Activity Report 2012

Team SERPICO

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

RESEARCH CENTER
Rennes - Bretagne-Atlantique

THEME
Computational Biology and Bioinformatics

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Team SERPICO

Keywords: Biological Images, Computational Biology, Image Processing, Statistical Methods, Tracking, Motion Estimation

Creation of the Team: January 01, 2010 .

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2. Overall Objectives

2.1. Scientific context and motivations

Light microscopy, especially fluorescence microscopy, has taken a prominent role in life science research due to its ability to investigate the 3D interior of cells and organisms. It enables to visualize, in vitro and in vivo, particular biomolecules and proteins (gene expression) with high specificity through fluorescent labeling (GFP - Green Fluorescence Protein probes) both at the microscopic and nanoscopic scales. Nevertheless, the mechanisms of life are very complex and driven by multimolecular interactions: mitotic spindle, cell signaling complexes, intracellular transport, cell morphogenesis and motility... A dynamical quantitative and integrated description of molecular interactions and coordination within macromolecular complexes at different scales appears essential today for the global understanding of live mechanisms. A long-term research will consist in inferring the relationships between the dynamics of macromolecules and their functions. This constitutes one of the challenges of modern biology. The proposed mathematical models and algorithms are mainly developed to identify molecular processes in fundamental biology but they have also a strong potential for applications in biotechnology and medicine: disease diagnosis, detection of genomic instabilities, deterioration of cell cycle, epigenetic mechanisms and cancer prevention.

2.2. Objectives in cell imaging

Facing the amount of information provided by high-throughput multidimensional microscopy, the SERPICO team investigates computational and statistical models to better elucidate the role of specific proteins inside their multiprotein complexes and to help to decipher the dynamic coordination and organization of molecular complexes at the single cell level. We investigate image processing methods, mathematical models, and algorithms to build an integrated imaging approach that bridges the resolution gaps between the molecule and the whole cell, in space and time [45]. We address the following topics:

- image superresolution/image denoising required to preserve cell integrity (photo-toxicity versus exposure time) and image analysis in multidimensional microscopy;
- spatio-temporal modelling of molecular species and multiscale architectures (e.g. multiscale registration of electron and light microscopy images to study molecule interactions in space and time);
- computational simulation and modelling of molecule trafficking at different spatial and temporal scales (e.g. biophysical model assimilation for dynamic representation in video-microscopy and prediction in biology).

We focus on the cellular and molecular mechanisms involved in membrane transport and trafficking at the scale of a single cell.

2.3. Main challenges in image processing for high-throughput, dynamic and multimodal microscopy

In most cases, modern microscopy in biology is characterized by a large number of dimensions that fits 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 bio-molecular species. Dynamic microscopy is also characterized by the nature of the observable objects (cells, organelles, single molecules, ...), eventually 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 a comparative analysis of multiple image series acquisitions is massive (up to few Giga bytes per hour). Therefore, it becomes necessary to facilitate and rationalize the production of those multidimensional data, to improve post acquisition analysis (i.e. image processing) which are limiting factors in front of the data, and to favor the organization and the interpretation of the information associated to this data corpus. It motivates and requires innovative mathematical tools and concepts: data fusion, image registration, superresolution, data mining, life dynamics modelling, ...

2.4. Organization and collaborations

In collaboration with UMR 144 CNRS Institut Curie (“Subcellular Structure and Cellular Dynamics” Unit) and PICT-IBiSA (Cell and Tissue Imaging Facilities), the members of the SERPICO team participate several projects (PhD and post-doc supervision, contracts...) with biologists in the field of cell biology and microscopy. We have promoted and designed non-parametric methods since prior knowledge cannot be easily taken into account for extracting unattended but desired information from image data. We have proposed user-friendly algorithms for processing 3D or 4D data.

To reinforce the interactions between cell biology, imaging instrumentation and applied mathematics and to improve visibility, the SERPICO team was created in 2010 at Inria Rennes with the aim to settle an EPC in 2013 in collaboration with CNRS-INSB and Institut Curie. The scientific projects are complementary to the other on going and planned projects of the UMR 144 CNRS Institut Curie Unit; a subset of projects is 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 correlative approaches with electronic microscopy. Our projects rely partially on the results and advances of these instrumental projects and a positive synergy is foreseen.

3. Scientific Foundations

3.1. Glossary

WF Optical Wide-Field microscopy.

SDC (Spinning-Disk Confocal microscopy): illumination of the sample with a rotating pattern of several hundred of pinholes for complete simultaneous confocal illumination.

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 [20].

SIM (Structured Illumination Microscopy): high-resolution light microscopy using structured patterns and interference analysis [30].

TIRF (Total Internal Reflectance): 2D optical microscopy using evanescent waves and total reflectance [19].

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.

3.2. Image restoration for high-resolution microscopy

In order to produce images compatible with the dynamic processes in living cells as seen in video-microscopy, we study the potential of non-local neighborhood filters and image denoising algorithms (e.g. ND-SAFIR software) [6], [2], [7], [4]. The major advantage of these approaches is to acquire images at very low SNR while recovering denoised 2D+T(ime) and 3D+T(ime) images [1]. Such post-acquisition processing can improve the rate of image acquisition by a factor of 100 to 1000 times [5], reducing the sensitivity threshold and allowing imaging for long time regime without cytotoxic effect and photodamages. This approach has been successfully applied to WF, SDC [1], TIRF [19], fast live imaging and 3D-PALM using the OMX system in collaboration with J. Sedat and M. Gustafsson at UCSF [5]. The ND-SAFIR software (see Section 5.1) has been licensed to a large set of laboratories over the world (see Figure 2). New information restoration and image denoising methods are currently investigated to make SIM imaging compatible with the imaging of molecular dynamics in live cells. Unlike other optical sub-diffraction limited techniques (e.g. STED [32], PALM [20]) SIM has the strong advantage of versatility when considering the photo-physical properties of the fluorescent probes [30]. Such developments are also required to be compatible with "high-throughput microscopy" since several hundreds of cells are observed at the same time and the exposure times are typically reduced.

3.3. Dynamic analysis and trajectory computation

3.3.1. Motion analysis and tracking

In time-lapse microscopy, the challenge is to detect and track moving objects. Classical tracking methods have limitations as the number of objects and clutter increase. It is necessary to correctly associate measurements with tracked objects, i.e. to solve the difficult data association problem [37]. Data association even combined with sophisticated particle filtering techniques [40] or matching techniques [38] is problematic when tracking several hundreds of similar objects with variable velocities. Developing new optical flow and tracking methods and models in this area is then very stimulating since the problems we have to solve are really challenging and new for applied mathematics. The goal is to formulate the problem of optical flow estimations in ways that take physical causes of brightness violations into account [26], [31]. In addition, the interpretation of computed flow fields enables to provide spatio-temporal signatures of particular dynamic processes and could help to complete the traffic modelling.

3.3.2. Event detection

Several approaches can be considered for the automatic detection of appearing and vanishing particles (or spots) in WF and TIRF microscopy images. The difficulty is to distinguish motions due to trafficking from the appearing and vanishing spots. Ideally this could be performed by tracking all the vesicles contained in the cell [40], [29]. Among the methods proposed to detect particles in microscopy images [43], [39], none is dedicated to the detection of a small number of particles appearing or disappearing suddenly between two time steps. Our way of handling small blob appearances/dis-appearances originates from the observation that two successive images are redundant and that occlusions correspond to blobs in one image which cannot be reconstructed from the other image [1] (see also [24]).

