IN PARTNERSHIP WITH:
CNRS

Institut Curie

Activity Report 2018

Project-Team SERPICO

Space-timE RePresentation, Imaging and cellular dynamics of molecular COmplexes

IN COLLABORATION WITH: UMR 144 - Compartimentation et dynamique cellulaires

RESEARCH CENTER
Rennes - Bretagne-Atlantique

THEME
Computational Biology

Table of contents

Project-Team SERPICO

Creation of the Team: 2010 January 01, updated into Project-Team: 2013 July 01

Keywords:

Computer Science and Digital Science:

A3.1.1. - Modeling, representation
A3.3. - Data and knowledge analysis
A3.3.3. - Big data analysis
A3.4. - Machine learning and statistics
A3.4.1. - Supervised learning
A3.4.5. - Bayesian methods
A3.4.6. - Neural networks
A3.4.7. - Kernel methods
A3.4.8. - Deep learning
A5.3. - Image processing and analysis
A5.3.2. - Sparse modeling and image representation
A5.3.3. - Pattern recognition
A5.3.4. - Registration
A5.4.1. - Object recognition
A5.4.4. - 3D and spatio-temporal reconstruction
A5.4.5. - Object tracking and motion analysis
A5.4.6. - Object localization
A5.9.1. - Sampling, acquisition
A5.9.2. - Estimation, modeling
A5.9.3. - Reconstruction, enhancement
A5.9.5. - Sparsity-aware processing
A5.9.6. - Optimization tools
A6.1.2. - Stochastic Modeling
A6.1.3. - Discrete Modeling (multi-agent, people centered)
A6.1.4. - Multiscale modeling
A6.1.5. - Multiphysics modeling
A6.2.3. - Probabilistic methods
A6.2.4. - Statistical methods
A6.2.6. - Optimization
A6.3. - Computation-data interaction
A6.3.1. - Inverse problems
A6.3.2. - Data assimilation
A6.3.3. - Data processing
A6.3.4. - Model reduction
A6.3.5. - Uncertainty Quantification
A9.2. - Machine learning
A9.3. - Signal analysis

Other Research Topics and Application Domains:

- B1.1.1. - Structural biology
- B1.1.7. - Bioinformatics
- B1.1.8. - Mathematical biology
- B2.2.3. - Cancer
- B2.6. - Biological and medical imaging

1. Team, Visitors, External Collaborators

Research Scientists

Charles Kervrann [Team leader, Inria, Senior Researcher, HDR]
Patrick Bouthemy [Inria, Senior Researcher, HDR]

PhD Students

Anca-Georgiana Caranfil [Univ de Rennes I, MESR grant]
Yunjiao Lu [INRA, INRA grant, from Oct 2017]
Léo Maczyta [Inria, granted by DGA & Région Bretagne, from Oct 2017]
Sandeep Manandhar [Inria, CORDI-S grant]
Emmanuel Moebel [Inria, granted by Fourmentin-Guilbert & Région Bretagne]
Juan Manuel Perez Rua [Technicolor, granted by CIFRE, until Jan 2018]
Antoine Salomon [Inria, granted by ANR DALLISH, from Nov 2017]
Bertha Mayela Toledo Acosta [granted by Conacyt, Mexico, until May 2018]

Technical staff

Hoai Nam Nguyen [Inria, ADT-FastTrack, from May 2018 until Oct 2018]
Sylvain Prigent [CNRS, CNRS-UMR 144 Institut Curie, from Dec 2018]

Administrative Assistant

Huguette Bechu [Inria]

2. Overall Objectives

2.1. Glossary

FLIM (Fluorescence Lifetime Microscopy Imaging): imaging of fluorescent molecule lifetimes.

PALM (Photo-Activated Localization Microscopy): high-resolution microscopy using stochastic photo-activation of fluorophores and adjustment of point spread functions [37].

SIM (Structured Illumination Microscopy): high-resolution light microscopy using structured patterns and interference analysis [40].

TIRF (Total Internal Reflectance): 2D optical microscopy using evanescent waves and total reflectance [36].

Cryo-EM (Cryo-Electron Tomography): 3D representation of sub-cellular and molecular objects of 5-20 nanometres, frozen at very low temperatures, from 2D projections using a transmission electron microscope.

2.2. Scientific context and motivations

During the past two decades, biological imaging has undergone a revolution in the development of new microscopy techniques that allow visualization of tissues, cells, proteins and macromolecular structures at all levels of resolution, physiological states, chemical composition and dynamics. Thanks to recent advances in optics, digital sensors and labeling probes (e.g., Colored Fluorescence Protein), one can now visualize subcellular components and organelles at the scale of several hundreds of nanometers to a few dozens nanometers. As a result, fluorescent microscopy and multimodal imaging (fluorophores at various wavelengths) have become the workhorse of modern biology. All the technological advances in microscopy have created new issues and challenges for researchers in quantitative image processing and analysis. Since the digital processing is now part of the imaging loop, image processing may even drive imaging. A brilliant example of this shift in paradigm is super-resolution localization microscopy (PALM, STED), which was awarded the 2014 Nobel Prize in Chemistry.

2.3. Challenges in biological image processing and quantitative microscopy

In most cases, modern microscopy in biology is characterized by a large number of dimensions that fit perfectly with the complexity of biological features: two or three spatial dimensions, at macro to nano-scales, and one temporal dimension, sometimes spectrally defined and often corresponding to one particular biomolecular species. Dynamic microscopy is also characterized by the nature of the observable objects (cells, organelles, single molecules, ...), by the large number of small size and mobile elements (chromosomes, vesicles, ...), by the complexity of the dynamic processes involving many entities or group of entities sometimes interacting, by particular phenomena of coalescence often linked to image resolution problems, finally by the association, dissociation, recomposition or constitution of those entities (such as membrane fusion and budding). Thus, the corpus of data to be considered for any analysis involving multiple image series acquisitions is massive (up to few GigaBytes per hour). Therefore, it becomes necessary to facilitate and rationalize the production of those multidimensional data, to improve post acquisition analysis, and to favor the organization and the interpretation of the information extracted from this data corpus. It motivates innovative methods and concepts for data fusion, image registration, super-resolution, data mining... More importantly, modern microscopy has led to recent breakthroughs, related to the potential interactions between molecules in the cell. A long-term research consists now in inferring the relationships between the dynamics of macromolecules and their functions. Research on computational biology and quantitative bioimaging lies at the core of the activities of Serpico team.

2.4. Objectives of Serpico in cell imaging

In order to tackle the aforementioned challenges, the Serpico team aims to develop innovative approaches and paradigms for image reconstruction, 3D molecule tracking and motion estimation, and biophysical parameter estimation to face the huge data volumes acquired with cutting-edge microscopy set-ups. To this end, applied mathematics, image processing and analysis have to be considered in association with biophysics and biology. To be successful, a sustained synergy between all these scientific domains is necessary. To improve state-of-the-art methods and solve important problems in computational bioimaging, the members of Serpico especially address the following topics:

- Image restoration/reconstruction motivated by preserving cell integrity (photo-toxicity versus exposure time) and image analysis in multidimensional microscopy;
- Motion analysis and computation of molecule trajectories in live-cell imaging to study molecular interactions in space and time;
- Computational simulation, modelling and estimation of molecule trafficking and interactions at different spatial and temporal scales.

The resulting mathematical models and algorithms will help biologists to decipher molecular processes in fundamental biology and will be exploited for health applications: disease diagnosis, detection of genomic instabilities, deterioration of cell cycle, cancer prevention.

We have successfully developed statistical and variational aggregation methods for image denoising and optical flow, and elaborated powerful methods for image colocalization, diffusion estimation, trajectory estimation-classification, and multimodal registration. An additional issue was the design and distribution of software tools for the biological image analysis and microscopy communities. Finally, the team has focused on the cellular and molecular mechanisms involved in molecule and protein transport and trafficking at the scale of a single cell. Our contributions are detailed in the next sections along three research axes.

2.5. Organization and collaborations

In collaboration with UMR 144 CNRS-Institut Curie (“Space Time imaging of Endomembranes and organelles Dynamics” team) and PICT-IBiSA (Cell and Tissue Imaging Facilities), the members of the Serpico team have participated in several projects (PhD and post-doc supervision, contracts...) in the field of cell biology and microscopy. We have promoted non-parametric methods since prior knowledge cannot be easily taken into account for extracting unattended but desired information from image data. We have also proposed user-friendly algorithms for processing 2D and 3D image sequences. The projects of Serpico were in line with several studies led in the UMR 144 CNRS-Institut Curie Unit. A subset of studies was related to instrumentation in electronic and photonic microscopy (PICT-IBiSA platform) including computational aspects on the reconstruction and enhancement of images related to sub-diffraction light microscopy and multimodal approaches. Serpico projects relied partially on the advances of these instrumental projects and a positive synergy was established.

3. Research Program

3.1. Statistics and algorithms for computational microscopy

Fluorescence microscopy limitations are due to the optical aberrations, the resolution of the microscopy system, and the photon budget available for the biological specimen. Hence, new concepts have been defined to address challenging image restoration and molecule detection problems while preserving the integrity of samples. Accordingly, the main stream regarding denoising, deconvolution, registration and detection algorithms advocates appropriate signal processing framework to improve spatial resolution, while at the same time pushing the illumination to extreme low levels in order to limit photo-damages and phototoxicity. As a consequence, the question of adapting cutting-edge signal denoising and deconvolution, object detection, and image registration methods to 3D fluorescence microscopy imaging has retained the attention of several teams over the world.

In this area, the Serpico team has developed a strong expertise in key topics in computational imaging including image denoising and deconvolution, object detection and multimodal image registration. Several algorithms proposed by the team outperformed the state-of-the-art results, and some developments are compatible with “high-throughput microscopy” and the processing of several hundreds of cells. We especially promoted non local, non-parametric and patch-based methods to solve well-known inverse problems or more original reconstruction problems. A recent research direction consists in adapting the deep learning concept to solve challenging detection and reconstruction problems in microscopy. We have investigated convolution neural networks to detect small macromolecules in 3D noisy electron images with promising results. The next step consists in proposing smart paradigms and architectures to save memory and computations.

More generally, many inverse problems and image processing become intractable with modern 3D microscopy, because very large temporal series of volumes (200 to 1000 images per second for one 3D stack) are acquired for several hours. Novel strategies are needed for 3D image denoising, deconvolution and reconstruction since computation is extremely heavy. Accordingly, we will adapt the estimator aggregation approach developed for optical flow computation to meet the requirements of 3D image processing. We plan to investigate regularization-based aggregation energy over super-voxels to reduce complexity, combined to modern optimization algorithms. Finally, we will design parallelized algorithms that fast process 3D images, perform energy minimization in few seconds per image, and run on low-cost graphics processor boards (GPU).

3.2. From image data to motion descriptors: trajectory computation and dynamics analysis

Several particle tracking methods for intracellular analysis have been tailored to cope with different types of cellular and subcellular motion down to Brownian single molecule behavior. Many algorithms were carefully evaluated on the particle tracking challenge dataset published in the Nature Methods journal in 2014. Actually, there is no definitive solution to the particle tracking problem which remains application-dependent in most cases. The work of Serpico in particle motion analysis is significant in multiple ways, and inserts within a very active international context. One of the remaining key open issues is the tracking of objects with heterogeneous movements in crowded configurations. Moreover, particle tracking methods are not always adapted for motion analysis, especially when the density of moving features hampers the individual extraction of objects of interest undergoing complex motion. Estimating flow fields can be more appropriate to capture the complex dynamics observed in biological sequences. The existing optical flow methods can be classified into two main categories: i/ local methods impose a parametric motion model (e.g. local translation) in a given neighborhood; ii/ global methods estimate the dense motion field by minimizing a global energy functional composed of a data term and a regularization term.

The Serpico team has developed a strong expertise in key topics, especially in object tracking for fluorescence microscopy, optical flow computation and high-level analysis of motion descriptors and trajectories. Several algorithms proposed by the team are very competitive when compared to the state-of-the-art results, and our new paradigms offer promising ways for molecule traffic quantification and analysis. Amongst the problems that we currently address, we can mention: computation of 3D optical flow for large-size images, combination of two frame-based differential methods and sparse sets of trajectories, detection and analysis of unexpected local motion patterns in global coherent collective motion. Development of efficient numerical schemes will be central in the future but visualization methods are also crucial for evaluation and quality assessment. Another direction of research consists in exploiting deep learning to 3D optical flow so as to develop efficient numerical schemes that naturally capture complex motion patterns. Investigation in machine learning and statistics will be actually conducted in the team in the two first research axes to address a large range of inverse problems in bioimaging. Deep learning is an appealing approach since expertise of biologists, via iterative annotation of training data, will be included in the design of image analysis schemes.

