
Activity Report 2011

# Team SERPICO

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

RESEARCH CENTER
**Rennes - Bretagne-Atlantique**

THEME
**Computational Biology and Bioinformatics**

# Table of contents

# Team SERPICO

**Keywords:** Biological Images, Computational Biology, Image Processing, Statistical Methods

# 1. Members

**Research Scientists**

Charles Kervrann [Team leader, Inria Senior Researcher, HdR]
Patrick Bouthemy [Inria Senior Researcher, from 01/10/2011, HdR]
Pierre Hellier [Inria Junior Researcher, until 31/07/2011, HdR]

**PhD Students**

Denis Fortun [Inria grant, from 01/11/10]
Philippe Roudot [Inria grant, from 15/10/10]

**Administrative Assistant**

Huguette Béchu [TR Inria, shared with Fluminance and Temics project-teams]

**Other**

Tristan Lecorgne [Engineer (IJD), Inria, from 15/10/11]

# 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 today essential 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, which constitutes one of the challenges of the modern biology. The 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

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 [43]. 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 the biogenesis of specialized organelles in epidermal cells with main functions in the immune system and in skin pigmentation and photoprotection.

## 2.3. 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 participates to several projects (PhD and post-doc supervision, contracts...) with biologists in the field of cell biology and microscopy. They 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. They 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 2012 in collaboration with CNRS-INSB and Institut Curie. SERPICO scientific projects are complementary to the other on-going and planned projects of the UMR 144 CNRS Institut Curie Unit; 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. Image restoration for high-resolution microscopy

WF Optical Wide-Field microscopy.

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

FRET (Förster Resonance Energy Transfer): energy transfer between neighbouring molecules.

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 [27].

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.

In order to produce images compatible with the dynamic processes in living cells as seen in video-microscopy, we study the potential of denoising approaches and non-iterative algorithms [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 treatment 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 wide-field, Nipkow disk based confocal [1], TIRF (Total Internal Reflection Fluorescence [19] microscopy), 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 1). New developments are required in the future to be compatible with "high-throughput microscopy" since we need to analyse several hundred of cells at the same time and since the exposure times are typically reduced.

Meanwhile, improving the resolution beyond 200 nm diffraction limit while retaining the advantages of light microscopy and the specificity of molecular imaging is a long-standing goal in optics. Recent advances have been achieved using 3D-SIM (Structured Illuminated Microscopy) [27]. While being probably less effective in "breaking the resolution barrier" than other optical sub-diffraction limited techniques (e.g. STED [29], PALM [20]), SIM approach has the strong advantage of versatility when considering the photo-physical properties of the fluorescent probes. Nevertheless, in their classical form, SIM is poorly compatible with time regimes expected in most live cell imaging, which restrict their application to fixed samples. Advances in information restoration and image denoising should make SIM imaging compatible with the imaging of molecular dynamic in live cells.

## 3.2. Dynamic analysis and trajectory computation

### *3.2.1. 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 [33]. Data association even combined with sophisticated particle filtering techniques [38] or matching techniques [35] 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 [25], [28]. 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.

### *3.2.2. Event detection*

Several approaches can be considered for the automatic detection of appearing and vanishing particles (or spots) in wide-field (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 [38], [26]. Among the methods proposed to detect particles in microscopy images [39], [37], 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 [23]). Complementary approaches in the line of work described in [12] are currently investigated to provide new results in wide-field and total internal reflection microscopy.

## 3.3. 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 [30], [43] aims at modelling system as a whole in an integrative perspective instead of focusing on independent biophysical processed. One of the goals of these approaches is the cell in silico as investigated in 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 [32] 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) [41] 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. Image processing in high-throughput 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 nD 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).

## 4.2. Data management and storage

In cell and molecular biology [43], new challenges arise to acquire a complete and quantified view from the scale of a “single” cell to the scale of a multi-cellular structure, within the whole organism. In the future, image analysis will be central to the successful use of optical microscopy in this post-genomics biology. Nevertheless, one major difficulty lies in correlating and/or fusing multi-modalities, now routinely used in biology laboratories: optical imaging (spinning-disk confocal, TIRF, SIM, PALM, STED, FLIM-FRET, MP, SPIM/DSLM), ionic imaging (NanoSIMS), atomic force imaging (AFM) and electron imaging (Cryo-EM, Tomo EM). Moreover, in the emerging era of high-throughput microscopy (biochemical screens, cell-based screening), systematic and accurate correlation and analysis of these data cannot be performed manually since the image sequences are composed of several hundred of 3D stacks. Efficient storage, fast retrieval and secure sharing of microscopy images are then others on-going challenges. Our goal is to build an image database with a built-in query system to annotate, retrieve, process and integrate analysis from different imaging modalities. The combination of complementary skills (image processing and analysis software, image data management) will yield a full integration of available information, from image acquisition and analysis to bioinformatics analysis and mathematical modelling in systems biology.

## 4.3. Light and electron microscopy for functional and structural analysis

In the post-genomic era, high resolution of protein structures becomes extremely important for accurate interpretations of biological functions at the molecular level. Meanwhile, microscopic imaging at both the light

and electron microscopic levels provides unique multiscale information on protein localization and interactions. It extends and enriches data obtained from molecular and biochemical techniques. Recently, correlative microscopy has been designed and built to combine the advantages of light fluorescence microscopy with the high resolving power of electron microscopy [34]. Unfrotunately, there is probably no universal similarity measure for multimodal/multiscale image registration in light (LM) and electron (EM) microscopy. In this area, we investigate mutual information [44] and other statistical criteria to correlate intensities in EM and LM images. Data fusion and LM-EM image matching are challenging issues and correspond to a large variety of scales. It is worth noting that light microscopy images are relatively blurred when compared to EM images [42]. The definition of the space of transformations is also an open issue since rigid and non-rigid registration is required to compensate distortions and scale. By correlating different optical imaging methods (Photo Activation-LM, Structured Illumination-LM, PALM, FLIM-FRET) to the subcellular organization uniquely obtained by Electron Microscopy, we hope to overpass current knowledge on specific mechanisms involved in endosomal sorting, specialization and crosstalks between endosomes and these particular organelles.