3.4. Computational simulation and modelling of membrane transport

Mathematical biology is a field in expansion, which has evolved into various branches and paradigms to address problems at various scales ranging from ecology to molecular structures. Nowadays, system biology [33], [45] aims at modelling systems as a whole in an integrative perspective instead of focusing on independent biophysical processes. One of the goals of these approaches is the cell in silico as investigated at Harvard Medical School (http://vcp.med.harvard.edu/) or the VCell of the University of Connecticut Health Center (http://www.nrcam.uchc.edu/). Previous simulation-based methods have been investigated to explain the spatial organization of microtubules [35] but the method is not integrative and a single scale is used to describe the visual patterns. In this line of work, we propose several contributions to combine imaging, traffic and membrane transport modelling in cell biology.

In this area, we focus on the analysis of transport intermediates (vesicles) that deliver cellular components to appropriate places within cells. We have already investigated the concept of Network Tomography (NT) [44] mainly developed for internet traffic estimation. The idea is to determine mean traffic intensities based on statistics accumulated over a period of time. The measurements are usually the number of vesicles detected at each destination region receiver. The NT concept has been investigated also for simulation [3] since it can be used to statistically mimic the contents of real traffic image sequences. In the future, we plan to incorporate more prior knowledge on dynamics to improve representation. An important challenge will be to correlate stochastic and dynamical 1D and in silico models studied at the nano-scale in biophysics, to 3D images acquired in vivo at the scale of few hundred nanometres. A difficulty is related to the scale change and statistical aggregation problems (in time and space).

4. Application Domains

4.1. Biological pilot models: Birbeck granule and Melanosome biogenesis

In the past recent years, research carried at UMR 144 CNRS Institut Curie 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:

- multiple sorting and structural events involved in the biogenesis of these organelles;
- complexity of the endo-melanosomal network of these highly specialized cells;
- complex molecular architecture organizing and coordinating their dynamics;
- 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 within the overall biological environment as seen 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 in a single workflow, 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.

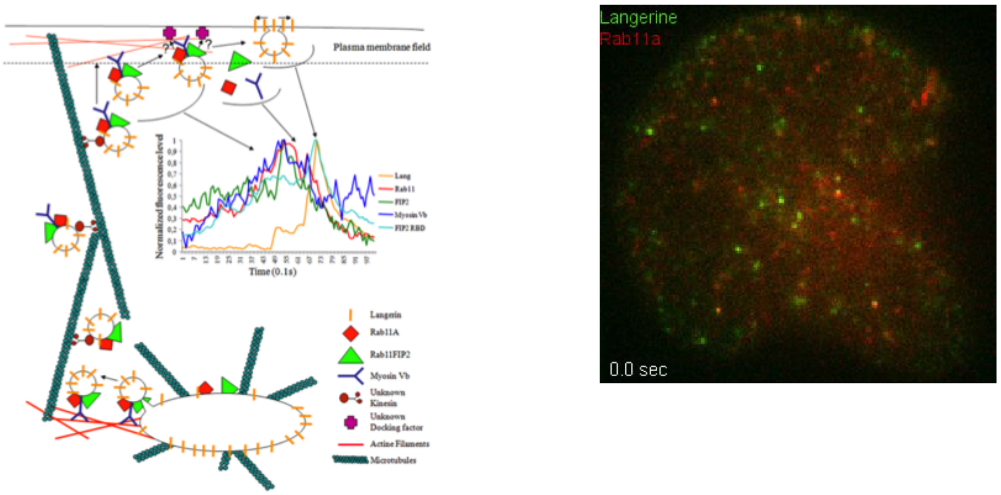

Figure 1. Rab11/Langerin (TIRF) and Birbeck granules: from interactions and trafficking to membrane biogenesis.

5. Software

5.1. Software for live cell imaging

Participants: Charles Kervrann, Patrick Bouthemy, Tristan Lecorgne.

Motion2d: parametric motion model estimation

The MOTION2D software written in C++ (APP deposit number: FR.001.520021.001.S.A.1998.000.21000 / release 1.3.11, January 2005) and JAVA (plug-in IMAGEJ (http://rsbweb.nih.gov/ij/) 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. 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.

Free academic software distribution: Motion2D Free Edition is the version of Motion2D available for development of Free and Open Source software only. More information on Motion2D can be found at http://www.irisa.fr/vista/Motion2D and the software can be downloaded at the same Web address.
On-line demo: Mobyle@GenOuest Bioinformatics http://mobyle.genouest.org/cgi-bin/Mobyle/portal.py#forms:Motion2D (see Fig. 5).

Partner: Fabien Spindler (Inria Lagadic team).

ND-Safir and Fast2D-SAFIR: Image denoising software

The ND-SAFIR software (APP deposit number: IDDN.FR.001.190033.002.S.A.2007.000.21000 / new release 3.0 in 2013) written in C++, JAVA and MATLAB, removes additive Gaussian and non-Gaussian noise in still 2D or 3D images or in 2D or 3D image sequences (with no motion computation) (see Figure 2) [4]. The method is unsupervised and is based on a pointwise selection of small image patches of fixed size (a data-driven adapted way) in spatial or space-time neighbourhood of each pixel (or voxel). The main idea is to modify each pixel (or voxel) using the weighted sum of intensities within an adaptive 2D or 3D (or 2D or 3D + time) neighbourhood and to use image patches to take into account complex spatial interactions. The neighbourhood size is selected at each spatial or space-time position according to a bias-variance criterion. The algorithm requires no tuning of control parameters (already calibrated with statistical arguments) and no library of image patches. The method has been applied to real noisy images (old photographs, JPEG-coded images, videos, ...) and is exploited in different biomedical application domains (time-lapse fluorescence microscopy, video-microscopy, MRI imagery, X-ray imagery, ultrasound imagery, ...).

The FAST-2D-SAFIR software (APP deposit number: IDDN.FR.001.190033.001.S.A.2007.000.21000) written in C++ removes mixed Gaussian-Poisson noise in large 2D images, typically $10^3 \times 10^3$ pixels, in a few seconds. The method is unsupervised and is a simplified version of the method related to the SAFIR-nD software. The software dedicated to microarrays image denoising, was licensed to the INNOPSYS company which develops scanners for disease diagnosis and multiple applications (gene expression, genotyping, aCGH, ChIP-chip, microRNA, ...).

On-line demo: Mobyle@GenOuest Bioinformatics
http://mobyle.genouest.org/cgi-bin/Mobyle/portal.py#forms::NDSafir
Free download binaries: Binaries of the software ND-SAFIR are freely and electronically distributed. Developed in standard C/C++ under Linux using the CImg library, it has been tested over several platforms such as Linux/Unix, Windows XP and Mac OS.
Academic licence agreements: Institut Curie, CNRS, ENS Ulm, Oxford university, Weizmann Institute, UCSF San-Francisco, Harvard university, Berkeley university, Stanford university, Princeton university, Georgia-Tech, Kyoto university, IMCB Singapore ...
Partners: J. Boulanger, J. Salamero (UMR 144 CNRS Institut Curie), P. Elbau (RICAM Linz, Austria), J.B. Sibarita (UMR 5091 University of Bordeaux 2).

