



INSTITUT NATIONAL DE RECHERCHE EN INFORMATIQUE ET EN AUTOMATIQUE

Team Serpico

*Space-time RePresentation, Imaging and
cellular dynamics of molecular COmplexes*

Rennes - Bretagne-Atlantique

Theme : Computational Biology and Bioinformatics

Activity
R *eport*

2010

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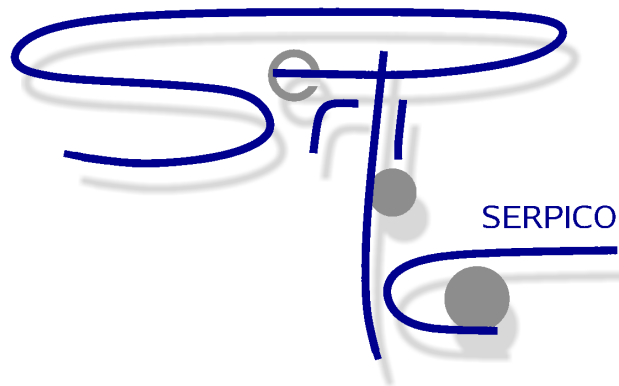


Figure 1. *SERPICO: Space-time RePresentation, Imaging and cellular dynamics of molecular COmplexes*

1. Team

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2. Overall Objectives

2.1. Introduction

Multidimensional and multimodal light microscopy combined with GFP (Green Fluorescence Protein) tagging has taken a prominent role in life science research due to its ability to study *in vitro* and *in vivo* biomolecules in the cell compartments and cell domains. Accordingly, the long-term goal SERPICO team is 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 [56]. We address the following themes:

- image superresolution/image denoising to preserve cell integrity (photo-toxicity versus exposure time) and image analysis in multidimensional microscopy;
- spatio-temporal modelling of molecular species and multiscale architectures / multiscale registration of electron and light microscopy images to study molecule interactions in space and time;

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- computational simulation and modelling of molecule trafficking at different spatial and temporal scales / 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. The mathematical theories and algorithms are mainly developed to identify other 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.

During the period (2006-2009), in collaboration with UMR 144 CNRS Institut Curie (“Subcellular Structure and Cellular Dynamics” Unit and PICT-IBiSA (Cell and Tissue Imaging Facilities), people involved in the VISTA EPI participated to several projects (PhD and post-doc supervision, contracts...) with biologists in the field of cell biology and microscopy. In particular, they have already developed methodologies and tools for processing complex image data. With originality they have promoted and developed non-parametric methods since prior knowledge cannot be easily taken into account for extracting unexpected but desired information from image data. They have proposed user-friendly algorithms for processing 3D or 4D data. These algorithms are non-iterative, do not require the adjustment of parameters and can be used by biologists.

To reinforce the interactions between cell biology, imaging instrumentation and applied mathematics and to improve visibility, the SERPICO team has been created in 2010 at INRIA Rennes, in collaboration with CNRS and Institut Curie. The scientific projects are complementary to the other on-going and planned projects of the Institut Curie CNRS UMR 144 Unit; a part of the existing projects is related to instrumentation in electronic and photonic microscopy (PICT-IBiSA platform) including some 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 result and advances of these instrumental projects and a positive synergy is foreseen.

2.2. Highlights

- SERPICO, the first team in “biological imaging” at INRIA, has been created officially on January 1st 2010.
- Charles Kervrann defended his “Habilitation à Diriger des Recherches” (HdR) entitled “*Modèles statistiques pour l’analyse d’images, algorithmes et applications en imagerie bio-cellulaire et moléculaire*” on June 29st, 2010 at University of Rennes 1 (committee : Nicholas Ayache, Gilles Celeux, Rémi Gribonval, Jean-Michel Morel, Jean Salamero, Christoph Schnörr).
- Pierre Hellier defended his “Habilitation à Diriger des Recherches” (HdR) entitled “*Image processing methods for 3D intraoperative ultrasound*” on June 30st, 2010 at University of Rennes 1 (committee : Nicholas Ayache, Dave Hawkes, Étienne Mémin, Xavier Morandi, Wiro Niessen, Jean-Philippe Thiran, Jocelyne Troccaz).

3. Scientific Foundations

3.1. Cell and Tissue Imaging facility (PICT)-Institut Curie

Glossary

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.

FLIM (Fluorescence Lifetime Microscopy Imaging) imaging of fluorescent molecule lifetimes (from picto to nano seconds).

FRAP (Fluorescence Recovery After Photobleaching) molecular diffusion analysis after local photoextinction.

FRET (Förster Resonance Energy Transfer) energy transfer and interaction measurement between neighbouring molecules.

Multipoint Spinning Disk Confocal fast confocal microscopy using rotative disks.

PALM (Photo-Activated Localization Microscopy) high-resolution microscopy using stochastic photo-activation of fluorophores and adjustment of point spread functions [36].

SIM (Structured Illumination Microscopy) high-resolution (wide-field) microscopy using structured patterns and interference analysis (Moiré patterns) [39].

TIRF (Total Internal Reflectance Microscopy) 2D optical microscopy using evanescent waves and total reflectance [34].

The PICT-IBiSA Institut Curie (<http://pict-ibisa.curie.fr/>) gathers highly sophisticated equipments to provide research groups from inside and outside the Institut Curie (350 individual users in 2010; external 15%; about 60 000 Hours on all machines, with strong disparities) with a workflow of state to the art imaging methods to study at different scales molecules, organelles, cells, whole organisms and tissues in normal and pathological states, at high spatial and temporal resolution. Main fields of research extend from physics, genetics, cell biology, immunology and development with a special focus on Cancer. The PICT-IBiSA was and is still partner of a number of national (ANR INNOVEO, ANR MICAD, ACI ModynCELL5D, and International program (EAMnet, FP5, CAmiNEMS FP7).