# 5. Software

## 5.1. nD-SAFIR: Image denoising software

**Participants:** Charles Kervrann, Patrick Bouthemy.

The ND-SAFIR software (APP deposit number: IDDN.FR.001.190033.002.S.A.2007.000.21000 / new release 3.0 in 2012) written in C++, JAVA and MATLAB, removes additive Gaussian and non-Gaussian noise in still 2D or 3D images or in a 2D or 3D image sequences (with no motion computation) (see Figure 1) [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 associate with each pixel (or voxel) 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, ...).

**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)

## 5.2. Fast2D-SAFIR: Fast denoising of large 2D images

**Participant:** Charles Kervrann.

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 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, ...).

## 5.3. PBED: Patch-based event detection

**Participant:** Charles Kervrann.

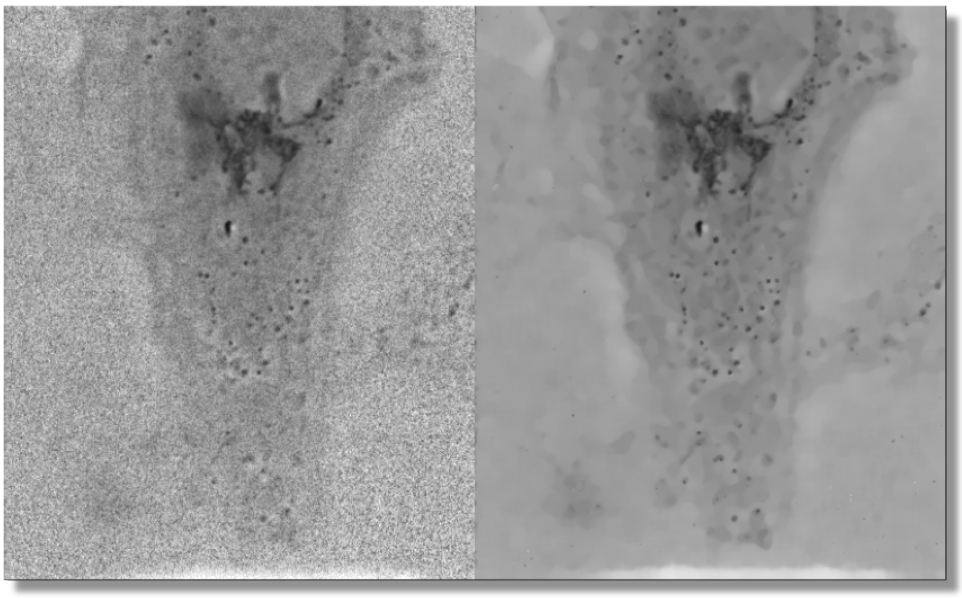

*Figure 1.* ND-SAFIR *software: denoising of a 3D image sequence in wide-field microscopy (GFP-Rab6A (Hela cell), UMR 144 CNRS Institut Curie)*

The PBED software written in C++ automatically quantifies in space and time the number of sudden and transient events observed in fluorescence (WF, TIRF) microscopy. The algorithm parameters are calibrated from the comparison of image patches expected to distinguish sudden appearing/vanishing fluorescent spots/particles from other motion behaviors such as lateral movements [1] and [23]. Two statistical procedures are proposed respectively to control the number of false alarms (Benjamini-Hochsberg, Bonferonni). The algorithm is mainly used to statistically explore the effect of several biological perturbations on the rate of transient events detected on the pilot biological model (e.g. Langerin-YFP endocytic-recycling trans-membrane protein).

**Partners:** J. Boulanger, A. Gidon, A. Chessel, B. Cinquin, J. Salamero (UMR 144 CNRS Institut Curie)

## 5.4. HullkGround: Background subtraction by convex hull estimation

**Participant:** Charles Kervrann.

The HULLKGROUND software (APP deposit number: IDDN.FR.001.400005.000.S.P.2009.000.21000) written in JAVA (plug-in IMAGEJ (http://rsbweb.nih.gov/ij/) 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 $\alpha$ shapes-based scale-space method [24].

**Partners:** A. Chessel and J. Salamero (UMR 144 CNRS Institut Curie)

## 5.5. TubuleJ: Straightening of microtubule cryo-EM projection views

**Participant:** Charles Kervrann.

The TUBULEJ software (APP deposit number: IDDN.FR.001.240023.000.S.P.2011.000.21000) written in JAVA (plug-in IMAGEJ (http://rsbweb.nih.gov/ij/) 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.

**Partners:** S. Blestel and D. Chrétien (UMR 6026 CNRS University of Rennes 1)

## 5.6. Cryo-Seg: Segmentation of tomograms in cryo-electron microscopy

**Participant:** Charles Kervrann.

The CRYO-SEG software written in C++ has been developed to detect microtubule structures and helical structures in 2D cryo-electron microscope images (see Figure 2). 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 6026 CNRS University of Rennes 1)