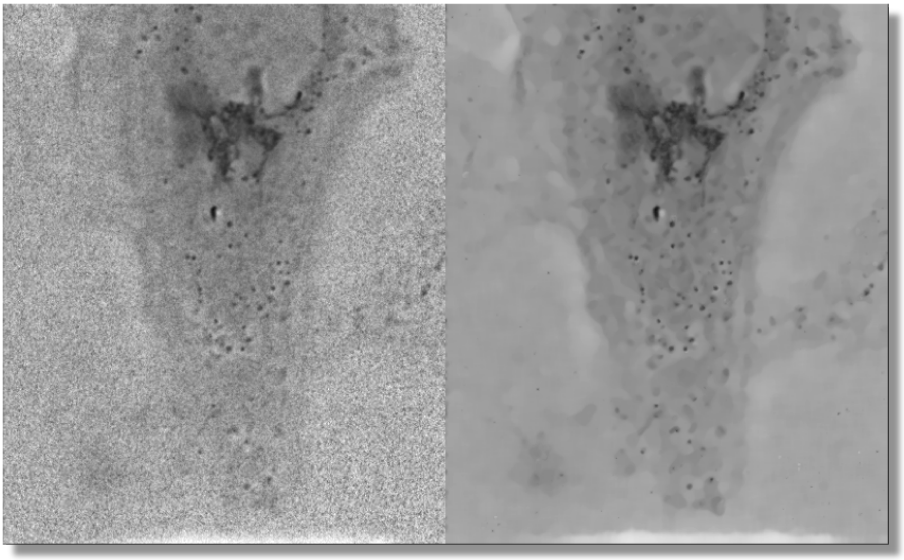

Figure 2. ND-SAFIR *software: denoising of a 3D image sequence in wide-field (WF) microscopy (GFP-Rab6A (Hela cell), UMR 144 CNRS Institut Curie).*

HullkGround: Background subtraction by convex hull estimation

The HULLKGROUND software (APP deposit number: IDDN.FR.001.400005.000.S.P.2009.000.21000) written in JAVA (plug-in IMAGEJ, see Fig. 3) 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 α shapes-based scale-space method [25].

On-line demo: Mobyle@GenOuest Bioinformatics http://mobyle.genouest.org/cgi-bin/Mobyle/portal.py#forms::Hullkground
Partners: A. Chessel and J. Salamero (UMR 144 CNRS Institut Curie)

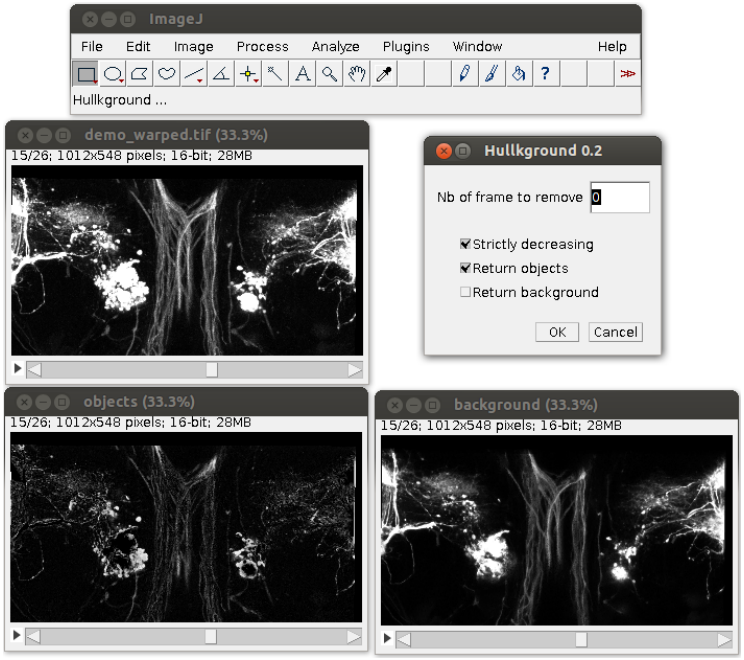

Figure 3. HULLKGROUND *software: plug-in* IMAGEJ.

5.2. Software for Cyo-electron tomography

Participant: Charles Kervrann.

TubuleJ: Straightening of microtubule cryo-EM projection views

The TUBULEJ software (APP deposit number: IDDN.FR.001.240023.000.S.P.2011.000.21000) written in JAVA (plug-in IMAGEJ) is devoted to the analysis of microtubules and helical structures in 2D cryo-electron microscope images. The software straightens curved microtubule images by estimating automatically points locations on the microtubule axis. The estimation of microtubule principal axis relies on microtubule cylindrical shape analyzed in the Fourier domain. A user-friendly interface enables to filter straight fiber images by selecting manually the layer lines of interest in the Fourier domain. This software can be used to generate a set of 2D projection views from a single microtubule projection view and a few parameters of this microtubule structure. These projection views are then back projected, by using the IMOD plug-in (http://rsbweb.nih.gov/ij/), to reconstruct 3D microtubules.

On-line demo: see http://equipes.igdr.univ-rennes1.fr/en/tips/Software/TubuleJ/

Partners: S. Blestel and D. Chrétien (UMR 6290 CNRS University of Rennes 1)

Cryo-Seg: Segmentation of tomograms in cryo-electron microscopy

The CRYO-SEG software written in C++ and JAVA (plug-in MAGEJ) has been developed to detect microtubule structures and helical structures in 2D cryo-electron microscope images (see Figure 4). Cryo-electron tomography allows 3D observation of biological specimens in their hydrated state. Segmentation is formulated as Maximum A Posteriori estimation problem and exploits image patches to take into account spatial contexts (Markov Random Fields). Because of the contrast anisotropy in the specimen thickness direction, the whole tomogram is segmented section by section, with an automatic update of reference patches. This algorithm has been evaluated on synthetic data and on cryo-electron tomograms of in vitro microtubules. On real data, this segmentation method extracts the most contrasted regions of microtubules, and 3D visualization is improved.

Partners: S. Blestel and D. Chrétien (UMR 6290 CNRS University of Rennes 1)

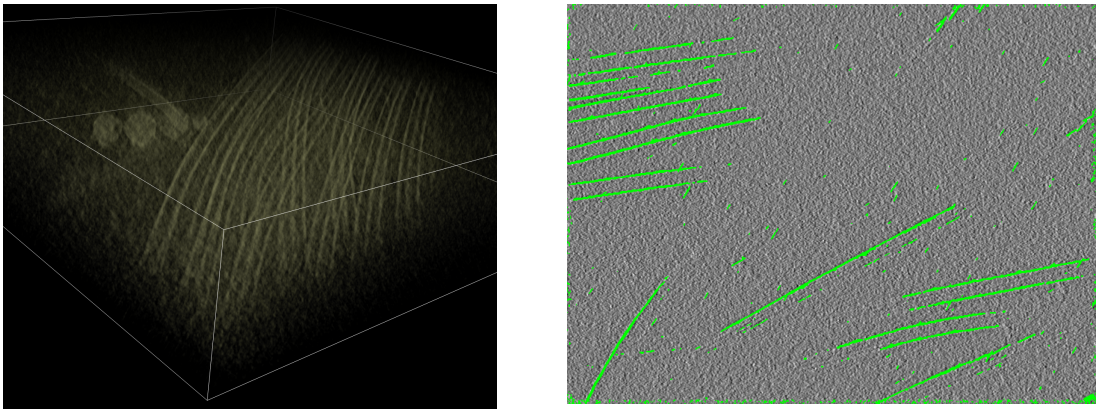

Figure 4. CRYO-SEG *software: Segmentation of 3D microtubles in a cryo-EM tomogram (left) and 2D view (right) (UMR 6290 CNRS University of Rennes 1).*

5.3. Image Processing software distribution

Participants: Tristan Lecorgne, Charles Kervrann.

