IN PARTNERSHIP WITH:
CNRS

Institut Curie

Activity Report 2019

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

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

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1. Team, Visitors, External Collaborators

Research Scientists

Charles Kervrann [Team leader, Inria, Senior Researcher, HDR]
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Léo Maczyta [Inria]
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Emmanuel Moebel [Inria, until Jan 2019]
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Technical staff

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External Collaborators

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Sylvain Prima [Invited researcher, Inria, untile Dec 2019]

2. Overall Objectives

2.1. Glossary

LLSM (Lattice Light Sheet Microscopy): high resolution Structured Illumination Microscopy that uses Light Sheet Bessel Beam illumination [47].

TIRF (Total Internal Reflectance): 2D optical microscopy using evanescent waves and total reflectance [45].

STORM (Stochastic Optical Reconstruction Microscopy): high-resolution microscopy using stochastic photo-activation of fluorophores and adjustment of point spread functions.

PALM (Photo-Activated Localization Microscopy): high-resolution microscopy using stochastic photo-activation of fluorophores and adjustment of point spread functions [46].

Cryo-ET (Cryo-Electron Tomography): 3D representation of sub-cellular and molecular objects of 5-20 nanometers, 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 tens nanometers, in live. As a result, fluorescent microscopy and multimodal imaging (fluorophores at various wavelengths) have become the workhorse of modern biology. As a matter of fact, taking into account all the publications in the 10 most relevant journals in fundamental biology (those with highest IF) for the last 2018-2019 years, the ratio of experimental figures based on BioImage data is close to 70% (GBI EoE.V, Singapour, Sep 2019). 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, modeling 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 CNRS-UMR 144 Institut Curie (and in cooperation with the "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 CNRS-UMR 144 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 analyzing 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

In the past recent years, research carried out out together with the “Space Time imaging of Endomembranes and organelles Dynamics” team at CNRS-UMR 144Institut Curie contributed to a better understanding of the intracellular compartmentation, particularly in specialized model cells such as melanocytes and Langerhans cells of the epidermis, of the components and structural events involved in the biogenesis of their specialized organelles: melanosomes and Birbeck granules, respectively and to the understanding on how the dynamics of those structures relate to their physiological functions. These studies have started to highlight: i/ the measurement of 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, an issue that become more and more accessible, thanks to improvement in all domains related to live imaging. We need to address the following topics:

1. developing new bioimaging approaches to observe and statistically analyze such coordinated dynamics in live material;

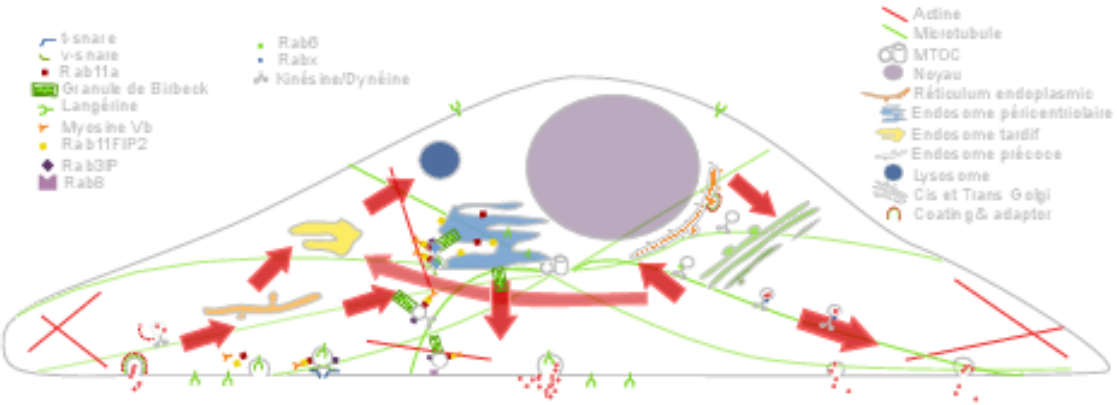

Figure 1. Cargo Langerin Trafficking controlled by Rab11A/Rab11FIP2/MyoVb platform.

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, how different machineries evolve together and thus coordinate (e.g. see Fig. 1). They help to decipher cell organization and function at different scales through an integrative workflow of methodological and technological developments. New approaches, such as optogenetics may even help controlling cell functions.

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 differentiation and cell transformation. Our methodological goal is also to link dynamics information obtained through diffraction limited light microscopy, at a time regime compatible with live cell imaging and close to biochemical molecular interactions. The overview of ultrastructural organization will be achieved by complementary electron microscopy methods which have also undergone a revolutionary improvement over the last decade. Image visualization and quantitative analysis are of course essential issues in this context.

4.2. Imaging and analysis of cytoskeleton 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 from primary tumor in blood (extravasation) and eventually the colonization of other organs and the formation of secondary tumors.

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. Another issue is of course to measure and understand how membrane protrusion and retraction keep the cell in homeostasis, which of course relate to membrane traffic.

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 how molecular trafficking is coordinated with cytoskeleton changes in these fundamental cellular functions.

5. Highlights of the Year

5.1. Highlights of the Year

- The SERPICO team organized the 7th International Conference on “Quantitative BioImaging” (QBI, https://www.quantitativebioimaging.com/qbi2019/) in January 2019 (350 attendees) in Rennes. The Quantitative BioImaging conference encourages scientific communication between researchers with interest in quantitative imaging in biological and biomedical sciences. A particular emphasis is to promote interdisciplinary interactions between physicists, computer scientists, chemists, mathematicians, and biologists.
- Emmanuel Moebel and Sandeep Manandhar defended their PhD theses in 2019.
- The DeepFinder algorithm was ranked first at the international SHREC’19 Challenge: "classification in cryo-electron tomograms" (Eurographics Workshop on 3D Object Retrieval – SHREC - 3D Shape Retrieval Contest (2019), Genova, Italy).

6. New Software and Platforms

6.1. 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. Colocalization amounts here 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 - Hollings Cancer Center at the Medical University of South Carolina, Charleston SC, USA
- Contact: Charles Kervrann
- Publication: Testing independence between two random sets for the analysis of colocalization in bioimaging
- URL: http://icy.bioimageanalysis.org/plugin/GcoPS

6.2. THOTH

Testing HypOtheses for diffusion TricHotomy

KEYWORDS: Photonic imaging - Fluorescence microscopy - Biomedical imaging - Classification - Statistical categorisation techniques - Statistics - Image sequence - Visual tracking

FUNCTIONAL DESCRIPTION: The THOTH software classifies biomolecule trajectories of biomolecules (computed with tracking algorithms) into three groups of diffusion: (i) free diffusion, (ii) subdiffusion or (iii) superdiffusion. Brownian motion corresponds to the NULL hypothesis. THOTH is a nonparametric three-decision test whose alternatives are subdiffusion and superdiffusion. Single and multiple testing procedures control respectively the type I error and the false discovery rate. THOTH 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: Statistical analysis of particle trajectories in living cells
- URL: https://team.inria.fr/serpico/software/thot/

6.3. SparseVolution

Sparse Variation for 2D Image Decovolution

KEYWORDS: Fluorescence microscopy - Image processing - Deconvolution - Inverse problem

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 the first (Total Variation) or second (Hessian Variation) derivatives to favor 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 algorithm has been dedicated to deconvolve very large 2D (e.g. 20 000 x 20 000) images or 3D images.

- Participants: Hoai Nam Nguyen, Charles Kervrann, Sylvain Prigent and Cesar Augusto Valades Cruz
- Partners: Innopsys - UMR 144 CNRS - Institut Curie
- Contact: Charles Kervrann

6.4. AiryscanJ

Reconstruction of AiryScan microscopy images

KEYWORDS: Image reconstruction - Fluorescence microscopy - Photonic imaging - Deconvolution - Image analysis

FUNCTIONAL DESCRIPTION: The AiryscanJ software enables to reconstruct a high resolution image from an array of multiple raw images acquired with the Airyscan technology (32 detectors). Airyscanning is a recent technique based on confocal laser scanning microscopy. The AiryscanJ software gathers four reconstruction methods (ISM, IFED, ISFED, ISM-Deconvolution) to compute a high resolution image: 1/ ISM amounts to summing the preliminarily registered raw images. 2/ IFED is the weighted difference between the inner detectors and the outer detector. 3/ ISFED is the weighted difference between the registered outer detectors and the original outer detectors. AiryscanJ automatically estimates the parameter controlling the IFED and ISFED algorithms. 4/ ISM-Deconvolution allows reconstructing a high resolution image by applying a deconvolution algorithm on the ISM image.