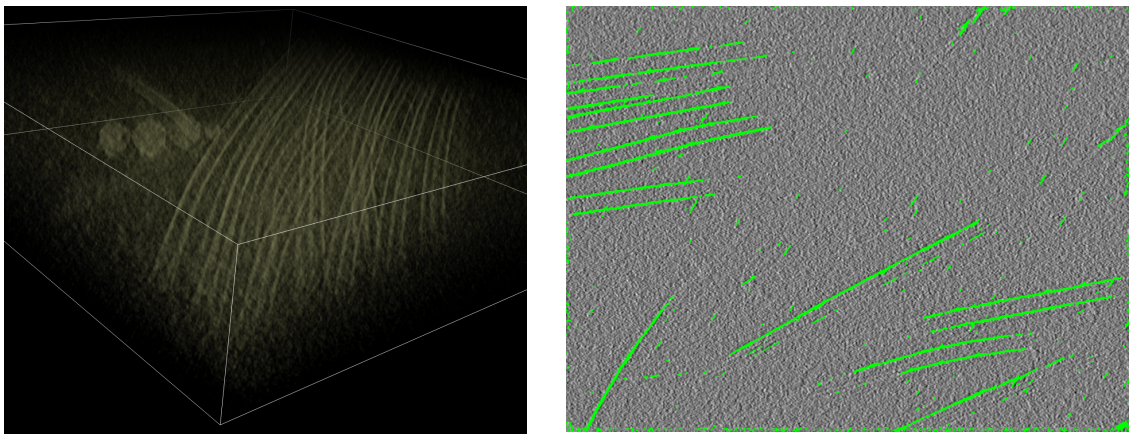

*Figure 2.* CRYO-SEG *software: Segmentation of 3D microtubles in a cryo-EM tomogram (left) and 2D view (right) (UMR 6026 CNRS University of Rennes 1)*

# 6. New Results

## 6.1. Aggregation methods for optical flow computation

**Participants:** Charles Kervrann, Denis Fortun.

We address the problem of optical flow estimation, that is recovering the dense apparent motion of the pixels in a sequence of images. It is a fundamental computer vision task at the basis of a large variety of applications: object tracking, video compression, motion segmentation, movement detection, 3D reconstruction . . . Most of state-of-the art methods rely on a common global variational framework [22], [31]. Computing optical flow amounts to minimizing a global energy. Our experiments demonstrated that the restriction of the minimization to local regions yields significant improvements of the estimation. Motivated by this fact, we developed a novel method to take advantage of this local approach by deriving the global estimate of the flow field from an aggregation of several local estimates. Our work can thus be seen as a general semi-local framework which can be used to improve the performance of any global variational method (Figure 3). We evaluated the performance of our approach on real and synthetic sequences. We investigate adaptations of this methodology to time-lapse fluorescence microscopy and we have recently performed comparisons with usual correlation techniques.

## 6.2. Lifetime estimation of moving vesicles in FLIM microscopy

**Participants:** Charles Kervrann, Philippe Roudot.

Fluorescence lifetime imaging microscopy (FLIM) is a widely spread imaging technique for sensing fluorophore environment in a living biological sample (like pH, ions...). Fluorescence lifetime (i.e. the average time a fluorophore stays in excited state before relaxing to its ground state possibly emitting a photon) is particularly useful to detect the Förster resonance energy transfer (FRET) which quantifies spatial proximity between molecules. We have proposed a statistical framework that exploits the intensity model of the frequency-domain FLIM output to jointly estimate trajectories and lifetimes of tracked vesicles. The proposed tracker, inspired from template cross-correlation or gaussian fitting, combines lifetime estimation and robust M-estimation in a efficient and fast way. Estimation of movement and lifetime are decoupled and alternatively performed, while particle/spot detection is performed on the first frame (Figure 4). To improve the results on real image sequences depicting moving vesicles, the background (cytoplasmic auto-fluorescence) model parameters and the scale parameters involved in the M-estimation procedure are estimated in our approach.

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

## 6.3. Repetitive and transient event detection in fluorescence video-microscopy

**Participants:** Charles Kervrann, Pierre Hellier.

Progresses in imaging dynamic behaviours of molecules including fast video microscopy and the application of evanescent wave microscopy have allowed to image intracellular vesicular movements, exocytosis and endocytosis of fluorescent-tagged proteins. For an unbiased quantification of repetitive and transient events, we have proposed an approach which is versatile enough, to be applicable to diverse although complementary modes of microscopy. The proposed detection method described in [21], [11], [16] can be decomposed into three main steps: i) a first pre-processing step is dedicated to the normalization of the image sequence; ii) the second step is the patch-based detection procedure to detect unusual patterns; iii) a third post-processing step allows us to cluster and count detected events in space and time. In a more recent case study, we have used this approach to analyse image sequences depicting M10 cells stably expressing Langerin-YFP and to get deeper insights in the recycling pathway and dynamics of this molecule.

**Partners:**: A. Gidon, B. Cinquin, A. Chessel, J. Boulanger and J. Salamero (UMR 144 CNRS Institut Curie)

## 6.4. Atlas creation of fluorescence microscopy images

**Participant:** Pierre Hellier.

In this work, we consider the analysis of fluorescence images over time to account for two artifacts: fluorescence decreasing over time and geometric misalignment. A single exponential function is most commonly used to represent measured fluorescence decay profiles due to photobleaching. Accordingly, homologous points need to be geometrically aligned over time. Unfortunately the living cell exhibits slow motion over time. We have considered the iterative estimation of both geometrical alignment and intensity correction by the creation of a 3D atlas.