The objective is to disseminate the distribution of SERPICO image processing software for biologist users:

- *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 web portal*: An online version of the software has been released using the Mobyle framework (see http://mobyle.pasteur.fr/) developed at Institut Pasteur. The main role of this web portal is to demonstrate the performance of the programs developed by the team. The web interface makes our image processing methods available for biologist users without any installation or configuration (see ND-SAFIR, HULLKGROUND, MOTION2D (see Fig. 5) at http://mobyle.genouest.org/). The size of submitted images is limited by network bandwidth. We use the computing facility of the GenOuest platform to run calculations.
- IMAGEJ *plug-ins*: 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, HULLKGROUND (see Fig. 3), MOTION2D, HOTSPOTDETECTION and OPTICALFLOW.

- *Institut Curie database*: Institut Curie is currently acquiring a new database system to store mass of data. The database can be searched via meta-data and includes menu selections that enable to run remote processing. We have integrated ND-SAFIR in the interface environment to allow the database users to denoise images easily.

Partners: C. Deltel (Inria Rennes SED) and Perrine Paul-Gilloteux (UMR 144 PICT IBiSA CNRS Institut Curie)

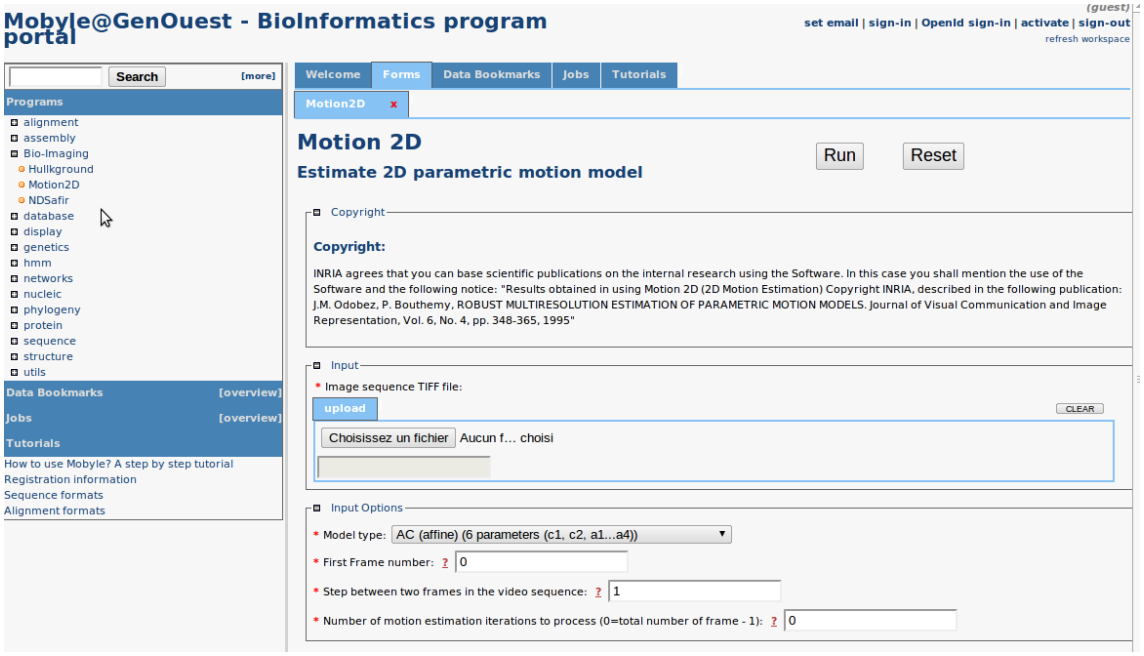

Figure 5. Motion2D on Mobyle@GenOuest Bioinformatics.

6. New Results

6.1. Robust parametric stabilization of moving cells

Participants: Solène Ozeré, Patrick Bouthemy, Charles Kervrann.

Paper under review.

Analysing the dynamic behaviour of individual particles (e.g., proteins, vesicles) inside a cell is of primary importance in cell biology. However, the motion of these particles observed in live cell microscopy image sequences is the addition of the global movement of the cell and their own single motions. Hence, automatically stabilizing the cell (or a group of cells), i.e. compensating for its global motion or equivalently registering its successive positions over time, is previously required. We have proposed a cell stabilization method based on a realtime robust multiresolution scheme (Motion2D software [36]). It can simultaneously handle the estimation of 2D parametric global motions (e.g., affine motion model) and of temporal intensity variations. Three temporal intensity models have been investigated: constant additive model, exponential decay model (corresponding to the photobleaching effect), continuity equation. We have carried out experiments on three biological situations: development of cells, displacements of endosomes, protein recruitment by the Golgi.

We have demonstrated the accuracy of our method on these challenging examples and its capacity to efficiently reveal the own motion of subcellular particles. It yields better results than the StackReg method (http://bigwww.epfl.ch/thevenaz/stackreg/), classically used in the field, in cases involving strong local dynamics (see Fig. 6).

Partners:: Perrine Paul-Gilloteaux (UMR 144 CNRS PICT IBiSA Institut Curie)

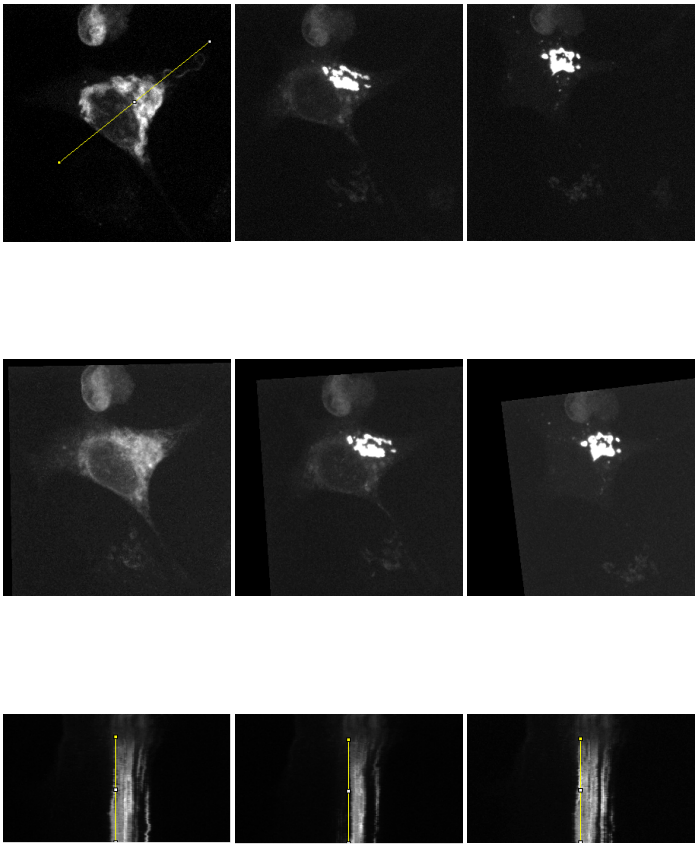

Figure 6. Real-time imaging of the synchronized trafficking of ManII-SBP-EGFP [21]. HeLa cells were transfected to express Ii-streptavidin as a hook and ManII-SBP-EGFP as a reporter. Release of the reporter was induced by addition of biotin and monitored using a spinning disk confocal microscope (F. Pérez, UMR 144 CNRS Institut Curie): 1st row: original images. 2nd row: images out of the motion-compensated sequence at time $t = 20, 60$ and 140 computed with our method; 3rd row: kymographs of the backwarped sequence computed respectively with the baseline motion equation (left), with the exponential decay (middle) and with StackReg *software (right).*

6.2. Motion classification for interpreting subcellular dynamics

Participants: Antoine Basset, Patrick Bouthemy, Charles Kervrann.