- Participants: Sylvain Prigent, Stephanie Dutertre and Charles Kervrann
- Partners: Université de Rennes 1 - CNRS
- Contact: Charles Kervrann
- URL: https://gitlab.inria.fr/serpico/airyscanj_bin

6.5. DeepFinder

Deep learning for macromolecule identification within 3D cellular cryo-electron tomograms

KEYWORDS: Image analysis - Deep learning - Cryo-electron microscopy - Object detection

FUNCTIONAL DESCRIPTION: DeepFinder is a computational approach that uses artificial neural networks to accurately and jointly localize multiple types and/or states of macromolecules in 3D cellular cryo-electron tomograms. DeepFinder leverages deep learning and outperforms the commonly-used template matching method on ideal data. On synthetic image data (SHREC 2019 challenge), DeepFinder is very fast and produces superior detection results when compared to other competitive deep learning methods, especially on small macromolecules. On experimental cryo-ET data depicting ribosomes, the detection results obtained by DeepFinder are consistent with expert annotations. We have got a high overlap of 86% and a similar structure resolution determined by subtomogram averaging.

- Participants: Emmanuel Moebel, Antonio Martinez and Charles Kervrann
- Partners: Max Planck Institute Martinsried - Fondation Fourmentin-Guilbert
- Contact: Charles Kervrann
- URL: https://gitlab.inria.fr/serpico/deep-finder

6.6. CPAnalysis

Change point detection algorithm for detecting switches of diffusion along 2D-3D single particle trajectories

KEYWORDS: Statistical modeling - Statistical physics - Fluorescence microscopy - Motion analysis

FUNCTIONAL DESCRIPTION: The change point detection algorithm is designed for detecting switches of diffusion along a given 2D-3D particle trajectory. We consider that the particle can switch between three main motion modes: 1/ Superdiffusion which occurs when the particle is transported via molecular motors along the cytoskeleton, 2/ Free diffusion (or Brownian motion) which arises when the particle evolves freely inside the cytosol, 3/ Subdiffusion observed when the particle is confined in a domain or evolves in a crowded area. The algorithm is a sequential nonparametric procedure based on a nonparametric three-decision test computed on local sliding windows along the trajectory.

- Participants: Antoine Salomon, Vincent Briane, Myriam Vimond, Jean Salamero and Charles Kervrann
- Partners: ENSAI - UMR 144 CNRS - Institut Curie
- Contact: Charles Kervrann
- Publication: A sequential algorithm to detect diffusion switching along intracellular particle trajectories
- URL: https://team.inria.fr/serpico/software/cpanalysis/

6.7. FlowScope

Optical flow computation for 3D fluorescence microscopy

KEYWORDS: Motion analysis - Fluorescence microscopy - Image analysis - Data visualization

FUNCTIONAL DESCRIPTION: The FLOWSCOPE software is able to estimate 3D motion between two fluorescence microscopy volumes. The underlying variational method amounts to minimizing an energy functional made up of two terms: a data term and a regularization term. The data term is derived from the continuous form of Census signature and the smoothness of the flow field is imposed by a L2 regularization term. The method is implemented for a single core CPU. FLOWSCOPE outputs three separate files corresponding to the motion vector components. The flow fields can be visualized with an appropriate color code named 3PHS. The 3PHS map projects the flow field onto the three orthogonal planes selected by the user. The projections of the vector field is color coded in the Hue (direction) and the Saturation (amplitude) spaces.

- Participants: Patrick Bouthemy, Charles Kervrann, Sylvain Prigent, Leo Maury and Philippe Roudot
- Partner: Danuser lab, University of Texas Southwestern, Dallas, USA
- Contact: Sandeep Manandhar
- Publication: 3D Flow Field Estimation and Assessment for Live Cell Fluorescence Microscopy
- URL: https://gitlab.inria.fr/smanandh/flowscope

6.8. MWR

Missing Wedge Restoration (MWR) and Noise Removal in 3D Cryo-Tomography

KEYWORDS: Image analysis - Inverse problem - Cryo-electron microscopy - Monte-Carlo methods - Bayesian estimation

FUNCTIONAL DESCRIPTION: The Missing Wedge Restoration (MWR) software enables to reduce the high amount of noise and artifacts observed in 3D cellular cryo-tomograms, induced by the presence of a missing wedge (MW) in the spectral domain. MWR takes as input a 3D tomogram derived from limited-angle tomography, and gives as output a 3D denoised and artifact compensated volume. The artifact compensation is achieved by filling up the MW with meaningful information. A Minimum Mean Square Error (MMSE) estimator is computed by applying a dedicated Markov Chain Monte-Carlo (MCMC) sampling procedure based on the Metropolis-Hasting algorithm. MWR can be used to enhance visualization or as a pre-processing step for image analysis, including segmentation and classification of macromolecules.

- Participants: Emmanuel Moebel and Antonio Martinez
- Partners: Max Planck Institute Martinsried - Fondation Fourmentin-Guilbert
- Contact: Charles Kervrann
- Publication: A Monte Carlo framework for missing wedge restoration and noise removal in cryo-electron tomography
- URL: https://gitlab.inria.fr/serpico/mwr

6.9. Platforms

6.9.1. Mobyle@serpico platform and software distribution

Participants: Sylvain Prigent, Léo Maury, Charles Kervrann.

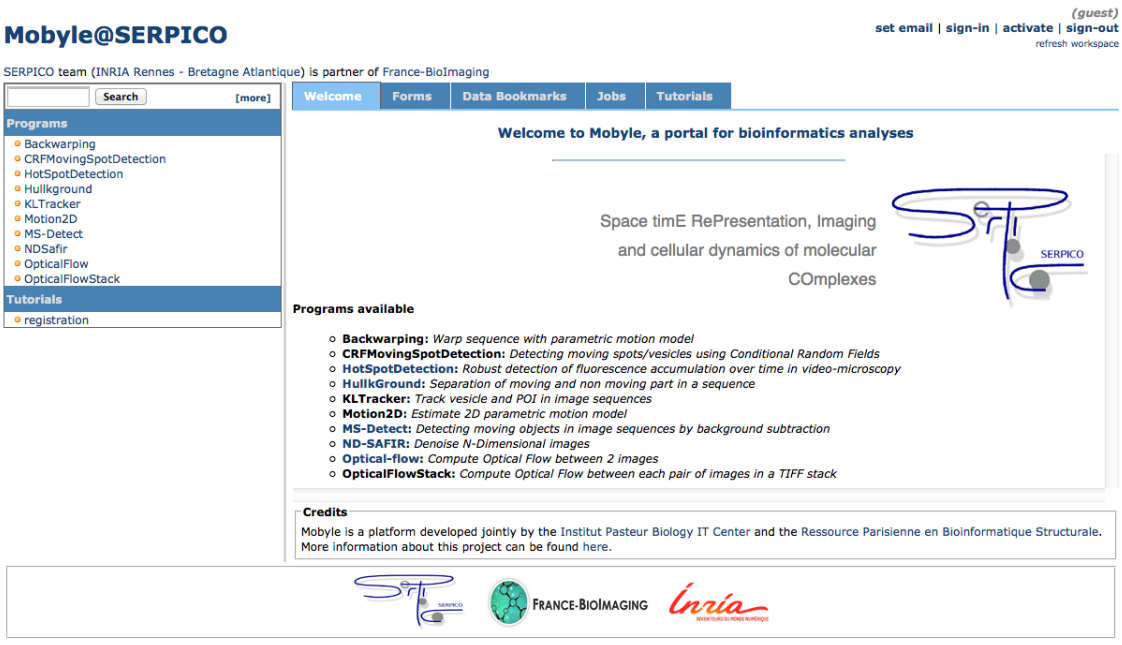

Figure 2. Mobyle@serpico web portal.

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/). A few of them are freely available on the team website https://team.inria.fr/serpico/software/ under a proprietary license.

Mobyle@serpico web portal: An on-line version (http://mobyle-serpico.rennes.inria.fr) of the image processing algorithms has been developed 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[9], ATLAS[1], HULLKGROUND[48], KLTRACKER[52], MOTION2D[51], MS-DETECT[49], ND-SAFIR[4], OPTICALFLOW and FLUX ESTIMATION [9]. The web interface makes our image processing methods available for biologists at Mobyle@serpico 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 [4], HULLKGROUND [48], MOTION2D [51], ATLAS [1]. The C-CRAFT [9], QUANTEV and GCOPS [19] algorithms have been developed for the image processing ICY platform (http://icy.bioimageanalysis.org/).