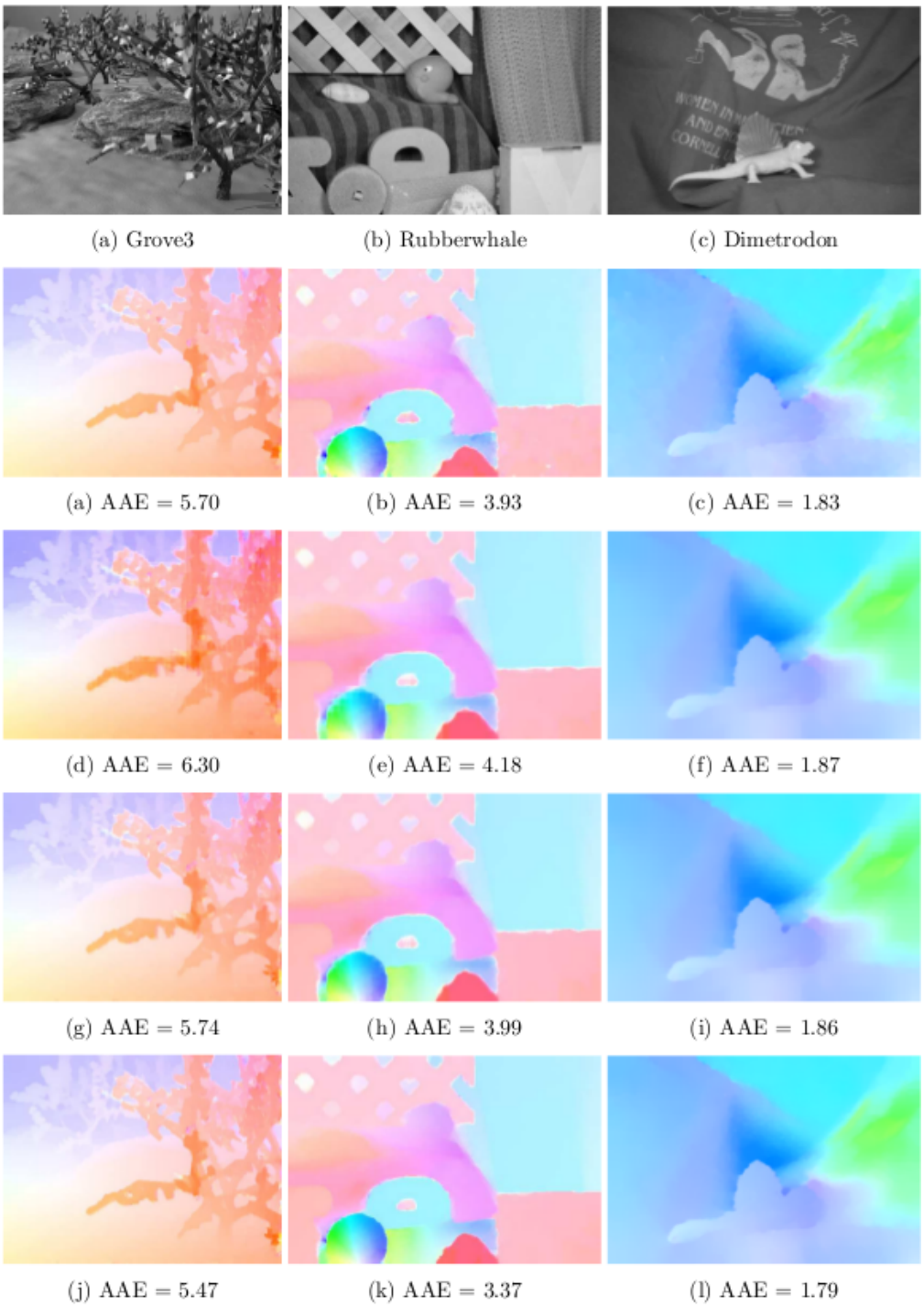


*Figure 3. Average Angular Error (AAE) of vector flows obtained with the global variational method and three aggregation methods applied to three image sequences of the Middlebury (http://vision.middlebury.edu/flow/) database: row #1: first frame of the sequence; row #2: global variational method; row #3: mean aggregation; row #4: confidence-based weighted average; row #5: graph cut aggregation.*

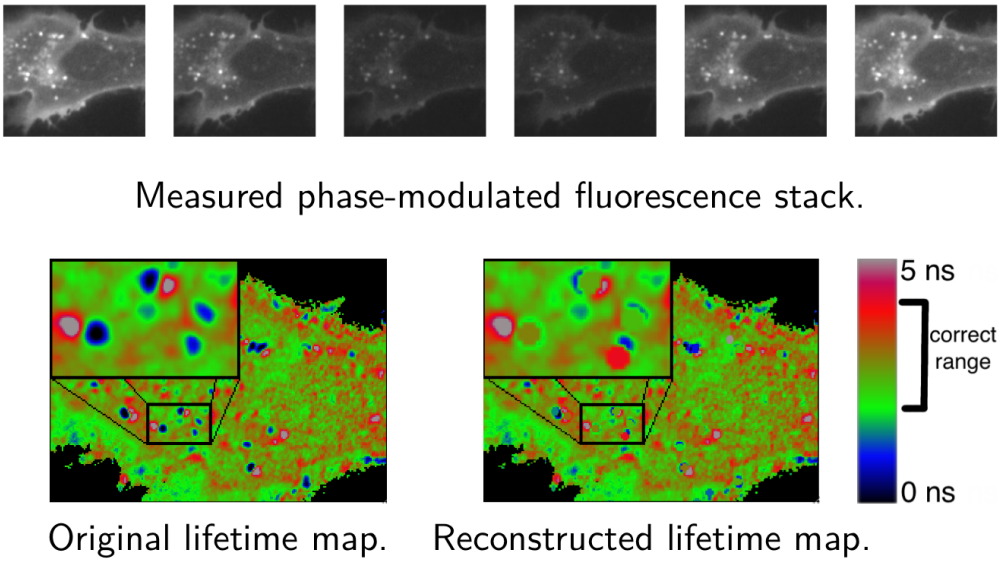


*Figure 4. Lifetime map after spot/particle motion compensation on a phase-modulated fluorescence image stack.*

## 6.5. Averaging of 3D volumes and denoising for the analysis of cryo-electron tomograms

**Participant:** Charles Kervrann.

Trichocysts are large vesicles secreted by the ciliated protozoa, Paramecium. They are characterized by the presence of large three-dimensional crystals of proteins. Under chemical or physical stimuli, or facing a predator, trichocysts undergo an exocytosis, right after fusion of their membrane with the unicellular organism plasma membrane. The crystalline mesh changes its conformation from a condensed to an extended shape in a few milliseconds. Nowadays, cryo-electron tomography (cryo-ET) allows one to visualize those crystals and so, to analyse their three-dimensional organization. However, two main impediments remain with this method. Samples are very sensitive to electron radiation involving the spreading out of the electron dose on the whole tilt series, causing the emergence of background noise in the images. Moreover, a lack of data occurs during image acquisition, called the "missing wedge", due to uncovered angles at the moment of the acquisition of the tilt series. After tomogram reconstruction of four trichocysts, we have tested usual denoising methods (anisotropic diffusion, Fourier coefficient thresholding) and an unpublished patch-based denoising method inspired from the nD-safir software (5.1). The denoising methods improved the alignment of different crystal sub-volumes. The sub-volume averaging allowed us to fill in partially the "missing wedge" and then, to obtain a more faithful three-dimensional crystal reconstruction [17], [15].