3.3. Biological and biophysical models and spatial statistics for quantitative bioimaging

A number of stochastic mathematical models were proposed to describe various intracellular trafficking, where molecules and proteins are transported to their destinations via free diffusion, subdiffusion and ballistic motion representing movements along the cytoskeleton networks assisted by molecular motors. Accordingly, the study of diffusion and stochastic dynamics has known a growing interest in bio-mathematics, biophysics and cell biology with the popularization of fluorescence dynamical microscopy and super-resolution imaging. In this area, the competing teams mainly studied MSD and fluorescence correlation spectroscopy methods.

In the recent period, the Serpico team achieved important results for diffusion-related dynamics involved in exocytosis mechanisms. Robustness to noise has been well investigated, but robustness to environmental effects has yet to be effectively achieved. Particular attention has been given to the estimation of particle motion regime changes, but the available results are still limited for analysing short tracks. The analysis of spatiotemporal molecular interactions from set of 3D computed trajectories or motion vector fields (e.g., co-alignment) must be investigated to fully quantify specific molecular machineries. We have already made efforts in that directions this year (e.g., for colocalization) but important experiments are required to make our preliminary algorithms reliable enough and well adapted to specific transport mechanisms.

Accordingly, we will study quantification methods to represent interactions between molecules and trafficking around three lines of research. First, we will focus on 3D space-time global and local object-based co-orientation and co-alignment methods, in the line of previous work on colocalization, to quantify interactions between molecular species. In addition, given N tracks associated to N molecular species, interaction descriptors, dynamics models and stochastic graphical models representing molecular machines will be studied in the statistical data assimilation framework. Second, we will analyse approaches to estimate molecular mobility, active transport and motion regime changes from computed trajectories in the Lagrangian and Eulerian settings. We will focus on the concept of super-resolution to provide spatially high-resolved maps of diffusion and active transport parameters based on stochastic biophysical models and sparse image representation. Third, we plan to extend the aggregation framework dedicated to optical flow to the problem of diffusion-transport estimation. Finally, we will investigate data assimilation methods to better combine algorithms, models, and experiments in an iterative and virtuous circle. The overview of ultrastructural organization will be achieved by additional 3D electron microscopy technologies.

4. Application Domains

4.1. Modeling and analysis of membrane transport and molecule trafficking at the single cell scale

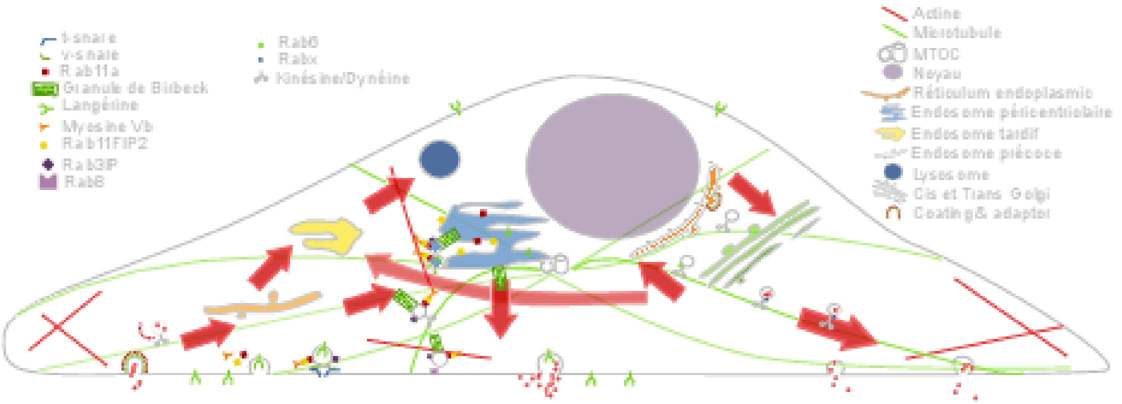


Figure 1. Cargo Langerin Trafficking controlled by Rab11A/Rab11FIP2/MyoVb platform.

In the past recent years, research carried at UMR 144 CNRS-Institut Curie ("Space Time imaging of Endomembranes and organelles Dynamics" team) contributed to a better understanding of the intracellular compartimentation of specialized model cells such as melanocytes and Langerhans cells, the components and structural events involved in the biogenesis of their specialized organelles: melanosomes and Birbeck granules, respectively. These studies have started to highlight: i/ multiple sorting and structural events involved in the biogenesis of these organelles; ii/ complexity of the endo-melanosomal network of these highly specialized cells; iii/ complex molecular architecture organizing and coordinating their dynamics; iv/ intracellular transport steps affected in genetic diseases, among which the Hermansky Pudlak syndrome (HPS) or involved in viral infection (HIV and Langerin in Langerhans cells).

In this context, the central aim of SERPICO is to understand how the different machineries of molecular components involved are interconnected and coordinated to generate such specialized structures. We need to address the following topics:

1. developing new bioimaging approaches to observe and statistically analyze such coordinated dynamics in live material;
2. correlating this statistically relevant spatiotemporal organization of protein networks with the biological architectures and at the ultrastructural level;

3. modeling intracellular transport of those reference biological complex systems and proposing new experimental plans in an iterative and virtuous circle;
4. managing and analyzing the workflow of image data obtained along different multidimensional microscopy modalities.

These studies are essential to unravel the complexity of the endomembrane system and how different machineries evolve together (e.g. see Fig. 1). They help to control cell organization and function at different scales through an integrative workflow of methodological and technological developments.

At long term, these studies will shed light on the cellular and molecular mechanisms underlying antigen presentation, viral infection or defense mechanisms, skin pigmentation, the pathogenesis of hereditary genetic disorders (lysosomal diseases, immune disorders) and on the mechanisms underlying cell transformation. Our methodological goal is also to link dynamics information obtained through diffraction limited light microscopy, eventually at a time regime compatible with live cell imaging. The overview of ultrastructural organization will be achieved by complementary electron microscopical methods. Image visualization and quantitative analysis are of course important and essential issues in this context.

4.2. Imaging and analysis of cytokskeleton dynamics during cell migration

The ability to migrate in space is among the most fundamental functions of eukaryotic cells and thus is one of the best-studied phenomena in biology. During embryonic development, cell movements result in a massive reorganization of the embryo, from a simple spherical ball of cells into a multi-layered organism; many of the cells at or near the surface of the embryo move to a new, more interior location. Moreover, inadequate or inappropriate migration of immune cells is also critically important for the delivery of protective immune responses to tissues and for wound healing. Finally, cell migration may facilitate the dissemination of tumor cells in blood and organs and eventually the formation of secondary tumors and metastases.

It has been established that the cytoskeleton, composed of actin filaments, microtubules and intermediate filaments (elongated structures with a diameter of a few dozens of nanometers), is essential for several cell mechanisms, including cell migration, cell division and molecule trafficking:

i/ the actin filaments promote cell protrusion, adhesion and retraction;

ii/ the microtubules are the support of molecule traffic and cell polarization;

iii/ the intermediate filaments are hypothesized to control microtubule organization.

Nevertheless, the mechanical and chemical states of migrating cells under various external conditions remain largely unknown. In the last decade, high-resolution microscopy methods led to the discovery of novel aspects of cell migration. Most approaches and models are limited to migration in 2D, justified by the flatness of the cell-motile mechanisms. However, the mechanical patterns that govern migration in 2D models are often not essential for efficient migration in 3D. Accordingly, recent very challenging 3D models of cells moving on flat surfaces have begun to emerge. The key challenge, however, is to understand how a 3D motile cell crawls through the 3D extracellular matrix.

The objective of SERPICO is to develop high-end signal processing and computer vision tools to unfold the dynamical coordination of microtubules, actin filaments and intermediate filaments in 3D, involved in cell migration, cell division and molecule trafficking.

5. Highlights of the Year

5.1. Highlights of the Year

The joint project team Serpico (Inria, CNRS-INSB, Institut Curie, UPMC, PSL Research University) is officially created in 2018.

The Serpico team will be the organizer of the 7th International Conference on “Quantitative BioImaging” (QBI) in January 2019 (300 attendees) in Rennes.

Bertha Mayela Toledo Acosta defended her PhD thesis in 2018.

6. New Software and Platforms

6.1. ATLAS

KEYWORDS: Image segmentation - Object detection - Photonic imaging - Image analysis - Fluorescence microscopy

FUNCTIONAL DESCRIPTION: The ATLAS software enables to detect spots in 2D fluorescence images. The spot size is automatically selected and the detection threshold adapts to the local image contrasts. ATLAS relies on the Laplacian of Gaussian (LoG) filter, which both reduces noise and enhances spots. A multiscale representation of the image is built to automatically select the optimal LoG variance. Local statistics of the LoG image are estimated in a Gaussian window, and the detection threshold is pointwise inferred from a probability of false alarm (PFA). The user only has to specify: i/ size of the Gaussian window, ii/ PFA value. The Gaussian window must be about the size of the background structures, increasing the PFA increases the number of detections.

- Participants: Antoine Basset, Patrick Bouthemy, Charles Kervrann, Jérôme Boulanger and Jean Salamero
- Partner: UMR 144 CNRS - Institut Curie
- Contact: Patrick Bouthemy
- Publication: Adaptive spot detection with optimal scale selection in fluorescence microscopy images
- URL: http://mobyle-serpico.rennes.inria.fr/cgi-bin/portal.py#forms::ATLAS

6.2. C-CRAFT

KEYWORDS: Fluorescence microscopy - Photonic imaging - Image analysis - Detection - 3D - Health - Biology - Segmentation

FUNCTIONAL DESCRIPTION: The C-CRAFT software enables to jointly segment small particles and estimate background in 2D or 3D fluorescence microscopy image sequences. The vesicle segmentation and background estimation problem is formulated as a global energy minimization problem in the Conditional Random Field framework. A patch-based image representation is used to detect spatial irregularity in the image. An iterative scheme based on graph-cut algorithm is proposed for energy minimization.

- Participants: Thierry Pécot, Charles Kervrann, Patrick Bouthemy and Jean Salamero
- Partner: UMR 144 CNRS - Institut Curie
- Contact: Charles Kervrann
- Publication: Background Fluorescence Estimation and Vesicle Segmentation in Live Cell Imaging with Conditional Random Fields
- URL: http://mobyle-serpico.rennes.inria.fr/cgi-bin/portal.py#forms::C-CRAFT

6.3. F2D-SAFIR

KEYWORDS: Biomedical imaging - Photonic imaging - Fluorescence microscopy - Image processing

FUNCTIONAL DESCRIPTION: The F2D-SAFIR software removes mixed Gaussian-Poisson noise in large 2D images, typically 10000 x 10000 pixels, in a few seconds. The method is unsupervised and is a simplified version of the method related to the ND-SAFIR software. The software is dedicated to microarrays image denoising for disease diagnosis and multiple applications (gene expression, genotyping, aCGH, ChIP-chip, microRNA, ...).

- Participant: Charles Kervrann
- Partner: INRA
- Contact: Charles Kervrann

6.4. GcoPS

KEYWORDS: Photonic imaging - Fluorescence microscopy - Image processing - Statistic analysis
FUNCTIONAL DESCRIPTION: The GCOPS (Geo-Co-Positioning System) software is dedicated to the co-localization of fluorescence image pairs for both conventional and super-resolution microscopy. The procedure is only controlled by a p-value and tests whether the Pearson correlation between two binary images is significantly positive. It amounts to quantifying the interaction strength by the area/volume of the intersection between the two binary images viewed as random distributions of geometrical objects. Under mild assumptions, it turns out that the appropriately normalized Pearson correlation follows a standard normal distribution under the null hypothesis if the number of image pixels is large. Unlike previous methods, GcoPS handles 2D and 3D images, variable SNRs and any kind of cell shapes. It is able to co-localize large regions with small dots, as it is the case in TIRF-PALM experiments and to detect negative co-localization. The typical processing time is two milliseconds per image pair in 2D and a few seconds in 3D, with no dependence on the number of objects per image. In addition, the method provides maps to geo-co-localize molecule interactions in specific image regions.

- Participants: Thierry Pécot, Frédéric Lavancier, Charles Kervrann and Liu Zengzhen
- Partners: Université de Nantes - UMR 144 CNRS - Institut Curie
- Contact: Charles Kervrann
- Publication: A Fast Automatic Colocalization Method for 3D Live Cell and Super-Resolution Microscopy
- URL: http://icy.bioimageanalysis.org/plugin/GcoPS

6.5. Hullkground

KEYWORDS: Biomedical imaging - Photonic imaging - Fluorescence microscopy - Image processing
FUNCTIONAL DESCRIPTION: The HullkGround software decomposes a fluorescence microscopy image sequence into two dynamic components: i) an image sequence showing mobile objects, ii) an image sequence showing the slightly moving background. Each temporal signal of the sequence is processed individually and analyzed with computational geometry tools. The convex hull is estimated automatically for each pixel and subtracted to the original signal. The method is unsupervised, requires no parameter tuning and is a simplified version of the shapes-based scale-space method.