PICT-IBiSA hosts and manages the French Nikon Imaging Center@Institut Curie-CNRS <http://nimce.curie.fr/>, since 2007. The platform includes equipments and expertise provided by researchers and engineers, within different departments of the Institut Curie on the Paris, (UMR144/ INSERM U 932; UMR168; UMR218; UMR3215/Inserm U934) and Orsay sites (UMR 3348 / CNRS UMR 3348; U759). PICT is organized around four major imaging axes: optical microscopy, Nano-SIMS, electron microscopy (EM) and image data processing. Optical microscopy extends from 3D deconvolution microscopy to high resolution photonic setup (2 FLIM-FRET systems (full open access: FOA), Dual TIRF-PALM (FOA), n-SIM (FOA), Ultra Fast Live Optical Microscopy-OMX (installed; progressively in FOA), Seven nD Spinning systems (6 in Partial or Full Open Access...)) with a particular highlight on fast dynamic imaging of molecular processes at the single cell level and in model organisms. Nano-SIMS provides chemical and isotopic maps of molecules within the cell. To a lower but increasing extend, “High Content Screening” (HCS) imaging is also developed within the centralized platform Biophenics at Institut Curie. One unique feature is the three complementary modalities and expertise in EM: Cellular/Molecular, Cryo and 3D electron microscopy. Optical and electron microscopy are combined to delineate biological questions at different spatial, dynamical and structural scales. The team trains users and is in charge of evaluation of new available systems. The platform develops new hardware solutions, applications, algorithms and software. The members organize and teach at national and international workshops and are involved in University modules.

3.2. Objectives and motivations

Light microscopy, and particularly fluorescence microscopy, has taken a prominent role in life science research due to its ability to study the 3D interior of cells and organisms, and to visualize, in vitro and in vivo, particular biomolecules and proteins (gene expression) with great specificity through fluorescent (GFP (Green Fluorescence Protein) probes) labeling both at the microscopic and nanoscopic scales. Nevertheless, the mechanisms of life are very complex and driven by multimolecular interactions. Most of these interactions take place within “molecular machines” resulting of the assembly of multiple macromolecular species. Furthermore, cellular functions must be thought as an integration of molecular activities in space and time. Molecular structures of the cytoskeleton such as the mitotic spindle, the cell signaling complexes, the nuclear envelop, the structures involved in intracellular transport, those responsible for adhesion or for cell morphogenesis and motility, all illustrate the notion of dynamic molecular assembly. Biological gatherings exist at different scales: molecular, subcellular and cellular, at the level of tissue and beyond.

In all cases, they constitute sub-systems or integrated modules whose activities are themselves controlled by signalization and/or regulation via a large number of molecular networks. 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.

In computational biology, three types of data mainly support the investigation: genomic data, transcription and expression data, and structural data. Nevertheless, over the last few years it became imperative to develop new approaches and reinforce the technologies that are necessary for the measurement of the dynamics of the biochemical and molecular processes in the 3D space. Accordingly, dynamic imaging in microscopy is exploited as an investigation tool to understand the function of particular biomolecules of interest. In combination with the amount of information provided by high-throughput multidimensional microscopy and automated image analysis methods, the SERPICO team proposes then to design computational and statistical models to better elucidate the role of specific proteins inside their multiprotein complexes. An important task is then to provide computational methods and mathematical models to automatically extract, organize and model dynamic information at the scale of a single scale, present in temporal series of images as they are obtained in nD microscopy. Another important aim is to foster dedicated technological developments to build an integrated imaging approach that bridges the resolution gaps between the molecule and the whole cell, including their temporal behavior. While we focus on particular biological models of endo-membrane biogenesis and trafficking in the endosomal-recycling pathway of specialized cells, we are convinced that most of our results and development will apply in different field of cell and integrative biology. Our research means intensive technological development linked to mathematical formulation and iterative studies between experimentalists (biologists and physicists) and theorists (mathematicians and computer scientists).

3.3. Image analysis in high-throughput and multidimensional microscopy

The last two decades have witnessed the explosion of the digital imaging for the understanding of such living cell activity and molecular structure organization. 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 an experiment constituted by multiple image series acquisitions is massive (up to few Giga-bytes per hour). Therefore, it becomes absolutely necessary in a wide number of modern biology and biomedical fields 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. The accurate management and analysis will necessitate innovative mathematical tools and concepts (data fusion, image registration, superresolution, data mining, life dynamics modelling).

3.4. Correlative Light (LM) and Electron (EM) Microscopy (CLEM) for spatio-temporal organization modelling of molecular species and multiscale architectures

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 level provides multiscale unique information on protein localization and interactions,

and extends and enriches that 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 [49]. The 3D reconstruction of macromolecular structures from 2D EM images of vitrified biological samples (Cryo-EM) has proved to be an effective technique to investigate the structure of native cells nanometer-scale resolution and preserve the whole integrity of the cell. Nevertheless, the high magnification available with EM comes with a limited field of view. One major limitation within CLEM strategies are the time interval between cell selection under the light microscope and the fixation step (chemical fixation but also transfer to the High Pressure Freezing apparatus) that takes minutes and up to 15-20 seconds, which is too slow to fix rapid intracellular movements at the exact time of interest. Therefore one needs a gentle and time efficient way to locate structures of interest, register multimodal images, improve image contrast and remove noise for a better interpretation of the image contents. Sub-diffraction limited imaging techniques and numerical super-resolution methods can be used to bridge the gap in between. In this area, the ambition of the SERPICO team is to design methods able to handle spatial resolutions ranging from 4 nm in EM to 100-200 nm in LM.

In collaboration with University of Rennes 1 - UMR 6026, we have already investigated methods to analyze in Cryo-TEM the dynamics of microtubules known to be involved in intracellular transporting, cell motility, meiosis and mitosis. In another line of work in cell biology (UMR 144 CNRS Institut Curie), the studies exploiting light and electron microscopy methods have started to reveal the morphogenetic processes and sub-cellular mechanisms involved in the biogenesis of specialized organelles of epidermal cells, melanosomes in melanocytes [48] and Birbeck granules in Langerhans cells. 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 mechanisms involved in endosomal sorting, specialization and crosstalks between endosomes and these particular organelles.

3.5. Computational simulation and modelling of membrane transport at different spatial and temporal scales

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 [44], [56] aims at modelling system 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 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 [45] 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 and traffic and membrane transport modelling in cell biology.

4. Application Domains

4.1. 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 denoising approaches and non-iterative algorithms [6], [1], [7], [3]. The major advantage of these approaches is to get images at very low levels of signal and then to restore the denoised 2D+T(ime) and 3D+T(ime) signals [12]. Such post-acquisition treatment can improve the rate of image acquisition by a factor of 100 to 1000 times [14], reducing the sensitivity threshold and allowing imaging for long time regime without cytotoxic effect and photodamages. The two last years, this approach developed has been successfully applied to wide-field, Nipkow disk based confocal [12], TIRF (Total Internal Reflection Fluorescence [34] microscopy and fast live imaging and 3D-PALM using the OMX system in collaboration with J. Sedat and M. Gustafsson at UCSF [14]. In this latter case, the denoising algorithm speeded up the processing time of PALM reconstruction up to 100 times depending on the noise level [18]. The ND-SAFIR software (see Section 5.2) has been licensed to several laboratories over the world (see Figure 3).