**Partners:** E. Pollet, A. Guesdon and D. Chrétien (UMR 6026 CNRS University of Rennes 1)

## 6.6. Analysis of spatio-temporal dynamics of cytoplasmic actin under geometrical confinement

**Participant:** Charles Kervrann.

The generation, cell-cycle regulation and maintenance of such cellular functions are often correlated with symmetry breaking and spatiotemporal reorganization of F-actin assembly. In this study, we analyzed the spatiotemporal evolution of actin filaments using Xenopus meiotic extracts artificially confined within a geometry mimicking the cell boundary. It turns out the confinement of the cytoplasm generates symmetry breaking in the spatial organization of actin filaments. Combination of quantitative image analysis and biochemical perturbations show that both spatial localization of F-actin nucleators and actin turnover play a decisive role in generating symmetry breaking. In this project, we proposed to combine an optical flow-based tracker (Kanade-Lucas-Tomasi tracker [40], [36]) to a photobleaching correction method in order to extract quantitative spatiotemporal characteristics of the actin dynamics.

**Partners:** Z. Gueroui (BioPhysics team, UMR 8640 Ecole Normale Superieure, Paris) and M. Pinot (UMR 144 CNRS Institut Curie, Paris)

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

**Participant:** Charles Kervrann.

Modifications of plasma membrane physical properties are also known to be involved in the perception and response to environmental modifications such as temperature, mechanical and osmotic stress in various organisms. We have analysed a recently designed probe, di-4-ANEPPDHQ, that can change its fluorescent properties depending on whether it is residing in ordered or disordered phases of the tobacco cell plasma membranes. We performed a spatial analysis (covariograms, Markov Random Fields) of small (<200 nm) ordered domains observed in multispectral confocal microscopy. We focused on binary images, assumed to be realizations of a MRF-Ising model, depicting the spatial organization of ordered domains. Maximum pseudo-likelihood methods were investigated to estimate parameters able to describe the spatial properties of ordered domains. We analyzed the modifications of the whole plasma membrane fluidity, and the distribution of ordered domains occurring in the few minutes following addition of the elicitor of defense reaction cryptogein to tobacco cells.

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

# 7. Contracts and Grants with Industry

## 7.1. Contracts with Industry

J. Boulanger, C. Kervrann, P. Bouthemy. Commercial software license agreement with Photometrics company: “Software for denoising n-dimensional images”.

# 8. Partnerships and Cooperations

## 8.1. Regional Initiatives

### *8.1.1. Computing and storage facilities*

**Participant:** 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 might be desirable.

**Funding:** Rennes-Metropole - “Allocation Installation Scientifique”

### 8.1.2. Cryo-Soft project

**Participant:** Charles Kervrann.

The goal of this project is to develop image processing softwares (TubuleJ (5.5), Cryo-Seg (5.6), ...) and to design user-friendly interfaces for applications in cryo-electron microscopy.

**Funding:** University of Rennes 1 - "Emerging Scientific Challenges" program
**Partner:** UMR 6026 CNRS University of Rennes 1

## 8.2. National Initiatives

### 8.2.1. Quaero project

**Participant:** Charles Kervrann.

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 have conducted activities in object tracking and indexing for video-microscopy analysis (Denis Fortun's PhD grant (6.1)).

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

### 8.2.2. 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 (6.2))

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

### 8.2.3. 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 sequence of 3D volumes containing a population of growing axons.

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

### 8.2.4. Bio-IP toolbox

**Participants:** Charles Kervrann, Tristan Lecorgne, Pierre Hellier.

The goal of this project is to integrate software tools (Section 5) in user-friendly interfaces. The softwares have been developed in various bioimaging projects and some of them required GPU accelerations for facing high-content microscopy. The softwares and plug-in will be used by biologists to explore image data.

**Funding:** Inria ADT (2011-2013)
**Partner:** UMR 144 CNRS PICT IBiSA Institut Curie

### *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. ESFRI Euro-BioImaging initiative*

**Participant:** Charles Kervrann.

SERPICO participates to the ESFRI Euro-BioImaging project, one of the four new biomedical sciences 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 Initiatives

### *8.4.1. Inria International Partners*

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

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 RICAM Linz (Austria), P. Elbau, on non-local image denoising.

# 9. Dissemination

## 9.1. Animation of the scientific community

- *Technical program committees of conferences*
  - Charles Kervrann: reviewer for ISBI'2011, ISBI'2012, ICASSP'2011, ICASSP'2012, ICIP'2012, EMMCVPR'2011.
  - Pierre Hellier: reviewer for ISBI'2011, MICCAI'2011.
  - Patrick Bouthemy: reviewer for TAIMA'2011, ICRA'2011, ICPRAM'2012, ISBI' 2012.
- *Journal reviewing*

- Charles Kervrann: reviewer in 2011 for Image and Vision Computing, Medical Image Analysis, BMC Biophysics, IEEE Transactions on Image Processing, IEEE Transactions on Medical Imaging, IEEE Transactions on Pattern Analysis and Machine Intelligence, Journal of Mathematical Imaging and Vision.
- Pierre Hellier: reviewer in 2011 for Medical image Analysis, IEEE Transactions on Medical Imaging, Journal of Real-time Image processing, International Journal of Computer Assisted Radiology and Surgery.
- Patrick Bouthemy: reviewer in 2011 for for International Journal of Computer Vision, International Journal of Robotics Research, IEEE Transactions on Circuits and Systems for Video Technology, Traitement du Signal.