- Participants: Anatole Chessel, Charles Kervrann and Jean Salamero
- Partner: UMR 144 CNRS - Institut Curie
- Contact: Charles Kervrann
- URL: http://mobyle-serpico.rennes.inria.fr/cgi-bin/portal.py#forms::Hullkground

6.6. Motion2D

KEYWORDS: Image sequence - Motion model - 2D

FUNCTIONAL DESCRIPTION: The Motion2D software is a multi-platform object-oriented library to estimate 2D parametric motion models in an image sequence. It can handle several types of motion models, namely, constant (translation), affine, and quadratic models. Moreover, it includes the possibility of accounting for a global variation of illumination and more recently for temporal image intensity decay (e.g. due to photobleaching decay in fluorescence microscopy). The use of such motion models has been proved adequate and efficient for solving problems such as optic flow computation, motion segmentation, detection of independent moving objects, object tracking, or camera motion estimation, and in numerous application domains (video surveillance, visual servoing for robots, video coding, video indexing), including biological imaging (image stack registration, motion compensation in videomicroscopy). Motion2D is an extended and optimized implementation of the robust, multi-resolution and incremental estimation method (exploiting only the spatio-temporal derivatives of the image intensity function). Real-time processing is achievable for motion models involving up to six parameters. Motion2D can be applied to the entire image or to any pre-defined window or region in the image.

RELEASE FUNCTIONAL DESCRIPTION: Modifications and improvements in the PNG image file support. Support RAW and Mpeg2 video format as input (see CReader). The available video format which can be handled by the motion estimator are given by CReader::EReaderFormat. For the results, video sequences can be writen using the format specified by CWriter::EWriterFormat. Support Fedora 3 (g++ 3.4.2).

- Participants: Patrick Bouthemy, Jean Marc Odobez, Fabien Spindler, Thierry Pécot and Charles Kervrann
- Contact: Patrick Bouthemy
- URL: http://www.irisa.fr/vista/Motion2D/

6.7. ND-SAFIR

KEYWORDS: Fluorescence microscopy - Photonic imaging - Image analysis - Health - Biomedical imaging

SCIENTIFIC DESCRIPTION: ND-SAFIR is a software for denoising n-dimentionnal images especially dedicated to microscopy image sequence analysis. It is able to deal with 2D, 3D, 2D+time, 3D+time images have one or more color channel. It is adapted to Gaussian and Poisson-Gaussian noise which are usually encountered in photonic imaging. Several papers describe the detail of the method used in ndsafir to recover noise free images (see references).

- Participants: Jérôme Boulanger, Charles Kervrann, Patrick Bouthemy and Jean Salamero
- Partners: INRA - PiCT - UMR 144 CNRS - Institut Curie
- Contact: Charles Kervrann
- URL: http://serpico.rennes.inria.fr/doku.php?id=software:nd-safir:index

6.8. OWF

KEYWORDS: Image filter - Image processing - Statistics

FUNCTIONAL DESCRIPTION: The OWF software enables to denoise images corrupted by additive white Gaussian noise. In the line of work of the Non-Local means and ND-SAFIR algorithms, this adaptive estimator is based on the weighted average of observations taken in a neighborhood with weights depending on the similarity of local patches. The idea is to compute adaptive weights that best minimize an upper bound of the pointwise L2 risk. The spatially varying smoothing parameter is automatically adjusted to the image context. The proposed algorithm is fast and easy to control and is competitive when compared to the more sophisticated NL-means filters.

- Participants: Qiyu Jin, Ion Grama, Quansheng Liu and Charles Kervrann
- Partner: University of Bretagne-Sud
- Contact: Charles Kervrann
- Publication: Non-local means and optimal weights for noise removal
- URL: http://serpico.rennes.inria.fr/doku.php?id=software:owf

6.9. QuantEv

KEYWORDS: Photonic imaging - Fluorescence microscopy - Biomedical imaging - Image analysis - Image sequence - Statistic analysis
FUNCTIONAL DESCRIPTION: The QUANTEV software analyzes the spatial distribution of intracellular events represented by any static or dynamical descriptor, provided that the descriptors are associated with spatial coordinates. QUANTEV first computes 3D histograms of descriptors in a cylindrical coordinate system with computational cell shape normalization, enabling comparisons between cells of different shape. Densities are obtained via adaptive kernel density estimation, and we use the Circular Earth Mover's Distance to measure the dissimilarity between densities associated to different experimental conditions. A statistical analysis on these distances reliably takes into account the biological variability over replicated experiments.

- Participants: Thierry Pécot, Charles Kervrann, Jérôme Boulanger, Liu Zengzhen and Jean Salamero
- Partner: UMR 144 CNRS - Institut Curie
- Contact: Charles Kervrann
- Publication: QuantEv: quantifying the spatial distribution of intracellular events
- URL: http://mobyle-serpico.rennes.inria.fr/cgi-bin/portal.py#forms::QuantEv-Densities

6.10. TMA-Lib

KEYWORDS: Photonic imaging - Fluorescence microscopy - Biomedical imaging - Image processing
FUNCTIONAL DESCRIPTION: The TMA-LIB enables to jointly detect suing adaptive wavelet transform, segment with parametric active contours and restore (i.e., artifact correction and deconvolution) TMA (Tissue MicroArrays) images.

- Participants: Hoai Nam Nguyen, Charles Kervrann, Cyril Cauchois and Vincent Paveau
- Partner: Innopsys
- Contact: Charles Kervrann
- Publications: A variational method for dejittering large fluorescence line scanner images - Generalized Sparse Variation Regularization for Large Fluorescence Image Deconvolution - ATMAD : robust image analysis for Automatic Tissue MicroArray De-arraying

6.11. TOTH

KEYWORDS: Photonic imaging - Fluorescence microscopy - Biomedical imaging - Classification - Statistical categorisation techniques - Statistics - Image sequence - Visual tracking
FUNCTIONAL DESCRIPTION: The TOTH software classifies trajectories of biomolecules computed with tracking algorithms. Trajectories in living cells are generally modelled with three types of diffusion processes: (i) free diffusion, (ii) subdiffusion or (iii) superdiffusion. We used a test approach with the Brownian motion as the null hypothesis, and developed a non-parametric three-decision test whose alternatives are subdiffusion and superdiffusion. First, we built a single test procedure for testing a single trajectory. Second, we proposed a multiple test procedure for testing a collection of trajectories. These procedures control respectively the type I error and the false discovery rate. Our approach can be considered as an alternative to the Mean Square Displacement (MSD) method commonly used to address this issue. It gives more reliable results as confirmed by our Monte Carlo simulations and evaluations on real sequences of images depicting protein dynamics acquired with TIRF or SPT-PALM microscopy.

- Participants: Vincent Briane, Charles Kervrann and Myriam Vimond
- Partner: ENSAI
- Contact: Charles Kervrann
- Publication: A Statistical Analysis of Particle Trajectories in Living Cells
- URL: http://serpico.rennes.inria.fr/doku.php?id=software:thot:index

6.12. SparseVolution

Sparse Variation for 2D Image Decovolution

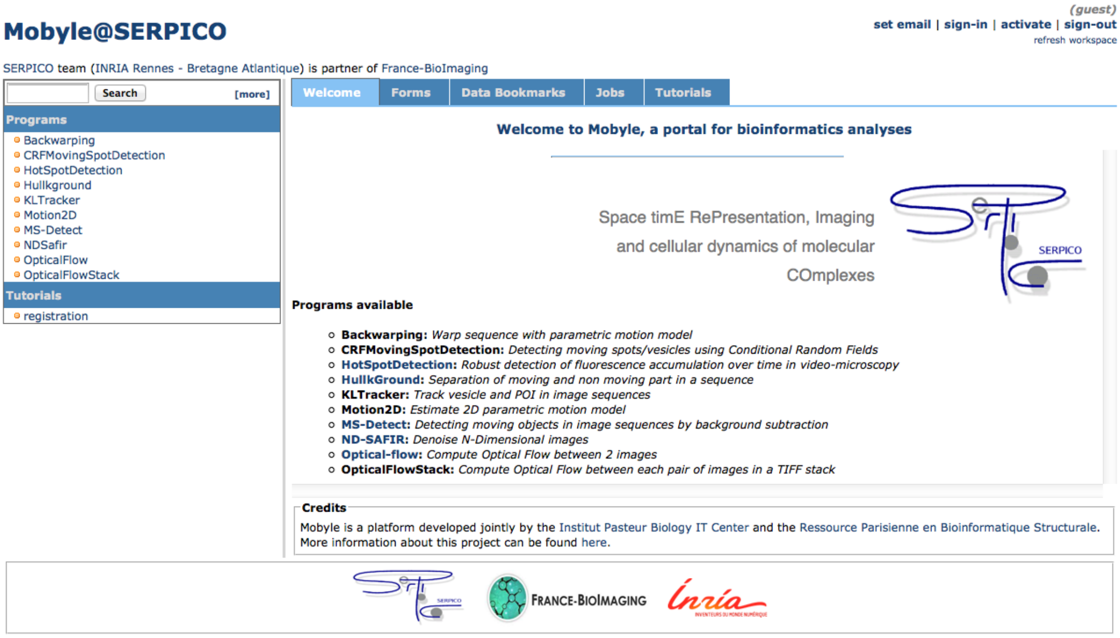
KEYWORDS: 2D - Fluorescence microscopy - Image processing - Problem inverse - Deconvolution

FUNCTIONAL DESCRIPTION: In order to improve the resolution of acquired fluorescence images, we introduced a method of image deconvolution by considering a family of convex regularizers. The considered regularizers are generalized from the concept of Sparse Variation which combines the L1 norm and Total Variation (TV) to favors the colocalization of high-intensity pixels and high-magnitude gradient. The experiments showed that the proposed regularization approach produces competitive deconvolution results on fluorescence images, compared to those obtained with other approaches such as TV or the Schatten norm of Hessian matrix. The final deconvolution algorithm has been dedicated to large 2D 20 000 x 20 000 images. The method is able to process a 512 x 512 image in 250 ms (Matlab) with a non optimized implementation.

- Participants: Hoai Nam Nguyen and Charles Kervrann
- Partner: Innopsys
- Contact: Charles Kervrann

6.13. Platforms

6.13.1. Mobyle@Serpico plateform and software distribution



SERPICO team (INRIA Rennes - Bretagne Atlantique) is partner of France-BioImaging

Welcome to Mobyle, a portal for bioinformatics analyses

Space timE RePresentation, Imaging and cellular dynamics of molecular COmplexes

Programs available

- **Backwarping:** *Warp sequence with parametric motion model*
- **CRFMovingSpotDetection:** *Detecting moving spots/vesicles using Conditional Random Fields*
- **HotSpotDetection:** *Robust detection of fluorescence accumulation over time in video-microscopy*
- **HullkGround:** *Separation of moving and non moving part in a sequence*
- **KLTracker:** *Track vesicle and POI in image sequences*
- **Motion2D:** *Estimate 2D parametric motion model*
- **MS-Detect:** *Detecting moving objects in image sequences by background subtraction*
- **ND-SAFIR:** *Denoise N-Dimensional images*
- **Optical-flow:** *Compute Optical Flow between 2 images*
- **OpticalFlowStack:** *Compute Optical Flow between each pair of images in a TIFF stack*

Credits

Mobyle is a platform developed jointly by the Institut Pasteur Biology IT Center and the Ressource Parisienne en Bioinformatique Structurale. More information about this project can be found here.

*Figure 2. Mobyle@*SERPICO *web portal.*

The objective is to disseminate the distribution of SERPICO image processing software in the community of cell biology and cell imaging.

Free binaries: software packages have been compiled for the main operating systems (Linux, MacOS, Windows) using CMake (see http://www.cmake.org/). They are freely available on the team website under a proprietary license (e.g. ND-SAFIR and HULLKGROUND are distributed this way at http://serpico.rennes.inria.fr/doku.php?id=software:index).

Mobyle@Serpico web portal: An on-line version (http://mobyle-serpico.rennes.inria.fr) of the image processing algorithms has been developped using the Mobyle framework (Institut Pasteur, see http://mobyle.pasteur.fr/). The main role of this web portal (see Fig. 2) is to demonstrate the performance of the programs developed by the team: QUANTEV, C-CRAFT[14], ATLAS[1], HULLKGROUND[38], KLTRACKER[44], MOTION2D[43], MS-DETECT[39], ND-SAFIR[6], OPTICALFLOW and FLUX ESTIMATION [14]. The web interface makes our image processing methods available for biologists at Mobyle@SERPICO (http://mobyle-serpico.rennes.inria.fr/cgi-bin/portal.py#welcome) without any installation or configuration on their own. The size of submitted images is limited to 200 MegaBytes per user and all the results are kept 15 days. The web portal and calculations run on a server with 2 CPU x 8 cores, 64 GigaBytes of RAM (500 MegaBytes for each user / Data is saved for 3 months).

ImageJ plugins: IMAGEJ (see http://rsb.info.nih.gov/ij/) is a widely used image visualization and analysis software for biologist users. We have developed IMAGEJ plug-in JAVA versions of the following software: ND-SAFIR [6], HULLKGROUND [38], MOTION2D [43], ATLAS [1]. The C-CRAFT algorithm [14] has been developped for the image processing ICY platform (http://icy.bioimageanalysis.org/).