We have just started to address the classification of motions of subcellular particles in light microscopy time-lapse image sequences. For the while, we are considering the following three general classes: diffused motion, obstructed motion and directed motion. We are investigating three approaches. First, we can design

likelihood ratio tests for deciding the relevant configuration on local patches. Second, we can define a short-term classification framework based on optical flow computed at time t. The third approach is a mid-term one exploiting pieces of trajectories (tracklets) computed by tracking a set of points.

Partners:: Jérôme Boulanger (UMR 144 CNRS Institut Curie)

6.3. Aggregation of patch-based estimations for illumination-invariant optical flow in live cell imaging

Participants: Denis Fortun, Charles Kervrann, Patrick Bouthemy.

Paper under review.

Live cell image sequences provide a large variety of challenging situations for motion estimation. We have developed a novel optical flow estimation method in the line of work of [11], based on a two-stage aggregation framework and designed to handle this diversity of issues. First, semi-local candidates are estimated with a combination of patch correspondences and illumination-invariant affine motion estimations. Then, one candidate is selected at each pixel in a graph-cut based global aggregation stage. This approach allows us to overcome usual limitations of existing methods such as loss of small structures with large displacements, dependency on illumination changes and over-smoothing of the motion discontinuities. The method jointly estimates the motion and illumination fields. We have compared our approach to state-of-the-art methods and have demonstrated its ability to outperform existing methods in challenging cases frequently arising in live cell imaging (see Fig. 7).

Partners:: Perrine Paul-Gilloteaux (UMR 144 CNRS PICT IBiSA Institut Curie)

6.4. Correlation and variational approaches for motion and diffusion estimation in fluorescence imaging

Participants: Denis Fortun, Charles Kervrann.

Paper under review.

In this work, we have compared a correlation-based approach and a variational method for both motion and diffusion estimation in representative cell biology studies in fluorescence imaging. The so-called Spatio-Temporal Image Correlation Spectroscopy (STICS) is widely used in fluorescence imaging to recover physical parameters (e.g. direction of flow or Brownian motion of molecules). We have investigated recent advances in variational dense motion estimation and we have proposed to adapt the variational framework to the estimation of diffusion (i.e. Brownian motion). We have demonstrated the influence of the regularization parameter in the variational approach and its ability to capture motion of individual or clusters of moving objects. We have evaluated the advantages and limits of the two approaches for different biological studies (see Fig. 8).

Partners:: Perrine Paul-Gilloteaux, Francois Waharte and Chen Chen (UMR 144 CNRS PICT IBiSA Institut Curie)

6.5. Noise modeling and denoising for intensified camera in fluorescence imaging

Participants: Philippe Roudot, Charles Kervrann.

Two papers under review.

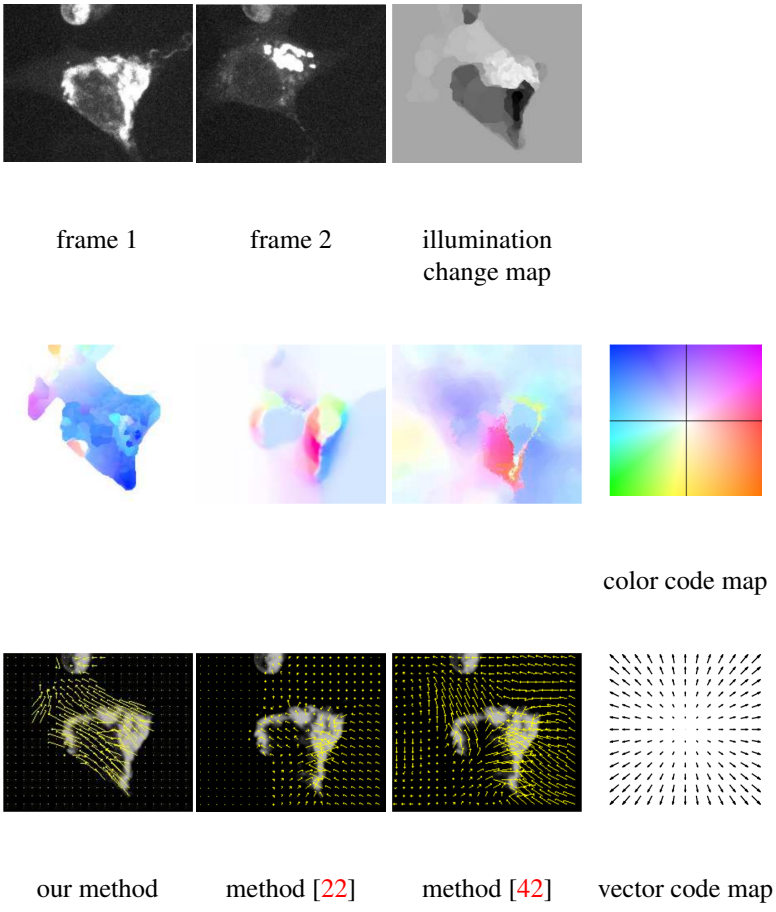

Figure 7. Comparison of our method with the methods of [22] and [42] on a sequence of "HeLa cells" (courtesy of F. Pérez UMR 144 CNRS Institut Curie, PICT-IBiSA).