- *PhD and HdR jury*
  - Charles Kervrann: L. Pizarro supervised by J. Weickert, (PhD, reviewer, University of Saarland, Germany), N. Noury supervised by M.O. Berger and F. Sur (PhD, reviewer, University of Henri Poincaré - Nancy 1), B. Cinquin supervised by J. Salamero and M. Réfrégiers (PhD, member of the jury, University of Paris-Diderot), J. Delon (HdR, member of the jury, ENS Cachan).
  - Patrick Bouthemy: M. Primet supervised by L. Moisan (PhD, reviewer, University of Paris 5), C. Guilmart supervised par S. Herbin (PhD, reviewer, ENS Cachan), A. Dame supervised by E. Marchand (PhD, president of the jury, University of Rennes 1), C. Wang supervised by N. Paragios (PhD, member of the jury, Ecole Centrale Paris), R. Perrier supervised by P. Sturm (PhD, member of the jury, Inria Grenoble Rhône-Alpes), T. Corpetti (HdR, member of the jury, University of Rennes 1).

- *Participations in seminars, invitations, awards*
  - Charles Kervrann was invited to give a talk entitled "Change detection methods in fluorescence live cell imaging for sub-cellular trafficking analysis" at the "BioImage Informatics II" conference, Janelia Farm, Howard Hugues Medical Institute, Ashburn, VA, November 2011.
  - P. Bouthemy gave a talk entitled "Conditional mixed-state MRF for simultaneous motion detection and background image reconstruction" at the seminar "Hidden Markov Models and extensions" of GDR ISIS, March 2011.

- *Responsibilities*

  Charles Kervrann:

  member of the IEEE BISP "Biomedical Image and Signal Processing" committee

  member of executive board of the GdR 2588 ("Microscopie Fonctionelle du Vivant") CNRS

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

  member of the 2011 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/) (imagery plateform, University of Rennes 1)

  Pierre Hellier

  consultancy work for Syneika startup

member of the Inria committee for communication and the Inria committee for technological development

Patrick Bouthemy

Director of the Inria Rennes - Bretagne Atlantique centre until 30/09/2011

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

member of the board of AFRIF (Association Francaise pour la Reconnaissance et l'Interprétation des Formes) and of Association GRETSI (Groupement de Recherche en Traitement du Signal et des Images)

- *Other activities*
  - SERPICO is involved in the french network GdR ISIS "Information, Signal and Images" CNRS and in the french network GdR 2588 "Microscopie Fonctionelle du Vivant" CNRS.
  - SERPICO organized the GdR 2558-ISIS/GIS Europia meeting on "Image Processing in Biological Imaging" at the Inria Rennes - Bretagne Atlantique centre on November 2011.

## 9.2. Teaching

Charles Kervrann:

Master 2 Recherche SISEA: "Geometric modelling for shapes and images", 5 hours, University of Rennes 1

Engineer Degree ENSAI: "Statistical models and image analysis", 20 hours, Ecole National de la Statistique et de l'Analyse de l'Information, Bruz

Patrick Bouthemy:

Master 2 Recherche SISEA : "Analysis of image sequences", 14 hours, University of Rennes 1

Engineer Degree and Master 2 Recherche IRIV : "Motion analysis", 11 hours, ENSPS, University of Strasbourg

PhD & HdR:

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

# 10. Bibliography

## Major publications by the team in recent years

[1] J. BOULANGER, A. GIDON, C. KERVRANN, J. SALAMERO. *A Patch-Based Method for Repetitive and Transient Event Detection in Fluorescence Imaging*, in "PLoS ONE", Oct 2010, vol. 5, n$^{o}$ 10, e13190 [*DOI :* 10.1371/JOURNAL.PONE.0013190], http://hal.inria.fr/inria-00541072/en.

[2] J. BOULANGER, C. KERVRANN, P. BOUTHEMY. *Space-time adaptation for patch based image sequence restoration*, in "IEEE Transactions on Pattern Analysis and Machine Intelligence", 2007, vol. 29, n$^{o}$ 6, p. 1096–1102.

[3] J. BOULANGER, C. KERVRANN, P. BOUTHEMY. *A simulation and estimation framework for intracellular dynamics and trafficking in video-microscopy and fluorescence imagery*, in "Medical Image Analysis", 2009, vol. 13, p. 132–142.

[4] J. BOULANGER, C. KERVRANN, P. BOUTHEMY, P. ELBAU, J.-B. SIBARITA, J. SALAMERO. *Patch-Based Nonlocal Functional for Denoising Fluorescence Microscopy Image Sequences*, in "IEEE Transactions on Medical Imaging", Feb 2010, vol. 29, n$^{o}$ 2, p. 442-453 [*DOI :* 10.1109/TMI.2009.2033991], http://hal.inria.fr/inria-00541082/en.

[5] P. M. CARLTON, J. BOULANGER, C. KERVRANN, J.-B. SIBARITA, J. SALAMERO, S. GORDON-MESSER, J. HABER, S. HAASE, L. SHAO, A. WINOTO, P. KNER, S. USAWA, Y. STRUKOV, M. GUSTAFSSON, Z. KAM, D. AGARD, J. W. SEDAT. *Fast live simultaneous multiwavelength four-dimensional optical microscopy*, in "Proc Natl Acad Sci USA", Sep 2010, vol. 107, n$^{o}$ 37, p. 16016-16022 [*DOI :* 10.1073/PNAS.1004037107], http://hal.inria.fr/inria-00540978/en/.

[6] C. KERVRANN, J. BOULANGER. *Optimal spatial adaptation for patch-based image denoising*, in "IEEE Transactions on Image Processing", 2006, vol. 15, n$^{o}$ 10, p. 2866–2878.