- **Contact:** Charles Kervrann, Charles Deltel (Inria Rennes SED).
- **Partner:** UMR 144 CNRS-Institut Curie and France-BioImaging.

6.13.2. IGRIDA-Serpico cluster

The IGRIDA-Serpico cluster of 200 nodes is opened for end-users for large scale computing and data sets processing (200 TeraBytes).

- **Batch Scheduler:** OAR
- **File management:** Puppet / Git / Capistrano
- **OS:** Linux Debian 7
- **User connexion:** public ssh key
- **Contact:** Charles Kervrann, Charles Deltel (Inria Rennes SED).

7. New Results

7.1. A Monte-Carlo approach for missing wedge restoration in cryo-electron tomography

Participants: Emmanuel Moebel, Charles Kervrann.

We investigated a Monte-Carlo approach to restore spectral information in the missing wedge (MW) in cryo-electron tomography (CET). The MW is known to be responsible for several types of imaging artifacts, and arises because of limited angle tomography: it is observable in the Fourier domain and is depicted by a region where Fourier coefficient values are unknown (see Fig. 3). The proposed computational method tackles the restoration problem by filling up the MW by iterating the two following steps: adding noise into the MW (step 1) and applying a denoising algorithm (step 2). The role of the first step is to propose candidates for the missing Fourier coefficients and the second step acts as a regularizer. Also, specific constraint is added in the spectral domain by imposing the known Fourier coefficients to be unchanged through iterations. We justified this approach in the Monte-Carlo simulation and Bayesian framework. In practice, different denoising algorithms (BM3D, NL-Bayes, NL-means...) can be applied. In our experiments, several transforms have been tested in order to apply the constraint (Fourier transform, Cosine transform, pseudo-polar Fourier transform). Convincing results have been achieved (see Fig. 3) using the Fourier Shell Correlation (FSC) as an evaluation metric.

Collaborators: Damien Larivière (Fondation Fourmentin-Guilbert),
Julio Ortiz (Max-Planck Institute, Martinsried, Germany).

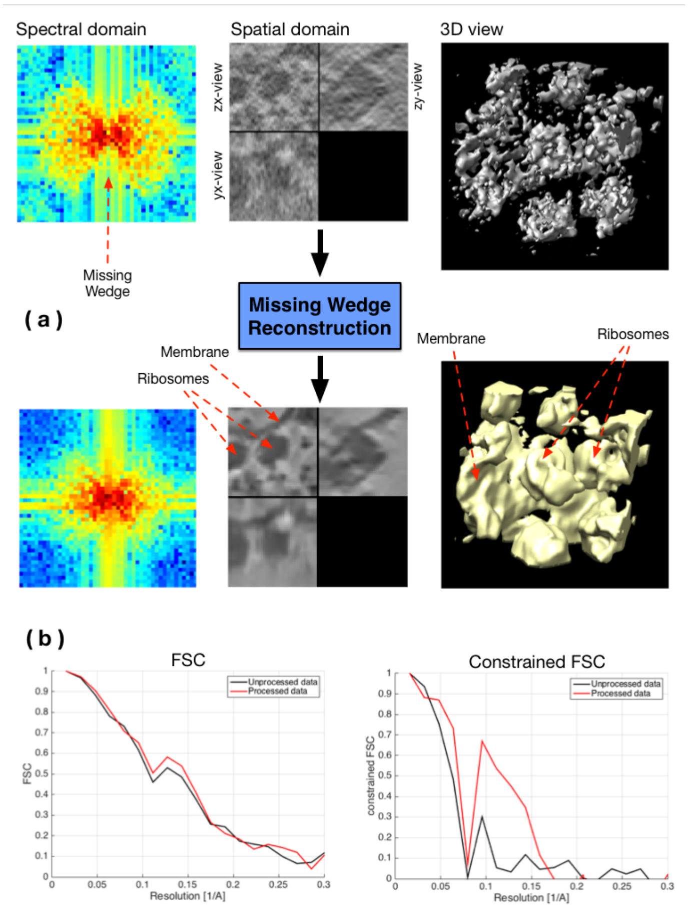


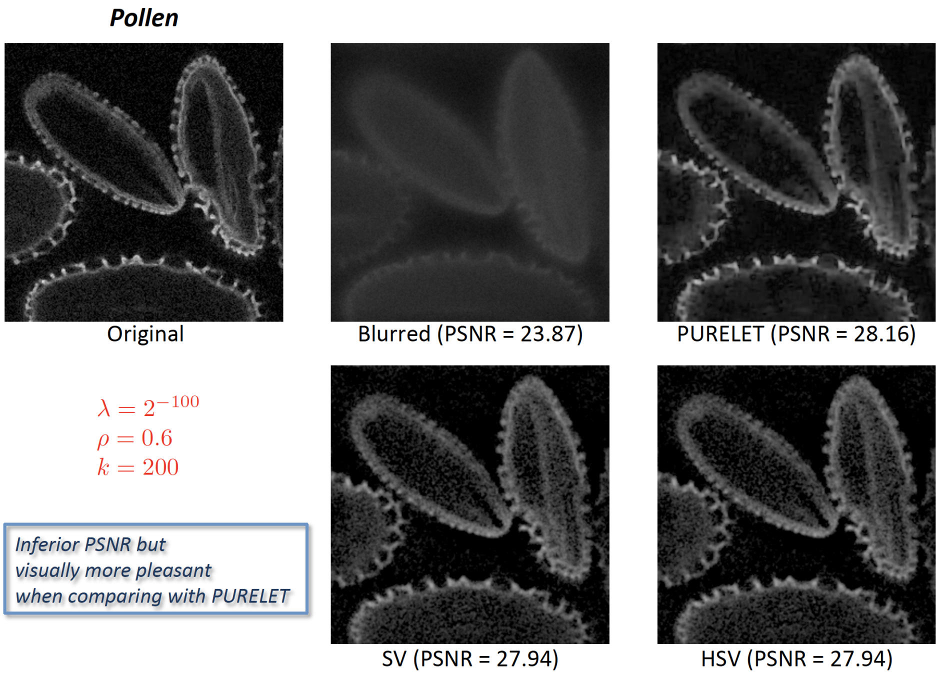
Figure 3. Experimental sub-tomogram containing ribosomes attached to a membrane. (a) Top row: original data in the spectral (left) and spatial (middle) domains and 3D view of the thresholded data (right). Bottom row: denoised data shown as above. (b) FSC and constrained FSC measures of the method input (in black) and output (in red).

7.2. Algorithms for deconvolving and denoising fluorescence 2D-3D microsocopy images

Participants: Hoai-Nam Nguyen, Charles Kervrann.

In this work, we proposed a restoration method for 2D and 3D fluorescence imaging using a novel family of convex regularizers. The proposed regularization functionals are based on the concept of sparse variation, that consists in penalizing jointly the image intensity and gradient at each pixel to favor the co-localization of non-zero intensities and gradients, by considering eventually higher-order differentiation operators. By construction, these regularizers possess interesting mathematical properties, namely convexity, invariance to scale, rotation, and translation as the well-known total variation regularization approach. It enables therefore to design efficient algorithms to solve the underlying deconvolution problem, which is in general large-scale in the context of fluorescence microscopy. We reformulated denoising or deconvolution (given the point spread function) as a minimization problem of a convex energy function composed of a quadratic data fidelity term and a sparse-variation-based regularity term under the constraint of positivity and maximum intensity value. In order to minimize this energy, we considered a primal-dual (proximal) algorithm based on the full splitting technique, which only involves first-order operators to cope with the large-scale nature of the problem. Experimental results on both 2D and 3D synthetic and real fluorescence images demonstrated that our method was able to produce very competitive deconvolution results, when compared to several competing methods such as the Schatten norm of the Hessian matrix, in terms of quantitative performance as well as visual quality and computational time. The method is able to process a 512×512 image in 250 ms (in Matlab) with a non optimized implementation and can process 3D images in a few minutes (with no code optimization technique and multithreading).

Collaborators: Cyril Cauchois (Innopsys company).



$\lambda = 2^{-100}$
$\rho = 0.6$
$k = 200$

Inferior PSNR but visually more pleasant when comparing with PURELET

Figure 4. Comparison (2D view) of sparse deconvolution (SparsEvolution) method with Purelet method [42] on the artificial blurred and noisy (Gaussian-Poisson noise) 3D pollen image ($256 \times 256 \times 32$) described in [42].

7.3. Colocalization and co-orientation in fluorescence imaging

Participant: Charles Kervrann.

In the context of bioimaging, colocalization refers to the detection of emissions from two or more fluorescent molecules within the same pixel of the image. It enables to quantify the protein-protein interactions inside the cell, just at the resolution limit of the microscope. Colocalization is an open problem for which no satisfying solution has been found up to now. Accordingly, we proposed an objective, robust-to-noise colocalization method (GcoPS – Geo-coPositioning System) which only requires the adjustment of a p-value that guarantees more reproducibility and more objective interpretation. It is based on the statistical analysis of the intersection (area or volume) between the two (2D or 3D) binary segmented images. In the context of super-localization imaging, we exploit the localization uncertainty of molecules to generate two input binary images. A the end, GcoPS handles 2D and 3D images, variable signal-to-noise ratios and any fluorescence image pair acquired with conventional or super-resolution microscopy. To our knowledge, no existing method offers the same robustness and precision level with such an easy control of the algorithm. In a recent study, we adapted the GcoPS framework to analyze the spatiotemporal molecular interactions from a set of 3D computed trajectories or motion vector fields (e.g., co-alignment), and then to fully quantify specific molecular machineries.

Collaborators: Frédéric Lavancier (University of Nantes, Laboratoire de Mathématiques Jean Leray),
Thierry Pécot (Hollings Cancer Center at the Medical University of South Carolina),
Jean Salamero and Liu Zengzhen (UMR 144 CNRS-Institut Curie).

7.4. Detection of transitions between diffusion models along biomolecule trajectories

Participants: Antoine Salomon, Charles Kervrann.

Recent advances in molecular biology and fluorescence microscopy imaging have made possible the inference of the dynamics of single molecules in living cells. When we observe a long trajectory (more than 100 points), it is possible that the particle switches mode of motion over time. Then, a goal is to estimate the temporal change-points, that is the distances at which a change of dynamics occurs. To address this issue, we proposed a non-parametric procedure based on test statistics [16], computed on local windows along the trajectory, to detect the change-points. This algorithm controls the number of false change-point detections in the case where the trajectory is fully Brownian. Our algorithm is user-friendly as there is only one parameter to tune, namely the sliding window size. A Monte Carlo study is proposed to demonstrate the performances of the method and also to compare the procedure to two competitive algorithms. Our method is much faster than previous methods which is an advantage when dealing with a large numbers of trajectories. With this computational approach, we analyzed real data depicting neuronal mRNPs (mRNAs in complex with mRNA-binding), and another very complex biological example, Gal-3 trafficking from the plasma membrane to different cellular compartments (acquired with Lattice Light Sheet microscopy). The analysis of multiple Gal-3 trajectories demonstrates nicely that there is not one typical signature. Biological trafficking events are very multifaceted. The algorithm was capable of identifying and characterizing the multistep biological movement, switching several times between subdiffusive, superdiffusive and Brownian motion.

Collaborators: Vincent Briane (UNSW Sydney, School of Medical Sciences, Australia),
Myriam Vimond (CREST ENSAI Rennes),
C.A. Valades Cruz and C. Wunder, (Institut Curie, PSL Research University, Cellular and Chemical Biology, U1143 INSERM / UMR 3666 CNRS).

7.5. Intracellular drift and diffusion coefficient estimation: a trajectory label-based approach

Participants: Antoine Salomon, Charles Kervrann.

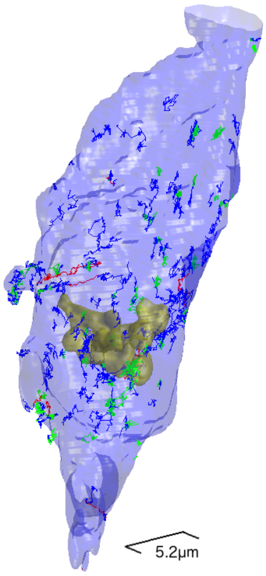


Figure 5. Change point detection over a set of three-dimensional trajectories of Galectin-3 proteins observed in HeLa cells with a Lattice Light Sheet microscope. The blue parts correspond to Brownian portions of the trajectory, red parts to superdiffusive portions, green parts to the subdiffusive portion. In light blue we plot the cell membrane and in yellow the Golgi apparatus for structural orientation.