Meanwhile, improving the resolution beyond 200nm diffraction limit while retaining the advantages of light microscopy and the specificity of molecular imaging is a long-standing goal. This goal has been recently achieved using 3D-SIM (Structured Illuminated Microscopy) [39]. 3D-SIM can produce multi-color 3D imaging reconstruction of fluorescently-labelled specimens with a lateral resolution approaching 100nm, which is unavailable in practice with conventional methods of 3D light microscopy. While being probably less effective in “breaking the resolution barrier” than the other optical sub-diffraction limited techniques (e.g. STED [43], PALM [36]), 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. Thanks to advances in information restoration and image denoising, it goes without saying that the SIM imaging will become compatible with the imaging of molecular dynamic in live cells. Accordingly, in collaboration with Roper Scientific, we pursue our previous efforts in content-based image denoising and machine learning to make SIM imaging more compatible with the imaging of molecular dynamic in live cells. These developments are required also 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.

4.2. Dynamic analysis and trajectory computation

4.2.1. Motion analysis and tracking

The challenge is to tackle the detection and linking of trajectories simultaneously. More generally, traditional tracking methods have difficulties as the number of objects and clutter increase. Typically, measurements from clutter and multiple objects confound tracking and it is then necessary to associate measurement with the correct object, i.e. to solve the difficult data association problem [46]. In video-microscopy, tracking methods that estimate trajectories of small objects (particles) may also encounter the same difficulties since the number of objects is large and the signal-to-noise ratio is low. So far data association even combined with sophisticated particle filtering techniques [52] or matching techniques [50] is problematic when tracking several hundreds of similar objects with variable velocities and for generating information on partial or complete trajectories. Moreover, optical flow [35] cannot be reliably estimated because of low signal-to-noise ratios in images. Developing new 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. In video-microscopy, we need to consider models of brightness variation that have time-dependent physical causes as investigated in [37], [40]. The goal is to formulate the problem of optical flow estimations in ways that take physical causes of brightness violations into account, leading to original methods for diffusion parameter estimation. In addition, the interpretation of flow fields enables to provide spatio-temporal signature of particular dynamic processes and could help to complete the traffic modelling.

4.2.2. Event and motion detection

Several approaches can be considered for the automatic detection of appearing and vanishing spots in wide-field (WF) and TIRF microscopy. 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 [52], [38]. Among the methods proposed to detect spots in an image [53], [51], no one was dedicated to the detection of spots appearing or disappearing suddenly between two time steps. More generally, in image analysis, detection of occluded areas is known to be also critical for motion estimation, especially when displacements are large. Theoretically, the pixels at the occlusion location should not be assigned any flow vector since there is no correspondence available in the other image. The quality of occlusion detection based on usual optical flow is then not always satisfactory for change handling in real challenging and noisy sequences. In our studies, we are only interested in the detection of a few events, that is one or two blobs by frame, and the previous methods are mostly inappropriate since the number of false alarms must be reduced. Our sense idea of handling small blob appearances/disappearances originates from the observation that two successive images are redundant, and the occlusions correspond to blobs in one image, which cannot be reconstructed from the other image [12], [22]. Complementary approaches in the line of work described in [33] are currently investigated to provide

new results and detect “packing” and “un-packing” events (Rab 11 proteins in Langerhans cells) in wide-field and total internal reflection microscopy.

4.3. Data management and storage

In cell and molecular biology [56], there is a need to address new challenges to acquire a complete and quantified view at 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/DSLIM), 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 intelligent image database with a built-in query system to annotate, retrieve, process and integrate analysis from different imaging modalities. The collaboration will bring each other skills (image processing and analysis software, image data management) together, towards a full integration of the image and data life-cycle, from image acquisition and analysis, to bioinformatics analysis and mathematical modelling in systems biology. In the meanwhile, user-friendly and fast algorithms must be developed to face the mass of data.

4.4. Multiscale image registration

There is probably no universal measure for multimodal/multi-scale image registration in light (M) and electron (EM) microscopy. In this area, we investigate mutual information and other statistical criteria to correlate intensities in EM and LM images. In usual approaches in medical imaging, pixel/voxel-based registration methods to match the source and target images, without image segmentation. The sum of squared intensity differences, intensity differences, and cross-correlation are the most successful criteria to match images at the same spatial resolution. The step beyond for CLEM is to assume that the two images to be registered are statistically very different. Information theory criteria (e.g. mutual information [57]) are more appropriate to analyze images coming from different modalities. Nevertheless, data fusion and LM and EM image matching is challenging since new in the literature in image processing and corresponding to a large variety of scales. 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. We will address these problems since light microscopy images are relatively blurred when compared to EM images as shown in [55].

4.5. Superresolution imaging

Sub-diffraction limited microscopic techniques fulfil some of the requirements such as to close the resolution gap between conventional LM and EM methods. Improving image quality in space and time using algorithms from micro- to nano-scales enables to progress and to establish new knowledge in cell biology. In this area, we develop specific “super-resolution” methods for image sequence reconstruction as introduced at the beginning of the last decade [42]. A high-resolution (HR) image can be useful for correlative microscopy, scale alignment or structural detail analysis. The purely numerical process that exploits series of low resolution (LR) images acquired by conventional light microscopy to reconstruct a single high-resolution (HR) image will be particularly investigated and is referred to “super-resolution imaging”. The more commonly-used SR approaches are based on the constraint that the SR image, once warped, blurred and sub sampled should give the LR images [42]. The challenge is to reconstruct a still high resolution TIRF image with details using patch-based methods [47]. The reconstructed HR image will be matched further to images acquired with superresolution SIM/PALM microscopy to bridge the gap of resolution. Because of the huge amount of these data, the algorithms need to be accurately developed to run in competitive time and able to handle massive amount of data that will be produced.

4.6. Traffic modelling, estimation and simulation

In this area, we focus on the analysis of transport intermediates (vesicles) that deliver cellular components to appropriate places within cells. In the previous works, we have already investigated an alternative approach to conventional tracking methods and based on the concept of Network Tomography (NT) [54] mainly developed for internet traffic estimation. In this approach, it is not necessary to track an object through a dynamic scene, just to determine when an object reaches a node, something that is generally easier than estimating a continuous trajectory. This approach simplifies the tracking process because it only requires detection of an object as it moves from one region to another. Instead, it determines mean traffic intensities based on statistics accumulated over a period of time. The measurements are usually the number of vesicles successfully detected at each destination region receiver or the vesicle time between the source and each destination. The NT concept has been investigated also for simulation [2] since it can be used to statistically mimic the contents of real traffic image sequences.