[7] C. KERVRANN, J. BOULANGER. *Local adaptivity to variable smoothness for exemplar-based image denoising and representation*, in "International Journal Computer Vision", August 2008, vol. 79, n$^{o}$ 1, p. 45–69.

## Publications of the year

### Articles in International Peer-Reviewed Journal

[8] B. CINQUIN, A. GIDON, A. CHESSEL, T. PÉCOT, J. BOULANGER, C. GUEUDRY, C. KERVRANN, J. SALAMERO. *Advanced microcopy to study tracking and spatiotemporal organization of intracellular membranes*, in "Bio-medical materials and engineering", 2011, http://hal.inria.fr/inria-00541284/en.

[9] F. COQUELLE, S. BLESTEL, C. HEICHETTE, I. ARNAL, C. KERVRANN, D. CHRÉTIEN. *Cryo-Electron Tomography of microtubules assembled in vitro from purified components.*, in "Methods in Molecular Biology -Clifton then Totowa-", July 2011, vol. 777, p. 193-208, http://hal.inria.fr/hal-00651384/en/.

[10] T. CRIVELLI, P. BOUTHEMY, B. CERNUSCHI-FRIAS, J.-F. YAO. *Simultaneous motion detection and background reconstruction with a conditional mixed-state markov random field*, in "International Journal of Computer Vision", September 2011, vol. 94, n$^{o}$ 3, p. 295–316 [*DOI :* 10.1007/s11263-011-0429-z], http://hal.inria.fr/hal-00651558/en/.

[11] A. GIDON, S. BARDIN, J. BOULANGER, F. WAHARTE, B. CINQUIN, L. HELIOT, H. DE LA SALLE, D. HANAU, C. KERVRANN, B. GOUD, J. SALAMERO. *Rab11A/MyosinVb/Rab11-FIP2 complex frames two late recycling steps of langerin from ERC to plasma membrane*, in "Traffic", 2011, to appear.

[12] C. KERVRANN, J. BOULANGER, T. PÉCOT, P. PÉREZ, J. SALAMERO. *Multiscale neighborhood-wise decision fusion for redundancy detection in image pairs*, in "SIAM Journal Multiscale Modeling & Simulation (accepted)", 2011, to appear, http://hal.inria.fr/inria-00487051/en.

### Conferences without Proceedings

[13] F. COQUELLE, A. GUESDON, M. ANGEVIN, T. CHENUEL, M. CHRÉTIEN, C. HEICHETTE, S. BLESTEL, C. KERVRANN, I. ARNAL, D. CHRÉTIEN. *Interaction of +TIPs with microtubule ends*, in "12th Congres

of the French Society for Microscopy", Strasbourg, France, Société Française des Microscopies, June 2011, http://hal.inria.fr/hal-00651851/en/.

[14] A. GUESDON, S. BLESTEL, C. KERVRANN, D. CHRÉTIEN. *How does GTP-hydrolysis destabilize the microtubule lattice ?*, in "12th Congres of the French Society for Microscopy", Strasbourg, France, Société Française des Microscopies, June 2011, http://hal.inria.fr/hal-00651848/en/.

[15] A. GUESDON, F. COQUELLE, S. BLESTEL, E. POLLET, B. VITRE, M. ANGEVIN, C. HEICHETTE, I. ARNAL, C. KERVRANN, D. CHRÉTIEN. *Cryo-electron tomography of macromolecular assemblies: from quantum dots to 3D crystals of proteins*, in "12th Congres of the French Society for Microscopy", Strasbourg, France, Société Française des Microscopies, June 2011, http://hal.inria.fr/hal-00651880/en/.

[16] C. KERVRANN, J. BOULANGER, A. CHESSEL, B. CINQUIN, J. SALAMERO. *Change detection methods in fluorescence live cell imaging for sub-cellular trafficking analysis*, in "BioImage Informatics II", Ashburn VA, États-Unis, Janelia Farm - Howard Hugues Medical Institute, September 2011, invited talk, http://hal.inria.fr/hal-00651919/en/.

[17] E. POLLET, C. FISCH, A. GUESDON, C. HEICHETTE, P. DUPUIS-WILLIAMS, C. KERVRANN, A. LEFORESTIER, D. CHRÉTIEN. *Structure of Paramecium trichocysts*, in "12th Congres of the French Society for Microscopy", Strasbourg, France, Société Française des Microscopies, June 2011, http://hal.inria.fr/hal-00651898/en/.

### Scientific Books (or Scientific Book chapters)

[18] T. CRIVELLI, B. CERNUSCHI-FRIAS, P. BOUTHEMY, J.-F. YAO. *Mixed-state Markov models in image motion analysis*, in "Machine Learning for Vision-Based Motion Analysis", SPRINGER (editor), Theory and Techniques Series: Advances in Pattern Recognition / Wang, L.; Zhao, G.; Cheng, L.; Pietikäinen, M. (Eds.), SpringerLink, 2011, vol. 1st Edition., 2011, XIV, 372 pages, p. Part 2, 77-115 [*DOI :* 10.1007/978-0-85729-057-1_4], http://hal.inria.fr/inria-00541253/en.

## References in notes

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

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

[21] J. BOULANGER, A. GIDON, C. KERVRANN, J. SALAMERO. *A Patch-Based Method for Repetitive and Transient Event Detection in Fluorescence Imaging*, in "PLoS ONE", Oct 2010, vol. 5, n$^{o}$ 10, e13190 [*DOI :* 10.1371/JOURNAL.PONE.0013190], http://hal.inria.fr/inria-00541072/en.

[22] T. BROX, A. BRUHN, N. PAPENBERG, J. WEICKERT. *High Accuracy Optical Flow Estimation Based on a Theory for Warping*, in "European Conference on Computer Vision (ECCV'04)", Prague, Czech Republic, 2004, vol. 4, p. 25-36.