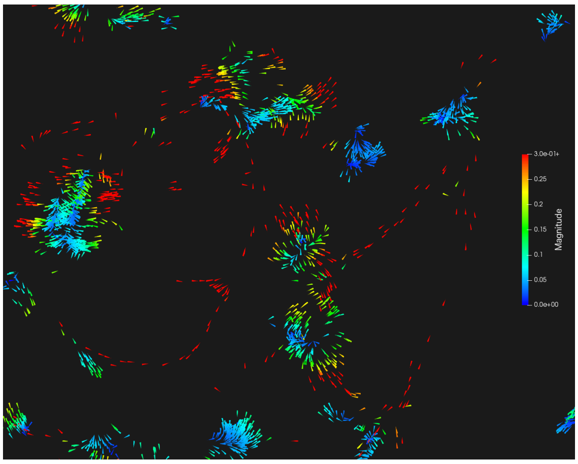


Figure 6. Estimation of a 2D drift field in 2D-TIRF microscopy of exocytosis biomolecules.

Dedicated computational methods for intracellular drift and diffusion map estimation rely on a scanning approach, using a sliding window (or cube, in the 3D case) in which all elementary particle movements taken from the trajectories inside the window/box are averaged [41]. This approach lacks precision, and a huge amount of trajectory data is needed to obtain significative results. In our new approach, we currently investigate several high-level features to obtain more satisfying results even from a small amount of data. First, we exploit classification of sub-trajectories (Brownian motion, superdiffusion, subdiffusion) obtained in the Lagrangian setting [16]. This classification is used to select trajectories of the same type in a local region and to guide weighting averaging. It allows us to calculate the drift separately on each type of movement, which avoids confusion between Brownian, confined and directed motions (see Fig. 6). Furthermore, the calculation is efficiently performed on trajectory-sliding kernels instead of scanning windows to save computing time in 2D and 3D. We considered square-box (sliding window), circle-box, cone-shaped, and Gaussian-shaped kernels.

Collaborators: Vincent Briane (UNSW Sydney, School of Medical Sciences, Australia),
Myriam Vimond (CREST ENSAI Rennes),
C.A. Valades Cruz and C. Wunder, (Institut Curie, PSL Research University, Cellular and Chemical Biology, U1143 INSERM / UMR 3666 CNRS).

7.6. 3D flow estimation in 3D fluorescence microscopy image sequences

Participants: Sandeep Manandhar, Patrick Bouthemy, Charles Kervrann.

Three-dimensional (3D) motion estimation for light-sheet microscopy is challenged by the heterogeneous scales and nature of intracellular dynamics. As typical examples in cell imaging, blebbing of a cell has small motion magnitude, while cell migration may show large displacement between frames. To tackle this problem, we have designed a two-stage 3D optical flow method. The first stage involves an extension of two-dimensional PatchMatch paradigm to 3D data, which in addition 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 is more robust to noise and to intensity variation between time steps. Only discrete displacements are estimated in this stage. Then, in a second stage, a 3D variational method enables to recover a sub-voxel dense 3D flow map. The variational approach still involves a data fidelity term based on the Census transform. The combination of the 3D PatchMatch and the 3D variational method is able to capture both large and small displacements. We assessed the performance of our method on data acquired with two different light sheet microscopes 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. 7, our method is able to successfully estimate various range of motion during cell migration and blebbing. A straightforward way to visualize the resulting 3D flow field is to use 3D glyphs (arrows), which represent vector direction and magnitude. However, it may not lead to easy understanding for visualization in 3D and over time. Consequently, we propose to visualize 2D projections of 3D flow field in 3 orthogonal planes (see Figure 7 a.1.2 and b.1.2). Using the standard Middlebury-style color coding for 2D optic flow, the motion field becomes easier to understand. This work is carried out in collaboration with UTSW Dallas int the frame of the Inria associated team CytoDI.

Collaborators: Philippe Roudot and Erik Welf (UTSW, Dallas, USA).

7.7. Connecting trajectories in TIRF images of rod-shaped bacteria

Participants: Yunjiao Lu, Charles Kervrann.

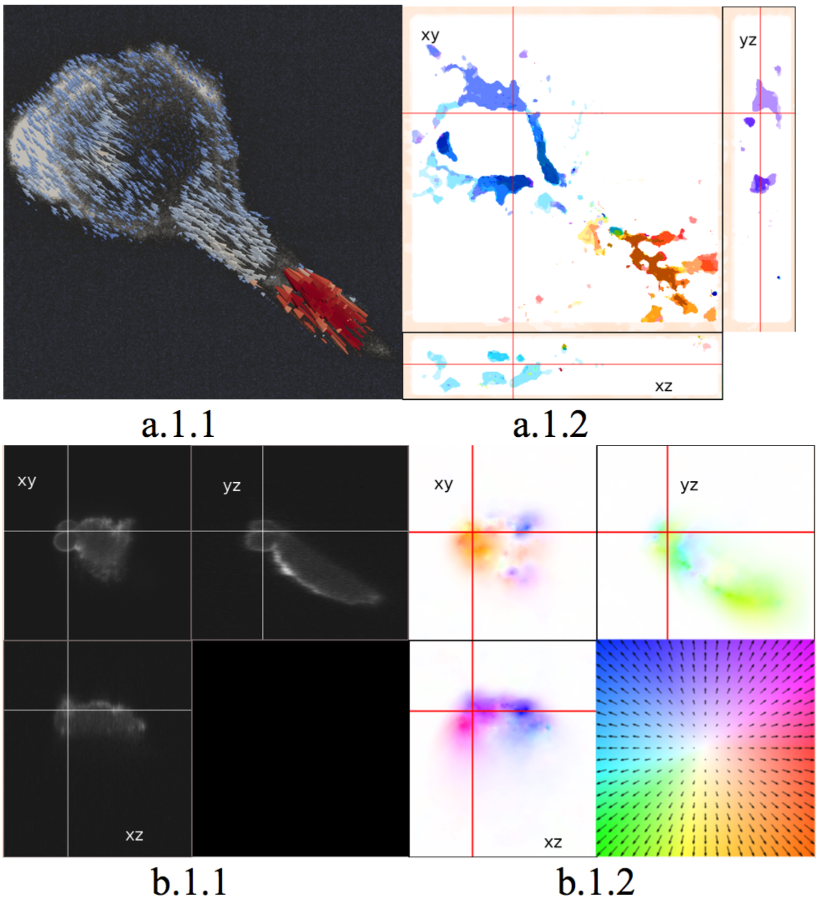


Figure 7. (a) Migration of MV3 melanoma cell in collagen; (a.1.1) 3D flow field in a slice of cell data represented with glyphs. Larger motion magnitude is coded in warm colors and smaller one in cold colors. (a.1.2) Motion map of collagen channel in 3 orthogonal planes (see b.1.2 for color code). (b) Blebbing of MV3 cell in a cover slip; (b.1.1) 3 orthogonal planes of cell data. (b.1.2) 3D computed flow field projected in 3 orthogonal planes. The motion map is color-coded as shown in lower-right corner of b.1.2. (input images by courtesy of Danuser lab, UTSW Dallas, USA).

In this work, we investigate the role of MreB protein involved in the cell wall construction in rod-shaped bacteria Bacillus. Previous work concerning the quantification of dynamics of MreB is performed from 2D TIRFM image sequences, ignoring the curvature of the rod-shaped cell wall and the thickness of TIRFM plane. To evaluate the effect of these approximations, we have developed a simulator to generate the trajectories on the surface of the cylinder. We assume that trajectories are modeled as:

$$dX = b(X)dt + \sqrt{2}B(X)dW,$$

where $b(X)$ is the drift tangent to the surface, W is a white noise, and $\sigma(X) = \frac{1}{2}B(X)B(X)^T$ is the diffusion tensor assumed to be isotropic. In our approach, the drift and diffusion tensor on the surface and on the projected plane are estimated respectively. Moreover, we propose to extrapolate the drift and diffusion tensor to the hidden region, to reconnect the segments we observe in the visible region, and then, to recover the entire trajectory on the surface of the cylinder in 3D (see Fig. 8).

Collaborators: Alain Trubuil and Pierre Hodara (INRA UR MAIAGE, Jouy-en-Josas),
Rut Carbllido-López and Cyrille Billaudeau (INRA, UR MICALIS, Jouy-en-Josas).

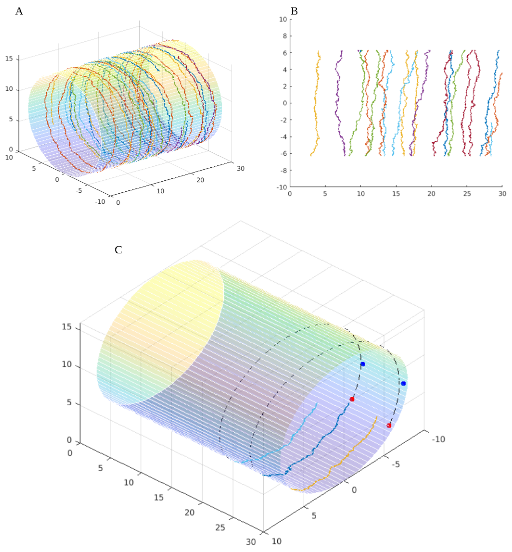


Figure 8. (A): Trajectories generated on the surface of the cylinder and (B): the view of TIRF microscope which is the projection of the dynamics on the cylinder near the support surface. The unity in the two figures is in pixel and in our TIRF images 1 pixel $\approx$ *64 nm. As the theoretical thickness of TIRF is 200 nm, the trajectories whose* z *coordinate is under 3.125 pixels are projected onto* $x - y$ *plan. Colors for different trajectories are random. (C): The connection result through the hidden region of three segments (light blue, dark blue and yellow in order of passage). Red points represent the extrapolated points using drift and diffusion tensor of segments on the surface, while blue points are the extrapolated points using projected drift and diffusion tensor on 2D plan.*

7.8. 3D registration for correlative light-electron microscopy

Participants: Bertha Mayela Toledo Acosta, Patrick Bouthemy.

Correlative light and electron microscopy (CLEM) enables the study of cells and subcellular elements in complementary ways by combining information on the dynamics and on the structure of the cell, provided a reliable registration between light microscopy (LM) and electron microscopy (EM) images is efficiently achieved. We have developed a general automatic registration method. Due to large discrepancies in appearance, field-of-view, resolution and position, a pre-alignment stage is required before any 3D fine registration stage. We first compute a 2D maximum intensity projection (MPI) of the LM stack along the z-axis, and we match 2D EM regions of interest (ROI), extracted from different EM slices, into the 2D LM-MPI image. From the resulting candidates, we estimate, using a robust criterion, the 2D xy-shift to pre-align the LM and EM stacks. Afterwards, a 3D affine transformation between 3D-LM-ROI and 3D-EM–ROI can be estimated using mutual information. We carried out experimental results on different real datasets of 3D correlative microscopy, demonstrating computational efficiency and overlay accuracy.

Collaborators: Xavier Heiligenstein (UMR 144 CNRS-Institut Curie),
Grégoire Malandain (Inria, Morpheme EPC, Sophia-Antipolis).

7.9. 3D Convolutional Neural Networks for macromolecule localization in cryo-electron tomograms of intact cells

Participants: Emmanuel Moebel, Charles Kervrann.

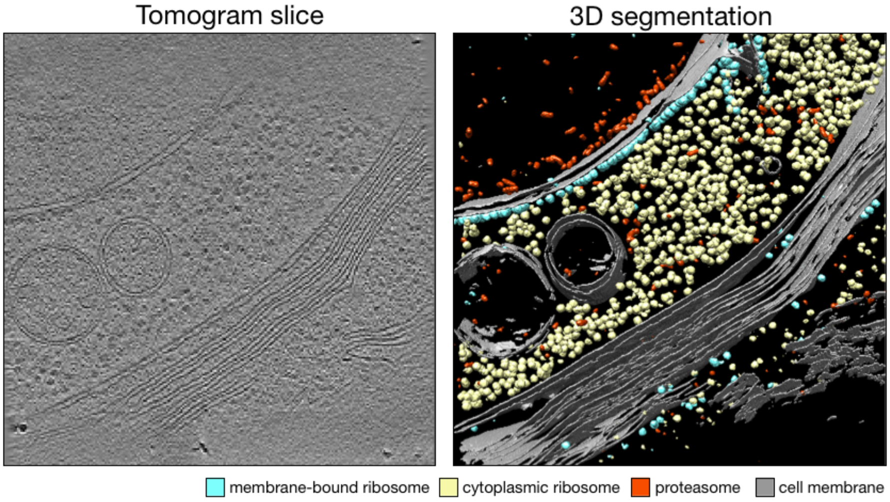


Figure 9. Experimental tomogram of a Chlamydomonas Reinhardtii cell (central slice) and the 3D segmentation obtained by our convolutional neural network. The network is able to identify 3 macromolecule classes (membrane-bound ribosome, cytoplasmic ribosome and proteasome) as well as the cell membrane (tomogram by courtesy of Max-Planck Institute, Martinsried, Germany).