New mathematical approaches that can deal with high degrees of complexity and uncertainty that are inherent in all biological systems are needed [44], especially for describing the interactions between the different components involved in the endo/exocytosis pathway. In particular, we plan to incorporate more prior knowledge on dynamics to improve representation. First, from simulations, we hope to determine meaningful features to be matched to real image data. Secondly, a family of nano-meter-scale proteins typically known as “motor proteins” performs the majority of intracellular transport. However, the physico-chemical models of molecular motor provide a two fine grained view of the traffic, the challenge will be to correlate stochastic and dynamical models in 1D and in silico studied at the nano-scale in biophysics, to 3D images acquired at the scale of few hundred nanometres in vivo. Consequently, the solution resides probably in the combination of several degrees of modelling adapted to the space-time scale of the considered processes. A major difficulty is then related to the scale change and statistical aggregation problems (in time and space).

5. Software

5.1. PBED - Patch-based event detection

Participants: Sophie Blestel, Charles Kervrann.

[In collaboration with J. Boulanger (RICAM, Austria), A. Gidon, A. Chessel, B. Cinquin, J. Salamero (UMR 144 CNRS Institut Curie)]

The PBED software (APP deposit in 2011) written in C++ enables to automatically quantify in space and time the number of sudden and transient events observed in fluorescence microscopy (see Figure 2). The algorithm parameters are calibrated from the comparison of image patches expected to distinguish sudden appearing/vanishing fluorescent spots from other motion behaviors such as lateral movements [12]. We analyzed the performances of two statistical control procedures and compared the proposed approach to a frame difference approach using the same controls on a benchmark of synthetic image sequences. We selected a molecular model related to membrane trafficking for evaluation, and we considered real image sequences obtained in cells stably expressing an endocytic-recycling trans-membrane protein, the Langerin-YFP, for validation. With this model we targeted the efficient detection of fast and transient local fluorescence concentration arising in image sequences from a data-base provided by two different microscopy modalities, wide field (WF) video microscopy using maximum intensity projection along the axial direction and total internal reflection fluorescence microscopy. Finally, the algorithm is used to statistically explore the effect of several perturbations on the rate of transient events detected on the pilot biological model.

5.2. nD-SAFIR - image denoising software

Participant: Charles Kervrann.

[In collaboration with J. Boulanger, P. Elbau (RICAM, Austria), P. Bouthemy (INRIA Rennes), J.B. Sibarita (UMR 5091 Université Bordeaux 2) and J. Salamero (UMR 144 CNRS Institut Curie)]

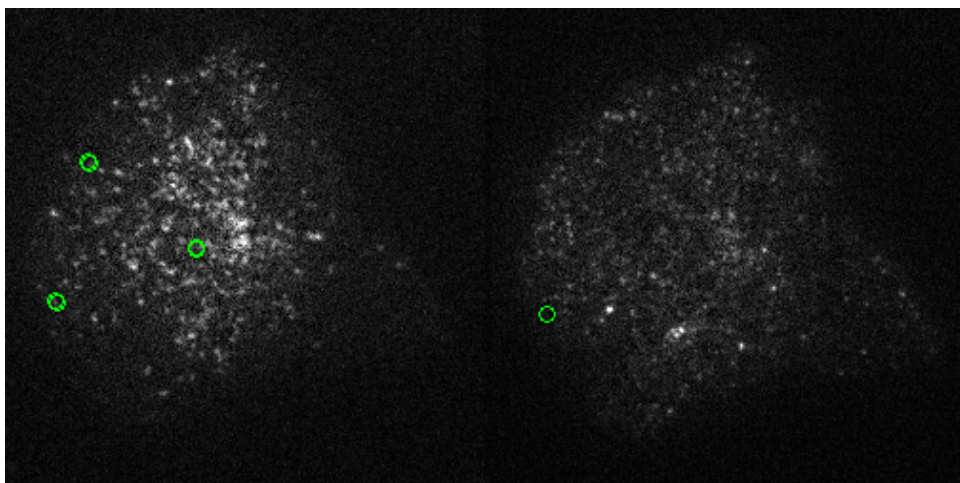


Figure 2. PBED software: detection of transitory events (sudden appearances of spots) for Rab11 (left) and Langerin (right) proteins in TIRF microscopy (M10 cell, UMR 144 CNRS Institut Curie)

The ND-SAFIR software (APP deposit number: IDDN.FR.001.190033.002.S.A.2007.000.21000) written in C++, JAVA and MATLAB, enables to remove additive Gaussian and non-Gaussian noise in a still 2D or 3D image or in a 2D or 3D image sequence (with no motion computation) (see Figure 3) [13]. The method is unsupervised and is based on a pointwise selection of small image patches of fixed size in (a data-driven adapted) 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 (fluorescence microscopy, video-microscopy, MRI imagery, X-ray imagery, ultrasound imagery, ...). This algorithm outperforms most of the best published denoising methods for still images or image sequences.

5.3. FAST-2D-SAFIR software - 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++ enables to remove 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 method is based on a locally piecewise constant modelling of the image with an adaptive choice of a window around each pixel. The restoration technique associates with each pixel the weighted sum of data points within the window. 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, ...).

5.4. HULLKGROUND software - Background subtraction by convex hull estimation

Participant: Charles Kervrann.

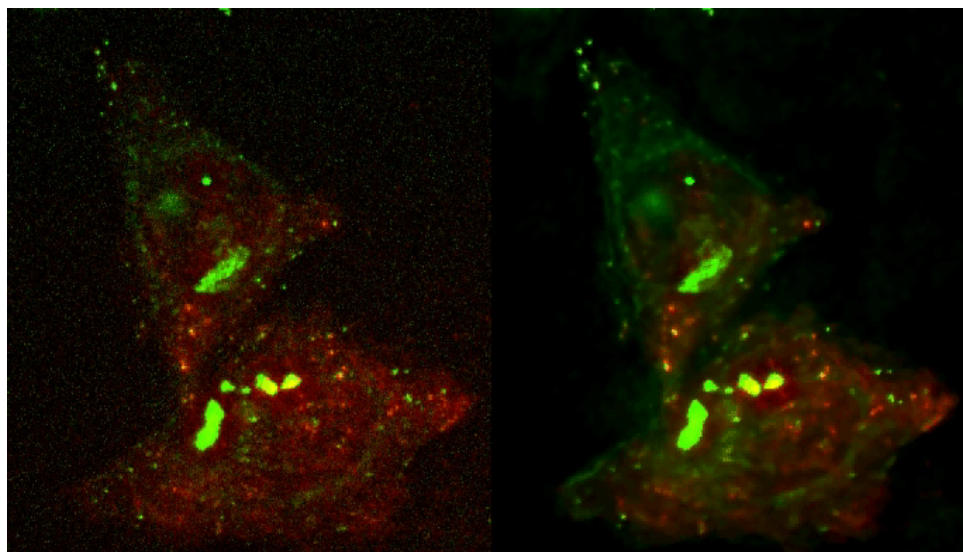


Figure 3. ND-SAFIR software: denoising of a 3D+T(ime) image sequence in spinning-disk confocal microscopy (green) (z-projection of 10 sections) and denoising of 2D+T(ime) image sequence in TIRF microscopy (red) (GFP-Rab6A (Hela cell), images are registered in the set-up, UMR 144 CNRS Institut Curie)