Partners: CNRS-UMR 144 Institut Curie & France-BioImaging (UMS 3714 CEMIBIO).

6.9.2. Bioimage-IT for bioimage management and processing

Participants: Sylvain Prigent, Cesar Augusto Valades Cruz, Léo Maury, Jean Salamero, Charles Kervrann.

New image acquisition systems generate large number of images and large volume images. Such data sets are hard to store, to process and to analyze for one user in a workstation. Many solutions exist for data management (e.g. Omero, OpenImadis), image analysis (e.g. Fiji, Icy, CellProfiler) and statistics (e.g R). Each of them has its specificities and several bridges have been developed between pieces of software. Nevertheless, in many use-cases, we need to perform analysis using tools that are available in different pieces of software and different languages. It is then tedious to create a workflow that brings the data from one tool to another. It needs programing skills and most of the time, a dedicated script using a dedicated file system for processed data management is developed. The aim of BioImage-IT is to create a "bandmaster" application that allow any scientist to annotate, process, and analyze data using only one single high level application. This BioImage-IT application is based on 3 components:

- an image annotation method based on a JSON file system,
- an image processing and analysis tools integration method based on Docker and XML commands description,
- an application with a graphical interface to easily annotate data, run processing tools, and visualize data and results.

This software architecture has three main goals. First, data are annotated using a file system. This means that data are not dependent on any software like a SQL database, and each experiment can then be stored in a different directory and can be moved from one server to another or to any drive with a simple copy pasting operation. Second, the processing tools are used as binary packages managed by the Docker technology. Docker enables to gently handle dependencies and several versions of the same tool. Any existing tool can then be integrated in its native programming language. Third, using a single "bandmaster" application allows one to automatically generate metadata for any processed data, improving the traceability and the repeatability of any experimental result.

BioImage-IT (https://project.inria.fr/bioimageit) is developed in the context of the France-BioImaging research infrastructure in coordination with the IPDM-FBI (Image Processing and Data Management) node in order to provide a standardized image processing tool set and data management for the imaging facilities.

Partners: CNRS-UMR 144 and U1143 INSERM/CNRS-UMR 3666, Institut Curie & France-BioImaging (UMS 3714 CEMIBIO).

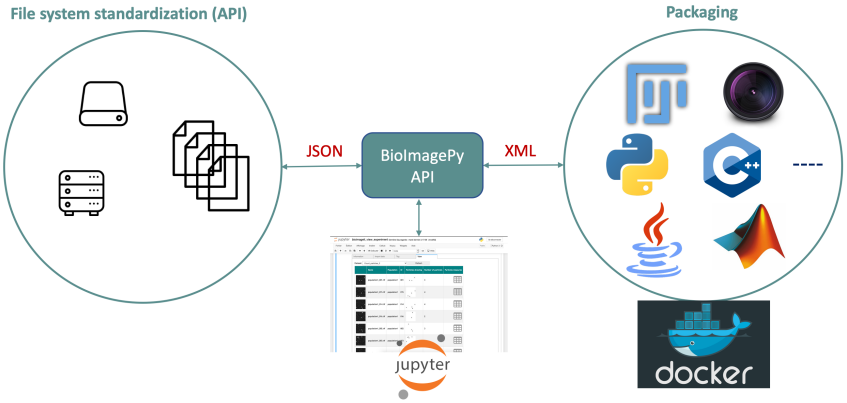

Figure 3. Scheme of the BioImage-IT components interactions.

7. New Results

7.1. Empirical SURE-guided microscopy super-resolution image reconstruction from confocal multi-array detectors

Participants: Sylvain Prigent, Charles Kervrann.

Recent confocal microscopes use an array detector instead of single point detector to take multiple views of the same sample. The microscope output is then an array of images, one image per detector. The array of images is then processed to build a single image with higher signal-to-noise ratio and higher resolution than a classical confocal microscope image. In the literature, several methods have been recently proposed to reconstruct the single high resolution image: i/ the ISM method combines array registration and Wiener deconvolution; ii/ the IFED method estimates a high resolution image by subtracting the outer detectors of the array to the inner detectors; iii/ the ISFED consists in subtracting the outer registered detectors and the outer raw images. In that context, we proposed a SURE-guided (Stein's unbiased risk estimation) estimation method to automatically select the parameter ϵ controlling the IFED and ISFED reconstruction algorithms (see Figure 4). We showed on real data that the proposed method is capable to achieve a resolution close to 100 nm without any deconvolution method.

Software: AiryscanJ (see Section 6.4).
Collaborator: S. Dutertre (IGDR – Institute Genetics & Development of Rennes).

7.2. Dense mapping of intracellular diffusion and drift from single-particle tracking data analysis

Participants: Antoine Salomon, Cesar Augusto Valades Cruz, Charles Kervrann.

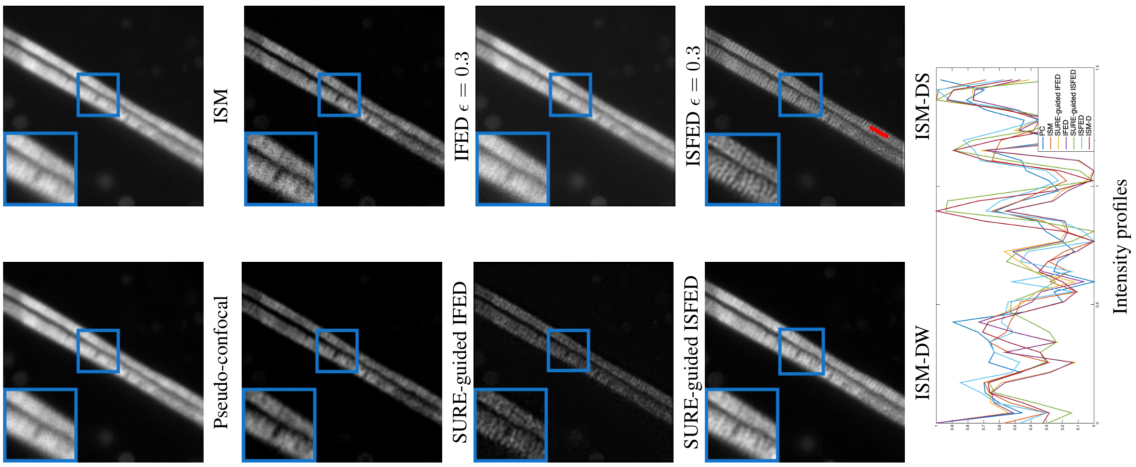

Figure 4. Results obtained on a c.elegans sample and intensity profiles for all the tested methods. The profile line is shown with a red line on the ISM-DS image (top right).

It is of primary interest for biologists to be able to locally estimate diffusion and drift inside a cell. In our framework, we assumed that particle motion is governed by the following Langevin equation: $\mathbf{dx} = \mathbf{b}(\mathbf{x})dt + \sigma(\mathbf{x})\mathbf{dw}$ where $\mathbf{x}$ denotes the 2D or 3D spatial coordinates of the particle, $\mathbf{b}$ the drift vector, σ the diffusion coefficient, and $\mathbf{w}$ the standard Gaussian white noise. In that context, we proposed a new mapping method inspired from [50] that developed a method providing results in the form of matrices by scanning the data by blocks, and from the framework in [5], dedicated to both classifying particle motion types and detecting potential motion switches along a trajectory. To avoid the calculation of both drift and diffusion in cell coordinates where no data is available, we replaced the scanning movement of an averaging window by a Gaussian window centered on trajectory points. Each drift vector and each diffusion coefficient are calculated at coordinates corresponding exactly to the coordinates given by the preliminary particle tracking, which provides more details. A nonparametric three-decision test enables to label trajectories or sub-trajectories [5]. This information is then used. to calculate drift and diffusion coefficient (or Kramers-Moyal coefficients) maps separately on each class of motion with the most appropriate diffusion models: confined motion (the particle is bound to a specific point), Brownian motion (the particle moves randomly), and directed motion (the particle moves in a given direction) (see Figure 5).

Software: THOTH and CPAnalysis (see Sections 6.2 and 6.6).