In this study, we focus on macromolecule localization and classification in cryo-electron tomography images. Cryo-electron tomography (cryo-ET) allows one to capture 3D images of cells in a close to native state, at sub-nanometer resolution. However, noise and artifact levels are such that heavy computational processing is needed to access the image content. We propose a deep learning framework to accurately and jointly localize multiple types and states of macromolecules in cellular cryo-electron tomograms. We compare this framework to the commonly-used template matching method on both synthetic and experimental data. On synthetic image data, we show that our framework is very fast and produces superior detection results. On experimental data, the detection results obtained by our method correspond to an overlap rate of 86% with the expert annotations, and comparable resolution is achieved when applying subtomogram averaging. In addition, we show that our method can be combined to template matching procedures to reliably increase the number of expected detections. In our experiments, this strategy was able to find additional 20.5% membrane-bound ribosomes that were missed or discarded during manual annotation.

Collaborators: Damien Larivière (Fondation Fourmentin-Guilbert),
Julio Ortiz, Antonio Martinez (Max-Planck Institute, Martinsried, Germany).

7.10. Data assimilation and modeling of cell division mechanism

Participants: Ancageorgiana Caranfil, Charles Kervrann.

In this work, we focus on the dynamics of the spindle during cell division mechanism. We aim at understanding the role and interaction of the molecular key players at different scales, and their individual and collective impact on the global mechanism at the cell level. Our approach consists in creating a biophysical model for this mechanism, and uses data assimilation to adjust the model and optimally integrate the information from the observations. The overall spindle behavior is led by the spindle poles behavior. We thus proposed a new biophysical model for the spindle pole functioning during anaphase, that explains the oscillatory behavior with a minimum number of parameters. By mathematically analyzing our model, we confirmed some previous findings, such as the existence of a threshold number of active force-generator motors required for the onset of oscillations. We also confirmed that the monotonic increase of motor activity accounts for their build-up and die-down. We determined boundaries for the motor activity-related parameters for these oscillations to happen. This also allowed us to describe the influence of the number of motors, as well as physical parameters related to viscosity or string-like forces, on features such as the amplitude and frequency of oscillations. Lastly, by using a Bayesian approach to confront our model to experimental data, we were able to estimate distributions for our biological and biophysical parameters. A statistical reduction model approach was preliminary applied to select the most influential model parameters. These results give us insights on variations in spindle behavior during anaphase in asymmetric division, and provide means of prediction for phenotypes related to misguided asymmetric division. Data assimilation will be further used to properly combine the information given by our model and the experimental data.

Collaborators: Yann Le Cunff and Jacques Pécréaux (IGDR Institute of Genetics & Development of Rennes).

7.11. Motion saliency in videos

Participants: Léo Maczyta, Patrick Bouthemy.

The problem we have addressed appertains to the domain of motion saliency in videos. More specifically, we aim to extract the temporal segments of the video where motion saliency is present. It is a prerequisite for computing motion saliency maps in relevant images. It turns out to be a frame classification problem. A frame is classified as dynamically salient if it contains local motion departing from its context. Temporal motion saliency detection is relevant for applications where one needs to trigger alerts or to monitor dynamic behaviors from videos. The proposed approach handles situations with a mobile camera, and involves a deep learning classification framework after camera motion compensation. We have designed and compared two methods respectively based on image warping, and on residual flow. A baseline that relies on a two-stream network to process temporal and spatial information, but that does not use camera motion compensation, was also defined. Experiments on real videos demonstrate that we can obtain an accurate classification in highly challenging situations, and get significant improvement over the baseline. In particular, we showed that the compensation of the camera motion produces a better classification. We also showed that for the limited training data available, providing the residual flow as input to the classification network produces better results than providing the warped images.

Collaborators: Olivier Le Meur (Percept team, Irisa).

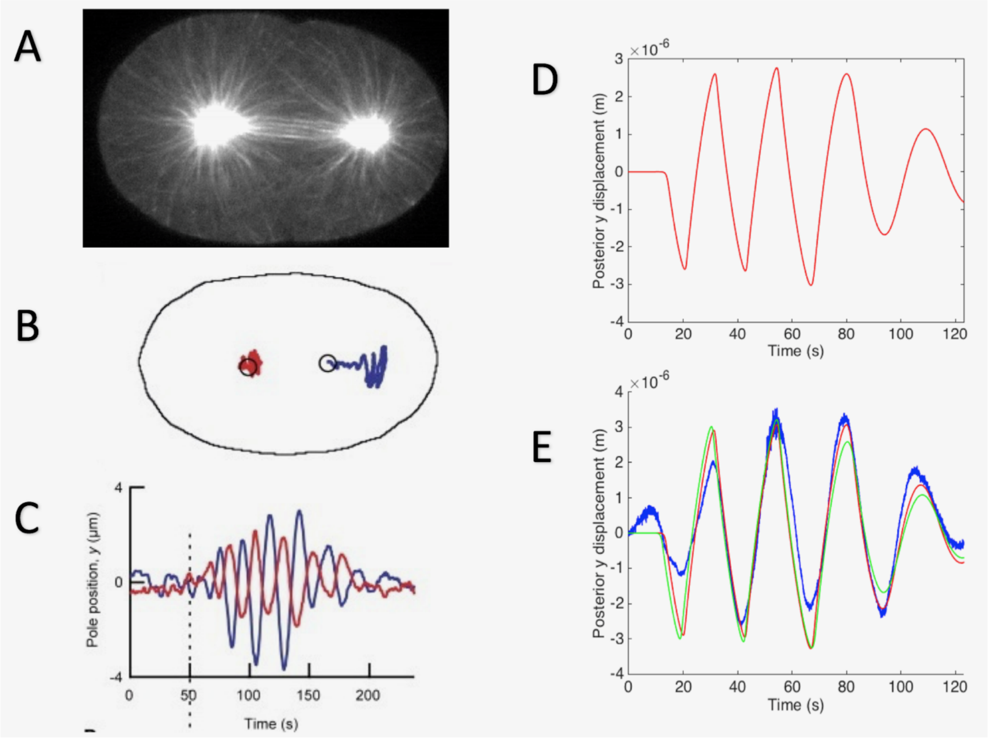


Figure 10. A. Cell division observed by fluorescence microscopy. B. and C. Tracking of the two spindle pole (anterior in red and posterior in blue). Oscillations of the poles during metaphase and anaphase. D. Simulation of oscillations using our model. E. Fitting of experimental data (in blue), and the two estimated curves with our method (minimum estimator in red, and mean estimator in green).

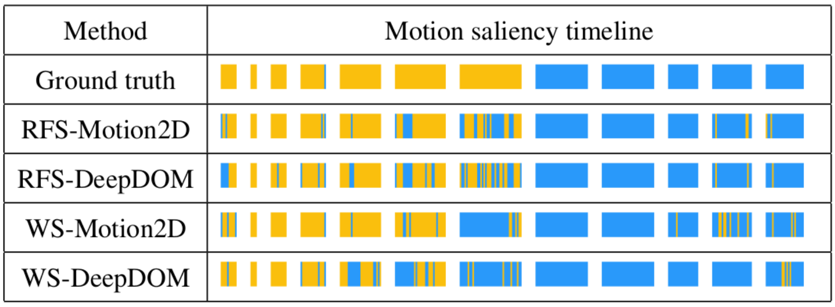


Figure 11. Timeline of the classification results supplied by the methods, for 12 videos of the testing set. Orange denotes salient frames, and blue non salient frames. Videos are separated by horizontal spaces. WS and RFS denote respectively the use of image warping and or residual flow in order to predict saliency. For dominant parametric motion estimation, the robust classical method Motion2D and the neural network DeepDOM were used.

8. Bilateral Contracts and Grants with Industry

8.1. Bilateral grants with industry

8.1.1. Fourmentin-Guilbert Foundation: Macromolecule detection in cryo-electron tomograms

Participants: Emmanuel Moebel, Charles Kervrann.

Collaborators: Damien Larivière (Fourmentin-Guilbert Foundation).

A three-year contract was established with Fondation Fourmentin-Guilbert to partly support the PhD thesis of Emmanuel Moebel. The Fondation Fourmentin-Guilbert strives for building a virtual E. coli bacteria. Information about the position of macromolecules within the cell is necessary to achieve such a 3D molecularly-detailed model. The Fondation Fourmentin-Guilbert supports cutting-edge *in-situ* cryo-electron tomography combined with image processing at the Max-Planck Institute of Biochemistry to map the spatial distribution of the ribosomes, and obtain structural information on the complexes they form *in-situ* with cofactors and other ribosomes. The objective of the project is to explore novel methods from the field of 3D shape retrieval for identifying and counting macromolecules within a tomogram. This project is also supported by Région Bretagne.

8.1.2. DGA contract on motion saliency analysis

Participants: Léo Maczyta, Patrick Bouthemy

Funding: DGA (National Defense Agency) (Oct 2017 - Sept 2020)

Collaborators:

This project funded by the DGA (Ministry of defense) concerns the PhD thesis (co-funding) carried out by Léo Maczyta. The goal is to develop motion saliency methods along three axes: temporal motion saliency detection, saliency map estimation, trajectory-based saliency detection.

9. Partnerships and Cooperations

9.1. Regional Initiatives

Région Bretagne: Identification, localization and enumeration of ribosomes within a tomogram by combining state-of-the-art denoising methods and object descriptor-based recognition (CATLAS, see Section 8.2.1) (PhD thesis of Emmnuel Moebel); motion saliency in video sequences (PhD thesis of Léo Maczyta).

BioGenOuest: Collaboration with S. Prigent (engineer) in charge of the organization of image processing services for Biogenouest bio-imaging facilities.

IGDR: Collaboration with J. Pecreaux, Y. Le Cunff (co-supervision of PhD thesis of A. Caranfil).

9.2. National Initiatives

9.2.1. France-BioImaging project

Participants: Charles Kervrann, Patrick Bouthemy.

The goal of the France-BioImaging project (http://france-bioimaging.org/) is to build a distributed coordinated French infrastructure for photonic and electronic cellular bioimaging, dedicated to innovation, training and technology transfer. High-computing capacities are needed to exhaustively analyse image flows. Serpico is co-head of the IPDM (Image Processing and Data Management) node of the FBI network composed of 6 nodes. In this context, we address the following scientific problems: i/ exhaustive analysis of bioimaging data sets; ii/ deciphering of key steps of biological mechanisms at organ, tissular, cellular and molecular levels through the systematic use of time-lapse 3D microscopy and image processing methods; iii/ storage and indexing of extracted and associated data and metadata through an intelligent data management system. Serpico recruited R&D engineers (2011-2016) to disseminate image processing software, to build the Mobyle@Serpico web portal and to manage the IGRIDA-Serpico cluster (200 nodes; batch scheduler: OAR; File management: Puppet/Git/Capistrano; OS: Linux Debian 7; User connexion: public ssh key) opened for end-users and dedicated to large scale computing and data sets processing (storage: 200 TeraBytes).

- **Coordinator:** CNRS (Jean Salamero, UMR 144 CNRS-Institut Curie).
- **Partners:** University of Paris-Diderot-Paris 7, Aix-Marseille University, University of Bordeaux, University of Montpellier, Institut Pasteur, Institut Curie, Inria, ENS Ulm, University of Paris Descartes, UPMC, Ecole Polytechnique, Inserm.
- **Funding:** Investissement d'Avenir Infrastructures Nationales en Biologie et Santé, ANR INBS-PIA 2011.
- **Total amount:** 26 000 Keuros (Inria Serpico: 606 Keuros).

9.2.2. ANR DALLISH project (2016-2020): Data Assimilation and Lattice LIght SHeet imaging for endocytosis/exocytosis pathway modeling in the whole cell

Participants: Charles Kervrann, Ancageorgiana Caranfil, Antoine Salomon.

Cutting-edge LLS microscopy represents the novel generation of 3D fluorescence microscopes dedicated to single cell analysis, generating extraordinarily high resolved and sharp, but huge 3D images and videos. One single live cell experiment in one single biological condition can result into up to one terabyte of data.The goal of the project is to develop new paradigms and computational strategies for image reconstruction and 3D molecule tracking/motion estimation. Furthermore, establishing correspondences between image-based measurements and features, stochastic motion models, and underlying biological and biophysical information remains a challenging task. In a larger perspective, the quantitative description of image data corresponding to protein transport will be a prerequisite for understanding the functioning of a cell in normal and pathological situations including cancer, viral infection and neurodegenerative diseases.

- **Coordinator:** Inria (Charles Kervrann)
- **Partners:** Inria (Serpico, Beagle, Fluminance teams), INRA MaIAGE Unit Jouy-en-Josas, Institut Curie (UMR 144 CNRS & U1143 Inserm UMR 3666) Paris
- **Funding:** ANR (Agence Nationale de la Recherche) PRC (Collaborative Research Project)
- **Total amount:** 440 Keuros (Inria Serpico: 170 Keuros).

9.2.3. Inria Project Labs, Exploratory Research Actions and Technological Development Actions

Participants: Charles Kervrann, Patrick Bouthemy.