[In collaboration with A. Chessel and J. Salamero (UMR 144 CNRS Institut Curie)]

The HULLKGROUND software (APP deposit number: IDDN.FR.001.400005.000.S.P.2009.000.21000) written in JAVA enables to decompose a fluorescence microscopy image sequence into two components: 1/ an image sequence showing mobile objects; 2/ 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 (see Figure 4).

5.5. TubuleJ software - A plugin for automatic straightening, filtering and 3D reconstruction of microtubule cryo-EM projection views

Participants: Charles Kervrann, Sophie Blestel.

[In collaboration with D. Chrétien (UMR 6026, Rennes)]

The TUBULEJ software (APP deposit in 2011) written in JAVA has been developed to analyze microtubule structures and helical structures in 2D cryo-electron microscope images. The software enables to straighten curved microtubule images by estimating automatically points locations on the microtubule axis. The local center estimation method relies on microtubule cylindrical shape analyzed in the Fourier domain. A user friendly interface is provided to filter straight fiber images by selecting manually the layer lines of interest in the Fourier domain. Third, this plugin 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 for example the IMOD plugin of IMAGEJ (<http://rsbweb.nih.gov/ij/>), to reconstruct the 3D microtubule.

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

Participants: Sophie Blestel, Charles Kervrann.

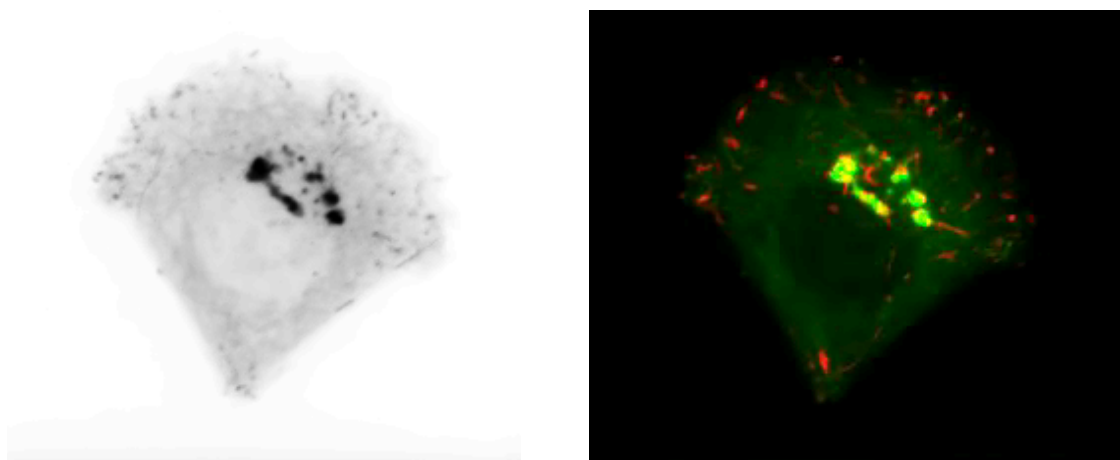


Figure 4. HULLGROUND software: separation of cytosolic (green) and vesicular components (red) from a “spinning-disk” confocal microscopy image sequence (GFP-Rab6 (micro-patterned HeLa cell), UMR 144 CNRS Institut Curie)

[In collaboration with D. Chrétien (UMR 6026, Rennes)]

The CRYO-SEG software (APP deposit in 2011) written in C++ has been developed to detect microtubule structures and helical structures in 2D cryo-electron microscope images (see Figure 5). Cryo-electron tomography allows 3D observation of biological specimens in their hydrated state. We have formulated the segmentation problem as a maximum a posteriori estimation problem and exploit image patches to take into account spatial contexts. 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.

6. New Results

6.1. Network tomography for tracking in fluorescence microscopy imaging

Participant: Charles Kervrann.

[In collaboration with T. Pécot (University of Columbus, USA), P. Bouthemy (INRIA Rennes), J. Boulanger (RICAM, Austria), J.B. Sibarita (UMR 5091 Université Bordeaux 2) and J. Salamero (UMR 144 CNRS Institut Curie)]

The study of protein dynamics is essential for understanding the multi-molecular complexes at subcellular levels. Green Fluorescent Protein (GFP)-tagging and time-lapse fluorescence microscopy enable to observe molecular dynamics and interactions in live cells and consequently to make progress in knowledge about protein dynamics. Original image analysis methods are then required to process challenging 2D or 3D image sequences. Tracking methods that estimate the whole trajectories of moving objects have been successfully experimented but can be applied for tracking a limited number of objects (a few dozens). To address the tracking problem of several hundreds of objects, we propose instead an original framework that provides general information about molecule transport that is about traffic flows between origin and destination regions detected in the image sequence. Traffic estimation can be accomplished by adapting the recent advances in Network Tomography commonly used in network communications [54]. NT-based approaches, devoted to