Collaborators: V. Briane (UNSW Sydney, School of Medical Sciences, Australia),
L. Leconte and J. Salamero (CNRS-UMR 144, Institut Curie, PSL Research University),
L. Johannes (U1143 INSERM / CNRS-UMR 3666, Institut Curie, PSL Research University),
E. Derivery (MRC laboratory of Molecular Biology, Cambridge, UK),
L. Muresan (Cambridge Advanced Imaging Centre, UK).

7.3. 3D tracking of endocytic and exocytic events using lattice light sheet microscopy

Participants: Cesar Augusto Valades Cruz, Ludovic Leconte, Jean Salamero, Charles Kervrann.

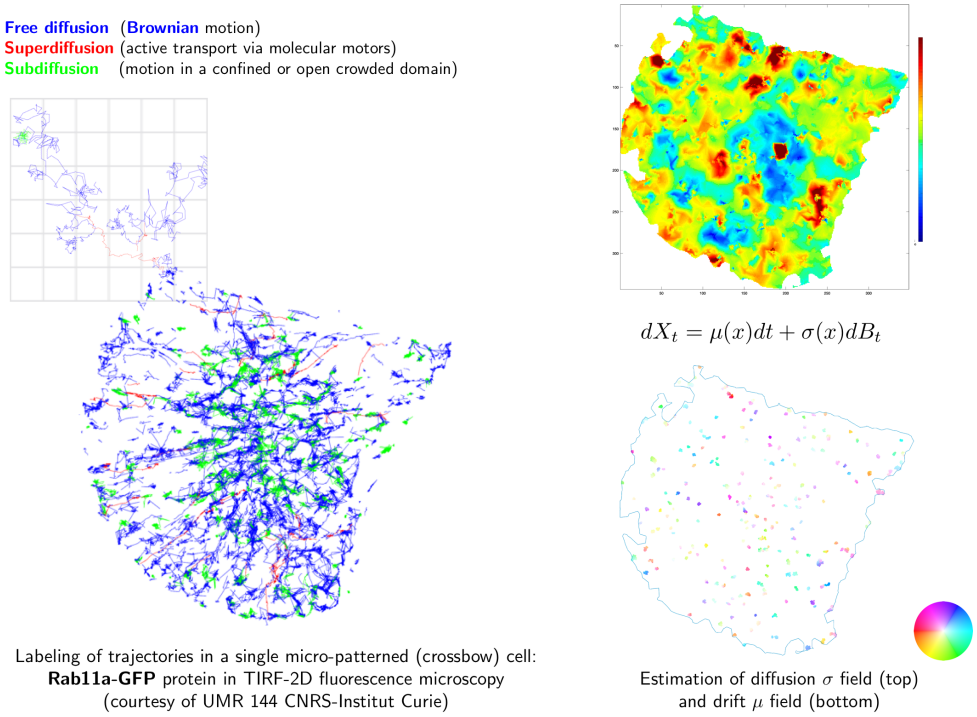

Figure 5. Intracellular diffusion and drift maps estimated from simulated tracking data (FluoSIM).

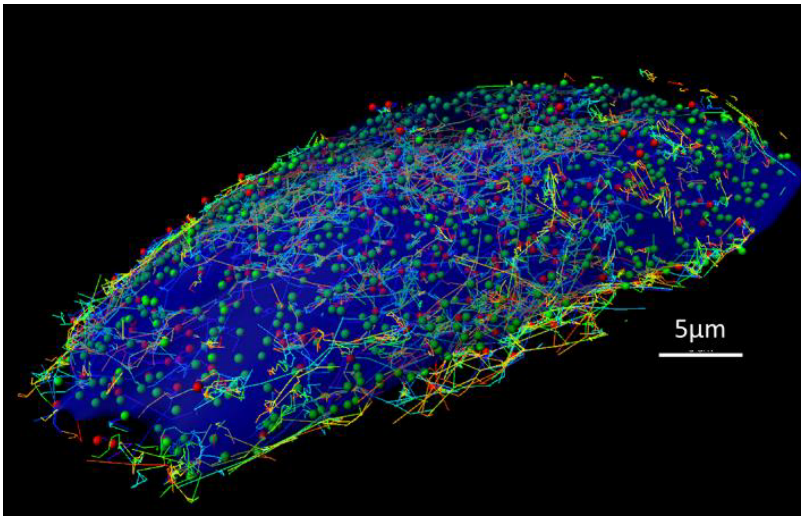

Figure 6. 3D tracking of Gal3-Atto647n (red) vs AP2-eGFP (green) adaptor protein in SUM159 cell.

The study of the whole cell dynamics of endocytic/exocytic-recycling events has proven difficult until recently because of lack of sensitivity, limited speed, photobleaching and phototoxicity associated with conventional imaging modalities. The Lattice Light Sheet Microscope (LLSM) allows to overcome these difficulties, yet reaching high spatial resolution. 3D images are captured for several minutes at a high acquisition frequency, and enables the study of signaling, transport, and stochastic self-assembly in complex environments. In addition, this imaging technique and 3D-tracking allow us to characterize the molecular machineries involved in the exocytosis and endocytosis mechanisms. We have the opportunity to observe a series of sequential events corresponding to the fusion with the plasma membrane (exocytosis) and the formation of endocytic carriers, including the trafficking of vesicles throughout the entire membrane system. We have got preliminary results of the coordination of vesicle recycling from the endosomal recycling compartment up to the plasma membrane using LLSM imaging and 3D tracking. In addition, we introduced a quantitative analysis of endocytosis dynamics of AP2 adaptor complex, Galectin-3 (see Figure 6) and Transferrin using single particle tracking analysis of 3D+time data. These case studies clearly demonstrated the advantage of lattice light sheet microscopy for imaging endocytic/exocytic events in single cells.

Software: THOTH and CPAnalysis (see Sections 6.2 and 6.6).
Collaborators: C. Wunder and L. Johannes (Institut Curie, PSL Research University, Cellular and Chemical Biology, U1143 INSERM / UMR 3666 CNRS).

7.4. 3D flow estimation in 3D fluorescence microscopy image sequences

Participants: Sandeep Manandhar, Patrick Bouthemy, Charles Kervrann.

We have proposed a variational approach for 3D optical flow computation from a pair of fluorescence microscopy volumes. This computational method has been extensively evaluated on appropriate simulated data. To simulate a volume pair with a ground truth flow field, we extended the Horn-and-Schunck optical flow method to 3D (3DHS). The computed flow field by 3DHS between two input images is then used to generate a new pair volume. The new source and target volumes along with the corresponding 3DHS flow fields serve as the dataset with the ground truth. The latter was used for the parameterization of our method and for the comparative study with the state-of-the art method proposed by Amat et al., 2012 [44].

Meanwhile, we proposed a novel error measure named SAE (for Structural Angular Error) for 3D optical flow, in absence of any ground truth flow field (see Figure 7). SAE measures the angular difference between the principal orientations of the structures present in the backward warped target and the source volumes at each voxel. We found out that the average of SAE (ASAE) and average of the end-point-error (a standard optical flow error in presence of ground truth) behave similarly.

We also integrated L_1 regularization in our variational approach. In contrast to our previous L_2 regularization approach, this method preserves discontinuities of the flow field. However, both of our methods are time demanding and not parallelizable in implementation. Then, we integrated our Census Signature-based data term with total variation regularization that also produces discontinuous flow fields. Consequently, we took the splitting approach for optimization. A gain of four times was obtained in the calculation speed with 12-core implementation of the new method, compared to our previous two methods, for still similar ASAE score.

We have also proposed two new methods for the visualization of the 3D flow fields, named 3DHSV and MIP-flow respectively. The 3DHSV method color codes the 2D projections of the flow field in the Hue and the Saturation color spaces, while mapping the off-the-plane motion to the Value space. MIP-flow also encodes the 2D projections to the Hue and the Saturation spaces. However, it only considers encoding the 2D projections of the 3D vectors corresponding to the maximum intensity points in the direction perpendicular to the projection plane. This work is carried out in collaboration with UTSW Dallas in the frame of the Inria associated team CytoDI.

Software: FlowScope (see Section 6.7).
Collaborators: P. Roudot, E. Welf and G. Danuser (UTSW Medical Center, Dallas, USA).