In the frame of the "Naviscope" IPL project, we plan to develop original and cutting-edge visualization and navigation methods to assist scientists, enabling semi-automatic analysis, manipulation, and investigation of temporal series of multi-valued volumetric images, with a strong focus on live cell imaging and microscopy application domains. We will build Naviscope upon the strength of scientific visualization and machine learning methods in order to provide systems capable to assist the scientist to obtain a better understanding of massive amounts of information. Such systems will be able to recognize and highlight the most informative regions of the dataset by reducing the amount of information displayed and guiding the observer attention. We will address the three following challenges and issues:

- Novel machine learning methods able to detect the main regions of interest, and automatic quantification of sparse sets of molecular interactions and cell processes during navigation to save memory and computational resources.
- Novel visualization methods able to encode 3D motion/deformation vectors and dynamics features with color/texture-based and non-sub-resolved representations, abstractions, and discretization, as used to show 2D motion and deformation vectors and patterns.
- Effective machine learning-driven navigation and interaction techniques for complex functional 3D+Time data enabling the analysis of sparse sets of localized intra-cellular events and cell processes (migration, division, etc.).

Finally, we will have also to overcome the technological challenge of gathering up the software developed in each team to provide a unique original tool for users in biological imaging, and potentially in medical imaging.

- **Coordinator:** Serpico Inria team (Charles Kervrann)
- **Partners:** Aviz Inria team (Saclay); Beagle Inria team (Lyon), Morpheme Inria team (Sophia-Antipolis); Parietal Inria team (Saclay); Mosaic Inria team (Lyon); MaIAGE INRA Unit (Jouy-en-Josas); Institut Curie CNRS-UMR 144 (Paris).
- **Funding:** Inria Project Lab (from 2018-2022)

9.3. European Initiatives

9.3.1. Major European Organizations with which the Team have followed Collaborations

ESFRI Euro-BioImaging initiative: SERPICO is involved in the ESFRI Euro-BioImaging project, one of the four new biomedical science projects in the roadmap of the European Strategic Forum on Research Infrastructures (ESFRI). The mission of Euro-BioImaging is to provide access, service and training to state-of-the-art imaging technologies and foster the cooperation and networking at the national and European level including multidisciplinary scientists, industry, regional, national and European authorities. SERPICO also is involved in the French initiative, the so-called “France-BioImaging” (FBI) network which gathers several outstanding cellular imaging centers (microscopy, spectroscopy, probe engineering and signal processing) as described in Section 9.2.1.

- **Coordinator:** EMBL (Jan Ellenberg, Heideldberg, Germany)
- **Partners:** 15 european countries in 2017
- **Funding:** Member states of the European Union

9.4. International Initiatives

9.4.1. Informal International Partners

Collaboration with Max-Planck Institute, Martinsried (Germany), Dr. Julio Ortiz and Antonio Martinez: Detection and segmentation of macromolecules in cryo-electron tomography (project in progress with Emmanuel Moebel and Charles Kervrann).

9.4.2. Inria Associate Teams Not Involved in an Inria International Labs

9.4.2.1. CytoDI Inria Associated-Team

Title: Quantitative Imaging of Cytoskeleton Dynamics in 3D

International Partner:

University of Texas, SouthWestern Medical Center, Dallas (United States) - Gaudenz Danuser

Start year: 2016

See also: http://serpico.rennes.inria.fr/doku.php?id=research:cytodi

Participants: Sandeep Manandhar, Patrick Bouthemy, Charles Kervrann.

The main scientific goal of the Associated-Team is the spatiotemporal characterization and comparison of cytoskeleton networks involved in cell migration and observed through live cell imaging in three dimensions (3D). Those networks include the cytoskeleton, i.e., microtubules (MT), intermediate filaments (IF), dynamically resolvable by Bessel Beam Light Sheet fluorescent microscopy. The goal will be achieved through the design of local and global descriptors of the spatial conformation and deformation of the cytoskeleton. Subsequently, general metrics to compare and classify the MT and IF networks will be investigated. This study will be carried out on oncogenically transformed lung cancer epithelial cells.

In 2018, the objective of the visit of Sandeep Manandhar (PhD student) at UTSW Dallas (March 1-31, 2018) was to i) get a deeper understanding of bioimaging capacities and limitations in the context of biological studies, 2) to refine the design and validation of his approaches in this context and 3) returning from his visit with a clear vision for biologically relevant tools. For the Danuser lab, the objective was 1) to evaluate the breakpoint of path-match-based approach toward heterogeneous motion 2) to fill the gap between object tracking and generic motion estimation for studies such as actin speckle or collagen.

9.5. International Research Visitors

9.5.1. Visits to International Teams

Leo Maczyta attended a summer school (one week): ICVSS 2018 (International Computer Vision Summer School), July, 8-14, Sicily, Italy.

10. Dissemination

10.1. Promoting Scientific Activities

10.1.1. Scientific Events Organization

10.1.1.1. General Chair, Scientific Chair

- Charles Kervrann is co-General Chair (with R. Ober, Texas A&M), and head of the organizing committee of the “Quantitative BioImaging” (QBI’2019) conference (350 attendees, Rennes, January 2019).

10.1.1.2. Member of the Organizing Committees

- Charles Kervrann was the organizer of a mini-symposium at the SIAM Image Science conference (June, Bologna, Italy).

10.1.2. Scientific Events Selection

10.1.2.1. Member of the Conference Program Committees

- Charles Kervrann: Associated Editor for the ISBI’2018 conference, member of the scientific committee of the JIONC workshop (GdR Ondes, since 2014).
- Patrick Bouthemy: Associate Editor for the ISBI’2019 conference.

10.1.2.2. Reviewer

- Charles Kervrann: reviewer for ISBI’2018 and ISBI’2019.

10.1.3. Journal

10.1.3.1. Member of the Editorial Boards

- Charles Kervrann is Associate Editor of the IEEE Signal Processing Letters journal.

- Patrick Bouthemy is co-editor in chief of the open access journal Frontiers in ICT, specialty Computer Image Analysis.

10.1.3.2. Reviewer - Reviewing Activities

- Charles Kervrann: IEEE Signal Processing Letters, Bioinformatics, J. Mathematical Imaging and Vision, Nature Communications.
- Patrick Bouthemy: IEEE Signal Processing Letters, Computer Vision and Image Understanding, IEEE Transactions on Circuits and Systems for Video Technology.

10.1.4. Invited Talks

- Charles Kervrann: Invited talk at the "Reverse Problems in Reconstructing Single Cell Molecular Dynamics" workshop (October, Pisa, Italy), at the GdR ImaBio-MIV Days (November, IGBMC Strasbourg), at the BioGenOuest General Meeting (December, La Gacilly); Invited seminars at Institut Pasteur (February, Paris), Institut Curie CNRS-UMR 144 (June, Paris), European Molecular Biology Laboratory - EMBL (November, Heidelberg, Germany).

10.1.5. Leadership within the Scientific Community

- Charles Kervrann is member of the executive board of the GdR MIV/ImaBio (2588 - Microscopie Fonctionnelle du Vivant) CNRS.
- Patrick Bouthemy is member of the board of AFRIF (Association Française pour la Reconnaissance et l'Interprétation des Formes).

10.1.6. Scientific Expertise

- Charles Kervrann was expert for the national project evaluation in the framework of DIM ELICIT Institut Pasteur - Région Ile-de-France in 2018.

10.1.7. Research Administration

- Charles Kervrann is member of the executive board of the project committee of the Inria Rennes - Bretagne Atlantique centre since 2010. He is Co-head of the “BioImage Informatics” node (ANR France-BioImaging project http://france-bioimaging.org/), National Research Infrastructure for Biology and Health) since 2011.
- Patrick Bouthemy has been head of Excellence Lab (Labex) CominLabs (http://www.cominlabs.ueb.eu) since April 2014. He is deputy member of the board of directors and member of the selection and validation committee of the Images & Réseaux competitivity cluster (http://images-et-reseaux.com/). He is the Inria representative in the steering committee of the DGA-Inria collaboration. He is member of the board of AFRIF (Association Française pour la Reconnaissance et l'Interprétation des Formes), and member of the Research Committee of IMT Atlantique.

10.2. Teaching - Supervision - Juries

10.2.1. Teaching

Charles Kervrann:

- Engineer Degree: Genomics and Informatics, 4.5 hours, Ecole Nationale Supérieure des Mines de Paris.
- Master: From Bioimage Processing to BioImage Informatics, 5 hours, coordinator of the module (30 hours), Master 2 Research IRIV, Telecom-Physique Strasbourg and University of Strasbourg.
- Master: Geometric Modeling for Shapes and Images, 6 hours, Master 2 Research SISEA, University of Rennes 1.
- Engineer Degree and Master 2 Statistics and Mathematics: Statistical Models and Image Analysis, 37 hours + 15 hours (TP, Emmanuel Moebel), 3rd year, Ecole Nationale de la Statistique et de l'Analyse de l'Information (ENSAI), Rennes.

Patrick Bouthemy:

- Master: Analysis of Image Sequences, 18 hours, Master 2 Research SISEA, ISTIC & University of Rennes 1.
- Master: Video Indexing, 3 hours, Master 2 Research Computer Science, ISTIC & University of Rennes 1.
- Engineer Degree and Master 2 Research IRIV: Motion Analysis, 12 hours, Telecom-Physique Strasbourg & University of Strasbourg.

10.2.2. Supervision

- Emmanuel Moebel, new strategies for the nonambiguous identification and enumeration of macromolecules in cryo-electron tomograms, started in November 2015, supervised by Charles Kervrann.
- Ancageorgiana Caranfil, data assimilation methods for cell division mechanisms and molecule trafficking analysis, started in December 2016, supervised by Charles Kervrann and Yann Le Cunff.
- Sandeep Manandhar, optical flow methods for 3D fluorescence imaging, started in October 2016, supervised by Patrick Bouthemy and Charles Kervrann.
- Yunjiao Lu, intracellular dynamics and super-resolution imaging: analysis of bacteria wall at the molecular scale, started in October 2017, supervised by Charles Kervrann and Rut Carballido-Lopez.
- Antoine Salomon, statistical aggregation for image analysis in fluorescence microscopy and super-resolution, started in November 2017, supervised by Charles Kervrann.
- Léo Maczyta, motion saliency in video sequences, started in October 2017, supervised by Patrick Bouthemy and Olivier Lemeur.

10.2.3. Juries

Referee/Reviewer of HdR thesis: Michèle Gouiffès (University of Paris-Sud)[P. Bouthemy]

Referee/Reviewer of PhD thesis: Yves Michels (University of Strasbourg, supervised by M. Tajine) [C. Kervrann], Anne-Sophie Macé (University of Paris Descartes, supervised by L. Moisan) [C. Kervrann].

President of PhD thesis jury: F. Deslandes (INRA, supervised by B. Laroche)[P. Bouthemy].

External examiner in international labs: Felix Zhou (PhD thesis, University of Oxford, supervised by Prof. X. Lu and J. Rittscher)[P. Bouthemy]

11. Bibliography

Major publications by the team in recent years

[1] A. BASSET, J. BOULANGER, J. SALAMERO, P. BOUTHEMY, C. KERVRANN. *Adaptive spot detection with optimal scale selection in fluorescence microscopy images*, in "IEEE Transactions on Image Processing", November 2015, vol. 24, n^{o} 11, 16 p. [*DOI :* 10.1109/TIP.2015.2450996], https://hal.inria.fr/hal-01248290

[2] A. BASSET, P. BOUTHEMY, J. BOULANGER, F. WAHARTE, J. SALAMERO, C. KERVRANN. *An extended model of vesicle fusion at the plasma membrane to estimate protein lateral diffusion from TIRF microscopy images*, in "BMC Bioinformatics", 2017, vol. 18, n^{o} 1, 352 p. [*DOI :* 10.1186/S12859-017-1765-Y], https://hal.inria.fr/hal-01561310

[3] J. BOULANGER, A. GIDON, C. KERVRANN, J. SALAMERO. *A patch-based method for repetitive and transient event detection in fluorescence imaging*, in "PLoS ONE", Oct 2010, vol. 5, n^{o} 10 [*DOI :* 10.1371/JOURNAL.PONE.0013190]

[4] J. Boulanger, C. Kervrann, P. Bouthemy. *Space-time adaptation for patch based image sequence restoration*, in "IEEE Transactions on Pattern Analysis and Machine Intelligence", 2007, vol. 29, n^o 6, pp. 1096–1102

[5] J. Boulanger, C. Kervrann, P. Bouthemy. *A simulation and estimation framework for intracellular dynamics and trafficking in video-microscopy and fluorescence imagery*, in "Medical Image Analysis", 2009, vol. 13, pp. 132–142

[6] J. Boulanger, C. Kervrann, P. Bouthemy, P. Elbau, J.-B. Sibarita, J. Salamero. *Patch-based nonlocal functional for denoising fluorescence microscopy image sequences*, in "IEEE Transactions on Medical Imaging", Feb 2010, vol. 29, n^o 2, pp. 442-453 [*DOI :* 10.1109/TMI.2009.2033991]