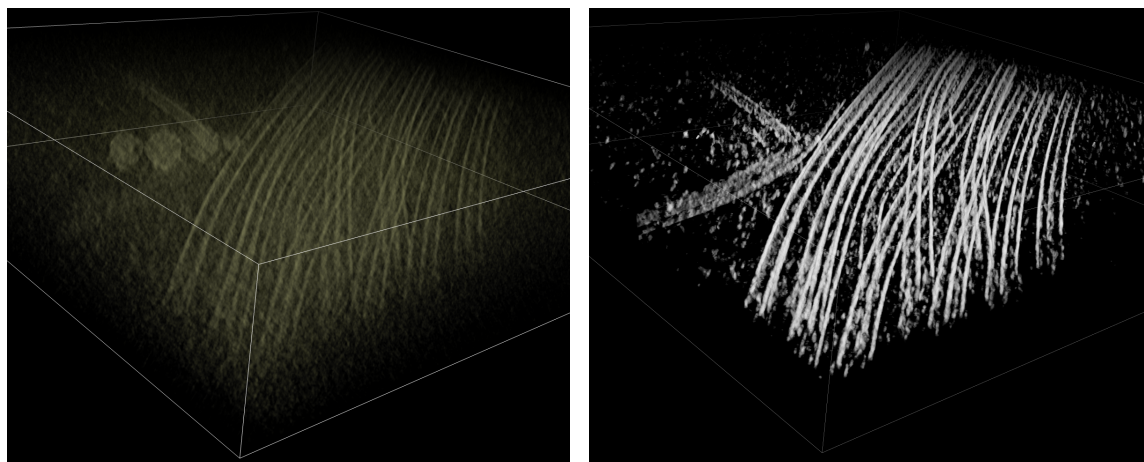


Figure 5. CRYO-SEG software: Conditional Random Field (CRF)-based segmentation of a 3D cryo-EM tomogram (microtubules / UMR 6026 CNRS University of Rennes 1)

statistical traffic analysis, simplify the tracking process because it only requires detection of an object as it moves from one region to another and avoids the difficult data association problem. This statistical method allows us to provide a global description of traffic flows. We just need to count the number of "objects/vesicles" in different image regions at each time step. In collaboration with UMR 144 CNRS Institut Curie, we extended the usual NT concept to non-binary routing from geodesic paths given the image sequence [11]. Unlike previous approaches, the new formulation can be considered as a probabilistic minimal paths modelling for object tracking. We showed that the origin-destination (OD) paths are not the minimal paths between the two extremities but formed as a set of minimal paths joining intermediate points. We also we proposed an estimation/optimization framework to derive counting measurements from image intensity (fluorescence). The traffic flow problem is also solved with additional parsimonious constraints. This approach has been developed for real image sequences and Rab proteins, known to be involved in the regulation of intracellular membrane trafficking.

6.2. Repetitive and transient event detection in fluorescence video-microscopy

Participant: Charles Kervrann.

[In collaboration with J. Boulanger (RICAM, Austria), A. Gidon, B. Cinquin and J. Salamero (UMR 144 CNRS Institut Curie)]

Endocytosis-recycling is an essential cellular trafficking process regulating the proper distribution and function of a large set of molecules, such as lipids, receptors, or adhesion transmembrane proteins. This dynamic process also participates to the homeostasis of intracellular membrane compartments. Progresses in imaging dynamics 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. In parallel, statistical image analysis has emerged as a basic methodology in the study of many biological phenomena. However, spatio-temporal analysis of transient events occurring at different sites of the cell has not been systematically performed. In addition, more formal tests are required in testing biological hypotheses, rather than visual inspection combined with more or less manual statistical analysis. For an unbiased quantification of repetitive and transient events, such as those observed during the trafficking of molecules travelling through the endosomal-recycling network of cells, their automatic detection becomes necessary. While requiring particular adjustments, our proposed approach is versatile enough, to be applicable

to diverse although complementary modes of microscopy. The proposed detection method described in [12], [32] 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.

This is first illustrated for both fast video and TIRF imaging techniques in collaboration with UMR 144 CNRS Institut Curie and RICAM (Austria). In our study, we focused on one particular Lectin receptor that constitutively recycles from internal compartments to the plasma membrane, it could be translated to many other studies of membrane trafficking in health and diseases such as diabetes, neurological, pigmentation or lysosomal defects. In a second case study, TIRF demonstrated that transient membrane concentration of fluorescently tagged proteins can occur close to or at the plasma membrane, suggesting that they may correspond to membrane sites specialized in endocytosis or exocytosis of the Langerin protein. Among diverse molecular behaviours reported by our previous studies, membrane concentration of fluorescently tagged proteins also gives rise to sudden spot appearance and disappearance. We worked on image sequences obtained from M10 cells stably expressing Langerin-YFP to get deeper insights in the recycling pathway and dynamics of this molecule (see Figure 2). First, fast 4D microscopy showed that as many as hundreds of such events may occur every minutes at different places within one cell. Then an alternative hypothesis would describe these transient concentrations as “packing” and “unpacking” of the Langerin molecules in a particular membrane structure, the Birbeck granules, whose biogenesis directly depends on the expression of Langerin, itself. Then, in order to quantify accurately the numbers, the frequencies and the half-lives of these transient membrane structures, a robust and automatic approach that could be adapted for either 3D or TIRF series of images, becomes necessary. Once combined with the use of biochemical or drug perturbation such as here the nocodazole, a drug inducing the depolymerisation of microtubules, these methods permit statistical analysis of these types of events in different experimental conditions. The gathering or the dispersion of many units of one type of them visualized here as spot appearing or vanishing, obviously correspond to a dynamics architecture involving the engagement of multiple partners.

6.3. 3D reconstruction in cryo-Electron Microscopy

Participants: Sophie Blestel, Charles Kervrann.

[In collaboration with D. Chrétien (UMR 6026, Rennes)]

In collaboration with UMR 6026 CNRS, we are interested in longitudinal projections of microtubules (i.e. perpendicular to the microtubule axis). Microtubules are composed of identical subunits that arrange together to form a helical lattice. Because of their symmetry, most of their information can be retrieved from their Fourier spectrum. However, due to their flexibility, microtubules are generally curved and their Fourier transform can no longer be used for symmetry analysis. We have proposed two sensitive contributions to automatically determine the local orientations and centres of short segments of microtubules in cryo-electron microscope images. Indeed, to our knowledge, the methods to determine the local centres of helices are not relevant for non centro-symmetric helices (e.g. 13-protofilament microtubules). The proposed algorithm exploits the helical symmetry of microtubules and the corresponding properties in the Fourier domain, so it can be easily extended to process other helical objects. Experimental results demonstrate that centre locations are estimated with an accuracy of lower than one pixel. We have applied the algorithm to automatically straighten images of curved microtubules with odd numbers of protofilaments, and to improve 3D reconstructions of microtubules using back-projection methods.

6.4. Segmentation of microtubules in cryo-Electron Tomograms

Participants: Sophie Blestel, Charles Kervrann.

[In collaboration with D. Chrétien (UMR 6026, Rennes)]

Cryo-electron tomography allows 3D observation of biological specimens in their hydrated state. Generally, cryo-tomograms have very low signal-to-noise ratios, and conventional image segmentation methods yield poor results. To address this problem, we have considered the Conditional Random Fields (CRF) framework and we have formulated the segmentation problem as a maximum a posteriori estimation problem. Segmentation is obtained by computing the global minimum of a non-convex energy functional defined, in the discrete setting, as the sum of a fidelity term and of a regularization term. We define an original fidelity term robust to noise based on a distance between patches. Segmentation is performed section by section, with an automatic update of the reference patches for the 2 classes: object and background. 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 method has been evaluated on synthetic data and on cryo-electron tomograms of *in vitro* microtubules (see Figure 5).