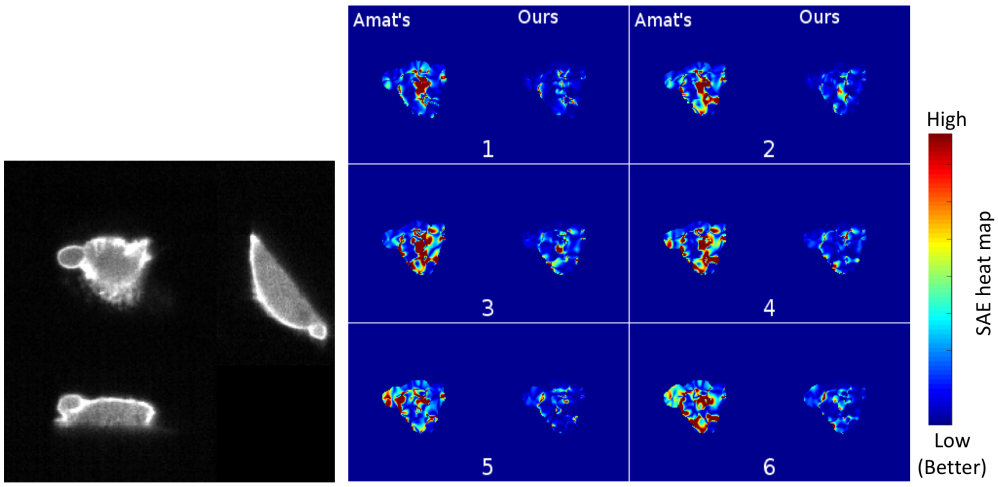

Figure 7. SAE maps (right) to compare our variational method to the Amat's method [44] applied to a 3D image pair (left) depicting a MV3 melanoma cell undergoing blebbing on a coverslip observed with Diagonally scanned Light-Sheet Microscopy (2.86 Hz sampling frequency, $300 \times 300 \times 83/50 \times 50 \times 30\mu m^3$*) (input images by courtesy of Danuser lab, UTSW Dallas, USA).*

7.5. Probabilistic overall reconstruction of membrane-associated molecular dynamics from partial observations in rod-shaped bacteria

Participants: Yunjiao Lu, Charles Kervrann.

Understanding the mechanisms that maintain the structure of rod-shaped bacteria is a challenging problem in cell biological research. Thanks to progress in molecular biology and microscopy (e.g Total Internal Reflection Fluorescence (TIRF) microscopy), we have the opportunity to observe the dynamics of the cell wall construction workers, that is the membrane-associated molecular machines (MMs). Due to the cylindrical form of the bacteria and the 2D selective visualization in TIRF microscopy, only around one third of the perimeter can be observed at a given time. Nevertheless, from the partial observed bacteria surface images, earlier studies showed that a fraction of the MMs performs directed motion, across the image field quasi-orthogonally to the cylinder axis.

Accordingly, we addressed the problem of the connection of motion segments on a cylindrical surface, assuming that one MM may re-enters into the observed region (OR), a certain period of time after having left the field of view. The directed MM motions are assumed as Brownian motion with drift. The birth and death events of the MMs are supposed to happen independently and uniformly on the surface. Given a set of observed segments entering and exiting the OR, we proposed a probabilistic framework to calculate the probabilities of the events of birth, death and re-entry, based on speed and diffusion of the motion and the time of exit and entry. Even though two third of the surface is hidden as shown in Figure 8, this framework allows us to derive a computational procedure aiming at connecting segments belonging to the same trajectory, and then recovering directed MMs dynamics on the whole surface. The performance of the method has been demonstrated on appropriate simulation data that mimics MMs dynamics observed in TIRF microscopy.

Collaborators: A. Trubuil and P. Hodara (INRA MaIAGE unit, Jouy-en-Josas),
R. Carballido-López and C. Billaudeau (INRA, UR MICALIS, Jouy-en-Josas).

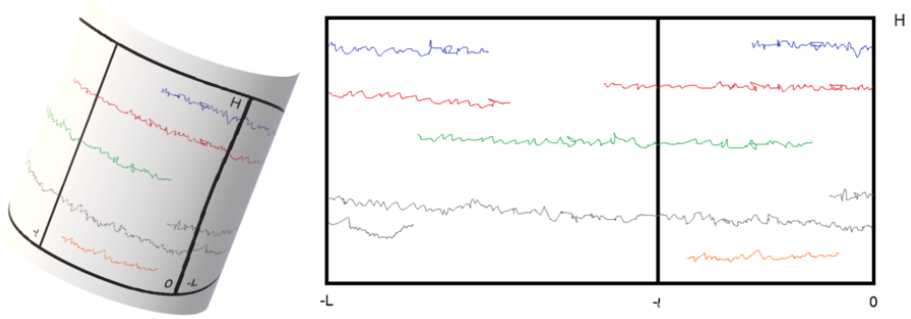

Figure 8. Trajectories on the cylinder and its 2D representation. The unobserved region is $(-L, -l] \times [0, H]$ *and the observed region is* $(l, 0] \times [0, H]$.

7.6. Data assimilation and modeling of cell division mechanism

Participants: Anca-Georgiana Caranfil, Charles Kervrann.

Asymmetric cell division is a complex process that is not yet fully understood. A very well-known example of such a division is the first division of C.elegans embryo. To improve our understanding of this process, we used mathematical modeling to study the first division of C.elegans embryo, both on wild type cells and under a wide range of genetic perturbations. Asymmetry is clearly visible at the end of the anaphase, as the mitotic spindle is off-center. The study of the mitotic spindle dynamics is, thus, a useful tool to gain insights into the general mechanics of the system used by the cell to correctly achieve asymmetric division. The overall spindle behavior is led by the spindle poles behavior. We proposed a new dynamic model for the posterior spindle pole that explains the oscillatory behavior during anaphase and confirms 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. By theoretically analyzing our model, 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 number 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 bio-physical 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. This model will be instrumental in probing the function of yet undocumented genes involved in controlling cell division dynamics.

Collaborators: Y. Le Cunff and J. Pécréaux (IGDR – Institute of Genetics & Development of Rennes).

7.7. Convolutional Neural Networks algorithms for calcium signal segmentation in astrocytes in 3D lattice light sheet microscopy

Participants: Anais Badoual, Charles Kervrann.

Astrocytes, glial cells of the central nervous system, are detectors and regulators of neuronal information processing. It is established that neuronal synapses are physical sites of intercellular contact that transmit and transform information in a very rapid and flexible way, playing a pivotal role for learning and memory formation as well as neurological diseases of the mammalian brain. Astrocytes are thought to integrate neuronal inputs and modulate information transfer between neurons. In particular, cytoplasmic calcium signaling in astrocytes is believed to be crucial for astrocyte-neuron communication. However, quantification

of intracellular calcium signals in astrocytes is hindered by the complexity of their cell shape, that consists of a cell body sprouting a highly ramified set of large to very fine protrusions called processes. Until recently, the quantification of intracellular propagation of calcium signal in astrocytes with fluorescent calcium indicators has been restricted to two dimensions, either 2D cell cultures or 2D slicing of a 3D setup. However it is not clear what amount of information is lost by ignoring the 3rd dimension in these experiments. The emergent 3D Lattice Light Sheet Microscopy (LLSM) is a powerful and promising technology (voxel size: 250nm x 250nm x 700nm; acquisition time: 200 frames per second) to give a much more complete and refined view of the dynamic behavior of calcium signaling in astrocytes inside living brain slices and in the intact mouse brain in vivo. Unfortunately, we lack image analysis tools to locate, segment, track and quantify the propagation of those 3D calcium signals in very ramified cell shapes.

In this context, we have started to develop an image processing tool for neurobiologists that 1) detects and segments calcium signals in 3D+time LLSM images, and 2) classifies these signals based on their 3D space-time morphological characterization. To do so, we focus on 3D convolutional network and machine learning techniques.

Collaborators: V. Nägerl and M. Arizono (Interdisciplinary Institute for Neuroscience, Bordeaux), H. Berry and A. Denisot (EPC BEAGLE, Inria Rhone-Alpes).

7.8. Geo-colocalization and coorientation in fluorescence super-resolution microscopy

Participants: Frédéric Lavancier, Reda Alami Chantoufi, Aymeric Lechevranton, Antoine Salomon, Charles Kervrann.

