New tools for the analysis and validation of cryo-EM maps and atomic models. *Acta Cryst.* **2018**, D74, 814-840.
https://doi.org/10.1107/S2059798318009324

L. Andronov, J. Michalon, K. Ouararhni, I. Orlov, A. Hamiche, J.-L. Vonesch & B. P. Klaholz. 3DClusterViSu: 3D clustering analysis of super-resolution microscopy data by 3D Voronoi tessellations. *Bioinformatics*, **2018**, 34, 3004–3012.
http://dx.doi.org/10.1093/bioinformatics/bty200 & https://www.biorxiv.org/content/early/2017/06/07/146456

O. von Loeffelholz, G. Papai, R. Danev, A. G. Myasnikov, S. K. Natchiar, I. Hazemann & J.-F. Ménétret, & B. P. Klaholz. Volta phase plate data collection facilitates image processing and cryo-EM structure determination. *J. Struct. Biol.*, **2018**, 202, 191-199.
http://dx.doi.org/10.1016/j.jsb.2018.01.003

I. Orlov, R. Drillien, D. Spehner, M. Bergoin, A. Abd-Alla, B. P. Klaholz. Structural features of the Salivary gland hypertrophy virus of the tsetse fly revealed by cryo electron microscopy and tomography. *Virology*, **2018**, 514, 165-169.
http://dx.doi.org/10.1016/j.virol.2017.11.016.

S. K. Natchiar, A. G. Myasnikov, H. Kratzat, I. Hazemann & B. P. Klaholz. Atomic model building and refinement into high-resolution cryo-EM maps. *Protocol Exchange*, **2017**
http://dx.doi.org/10.1038/protex.2017.122

S. K. Natchiar, A. G. Myasnikov, H. Kratzat, I. Hazemann & B. P. Klaholz. Visualization of chemical modifications in the human 80S ribosome structure. *Nature*, **2017**, 551, 472-477.
http://dx.doi.org/10.1038/nature24482

O. von Loeffelholz, S. K. Natchiar, N. Djabeur, A. G. Myasnikov, H. Kratzat, J.-F. Ménétret, I. Hazemann & B. P. Klaholz. Focused classification and refinement in high-resolution cryo-EM structural analysis of ribosome complexes. *Curr. Opin. Struct. Biol.*, **2017**, 46, 140-148.
http://dx.doi.org/10.1016/j.sbi.2017.07.007

I. Orlov, A. G. Myasnikov, L. Andronov, S. K. Natchiar, H. Khatter, B. Beinsteiner, J-F. Ménétret, I. Hazemann, K. Mohideen, K. Tazibt, R. Tabaroni, H. Kratzat, N. Djabeur, T. Bruxelles, F. Raivoniaina, L. di

Pompeo, M. Torchy, I. Billas, A. Urzhumtsev & B. P. Klaholz. The integrative role of cryo electron microscopy in molecular and cellular structural biology. *Biol Cell.,* **2017**, 109, 81-93.
http://dx.doi.org/10.1111/boc.201600042

L. Andronov, I. Orlov, Y. Lutz, J-L. Vonesch & B. P. Klaholz. ClusterViSu, a method for clustering of protein complexes by Voronoi tessellation in super-resolution microscopy. *Sci. Rep.* **2016**, 6, 24084.
http://dx.doi.org/10.1038/srep24084

L. Andronov, Y. Lutz, J-L. Vonesch & B. P. Klaholz. SharpViSu: integrated analysis and segmentation of super-resolution microscopy data. *Bioinformatics,* **2016**, 32, 2239-2241.
http://dx.doi.org/10.1093/bioinformatics/btw123

B. P. Klaholz. Structure sorting of multiple macromolecular states in heterogeneous cryo-EM samples by 3D multivariate statistical analysis. *Open Journal of Statistics*, Special Issue on Multivariate Statistical Analysis, **2015**, 5, 820-836.
http://dx.doi.org/10.4236/ojs.2015.57081

B. Beinsteiner, J. Michalon & B. P. Klaholz. IBiSS, a versatile and interactive tool for integrated sequence and 3D structure analysis of large macromolecular complexes. *Bioinformatics*, **2015**, 31, 3339-3344.
http://dx.doi.org/10.1093/bioinformatics/btv347

***

16h50 – 17h10

Single particle cryo electron microscopy of the promoter-bound transcription factor TFIID

Gabor Papai

Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch 67404, France

Transcription preinitiation complex assembly on the promoters of protein encoding genes is nucleated *in vivo* by TFIID composed of the TATA-box Binding Protein (TBP) and 13 TBP-associate factors (Tafs) providing regulatory and chromatin binding functions. Here we present the cryo-electron microscopy structure of promoter-bound yeast TFIID at a resolution better than 5 Å, except for a flexible domain. We position the crystal structures of several subunits and, in combination with cross-linking studies, describe the quaternary organization of TFIID. The compact tri lobed architecture is stabilized by a topologically closed Taf5-Taf6 tetramer. We confirm the unique subunit stoichiometry prevailing in TFIID and uncover a hexameric arrangement of Tafs containing a histone fold domain in the Twin lobe.