6.5. Image denoising in photoactivated localization microscopy

Participant: Charles Kervrann.

[In collaboration with J. Sedat (University of California San Francisco, USA) and J. Boulanger (RICAM, Austria)]

Photoactivated localization microscopy (PALM) and related fluorescent biological imaging methods are capable of providing very high spatial resolutions (up to 20 nm). Two major demands limit its widespread use on biological samples: requirements for photoactivatable/photoconvertible fluorescent molecules, which are sometimes difficult to incorporate, and high background signals from autofluorescence or fluorophores in adjacent focal planes in three-dimensional imaging which reduces PALM resolution significantly. We presented a high-resolution PALM method utilizing conventional EGFP as the photoconvertible fluorophore, improved algorithms to deal with high levels of biological background noise, and apply this to imaging higher order chromatin structure. We found that the emission wavelength of EGFP is efficiently converted from green to red when exposed to blue light in the presence of reduced riboflavin. The photon yield of redconverted EGFP using riboflavin is comparable to other bright photoconvertible fluorescent proteins that allow <20 nm resolution. We further found that image pre-processing using a combination of denoising and deconvolution of the raw PALM images substantially improved the spatial resolution of the reconstruction from noisy images. Performing PALM on *Drosophila* mitotic chromosomes labelled with H2AvD-EGFP, a histone H2A variant, revealed filamentous components of 70 nm. This is the first observation of fine chromatin filaments specific for one histone variant at a resolution approximating that of conventional electron microscope images (10-30 nm). As demonstrated by modelling and experiments described in [18] on a challenging specimen, the techniques described here facilitate super-resolution fluorescent imaging with common biological samples.

6.6. Tracking of actin network in asymmetrical cell division

Participant: Charles Kervrann.

[In collaboration with Z. Gueroui (BioPhysics team, UMR 8640, Ecole Normale Supérieure, Paris)]

A deeper understanding of asymmetrical cell division requires to unravel the physical origin of spindle migration. For instance, the “off-centering” process of spindles in mouse oocytes seems to depend mainly on actin filaments. In this project, the BioPhysics team investigates the symmetry breaking event of the actin network and the collective behaviour of actin filaments and motors. The long-term goal is to a mechanistic model for asymmetrical positioning in vertebrate oocytes. Recently, our collaborators developed a novel *in vitro* assay that showed how physical constraints due to the cell boundary could induce symmetry breakings in the spatial organization of microtubules and motors. Preliminary results demonstrated that a symmetry breaking process in the actin network occurs and eventually leads to the generation a steady state flow of actin filaments with a dynamic that is reminiscent of *in vivo* observations. We proposed an adaptation of commonly-used optical flow techniques to extract quantitative spatiotemporal characteristics of the actin dynamics and examine the basic principles underlying actin self-organization.

6.7. An Anthropomorphic Polyvinyl Alcohol Brain phantom based on Colin27 for use in Multimodal Imaging

Participant: Pierre Hellier.

[In collaboration with EPI Visages]

We have proposed a method for the creation of an anatomically and mechanically realistic brain phantom from polyvinyl alcohol cryogel (PVA-C) for validation of image processing methods for segmentation, reconstruction, registration, and denoising. PVA-C is material widely used in medical imaging phantoms for its mechanical similarities to soft tissues. The phantom was cast in a mold designed using the left hemisphere of the Colin27 brain dataset [41] and contains deep sulci, a complete insular region, and an anatomically accurate left ventricle. Marker spheres and inflatable catheters were also implanted to enable good registration and simulate tissue deformation, respectively. The phantom was designed for triple modality imaging, giving good contrast images in computed tomography, ultrasound, and magnetic resonance imaging. Multimodal data acquired from this phantom are made freely available to the image processing community (<http://pvabrain.inria.fr>) and will aid in the validation and further development of medical image processing techniques.

6.8. Real time ultrasound image denoising

Participant: Pierre Hellier.

Image denoising is the process of removing the noise that perturbs image analysis methods. In some applications like segmentation or registration, denoising is intended to smooth homogeneous areas while preserving the contours. In many applications like video analysis, visual servoing or image-guided surgical interventions, real-time denoising is required. We proposed a method for real-time denoising of ultrasound images: a modified version of the NL-means method is presented that incorporates an ultrasound dedicated noise model, as well as a GPU implementation of the algorithm. Results demonstrate that the proposed method is very efficient in terms of denoising quality and is real-time.

6.9. 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: intensity attenuation over time and geometric misalignment. Intensity attenuation due to photobleaching is classically accounted by an exponential decrease. To do so, homologous points need to be geometrically aligned over time. Unfortunately, the living cell exhibits some slow motion over the acquisition period. We consider here the iterative estimation of both geometrical alignment and intensity correction by the creation of a 3D atlas.

7. Contracts and Grants with Industry

7.1. Contracts with Industry

- J. Boulanger, C. Kervrann, P. Bouthemy. Commercial software license agreement (Roper Scientific company): “Software for denoising n-dimensional images”. INRA-INRIA Rennes, SERPICO Team, 2010-2011.

In collaboration with UMR 144 CNRS Institut Curie, we have developed an original and efficient spatio-temporal filtering method [13], [14] for significantly increasing the signal-to-noise ratio (SNR) in noisy fluorescence microscopic image sequences where small particles have to be tracked from frame to frame. The ND-SAFIR software (see Section 5.2) exploits 3D+time information to improve the signal-to-noise ratio of images corrupted by mixed Poisson-Gaussian noise. A variance stabilization transform is first applied to the image-data to introduce independence between the mean and variance. This pre-processing requires the knowledge of parameters related to the

acquisition system, also estimated in our approach. In a second step, a statistical patch-based framework is proposed for noise reduction and preservation of space-time discontinuities. The size of each neighbourhood is optimized to improve the performance of the pointwise estimator. In our experiments, the SNR is shown to be drastically improved and enhanced images can then be correctly segmented. In fluorescence video-microscopy, recent experiments demonstrated also that this method can be used as a pre-processing stage for image deconvolution, and allows us to increase the frame-rate of a factor 10 to 100, with the same SNR values. Finally, this novel approach can be used for biological studies where dynamics have to be analyzed in molecular and subcellular bio-imaging (see Figure 3).

- P. Hellier. Research collaboration (Syneika): “Neuronavigation on atlas and automatic target localization”. INRA-INRIA Rennes, SERPICO Team, 2011.