Colocalization aims at characterizing spatial associations between two fluorescently-tagged biomolecules by quantifying the co-occurrence and correlation between the two channels acquired in fluorescence microscopy. This problem remains an open issue in diffraction-limited microscopy and raises new challenges with the emergence of super-resolution imaging. In [19], we proposed an original method (GcoPS) that exploits the random sets structure of the tagged molecules to provide an explicit testing procedure. GcoPS requires the adjustment of a p-value that guarantees more reproducibility and more objective interpretation and takes as inputs two 2D or 3D binary segmented images. This year, we extended this approach to the estimation of local co-localization. This amounts to applying the statistical test on windows randomly drawn in the whole image. A multiple testing procedure allows us to compute a global partial colocalization score. Meanwhile, the excursion sets of colocalization score map estimated by Gaussian smoothing are very helpful to detect regions of interest corresponding to significant colocalization and anti-colocalization sites. This approach has been evaluated on STORM (Stochastic Optical Reconstruction Microscopy) images which provides several hundreds thousands of super-localized positions of individual molecules with an average accuracy of 10-20 nanometers (see Figure 9). Finally, the method has been successively extended to the geo-coorientation (or geo-coalignment) of 2D-3D vectors (optical flow, tensors) and trajectories to analyze the molecular interactions.

Software: GcoPS (see Section 6.1).

Collaborators: J. Salamero (CNRS-UMR 144, Institut Curie, PSL Research University), G. Bertolin (IGDR – Institute of Genetics & Development of Rennes), M. Lelek and C. Zimmer (Institut Pasteur, Paris).

7.9. Immersive and interactive visualization of 3D temporal data using a space time hypercube

Participants: Gwendal Fouché, Charles Kervrann.

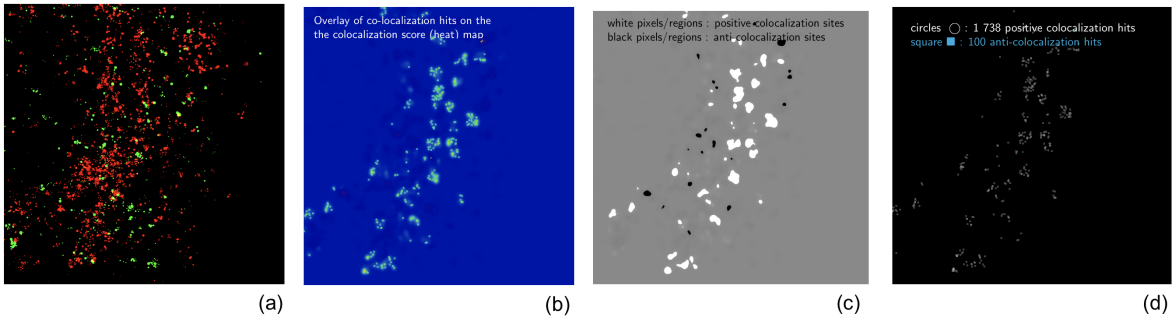

Figure 9. Illustration of geo-colocalization of two molecules (red/green channels) in STORM super-resolution microscopy (original image size: 4576 × 3564 pixels; pixel size: 3 nanometers). (a) Overlay of two channels (sub-region of the original pair); (b) colocalization hits overlaid on the score (heat) map; (c) excursion sets of detected colocalization (white) and anti-colocalization (black) sites overlaid on the score map; (d) detected co-localization (circles) and anti-co-localization (squares) hits.

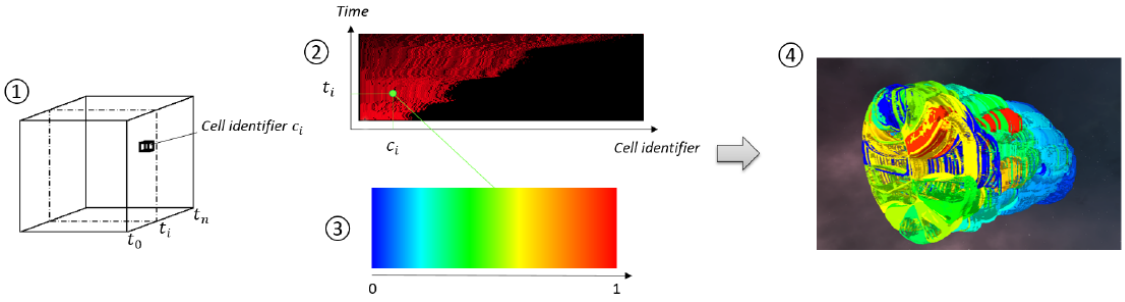

Figure 10. Flow diagram of the STC generation. In step 1, the user places the interactive clipping plan to get the desired cross-section. In step 2, camera parameters are automatically set in order to render the cross section at each time point. In step 3, the top image presents the output of the rendering operation, using the RGB channel to save cell identifiers; the bottom image is the result of an edge detection filter that will be useful for display. In step 4, the rendered images are stacked into a 3D texture. Each voxel contains a cell identifier, and its position in terms of depth indicates a time point t_i.

The analysis of multidimensional time-varying datasets, whose size grows as recording and simulating techniques advance, faces challenges on the representation and visualization of dense data, as well as on the study of temporal variations. In this context, we proposed an extension of the well-known Space-Time Cube (STC) visualization technique in order to visualize time-varying 3D spatial data acquired in 3D fluorescence microscopy, taking advantage of the interaction capabilities of Virtual Reality. The extended STC is based on a user-driven projection of the spatial and temporal information modeled as a 4D Space-Time Hypercube (STH). This projection yields a 3D STC visualization, which can also encode non-spatial quantitative data. Moreover, we proposed a set of tools allowing the user to manipulate the 3D STC that benefits from the visualization, exploration and interaction possibilities offered by immersive environments (see Figure 10). Finally, the extended STC has been integrated in a VR application for visualization of spatiotemporal biological data, illustrating the usage of the proposed visualization method for the morphogenesis analysis.

Collaborators: F. Argelaguet (EPC HYBRID, Inria Rennes),
E. Faure (Laboratory of Computer Science, Robotics and Microelectronics of Montpellier).

7.10. Unsupervised motion saliency map estimation based on optical flow inpainting

Participants: Léo Maczyta, Patrick Bouthemy.

We have addressed the problem of motion saliency in videos. Salient moving regions are regions that exhibit motion departing from their spatial context in the image, that is, different from the surrounding motion. In contrast to video saliency approaches, we estimate dynamic saliency based on motion information only. We propose a new unsupervised paradigm to compute motion saliency maps. The key ingredient is the flow inpainting stage. We have to compare the flow field in a given area, likely to be a salient moving element, with the flow field that would have been induced in the very same area with the surrounding motion. The former can be computed by any optical flow method. The latter is not directly available, since it is not observed. Yet, it can be predicted by a flow inpainting method. This is precisely the originality of our motion saliency approach.

Our method is then two-fold. First, we extract candidate salient regions from the optical flow boundaries. Secondly, we estimate the inpainted flow using an extension of a diffusion-based method for image inpainting, and we compare the inpainted flow to the original optical flow in these regions. We interpret the possible discrepancy (or residual flow) between the two flows as an indicator of motion saliency. In addition, we combine a backward and forward processing of the video sequence. The method is flexible and general enough, by relying on motion information only. Experimental results on the DAVIS 2016 benchmark demonstrate that the method compares favorably with state-of-the-art video saliency methods. Additionally, by estimating the residual flow, we provide additional information regarding motion saliency that could be further exploited (see Figure 11).

Collaborators: O. Le Meur (Percept team, IRISA, Rennes).

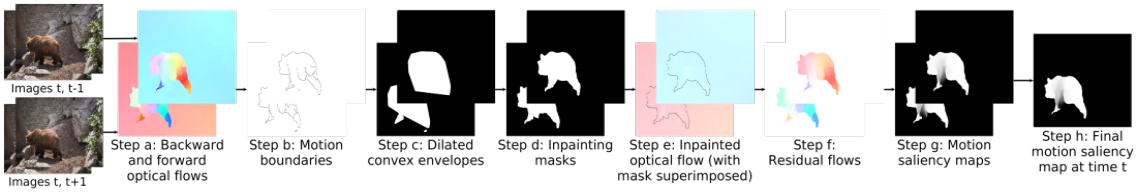

Figure 11. Workflow of the motion saliency estimation method.

8. Bilateral Contracts and Grants with Industry

8.1. Bilateral grants with industry

8.1.1. Contract with Fourmentin-Guilbert Foundation: Macromolecule detection in 3D cellular cryo-electron tomograms

Participants: Emmanuel Moebel, Charles Kervrann.
Duration: 5 months (Dec 2019 – Apr 2020).