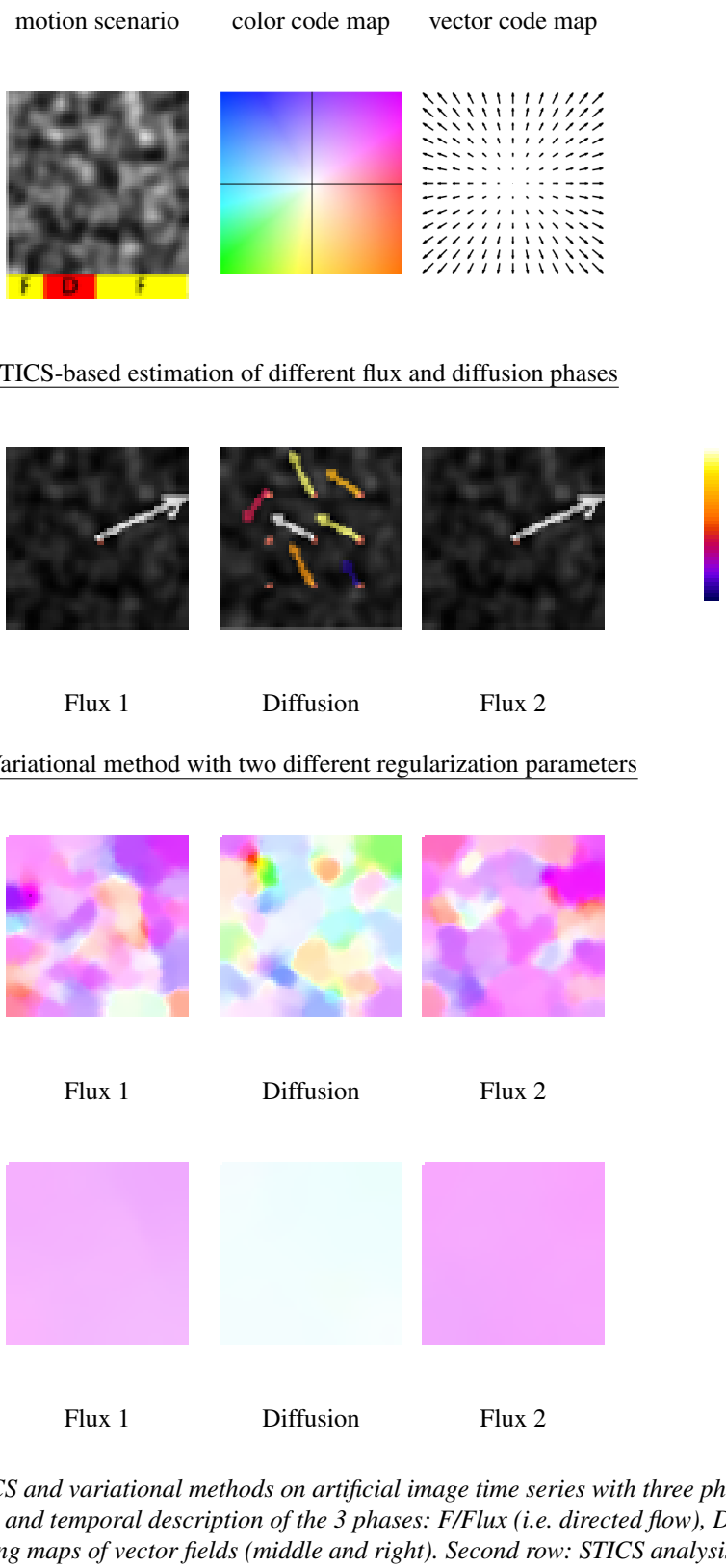

Figure 8. Analysis of STICS and variational methods on artificial image time series with three phases. First row: first frame of the sequence and temporal description of the 3 phases: F/Flux (i.e. directed flow), D/Diffusion (20 - 30 - 40 images) (left); coding maps of vector fields (middle and right). Second row: STICS analysis for each phase. The arrows show the direction of the displacement and the color code is used to represent orientation and magnitude of estimated velocities. Third and fouth rows: Variational estimation for image pairs of each phase with a low regularization parameter (third row) and a high regularization parameter (fourth row).

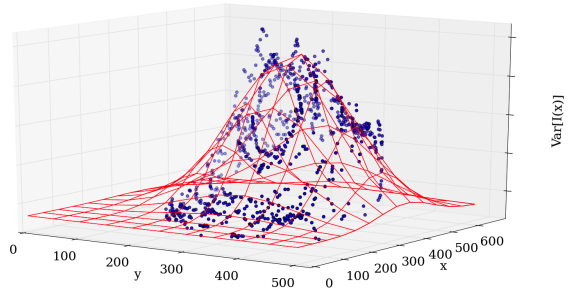

Figure 9. Variance prediction after noise model calibration using a fluorescein FD-FLIM reference stack acquired with a wide-field (WF) microscope (parametric model in red and measurements in blue).

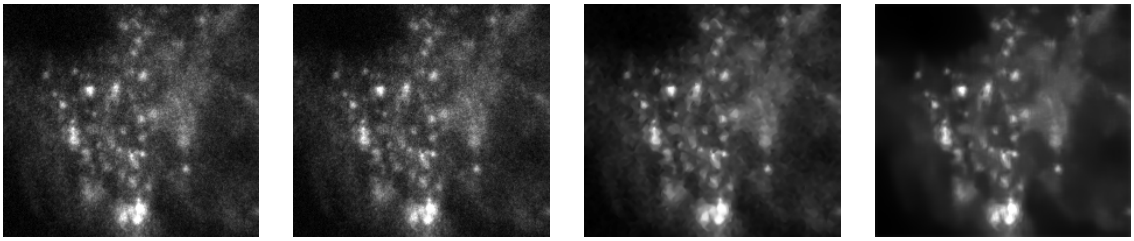

Figure 10. Denoising performance on a live cell image acquired in FD-FLIM (fluorescent tagged caveolin protein) using a confocal setup. From left to right: original image, results with BM3D *[27]* ND-SAFIR *[4] and our method.*

Image intensifiers are commonly used in low light level biological imaging, especially for fluorescence imaging. In this study, we have proposed a statistical framework for noise variance estimation dedicated to image sequences acquired with ICCD (Intensifier CCD). The model has been exploited for fluorescence lifetime estimation (Fluorescence lifetime imaging microscopy, FLIM) [13], [12] and image denoising. We have investigated an alternative approach to [41] and we have shown that intensifier gain variation cannot be neglected in the variance estimation as opposed to a CCD sensor gain. Additionally, we have suggested to correct the noise model spatially to cope with microscopical aberration which are common in experimental setups (see Fig. 9). Finally, we have proposed a novel denoising algorithm based on the NL-means filter [23] which does rely on variance stabilization. The novel patch-based filter is able to adapt to local intensity-based noise statistics (see Fig. 10).

Partners: F. Waharte and J. Boulanger (UMR 144 CNRS PICT IBiSA Institut Curie)

6.6. Microtubules modeling for variational assimilation analysis

Participants: Pierre Allain, Charles Kervrann.

In this project, we propose a bio-physical modeling of growing microtubules at the scale of a single cell. The theoretical advantage of such a modeling is to step aside empirically-based heuristics often carrying artificial parameters which can be hard to tune and to make sense in a data analysis context. We thus propose to model microtubules as rigid and growing cylinders alike (Nedelec and Foethk 2007) [34] but including Newtonian dynamics.

Using both this modeling and fluorescence microscopy, we aim at controlling simulated microtubules to satisfy in vitro observations. We plan to use variational assimilation with adjoint method in the future to achieve such an estimation. We believe that this approach should be able to provide information both on microtubules properties and on vesicle transport dynamics.

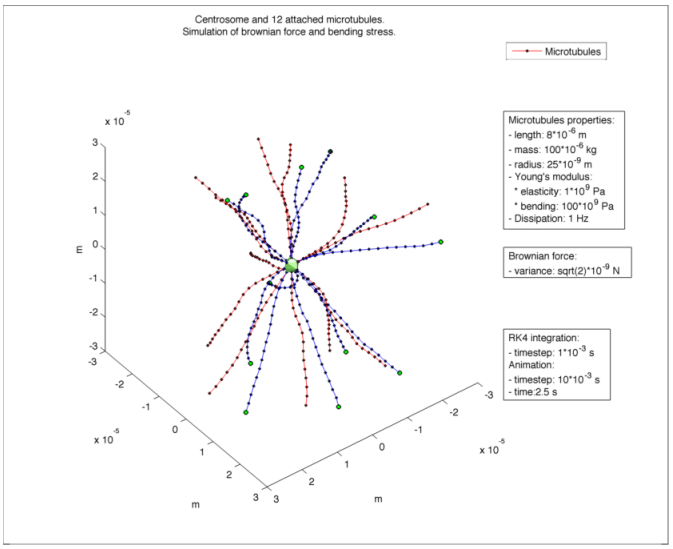

Figure 11. Simulation of growing microtubules in 3D.

6.7. Single versus dual-axis cryo-electron tomography for reconstruction of microtubules assembled in vitro

Participant: Charles Kervrann.

Single-axis cryo-electron tomography of vitrified specimens has become a method of choice to reconstruct in three dimensions macromolecular assemblies in their cellular context or prepared from purified components. In [9], we described a dual-axis acquisition scheme able to improve three-dimensional reconstructions of microtubules assembled in vitro. We showed that in single-axis tomograms, microtubules oriented close to the perpendicular of the tilt axis display diminished contrast, and ultimately transform into sets of parallel lines oriented in the direction of the electron beam when observed in cross-section. We analyzed projections in three-dimensional Fourier transform to demonstrate that imaging artifact is due to a decrease in the angular sampling of their equatorial components. Although the second orthogonal series of images does not fully complement the first one at the specimen level due to increased radiation damage, it still allows elongated features oriented in any directions to be correctly reconstructed, which might be essential for highly heterogeneous specimens such as cells.