Jeudi 8 Novembre

9h – 9h50

On the super-resolution capacity of fluorescence microscopy using random speckle illuminations

Jérôme Idier

Laboratoire des Sciences du Numérique de Nantes (LS2N, CNRS UMR 6004), F-44321 Nantes

Since Heintzmann and Gustaffsson's pioneering works in the early 2000s, Structured Illumination microscopy (SIM) has emerged as a high speed, widefield super-resolution technique, with about two-fold spatial resolution enhancement in both lateral and axial directions. However, SIM requires the careful control of the excitation pattern, which represents a true limit in thick samples. More recently, speckle illumination microscopy has been proposed, with a view to reduce the experimental complexity. Controlled illuminations are replaced by unknown, fully developped speckle patterns. Several publications report promising empirical results in terms of super-resolution capacity (e.g., Mudry 2012, KIM 2015, Negash 2016). In a collaboration with Institut Fresnel (Marseille) and the Center for Integrative Biology (Toulouse), we are currently producing practical evidences that the low toxicity and high temporal resolution of speckle-SIM enables the imaging of critical biological functions at the intracellular scale.

However, the theoretical capacity of speckle-SIM is far more complex to understand than classical SIM. In epi-illumination mode, it also thorougly differs from SOFI (superresolution optical fluctuation imaging), both in terms of theoretical and practical possibilities.

This presentation will mainly focus on a better statistical understanding of the possibilities of speckle-SIM. Computational aspects will also be addressed, since speckle-SIM does not lend to simple reconstruction algorithms as classical SIM.

***

9h50 – 10h10

Défloutage aveugle et calibrage de microscope

Valentin Debarnot[1], Paul Escande[2], Thomas Mangeat[3], Pierre Weiss[4]

[1] *ITAV, CNRS, Université de Toulouse*
[2] *CNRS, Université Aix-Marseille*
[3] *CBI, CNRS, Université de Toulouse*

Dans cet exposé, nous nous intéresserons à la restauration d'images floues en microscopie optique.

Une procédure assez standarisée de restauration d'images consiste à imager une micro-bille fluorescente, ce qui permet de calibrer le microscope en mesurant sa réponse impulsionnelle (PSF). On peut ensuite utiliser cette information pour réaliser une déconvolution non aveugle.

Dans beaucoup de situations, ce procédé est malheureusement insuffisant : premièrement, il est fréquent que la PSF varie spatialement. Deuxièmement, les paramètres du microscope peuvent varier entre la procédure de calibrage et la prise de l'échantillon.

L'opérateur de dégration dépend cependant d'un petit nombre de paramètres physiques tels que la température de la salle, de quelques vis de focalisation, ou d'un défaut géométrique d'alignement d'une lame par rapport au plan focal.

Dans ce travail, nous présenterons un algorithme permettant d'apprendre un sous-espace linéaire d'opérateurs de petite dimension qui approche l'ensemble des opérateurs que peut générer le système optique. Le principe de notre approche s'éloigne donc nettement des procédures standards : on apprend tout un sous-espace plutôt qu'un opérateur unique. Les outils théoriques utilisés sont l'analyse harmonique appliquée (décompositions en convolution-produit) ainsi que la décomposition de tenseurs de type Tucker2.

On montrera ensuite comment exploiter cette information pour construire des algorithmes de défloutage aveugle, où l'on contraint l'opérateur de dégradation à vivre dans le sous-espace admissible. Celui-ci étant de petite dimension, l'identifiabilité du système est nettement améliorée par rapport à des approches utilisant moins de connaissance préalable du système.

***

10h10 – 10h30

Binless: bayesian interaction and difference detection in Hi-C data

Yannick Spill

UMR 7242 "Biotechnologie et signalisation cellulaire", Régulation épigénétique de l'identité cellulaire (Équipe M. Weber), 300 Bd Sébastien Brant, 67412 Illkirch

3C-like experiments, such as 4C or Hi-C, have been fundamental in understanding genome organization. Thanks to these technologies, it is now known, for example, that Topologically Associating Domains (TADs) and chromatin loops are implicated in the dynamic interplay of gene activation and repression, and their disruption can have dramatic effects on embryonic development. However, the analysis of Hi-C experiments is both statistically and computationally demanding. Most methods are hindered by the high noise, large quantities of data and inadequate modelling of spatial dependency. In this talk, I will present a new way to represent Hi-C data, which leads to a more detailed classification of paired-end reads and, ultimately, to a new normalization and interaction detection method. This method, called Binless, uses a generalized additive model framework, and makes extensive use of the sparse fused lasso regression in a Bayesian setting. Binless is resolution-agnostic, and adapts to the quality and quantity of available data. I demonstrate its capacities to call interactions and differences using a large-scale benchmark, and dwell on the difficulties and open questions that remain both from the theoretical and from the applied perspective.