The objective of the project is to improve the DeepFinder software dedicated to the detection and identification of macromolecules within 3D cellular cryo-electron tomograms. In collaboration with Fourmentin-Guilbert Foundation, the goal is to build cellular atlases of several organisms from localizations of macromolecules (see Software DeepFinder in Section 6.9).

Funding: Fourmentin-Guilbert Foundation.
Collaborators: D. Larivière & E. Fourmentin (Fourmentin-Guilbert Foundation), A. Martinez & W. Baumeister (Max Planck Institute, Martinsried, Germany).

8.1.2. Contract with DGA: Motion saliency analysis in videos

Participants: Léo Maczyta, Patrick Bouthemy.
Duration: 36 months (Oct 2017 – Sep 2020).

This project funded by the DGA (Ministry of defense) and Région-Betagne 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 (see Section 7.10).

Funding: DGA (National Defense Agency) and Région-Bretagne.

8.1.3. Contract with GATACA Systems: Super-resolution microscopy and in live cell imaging

Participants: Jean Salamero, Ludovic Leconte, Charles Kervrann.
Duration: 36 months (Jan 2017 – Dec 2019).

The objective of the project is to transfer innovations for Multi-Angle TIRFM (using Azymuthal TIRFM from Ilas2) and collaborate as "β-Test site" for SIM in Nipkow disk microscopy (product: Live-SR).

Funding: GATACA Systems company.
Collaborators: C. Gueudry (GATACA Systems), J. Boulanger (MRC Laboratory of Molecular Biology, Cambridge Biomedical Campus, UK).

8.1.4. Contract with CryoCapCell SA: 3D LIVE CLEM (Correlative Light and Electron Microscopy) to decipher fates and functions of exosomes in vivo

Participant: Jean Salamero.
Duration: 24 months (Oct 2018 – Sep 2020).

The objective of the project is to link dynamic biogenesis of intracellular membrane compartments with their ultrastructures. It combines fast high resolution photonic imaging (MA-TIRFM and fast high pressure freezing for 3D cryoEM. It requires adapted registration methods in 3D, in order to navigate through the multiple scales.

Funding: DIM–ELICIT Empowering LIfe sCiences with Innovative Technologies (Région Ile de France).
Collaborators: G. Van Niel (coordinator, Institute of Psychiatry and Neuroscience of Paris), G. Raposo (CNRS-UMR 144 Institut Curie PSL Research), X. Heiligenstein (CryoCapCell SA).

9. Partnerships and Cooperations

9.1. Regional Initiatives

9.1.1. Motion saliency analysis in videos

Participants: Léo Maczyta, Patrick Bouthemy.
Duration: 36 months (Oct 2017 – Sep 2020).

See Section 8.1.2.

Funding: DGA (National Defense Agency) and Région-Bretagne.

9.2. National Initiatives

9.2.1. France-BioImaging project

Participants: Sylvain Prigent, Patrick Bouthemy, Charles Kervrann, Jean Salamero.
Duration: 2011 – 2024.

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 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) (see Section 6.13).

Funding: Investissement d'Avenir, ANR INBS-PIA 2011.
Coordinator: CNRS (J. Salamero, UMS 3714 CEMIBIO & CNRS-UMR 144, Institut Curie, PSL Research University).
Partners: CNRS, University of Paris-Diderot-Paris 7, Aix-Marseille University, University of Bordeaux, University of Montpellier, Institut Pasteur, Institut Curie, Inria, ENS Paris, University of Paris Descartes, UPMC, Ecole Polytechnique, INSERM.

9.2.2. ANR NucleoPLASTIC: Plasticity of the Nuclear Pore Complex

Participant: Jean Salamero.
Duration: 48 months (Oct 2015 – Sep 2019).

In this project, we have deciphered molecular/structural changes on the nuclear face of the Nuclear Pore Complex, their dynamics during cell division, and highlighted their role in the dynamics of association with the heart of the pore with consequences on maintaining the integrity of the genome. This was possible through the development of a 3D localization software GenLoc3D (https://team.inria.fr/serpico/software/genloc3d/, FIJI/ImageJ plug-in).

Funding: ANR (Agence Nationale de la Recherche).
Coordinator: C. Dargemont (INSERM, Hopital St Louis, Paris).
Partners: CNRS-UMR 144, Institut Curie, PSL Research, Paris.

9.2.3. ANR DALLISH project: Data Assimilation and Lattice LIght SHeet imaging for endocytosis/exocytosis pathway modeling in the whole cell

Participants: Antoine Salomon, Anca-Georgiana Caranfil, Sandeep Manandhar, Cesar Augusto Valades Cruz, Patrick Bouthemy, Ludovic Leconte, Jean Salamero, Charles Kervrann.
Duration: 48 months (Oct 2016 – Sep 2020).

Cutting-edge Light Lattice Sheet 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 motion estimation and tracking. 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 (see Sections 7.2–7.6 and 7.8).

Funding: ANR (Agence Nationale de la Recherche) PRC (Collaborative Research Project).
Coordinator: C. Kervrann.
Partners: Inria (SERPICO, BEAGLE, FLUMINANCE teams), INRA MaIAGE Unit Jouy-en-Josas, Institut Curie (CNRS-UMR 144 & U1143 INSERM / UMR 3666) Paris.

9.2.4. Inria Project Labs (IPL / DEFI), Exploratory Research Actions and Technological Development Actions

9.2.4.1. NAVISCOPE: image-guided NAvigation and VISualization of large data sets in live cell imaging and microCOPy

Participants: Gwendal Fouché, Cesar Augusto Valades Cruz, Ludovic Leconte, Anais Badoual, Jean Salamero, Charles Kervrann.
Duration: 60 months (2018 – 2022).

In the frame of the "Naviscope" IPL project (https://project.inria.fr/naviscope/), our objective is 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. Naviscope, built upon the strength of scientific visualization and machine learning methods, will 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 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 and deformation vectors and dynamics features with color and texture-based and non-sub-resolved representations, abstractions, and discretization, as used to display 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.) (see Section 7.9).

Meanwhile, we address 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.

Funding: Inria (IPL / DEFI).

Coordinator: C. Kervrann.
Partners: AVIZ Inria team (Saclay); BEAGLE Inria team (Lyon), HYBRID Inria team (Rennes), MORPHEME Inria team (Sophia-Antipolis); MOSAIC Inria team (Lyon), PARIETAL Inria team (Saclay), SERPICO Inria team (Rennes); MaIAGE INRA Unit (Jouy-en-Josas); CNRS-UMR 144, Institut Curie, PSL Research University (Paris).

9.3. European Initiatives

9.3.1. Collaborations in European Programs, Except FP7 & H2020

9.3.1.1. ESFRI initiative programm

SERPICO is involved in the ESFRI Euro-BioImaging (https://www.eurobioimaging.eu/) initiative, 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 is also 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: Turku University (J. Eriksson, Turku, Finland).
Funding: Member states of the European Union.
Partners: 15 European countries.

9.3.1.2. EIT Digital program
Participants: Sylvain Prigent, Charles Kervrann.
Duration: 12 months (Nov 2019 – Oct 2020).

SERPICO is involved in a European project which aims at developing a connected wearable device for diagnosis and treatment of photodermatoses. Using the data on skin sun sensitivity and UV exposure habits with machine learning algorithms will enable to make more precise optimal sun exposure predictions for patients. The wearable device will be useful for a larger population to increase awareness around overexposure to UV as a main cause of sun damage and worst-case skin cancer.

Funding: EIT Digital.
Inria coordinator: C. Kervrann.
Partners: UVisio and Nobleo Projects B.V., Eindhoven, The Netherlands.

9.4. International Initiatives

9.4.1. Inria International Partners

9.4.1.1. Informal International Partners

- Collaboration with Max-Planck Institute, Martinsried, Germany (with A. Martinez and W. Baumeister): Detection and segmentation of macromolecules in cryo-electron tomography (project in progress with E. Moebel and C. Kervrann) (see Sections 6.9 [30] and 6.12 [22]).
- Collaboration with University of Texas SouthWestern (UTSW) Medical Center, Dallas, United States (P. Roudot, E. Welf and G. Gaudenz): 3D optical flow for cell migration quantification (project in progress with S. Manandhar, P. Bouthemy and C. Kervrann) (see Sections 6.11 and 7.4 [21]).
- Collaborations with the MRC laboratory of Molecular Biology (with E. Derivery and J. Boulanger) and the Cambridge Advanced Imaging Centre (with L. Muresan), Cambridge, UK (project in progress with A. Salomon and C. Kervrann) (see Section 7.2).
- Collaboration with the PKU University, Institute of Molecular Medicine, Beijing (with L. Chen and Y.M. Liu): 3D reconstitution of the biogenesis of Endoplasmic Reticulum-plasma membrane contact sites (ER-PM MSCs upon Ca2+ store depletion or replenishment) (project in progress with C.A. Valades Cruz and J. Salamero).