Partners: Denis Chrétien, Audrey Guesdon and Sophie Blestel (UMR 6290 CNRS University of Rennes 1)

6.8. Analysis of lateral organization of ordered domains at the plasma membrane surface

Participant: Charles Kervrann.

Paper under review.

In this study, we have analysed a recently designed probe, di-4-ANEPPDHQ, that can change its fluorescent properties depending on whether it is residing in two distinct phases (ordered phase vs disordered phase) of the tobacco cell plasma membranes. We performed a spatial analysis of small (<200 nm) ordered domains observed in multispectral confocal microscopy. We focused on relevant binary images, assumed to be realizations of a MRF-Ising model, depicting the spatial organization of ordered domains. The Ising model depends on 2 parameters: the external field parameter h which controls the total fraction of the "ordered" phase and the interaction parameter which controls the spatial coupling. Maximum pseudo-likelihood methods were investigated to estimate parameters able to describe the spatial properties of ordered domains at the scale of 200 nm $\times$ 200 nm. Almost all estimates of the coupling parameter were positive excluding complete spatial randomness of ordered domains and showing a tendency to spatial aggregation at small distance. We then measured the strength of spatial aggregation through the calculation of the variability fraction explained by the spatial coupling. The mean fraction is low (0.5%) suggesting positive and limited interacting forces between neighbor ordered pixels. Altogether our simulations and analyses provided a probabilistic spatial characterization of PM ordered domains, indicating that recorded images showed a two-scale organization with spatial randomness at large scales (several micrometers) associated with spatial aggregation due to short-range interactions (up to 400 nm).

Partners: P. Gerbeau-Pissot, F. Simon-Plas (UMR 1088 PME INRA, Dijon) and K. Kiêu (MIA Unit INRA, Jouy-en-Josas)

6.9. Line detection in microarray scanner images

Participants: Alice Bergonzoni, Charles Kervrann.

In this study, we have studied two approaches to automatically detect straight lines in images (tool-slide) for calibrating scanners designed by Innopsys company. The Hough transform has been investigated and is able to produce satisfying results provided the algorithm parameters are carefully adjusted (see Fig. 12). To overcome this difficulty, we have evaluated the potential of *a contrario* approach [28] which is well ground theoretically and requires no object prior and parameter adjustment. According to the Helmholtz principle which is based on the *a contrario* approach, any structure is considered in a white noise image as a deviation from randomness.

A meaningful segment is detected when the expectation of its number of occurrences in a white noise image (i.e. number of false alarms) is low. We have evaluated the potential of this method and performed experiments using the LSD algorithm [46] inspired from [28].

Partners:: V. Paveau (Innopsys)

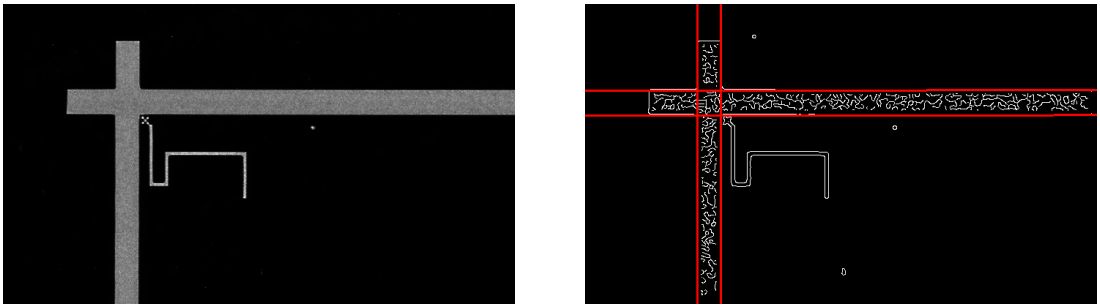

Figure 12. Detection of lines in an image (tool-slide) (pixel size: $3\mu m \times 3\mu m$).

7. Bilateral Contracts and Grants with Industry

7.1. Innopsys: Methods and algorithms for tissue microarrays image analysis

In collaboration with Magellium company and Institut Gustave Roussy, Innopsys plans to develop new image analysis software to be included in the INGRID platform developed by Megellium company. New statistical methods and algorithms will be investigated by SERPICO for:

- segmentation and detection of deformable cell contours and cell nuclei in 2D fluorescence tissue microarray images;
- deconvolution and superresolution of fluorescence microarray imaging.

The three-year contract supports the PhD thesis of Alice Bergonzoni (2013-2015).

8. Partnerships and Cooperations

8.1. Regional Initiatives

8.1.1. Computing and storage facilities

Participants: Tristan Lecorgne, Charles Kervrann.

The aim is to design a computing architecture to process bioimaging data sets and to deal with the data flow from the different imaging microscopy platforms. The software packages will manage the needs of end users in Rennes, where interactivity with the imaging devices and information systems are desirable.

Funding: Rennes-Metropole - “Allocation Installation Scientifique”

8.2. National Initiatives

8.2.1. Quaero project

Participants: Charles Kervrann, Patrick Bouthemy, Denis Fortun, Solène Ozeré.

Quaero is a European collaborative research and development program with the goal of developing multimedia and multi-lingual indexing and management tools for professional and public applications. SERPICO team participates in the Work Package 9 on Video Processing (WP9) of QUAERO Core Technology Cluster Project (CTC). Within WP9, former Vista project-team leaded three tasks: "Motion Recognition", "Object Tracking" and "Event Recognition". Since October 2010, SERPICO has conducted activities in object tracking and indexing for video-microscopy analysis (Denis Fortun PhD grant (6.3 and 6.4) and Solène Ozeré Internship (6.1)).

Funding: Quaero (no. Inria Alloc 3184), duration: 30 months
Partners: 24 academic and industrial partners leaded by Technicolor

8.2.2. ANR GreenSwimmers project

Participant: Charles Kervrann.

Biofilms are composed of spatially organized microorganisms (possibly including pathogens) embedded in an extracellular polymeric matrix. A direct time-lapse confocal microscopic technique was recently developed to enable the real-time visualization of biocide activity within the biofilm. It can provide information on the dynamics of biocide action in the biofilm and the spatial heterogeneity of bacteria-related susceptibilities that are crucial for a better understanding of biofilm resistance mechanisms. The approach is here to characterize the spatial and temporal exploration of the biofilm by microorganisms.

In this project, SERPICO will develop methods and software for the computation of mean velocity as well as other descriptors of swimmers bacteria dynamics inside biofilm image sequences. We will investigate spatio-temporal features and descriptors for comparison, classification, indexing and retrieval.

Funding: ANR, duration: 24 months
Partners: INRA, AgroParisTech, Naturatech company

8.2.3. LI-FLIM project

Participants: Charles Kervrann, Philippe Roudot.

The goal is to develop lifetime estimation methods of moving vesicles in FLIM microscopy. Grant to support collaboration between SERPICO team and UMR 144 CNRS PICT-IBiSA Institut Curie (P. Roudot's PhD (6.5))

Funding: GdR 2588 "Microscopie Fonctionnelle du Vivant" - Mobility grant
Partner: UMR 144 CNRS PICT IBiSA Institut Curie

8.2.4. DADA project

Participant: Charles Kervrann.