***

11h – 11h50

Computational diffraction microscopy for high resolution quantitative imaging

Guillaume Maire, Patrick Chaumet, Kamal Belkebir, Gabriel Soriano, Hugues Giovannini, Anne Sentenac

Institut Fresnel, Faculté de St Jérôme 13013 Marseille France
guillaume.maire@fresnel.fr

The resolution of diffraction (i.e. marker free) microscopy is fundamentally limited by the elastic light-matter interaction which states that, in the single scattering regime, the far-field scattered by an object illuminated under propagative waves conveys information on the object permittivity spatial frequencies up to $2/\lambda$ at most (where λ is the illumination wavelength in the background medium). In addition, this information is poorly restored with conventional analogical microscopes (such as brightfield or confocal) as the observed standard resolution limit, seen as the full width at half maximum of a point-like object is usually about $0.6\ \lambda$ instead of the theoretical reachable diffraction limit of 0.3λ [1].

A widely explored solution for improving the resolution consists in taking advantage of evanescent waves for the illumination or the detection via near-field probes or metamaterial lenses [2-3]. These techniques

ameliorate the resolution, albeit seldom beyond 0.3λ in practice, but at the expense of an increase in the experimental complexity and a restriction to surface imaging.

Another research avenue consists in extracting the most out of the sample scattered field using numerical reconstructions based on an accurate model of the sample-light interaction. This quantitative imaging approach has been implemented on different microscopy configurations which have all in common the recording of the sample scattered field for many incident angles, either with an interferometric approach [4-6] or using a ptychography-like technique [7]. It has been shown experimentally to achieve the best possible resolution of 0.3λ [6,8] in the single scattering regime and even much better for certain samples in the multiple scattering regime for which the diffraction limit does not hold [9].

A strong asset of quantitative imaging is that it can take advantage of *a priori* information on the sample to restrain the possible solutions of the inverse problem and possibly recover object spatial frequencies beyond the physically accessible domain. The most widespread constraint is the positivity of the sought parameter. Studied in numerous applications, from fluorescence microscopy to diffraction microscopy or microwave and acoustic imaging, it has been shown to improve the readability of the images, in particular by suppressing the deconvolution-induced oscillating behavior of the background, but has seldom brought any significant amelioration of the resolution.

In this talk, we push forward the performance of quantitative diffraction microscopy by imposing a binary behavior on the permittivity. Contrary to positivity, binary constraint is expected to have a major influence on the resolution by raising the indetermination between small objects of similar optical volume. We demonstrate experimentally that it permits a spectacular improvement of the resolution on complex samples [10].

References :

[1] (2010) O. Haeberlé, K. Belkebir, H. Giovannini and A. Sentenac, ‘Tomographic Diffractive Microscopy, basics, techniques and perspectives’, 57, 686-699

[2] D. W. Pohl, U. Ch. Fischer and U. T. Dürig 'Scanning near-field optical microscopy', J. of Microscopy, 152, 853-861, (1988)

[3] Liu Z, Lee H, Xiong Y, Sun C, Zhang' far-field optical hyperlens magnifying sub-diffraction-limited objects' Science 315, 1686, (2007)

[4] V. Lauer ‘New approach to optical diffraction tomography yielding a vector equation of diffraction tomography and a novel tomographic’ J. Microsc. \textbf{205}, 165--176 (2002).

[5] M. Debailleul, V. Georges, B. Simon, R. Morin, and O. Haeberlé ‘High-resolution three-dimensional tomographic diffractive microscopy of transparent inorganic and biological samples’Optics Letters Vol. 34, Issue 1, pp. 79-81 (2009)

[6] Yann Cotte, Fatih Toy, Pascal Jourdain,Nicolas Pavillon, Daniel Boss, Pierre Magistretti, Pierre Marquet and Christian Depeursinge, 'marker-free phase nanoscopy', Nature Photonics, 7, 113, (2013).

[7] L. Tian and L. Waller, ‘3d intensity and phase imaging from light field measurements in an led array microscope,’ Optica, 104--111 (2015).