9.5. International Research Visitors

9.5.1. Visits to International Teams

- Charles Kervrann visited the MRC laboratory of Molecular Biology and the Cambridge Advanced Imaging Centre (June, 1 week, Cambridge, UK).

10. Dissemination

10.1. Promoting Scientific Activities

10.1.1. Scientific Events: Organization

10.1.1.1. General Chair, Scientific Chair

- Charles Kervrann was 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).
- Jean Salamero was the scientific responsible and co-organizer of the first joint "Workshop on BioImaging" between Paris Sciences Lettres (PSL-Institut Curie) University and Peking University (PKU) (MoU for official "collaboration and exchange program PSL Qlife/PKU in the Bioimaging field 2020-2024).

10.1.2. Scientific Events: Selection

10.1.2.1. Member of the Conference Program Committees

- Charles Kervrann: member of the scientific committee of the international "Quantitative BioImaging" (QBI'2019, QBI'2020) conference, member of the scientific committee of the international NEUBIAS 2020 conference, and 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'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 Computer Science", section "Computer Vision and Image Analysis".

10.1.3.2. Reviewer - Reviewing Activities

- Charles Kervrann: Journal of Mathematical Imaging and Vision, IEEE Signal Processing Letters, Nature Communications, IEEE Transactions on Medical Imaging, OSA Biomedical Optics Express.
- Patrick Bouthemy: IEEE Signal Processing Letters.
- Jean Salamero: Nature Methods, Journal of Cell Sciences, Nature Communications, Biology of the Cell, ACS Photonics, Journal of Microscopy.

10.1.4. Invited Talks

Charles Kervrann:

- "Statistical methods for intracellular dynamics classification in live cell imaging", "Bioimage Processing" workshop, June, Cambridge, UK (see [29]).
- "Computational methods for intracellular dynamics in live cell imaging", "Random Walks and Intracellular Transport" workshop, Apr, Manchester, UK (see [28]).
- "A fast statistical colocalization method for 3D live cell imaging and super-resolution microscopy", "Statistical Modeling for Shapes and Imaging" workshop, March, Paris (see [27]).
- "Geolocalization and classification of macromolecules in cryo-electron microscopy with deep-learning", Journées Scientifiques de l'Université de Nantes: "la microscopie électronique pour les sciences du vivant", June, Nantes.
- "Computational methods for fluorescence microscopy", Institut Curie CNRS-UMR 168 Seminar, February, Paris.

Patrick Bouthemy:

- "Intensity-based methods for fully automated registration in 2D and 3D CLEM", COMULIS & BioImaging Austria/CMI conference, November, Vienna, Austria (see [26]).

Jean Salamero:

- "Live cell imaging in High Space-time Resolution" and BioImaging course (Co-Organizer), ReTUBI H2020) Super Resolution meeting and Imaging, October, Lisbon, Portugal.
- "Career Path for the Next Generation of BioImaging Scientist: Training Passport", Global BioImaging 2019 "EOE.V", September, Singapore.

Sylvain Prigent:

- "Portail analyse d'images France-BioImaging", 16èmes Assises Nationales des Plateformes du réseau RT-mfm, March, Rennes.
- "Portail analyse d'images France-BioImaging", 9èmes Journées Scientifiques et Techniques du Réseau des Microscopistes de l'INRA, November, Nantes.

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 and member of the scientific committee of the Interdisciplinary MiFoBio School CNRS (http://www.mifobio.fr).
- 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 member of the selection committee for an Assistant-Professor (Maitre de Conférences) position at the University of Paris-Descartes (Section CNU 26, Mathematics). He was reviewer for European Research Council (ERC Consolidator Grant).
- Patrick Bouthemy was member of the committee for professor promotion at IMT Atlantique.
- Jean Salamero was reviewer for European Research Council (Junior Grant), ANR and ITMO Cancer program.

10.1.7. Research Administration

Charles Kervrann:

- Member of the executive board of the project committee ("bureau du Comite´ des Projets") of the Inria Rennes - Bretagne Atlantique centre since 2010.
- 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:

- Head of Excellence Lab (Labex) CominLabs (http://www.cominlabs.ueb.eu) since April 2014.
- 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/).
- Inria representative in the steering committee of the DGA-Inria collaboration.
- Member of the Research Committee of IMT Atlantique.
- Head of the evaluation committee of the EORD profession of the DGA and head of the HCERES evaluation committee of the DTIS department of ONERA.
- Member of the ANR evaluation committee for the call for proposals regarding the Artificial Intelligence field.

Jean Salamero:

- Director of the National Research Infrastructure in Biology and Health, France BioImaging (2015-2020) (https://france-bioimaging.org/).
- Director of the Service Unit UMS 3714 (2015-2020) CEMIBIO CNRS-Institut Curie.
- Member of the Advisory Committee for the creation of the National Biomedical Imaging Center (NBIC@Beijing, Republic of China) since 2017.
- Member of the Advisory Board of EMBL Core Facilities.
- Member of the Steering Committee of the DIM-ELICIT program (Région Ile de France) since 2016.
- Member of the Qlife "Institut Convergences" since 2018.

10.2. Teaching - Supervision - Juries

10.2.1. Teaching

Charles Kervrann:

- Engineer Degree: Genomics and Informatics, 4.5 hours, MINES ParisTech.
- 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: Analysis of Image Sequences, 9 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.

10.2.2. Supervision

- Anca-Georgiana Caranfil (PhD in progress): Data assimilation methods for cell division mechanisms and molecule trafficking analysis (started in December 2016, supervised by C. Kervrann and Y. Le Cunff (IGDR, Rennes)).
- Gwendal Fouché (PhD in progress): Immersive interaction and visualization of temporal 3D data (started in October 2019, supervised by C. Kervrann, F. Argelaguet (EPC HYBRID, Inria, Rennes), and E. Faure (LIRMM, Montpellier)).
- Yunjiao Lu (PhD in progress): Intracellular dynamics and super-resolution imaging: analysis of bacteria wall at the molecular scale (started in October 2017, supervised by C. Kervrann, A. Trubuil and R. Carballido-Lopez (INRA, Jouy-en-Josas)).
- Léo Maczyta (PhD in progress): Motion saliency in video sequences (started in October 2017, supervised by P. Bouthemy and O. Lemeur (Team Percept, IRISA, Rennes)).
- Sandeep Manandhar (PhD defended [11]): Optical flow methods for 3D fluorescence imaging (defended in November 2019, supervised by P. Bouthemy and C. Kervrann).
- Emmanuel Moebel (PhD defended [12]): New strategies for the identification and enumeration of macromolecules in 3D images of cryo electron tomography (defended in January 2019, supervised C. Kervrann).
- Antoine Salomon (PhD in progress): Statistical aggregation for image analysis in fluorescence microscopy and super-resolution (started in November 2017, supervised by C. Kervrann).

10.2.3. Juries

Charles Kervrann:

- Reviewer of the HdR of E. Baudrier (University of Strasbourg, ICube – UMR 7357).
- Reviewer of the PhDs of A. Davy (University of Paris Saclay, Ecole Normale Supérieure Paris-Saclay, supervised by J.-M. Morel and A. Desolneux), P. Parutto (University of Paris Sciences et Lettres, Ecole Normale Supérieure, supervised by D. Holcman), J.D.K. Hansen (University of Copenhagen, DIKU, Denmark, supervised by F. Lauze).
- President of PhD thesis committee of M. Tassano (University of Paris-Descartes, supervised by J. Delon).
- Member of the PhD committee of P. Naylor (University of Paris Sciences et Lettres, MINES ParisTech, supervised by T. Walter).
- External examiner of the application of M. Arigovindan (Faculty assessment, promotion to the position of Associate Professor with tenure, Indian Institute of Science).

Patrick Bouthemy:

- President of the PhD thesis committee of A. Lopez (University of Rennes 1, supervised by J. Pettré and F. Chaumette).

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[12] E. MOEBEL. *New strategies for the identification and enumeration of macromolecules in 3D images of cryo electron tomography*, Université de Rennes 1, February 2019, https://hal.inria.fr/tel-02153877

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