[23] A. CHESSEL, B. CINQUIN, S. BARDIN, J. BOULANGER, J. SALAMERO, C. KERVRANN. *A detection-based framework for the analysis of recycling in TIRF microscopy*, in "IEEE International Symposium on Biomedical Imaging: From Nano to Macro (ISBI 2010)", Pays-Bas Rotterdam, IEEE (editor), IEEE Signal Processing

Society, Apr 2010, p. 1281-1284 [*DOI :* 10.1109/ISBI.2010.5490230], http://hal.inria.fr/inria-00541102/en.

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

[25] T. CORPETTI, E. MÉMIN, P. PÉREZ. *Dense estimation fluid flows*, in "IEEE Trans. Pattern Analysis and Machine Intelligence", 2002, vol. 24, n$^{o}$ 3, p. 365–380.

[26] A. GENOVESIO, T. LIEDL, V. EMILIANI, W. PARAK, M. COPPEY-MOISAN, J. OLIVO-MARIN. *Multiple particle tracking in 3D+t microscopy: method and application to the tracking of endocytosed quantum dots*, in "IEEE Trans. Image Processing", 2006, vol. 15, n$^{o}$ 5, p. 1062–1070.

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

[28] H. HAUSSECKER, D. FLEET. *Computing optical flow with physical models of brightness variation*, in "IEEE Trans. Pattern Analysis and Machine Intelligence", 2001, vol. 23, n$^{o}$ 6, p. 661–673.

[29] T. KLAR, S. JAKOBS, M. DYBA, A. EGNER, S. HELL. *Fluorescence microscopy with diffraction resolution barrier broken by stimulated emission*, in "Proc Natl Acad Sci USA", 2000, vol. 97, p. 8206–8210.

[30] C. LAVELLE, H. BERRY, G. BESLON, F. GINELLI, J. GIAVITTO, Z. KAPOULA, A. LE BIVIC, N. PEYRIERAS, O. RADULESCU, A. SIX, V. THOMAS-VASLIN, P. BOURGINE. *From molecules to organisms: towards multiscale integrated model of biological systems*, in "Theoretical Biology Insights", 2008, vol. 1, p. 13–22.

[31] E. MÉMIN, P. PÉREZ. *Hierarchical estimation and segmentation of dense motion fields*, in "Int. Journal of Computer Vision", February 2002, vol. 46, n$^{o}$ 2, p. 129–155.

[32] F. NÉDÉLEC. *Computer simulations reveal motor properties generating stable antiparallel microtubule interactions*, in "The Journal of Cell Biology", 2002, vol. 158, n$^{o}$ 6, p. 1005–1015.

[33] P. PÉREZ, J. VERMAAK, A. BLAKE. *Data fusion for visual tracking with particles*, in "Proc. IEEE", 2004, vol. 92, n$^{o}$ 3, p. 495–513.

[34] A. SARTORI, R. GATZ, F. BECK, A. RIGORT, W. BAUMEISTER, J. PLITZKO. *Correlative microscopy: bridging the gap between fluorescence light microscopy and cryo-electron tomography*, in "J. Structural Biology", 2007, vol. 160, n$^{o}$ 2, p. 135–145.

[35] I. SBALZARINI, P. KOUMOUTSAKOS. *Feature point tracking and trajectory analysis for video Imaging in cell biology*, in "J. Structural Biology", 2005, vol. 151, p. 182–195.

[36] J. SHI, C. TOMASI. *Good features to track*, in "IEEE Conference on Computer Vision and Pattern Recognition (CVPR'94)", Seattle, Washington, 1994, p. 593–600.

[37] I. SMAL, M. LOOG, W. NIESSEN, E. MEIJERING. *Quantitative comparison of spot detection methods in fluorescence microscopy*, in "IEEE Trans. Medical Imaging", 2010, vol. 29, n$^{o}$ 2, p. 282–301.

[38] I. SMAL, E. MEIJERING, K. DRAEGESTEIN, N. GALJART, I. GRIGORIEV, A. AKHMANOVA, M. VAN ROYEN, A. HOUTSMULLER, W. NIESSEN. *Multiple object tracking in molecular bioimaging by Rao-Blackwellized marginal particle filtering,*, in "Medical Image Analysis", 2008, vol. 12, n$^{o}$ 6, p. 764–777.

[39] D. THOMANN, D. RINES, P. SORGER, G. DANUSER. *Automatic fluorescent tag detection in 3D with super-resolution: application to the analysis of chromosome movement*, in "J. Microscopy", 2002, vol. 298, n$^{o}$ Part 1, p. 49–64.

[40] C. TOMASI, T. KANADE. *Detection and Tracking of Point Features*, Carnegie Mellon University, 1991, n$^{o}$ CMU-CS-91-132.

[41] Y. VARDI. *Network tomography: estimating source-destination traffic intensities from link data*, in "J American Statistical Association", 1996, vol. 91, p. 365–377.

[42] G. VICIDOMINI, M. GAGLIANI, M. CANFORA, K. CORTESE, F. FROSI, C. SANTANGELO, P. DI FIORE, P. BOCCACCI, A. DIASPRO, C. TACCHETTI. *High data output and automated 3D correlative light-electron microscopy method*, in "Traffic", 2008, vol. 9, p. 1828–1838.

[43] T. WALTER, D. SHATTUCK, R. BALDOCK, M. BASTIN, A. CARPENTER, S. DUCE, J. ELLENBERG, A. FRASER, N. HAMILTON, S. PIEPER, M. RAGAN, J. SCHNEIDER, P. TOMANCAK, J. HERICHE. *Visualization of image data from cells to organisms*, in "Nature Methods", 2010, vol. 7, n$^{o}$ 3 (Suppl), p. S26–41.

[44] W. WELLS, P. VIOLA, H. ATSUMI, S. NAKAJIMA, R. KIKINIS. *Multi-modal registration by maximization of mutual information*, in "Medical Image Analysis", 1996, vol. 1, n$^{o}$ 1, p. 35–51.