The accurate control of the growing and guidance of neuronal extensions to their target is a very important step for the maturation of the nervous system. The goal of this project (http://www-sop.inria.fr/members/Xavier.Descombes/DADA/home.html) is to develop new computational techniques to analyze image sequences of 3D volumes containing a population of growing axons (see Fig. 3) .

Funding: Inria ARC (2011-2012)
Partners: Inria Morpheme team and IBDC, laboratory from University of Nice Sophia Antipolis

8.2.5. France-BioImaging project

Participants: Charles Kervrann, Tristan Lecorgne.

The goal of the project 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. We address the following 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.

Funding: Investissement d'Avenir - Infrastructures Nationales en Biologie et Santé (2011-2016)
Partners: CNRS, Institut Jacques Monod, Institut Pasteur, Institut Curie, ENS Ulm, Ecole Polytechnique, INRA, INSERM

8.3. European Initiatives

8.3.1. Collaborations with Major European Organizations

ESFRI Euro-BioImaging initiative: SERPICO participates to 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. (3-year Preparatory Phase / start: December 2010). SERPICO also participates to the French counterpart, 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 8.2.5.

8.4. International Research Visitors

8.4.1. Visits to International Teams

- Collaboration with University of Saarland (Germany), Prof. J. Weickert, on optical flow computing (D. Fortun's visit in 2012, 3 months, Rennes-Metropole grant).
- Collaboration with Harvard Medical School (Boston, MA), Prof. G. Danuser, on object tracking in video-microscopy (P. Roudot's visit in 2012-2013, 3 months, Inria & CNRS grant).

8.4.2. Others

Collaboration with University of California - San Francisco (USA), J. Sedat and D. Agard, on image denoising in cryo-electron microscopy.

9. Dissemination

9.1. Scientific Animation

- *Technical program committees of conferences*
  - Charles Kervrann: PC member for ISBI'2012, ISBI'2013, reviewer for ICASSP'2012, ICASSP'2013, ICIP'2012, ICIP'2013, EMMCPRV'2013.
  - Patrick Bouthemy: PC member for ICPRAM'2013, MLDM'2013, TAIMA'2013, reviewer for ISBI'2012, ISBI'2013, ICRA'2013, ICPR'2012, EUSIPCO'2012, ACIVS'2012.
- *Journal reviewing*
  - Charles Kervrann: reviewer in 2012 for Image and Vision Computing, IEEE Transactions on Image Processing.

  - Patrick Bouthemy: reviewer in 2012 for International Journal of Computer Vision, IEEE Transactions on Circuits snd Systems for Video Technology, IEEE Computer Graphics and Applications, IEEE Signal Processing Letters, Signal Image and Video Processing, Results in Physics.

- *Participations in seminars, invitations, awards*
  - Charles Kervrann was invited to give a talk entitled "Patch-based Image Denoising in Light and Electron Microscopy" at the "BioImage Informatics" conference (Max Planck Institute, Dresden, Germany, November 2012) and at the "Statistics and Images" workshop (University of Strasbourg, November 2012); he was invited to give a talk entitled "Aggregation Methods for Optical Flow Computation" at the "SIAM Imaging Science" conference (Philadelphia, PA, May 2012) and at the "Mathematics and Image Processing" conference (University of Orleans, June 2012).
  - P. Bouthemy was invited to give a talk entitled "Estimation et interprétation du mouvement dans des séquences d'images", Interdisciplinary Mifobio School http://www.mifobio.fr/, plenary session (Seignosse, September 2012).
- *Responsibilities*

  Charles Kervrann:

  Member of the IEEE BISP "Biomedical Image and Signal Processing" committee,

  Member of executive board of the GdR 2588 ("Microscopie Fonctionnelle du Vivant") CNRS, member of the scientific committee of the Interdisciplinary MiFoBio School CNRS (http://www.mifobio.fr),

  Member of the executive board of the project committee of the Inria Rennes - Bretagne Atlantique centre,

  Member of the 2012 CORDIS post-doctoral fellowships committee (Inria Rennes - Bretagne Atlantique centre),

  Member of the Scientific Council of the INRA Rennes Research Centre,

  Deputy-head of the GIS Europia (http://gis-europia.univ-rennes1.fr/) (Rennes imagery platform).

  Patrick Bouthemy

  Deputy member of the board of directors and member of the Selection and Validation Committee of the Images & Réseaux competitivity cluster,

  Deputy member of the board of directors of IRT (Technological Research Institute) B-com,

  President of AFRIF (Association Francaise pour la Reconnaissance et l'Interprétation des Formes) and member of the board of the GRETSI (Groupement de Recherche en Traitement du Signal et des Images).
- *Other activities*
  - SERPICO is involved in the French network GdR 2588 "Microscopie Fonctionnelle du Vivant" CNRS,
  - SERPICO is member of the regional BioGenOuest GIS.

9.2. Teaching - Supervision - Juries

9.2.1. Teaching

Master: Charles Kervrann, Geometric Modeling for Shapes and Images, 7.5 hours, M2 SISEA, University of Rennes 1,

Engineer Degree: Charles Kervrann, Statistical Models and Image Analysis, 30 hours + 15 hours (TP, Denis Fortun), 3rd year, Ecole Nationale de la Statistique et de l'Analyse de l'Information ENSAI, Bruz,

Master: Patrick Bouthemy, Analysis of Image Sequences, 18 hours, M2 SISEA, ISTIC & University of Rennes 1,

Master: Patrick Bouthemy, Video Indexing, 9 hours, M2 Computer Science, ISTIC & University of Rennes 1,

Master & Engineer Degree: Patrick Bouthemy, Motion Analysis, 16.5 hours, M2 IRIV & 3rd year, ENSPS & University of Strasbourg.

9.2.2. Supervision

PhD in progress: Philippe Roudot, Lifetime estimation of moving vesicles in FLIM microscopy, started in October 2010, supervised by Charles Kervrann and Francois Waharte (UMR 144 CNRS PICT Institut Curie),

PhD in progress: Denis Fortun, Optical flow computing, aggregation methods and statistical methods: application to time-lapse fluorescence microscopy, started in October 2010, supervised by Charles Kervrann and Patrick Bouthemy,

PhD in progress: Antoine Basset, Event detection and recognition in video-microscopy and applications in cell biology, started in October 2012, supervised by Patrick Bouthemy and Charles Kervrann in collaboration with Jérôme Boulanger (UMR 144 CNRS Institut Curie),

PhD in progress: Alice Bergonzoni, Methods and algorithms for tissue microarrays image analysis, started in January 2013, supervised by Charles Kervrann and Vincent Paveau (Innopsys).

9.2.3. Juries

Referee of Habilitation thesis: A. Manzanera (ENSTA ParisTech) [Patrick Bouthemy], C. Wolf (INSA Lyon) [Patrick Bouthemy]

Referee of PhD thesis: A.S. Coquel (INSA Lyon) supervised by H. Berry and A. Lindner [Charles Kervrann],

Chair of PhD thesis juries: S. Postec (University of Bretagne-Sud) supervised by J. Froment [Charles Kervrann], P. Dérian (University of Rennes 1)) supervised by E. Mémin [Patrick Bouthemy], L. Coutard (University of Rennes 1) supervised by F. Chaumette [Patrick Bouthemy], M. Ullah (University of Rennes 1) supervised by I. Laptev and P. Pérez [Patrick Bouthemy].

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Major publications by the team in recent years

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Publications of the year

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