[7] P.M. Carlton, J. Boulanger, C. Kervrann, J.-B. Sibarita, J. Salamero, S. Gordon-Messer, J. Haber, S. Haase, L. Shao, L. Winoto, A. Matsuda, P. Kner, S. Usawa, Y. Strukov, M. Gustafsson, Z. Kam, D. Agard, J.W. Sedat. *Fast live simultaneous multiwavelength four-dimensional optical microscopy*, in "Proc Natl Acad Sci USA", Sep 2010, vol. 107, n^o 37, pp. 16016-16022 [*DOI :* 10.1073/pnas.1004037107]

[8] N. Chenouard, I. Smal, F. De Chaumont, M. Maska, I. F. Sbalzarini, Y. Gon, J. Cardinale, C. Carthel, S. Coraluppi, M. Winter, A. R. Cohen, W. J. Godinez, K. Rohr, Y. Kalaidzidis, L. Liang, J. Duncan, H. Shen, Y. Xu, K. Magnusson, J. Jalden, H. M. Blau, P. Paul-Gilloteaux, P. Roudot, C. Kervrann, F. Waharte, J.-Y. Tinevez, S. L. Shorte, J. Willemse, K. Celler, G. P. Van Wezel, H.-W. Dan, Y.-S. Tsai, C. Ortiz De Solorzano, J.-C. Olivo-Marin, E. Meijering. *Objective comparison of particle tracking methods*, in "Nature Methods", 2014, vol. 11, n^o 3, pp. 281–289 [*DOI :* 10.1038/nmeth.2808], https://hal.inria.fr/hal-00932869

[9] T. Crivelli, B. Cernuschi-Frias, P. Bouthemy, J.-F. Yao. *Motion Textures: Modeling, Classification, and Segmentation Using Mixed-State*, in "SIAM Journal on Imaging Sciences", December 2013, vol. 6, n^o 4, pp. 2484-2520 [*DOI :* 10.1137/120872048], https://hal.inria.fr/hal-00931667

[10] A. Gidon, S. Bardin, B. Cinquin, J. Boulanger, F. Waharte, L. Héliot, H. De La Salle, D. Hanau, C. Kervrann, B. Goud, J. Salamero. *A Rab11A/myosin Vb/Rab11-FIP2 complex frames two late recycling steps of langerin from the ERC to the plasma membrane*, in "Traffic", April 2012, vol. 13, n^o 6, pp. 815-833 [*DOI :* 10.1111/j.1600-0854.2012.01354.x], https://hal.inria.fr/hal-00782005

[11] C. Kervrann, J. Boulanger. *Optimal spatial adaptation for patch-based image denoising*, in "IEEE Transactions on Image Processing", 2006, vol. 15, n^o 10, pp. 2866–2878

[12] C. Kervrann, J. Boulanger. *Local adaptivity to variable smoothness for exemplar-based image denoising and representation*, in "International Journal of Computer Vision", August 2008, vol. 79, n^o 1, pp. 45–69

[13] C. Kervrann. *PEWA: Patch-based Exponentially Weighted Aggregation for image denoising*, in "NIPS - Neural Information Processing Systems", Montreal, Canada, Neural Information Processing Systems Foundation, December 2014, https://hal.inria.fr/hal-01103358

[14] T. Pécot, P. Bouthemy, J. Boulanger, A. Chessel, S. Bardin, J. Salamero, C. Kervrann. *Background Fluorescence Estimation and Vesicle Segmentation in Live Cell Imaging with Conditional Random Fields*, in "IEEE Transactions on Image Processing", February 2015, vol. 24, n^o 2, 14 p. [*DOI :* 10.1109/TIP.2014.2380178], https://hal.inria.fr/hal-01103126

Publications of the year

Doctoral Dissertations and Habilitation Theses

[15] B. M. TOLEDO ACOSTA. *Multimodal image registration in 2D and 3D correlative microscopy*, Rennes 1, May 2018, https://tel.archives-ouvertes.fr/tel-01868852

Articles in International Peer-Reviewed Journals

[16] V. BRIANE, C. KERVRANN, M. VIMOND. *Statistical analysis of particle trajectories in living cells*, in "Physical Review E ", June 2018, https://hal.inria.fr/hal-01961971

[17] H.-N. NGUYEN, V. PAVEAU, C. CAUCHOIS, C. KERVRANN. *A variational method for dejittering large fluorescence line scanner images*, in "IEEE Transactions on Computational Imaging", June 2018, vol. 4, n^o 2, 16 p. , https://hal.inria.fr/hal-01577139

[18] H.-N. NGUYEN, V. PAVEAU, C. CAUCHOIS, C. KERVRANN. *ATMAD : robust image analysis for Automatic Tissue MicroArray De-arraying*, in "BMC Bioinformatics", April 2018, vol. 19, n^o 148, 23 p. , https://hal.inria.fr/hal-01577238

[19] T. PÉCOT, L. ZENGZHEN, J. BOULANGER, J. SALAMERO, C. KERVRANN. *A quantitative approach for analyzing the spatio-temporal distribution of 3D intracellular events in fluorescence microscopy*, in "eLife", August 2018, https://hal.inria.fr/hal-01966817

[20] J.-M. PÉREZ-RÚA, O. MIKSIK, T. CRIVELLI, P. BOUTHEMY, P. H. S. TORR, P. PÉREZ. *ROAM: a Rich Object Appearance Model with Application to Rotoscoping*, in "IEEE Transactions on Pattern Analysis and Machine Intelligence", November 2018, https://hal.inria.fr/hal-01923059

Invited Conferences

[21] C. KERVRANN. *Computational methods for fluorescence microscopy and intracellular dynamics analysis*, in "Workshop on "Reverse Mathematical Methods for Reconstructing Molecular Dynamics in Single Cell"", Pisa, Italy, October 2018, https://hal.inria.fr/hal-01962608

[22] C. KERVRANN. *Computational methods for fluorescence microscopy and quantitative bioimaging*, in "NEUBIAS - Network of European Bioimage Analysts", Szeged, Hungary, January 2018, https://hal.inria.fr/hal-01962562

[23] C. KERVRANN. *GcoPS: a fast automatic colocalization method for 3D live cell imaging and super-resolution microscopy*, in "SIAM Conference on Imaging Science", Bologna, Italy, June 2018, https://hal.inria.fr/hal-01962601

International Conferences with Proceedings

[24] A. CARANFIL, Y. LE CUNFF, C. KERVRANN, J. PECREAUX. *Modelling oscillatory behavior in asymmetric division of C.elegans embryo*, in "Mathematical Biology Days", Besancon, France, June 2018, https://hal.inria.fr/hal-01970360

[25] C. KERVRANN. *GcoPS: a Geo-coPositionning System for live cell imaging and superresolution microscopy*, in "Quantitative BioImaging (QBI)", Gottingen, Germany, January 2018, https://hal.inria.fr/hal-01962578

[26] S. MANANDHAR, P. BOUTHEMY, E. WELF, P. ROUDOT, C. KERVRANN. *A sparse-to-dense method for 3D optical flow estimation in 3D light microscopy image sequences*, in "IEEE International Symposium on Biomedical Imaging (ISBI)", Washington DC, United States, April 2018, https://hal.inria.fr/hal-01960109

[27] E. MOEBEL, C. KERVRANN. *A Monte Carlo framework for denoising and missing wedge reconstruction in cryo-electron tomography*, in "4th International Workshop on Patch-based Techniques in Medical Imaging", Grenade, Spain, September 2018, https://hal.inria.fr/hal-01961938

[28] B. M. TOLEDO ACOSTA, X. HEILIGENSTEIN, G. MALANDAIN, P. BOUTHEMY. *Intensity-based matching and registration for 3D correlative microscopy with large discrepancies*, in "ISBI 2018 - IEEE 15th International Symposium on Biomedical Imaging", Washington, United States, IEEE, April 2018, pp. 493-496 [*DOI :* 10.1109/ISBI.2018.8363623], https://hal.inria.fr/hal-01930740

National Conferences with Proceedings

[29] L. MACZYTA, P. BOUTHEMY, O. LE MEUR. *Détection temporelle de saillance dynamique dans des vidéos par apprentissage profond*, in "RFIAP 2018 - Reconnaissance des Formes, Image, Apprentissage et Perception", Marne-la-Vallée, France, June 2018, pp. 1-8, https://hal.inria.fr/hal-01926351

Other Publications

[30] V. BRIANE, M. VIMOND, C. KERVRANN. *An overview of diffusion models for intracellular dynamics analysis*, December 2018, working paper or preprint, https://hal.inria.fr/hal-01966825

[31] V. BRIANE, M. VIMOND, C. A. VALADES CRUZ, A. SALOMON, C. WUNDER, C. KERVRANN. *A sequential algorithm to detect diffusion switching along intracellular particle trajectories*, December 2018, working paper or preprint, https://hal.inria.fr/hal-01966831

[32] F. LAVANCIER, A. POINAS, R. WAAGEPETERSEN. *Adaptive estimating function inference for non-stationary determinantal point processes*, 2018, working paper or preprint, https://hal.archives-ouvertes.fr/hal-01816528

[33] E. MOEBEL, C. KERVRANN. *A Monte Carlo framework for noise removal and missing wedge restoration in cryo-electron tomography*, December 2018, working paper or preprint, https://hal.inria.fr/hal-01966821

[34] E. MOEBEL, A. MARTINEZ, D. LARIVIÈRE, J. ORTIZ, W. BAUMEISTER, C. KERVRANN. *3D ConvNet improves macromolecule localization in 3D cellular cryo-electron tomograms*, December 2018, working paper or preprint, https://hal.inria.fr/hal-01966819

[35] J.-M. PÉREZ-RÚA, T. CRIVELLI, P. BOUTHEMY, P. PÉREZ. *Learning how to be robust: Deep polynomial regression*, November 2018, working paper or preprint, https://hal.inria.fr/hal-01923068

References in notes

[36] D. AXELROD. *Total Internal Reflection Fluorescent Microscopy in cell biology*, in "Traffic", 2004, vol. 2, pp. 4658–4668

[37] E. BETZIG, G. PATTERSON, R. SOUGRAT, O. LINDWASSER, S. OLENYCH, J. BONIFACINO, M. DAVIDSON, J. LIPPINCOTT-SCHWARTZ, H. HESS. *Imaging intracelluar fluorescent proteins at nanometer resolution*, in "Science", 2006, vol. 313, pp. 1642–1645

[38] A. CHESSEL, B. CINQUIN, S. BARDIN, J. SALAMERO, C. KERVRANN. *Computational geometry-based scale-space and modal image decomposition: application to light video-microscopy imaging*, in "Conf. on Scale Space and Variational Methods (SSVM'09)", Voss, Norway, June 2009, pp. 770–781

[39] T. CRIVELLI, P. BOUTHEMY, B. CERNUSCHI-FRIAS, J.-F. YAO. *Simultaneous motion detection and background reconstruction with a conditional mixed-state Markov random field*, in "International Journal of Computer Vision", 2011, vol. 94, n^{o} 3, pp. 295–316

[40] M. GUSTAFSSON, L. SHAO, P.M. CARLTON, R. WANG, I. GOLUBOVSKAYA, W. CANDE, D. AGARD, J.W. SEDAT. *3D resolution doubling in wide-filed fluorescence microscopy by structured illumination*, in "Biophysical J.", 2008, vol. 94, pp. 4957–4970

[41] N. HOZE, D. NAIR, E. HOSY, C. SIEBEN, S. MANLEY, A. HERRMANN, J.-B. SIBARITA, D. CHOQUET, D. HOLCMAN. *Heterogeneity of AMPA receptor trafficking and molecular interactions revealed by superresolution analysis of live cell imaging*, in "Proc. Natl. Academy Sciences", 2012, vol. 10, n^{o} 42, pp. 17052-17057

[42] J. LI, F. LUISIER, T. BLU. *PURE-LET image deconvolution*, in "IEEE Trans. Image Processing", 2018, vol. 27, n^{o} 1, pp. 92–105

[43] S. OZERE, P. BOUTHEMY, F. SPINDLER, P. PAUL-GILLOTEAUX, C. KERVRANN. *Robust parametric stabilization of moving cells with intensity correction in light microscopy image sequences*, in "10th International Symposium on Biomedical Imaging (ISBI)", IEEE, 2013, pp. 468–471

[44] M. PINOT, V. STEINER, B. DEHAPIOT, B.-K. YOO, F. CHESNEL, L. BLANCHOIN, C. KERVRANN, Z. GUEROUI. *Confinement induces actin flow in a meiotic cytoplasm*, in "Proceedings National Academy of Sciences USA", July 2012, vol. 109, n^{o} 29, pp. 11705–11710 [*DOI :* 10.1073/PNAS.1121583109], http://hal.inria.fr/inserm-00717415