[8] T. Zhang, Y. Ruan, G. Maire, D. Sentenac, A. Talneau, K. Belkebir, P. C. Chaumet, and A. Sentenac, 'Full-polarized tomographic diffraction microscopy achieves a resolution about one fourth of the wavelength ', Physical review letters, 11, 243904 (2013)

[9] J. Girard, G. Maire, H. Giovannini, A. Talneau, K. Belkebir, P. C. Chaumet and A. Sentenac, Nanometric resolution using far-field optical tomographic microscopy in the multiple scattering regime, Phys. Rev. A 82, 061801(R) (2010)

[10] Ting Zhang, Charankumar Godavarthi, Patrick C. Chaumet, Guillaume Maire, Hugues Giovannini, Anne Talneau, Marc Allain, Kamal Belkebir, and Anne Sentenac 'Far-field diffraction microscopy at $\lambda/10$ resolution’, Optica, 3, 609, (2016)

***

11h50 – 12h10

Microscopie ptychographique de Fourier: théorie, instrumentation et études de bruit

Anastasiia Bozhok

ICube, Université de Strasbourg, CNRS (UMR 7357), Illkirch

La microscopie ptychographique de Fourier est une nouvelle technique d'imagerie computationnelle qui vise à surmonter certaines limites physiques de la microscopie optique conventionnelle. Elle est basée sur le principe d'échantillonnage de l'éclairage et mise en place par une simple modification matérielle couplée à un traitement numérique des images acquises. Cette technique a été initialement proposée en 2013 et attire depuis lors de nombreux développements théoriques et expérimentaux.

L'idée principale est de remplacer la source de lumière standard par une matrice de LEDs que l'on allume séquentiellement. Cela permet de reconstruire une image à haute résolution spatiale à partir d'images prises avec différents angles d'éclairage. Par ailleurs, l'utilisation d'un objectif à faible ouverture numérique permet un large champ de vue et évite un compromis entre les dimensions de la zone observée et la résolution souhaitée. En plus de l'image d'intensité conventionnelle, cette méthode permet également d'accéder à des informations sur la phase de l'échantillon. Un autre avantage majeur d'un tel système est son faible coût.

Un grand champ de vision couplé à une haute résolution offre une possibilité d'imagerie des lames histologiques sans balayage mécanique. La phase obtenue présente un intérêt particulier pour les tissus transparents non colorés.

L'instrument est simple et peu coûteux, mais le jeu de données est dégradé par du bruit, des aberrations et une non-concordance avec le modèle instrumental. Ces dégradations doivent être prises en compte par la méthode de reconstruction. Nous avons en particulier étudié le bruit de la caméra pour justifier une fonction de coût utilisée dans le processus d'optimisation lors de la reconstruction.

Poursuivant l'idée de développer un système d'imagerie multimodale, nous discutons de la fusion de la microscopie ptychographique de Fourier avec la microscopie polarimétrique.

***

12h10 – 12h30

Microsphere-assisted microscopy for biological imaging

Stéphane Perrin, Paul Montgomery, Sylvain Lecler

Laboratoire ICube, UMR CNRS 7357 - Université de Strasbourg, FR-67412 ILLKIRCH

stephane.perrin@unistra.fr

Observation of biological elements through an optical microscope appears often restricted by the resolving power of the latter [1]. Indeed, a white-light microscope allows the visualisation of objects having a size larger than half of the wavelength (*e.g.*, bacteria and structures of plants [2]), in ideal cases (in reality, imperfections or misalignment of optical components increase the resolution limit). In 2011, Wang *et al.* introduced experimentally the phenomenon of two-dimensional super-resolution imaging through a glass microsphere [3]. They showed that microsphere-assisted microscopy distinguishes itself from others by being able to perform label-free and full-field acquisitions. In addition, with only slight modifications of a classical white-light microscopy, microsphere-assisted microscopy makes it possible to reach a lateral resolution of a few hundred nanometres which is adequate, for example, for the visualization of adenovirus using the fluorescence effect

[4]. Recently, we successfully demonstrated the label-free combination of microsphere-assisted microscopy with dark-field illumination in order to image translucid samples [5]. Random glass features, as well as brain cell morphology, have been retrieved. Future work should then progress towards the extension of 3D object inspection using interferometry [6].

References:

[1] Duffieux, P.M. *L'intégrale de Fourier et ses applications à l'optique*. Faculté des Sciences de Besançon, 1946

[2] Zuylen, J. The microscopes of Antoni van Leeuwenhoek. J. Microsc. 121, 309-328 (1981)

[3] Wang, Z., Guo,W., et al. Optical virtual imaging at 50 nm lateral resolution with a white-light nanoscope. Nature Commun. 2, 218 (2011)

[4] Li, L., Guo, W., et al. Label-free super-resolution imaging of adenoviruses by submerged microsphere optical nanoscopy. Light: Science & Applications 2, e104 (2013)

[5] Perrin, S., Li, H., et al. Transmission microsphere-assisted dark-field microscopy. Phys. Status Solidi RRL. doi:10.1002/pssr.201800445 (2018)

[6] Perrin, S., Leong-Hoi, A., *et al.* Microsphere-assisted phase-shifting profilometry. Appl. Opt. 56, 7249-7255 (2017)