7.2. National Initiatives

7.2.1. *Quaero project / object tracking and indexing in video-microscopy*

Participant: Charles Kervrann.

no. Inria Alloc 3184, duration 30 months

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. The program has been approved by European Commission on 11 March 2008. The program is planned for five years and is supported by the French government through OSEO with a total budget of 200 millions Euro. Quaero consortium involves 24 academic and industrial partners led by Thomson. Vista Team participated in the Work Package 9 on Video Processing (WP9) of QUAERO Core Technology Cluster Project (CTC). Within WP9 Vista led three tasks: “Motion Recognition”, “Object Tracking” and “Event Recognition”. Since October 2010, SERPICO conducts activities in object tracking and indexing for video-microscopy analysis. This grant funds Denis Fortun’s PhD.

7.2.2. *INRA grant (to support collaborations with INRIA) :Dynamic Modelling for Intracellular Transport in nD Imaging*

Participant: Charles Kervrann.

In collaboration with INRA MIA unit Jouy-en-Josas and Institut Curie UMR 144 CNRS Paris, this project, labeled within the arc INRIA program and contracted in January 2007, was supported by INRA in 2009 and 2010. The VISTA and SERPICO teams are the prime contractor of the project DynaMIT (started with ARC INRIA 2007-2008) that associates the following other groups: MIA (Mathématiques et Informatique Appliquées) unit from INRA Jouy-en-Josas, Institut Curie (“Compartmentation et Dynamique Cellulaires” Laboratory, UMR 144 CNRS). In this project, we develop new methods dedicated to the analysis of nD microscopy data and to the modelling of molecular and macromolecular mechanisms at the cell level. Our main objective is then to provide computational methods and mathematical models to automatically extract, organize and model dynamic information observed in temporal series of images in multi-dimensional (nD) microscopy. The central problem addressed by this project concerns the roles played by different molecular motors in Rab dynamics and a rich set of data (mostly image sequences in video-microscopy) support the analysis. The biologist partners use very powerful and novel molecular (RNA interference), mechanical (micro-patterning) and optical (FRAP, photoablation) tools to normalize and perturb the cell activity.

7.3. European Initiatives

7.3.1. *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).

8. Dissemination

8.1. Animation of the scientific community

8.1.1. Journal reviewing in 2010

- C. Kervrann: Medical Image Analysis, IEEE Transactions on Medical Imaging, NeuroComputing, IEEE Transactions on Image Processing, IEEE Transactions on Pattern Analysis and Machine Intelligence, IEEE Signal Processing Letters, International Journal on Computer Vision, Journal of Mathematical Imaging and Vision, Image Analysis and Stereology, SIAM Journal Imaging Science.
- P. Hellier: Medical image Analysis, IEEE Transactions on Medical Imaging, Journal of Real-time Image processing, International Journal of Computer Assisted Radiology and Surgery.

8.1.2. Technical program committees of conferences

- C. Kervrann: reviewer for ISBI’2010, ICPR’2010, MICCAI’ 2010, ICASSP’2011, ISBI’2011, EMM-CVPR’2011.
- P. Hellier: Area Chair of IPCAI’2010, reviewer for MICCAI’2010, ICPR’2010, ISBI’2011.

8.1.3. Ph.D. reviewing

Charles Kervrann was reviewer for the Ph.D. thesis of Luis Pizarro (thesis advisor: Joachim Weickert), Saarland University, January 2011.

Charles Kervrann was member of the Ph.D. committees of Joseph Salmon (thesis advisor: E. Le Pennec / University Paris VII), Alexandre Gidon (thesis advisors: B. Goud and J. Salamero, University Paris V). He was member of the HdR committee of Agnes Desolneux (University Paris V).

8.1.4. Project reviewing, consultancy, administrative responsibilities

- C. Kervrann is member of the Scientific Council of the INRA “Applied Mathematics and Informatics” Department (2006-2010), member of the GdR 2588 (“Functional Microscopy for Life Sciences”) CNRS committee since spring 2008 and member of the IEEE BISP “Biomedical Image and Signal Processing” committee since 2010. He is deputy head of the GIS Europa (<http://gis-europa.univ-rennes1.fr/>) (imagery platform) / University of Rennes 1) since November 2010 and member of the INRIA-Rennes project-team committee (“Comité des Projets”) since November 2010. Charles Kervrann reviewed two ANR “non-thematic” projects, served as an expert for the ITMO “Cell Biology and Cell Development”/ GdR 2588 committee and was member of the scientific evaluation committee of the GIS AGRAL / “Image Analysis and Modelling”, Dijon. He was member of the CORDIS post-doctoral fellowships committee (Centre INRIA Rennes 2010).
- P. Hellier was an expert for the Swiss National Science Foundation, conducted consultancy work for Syneika startup and is member of the INRIA committee for communication and the INRIA committee for technological development.

8.1.5. Other involvements

- SERPICO is involved in the french network GdR ISIS CNRS, “Information, Signal and Images”.
- SERPICO is involved in the french network GdR 2588 CNRS, “Functional Microscopy for Life Sciences”.

8.2. Teaching

- Master 2 Recherche SISEA “Signal, Télécommunications, Images”, University of Rennes 1 (C. Kervrann : “Geometric modelling for shapes and images” / 5h).
- ENSAI Rennes, 3rd year (C. Kervrann: “statistical models and image analysis” / 25h).
- Charles Kervrann participated as member of the scientific committee to the Interdisciplinary School Mifobio’10 (Seignosse, September 2008) organized by the GdR 2588 “Functional Microscopy for Life Sciences” CNRS.
- Master 2 SIBM “Signal et Images en Biologie et Médecine”, University of Rennes 1 (P. Hellier : “Deformation estimation in intraoperative imaging” / 3h).
- Master 1 SIBM “Signal et Images en Biologie et Médecine”, University of Rennes 1 (P. Hellier : “Statistical image analysis” / 4h).
- On-going PhD thesis supervision in the team:
 - Sophie Blestel, PhD student (2008-2011), supervised by Charles Kervrann and Denis Chrétien (UMR 6026 CNRS University of Rennes 1).
 - Philippe Roudot, PhD student (2010-2013), supervised by Charles Kervrann and Francois Waharte (UMR 144 CNRS Institut Curie).
 - Denis Fortun, PhD student (2010-2013), supervised by Charles Kervrann.
- External thesis supervision:
 - Laurence Mercier, PhD student at Montreal Neurological Institute, McGill University, P. Hellier is involved in the PhD committee.
- Science popularization:
 - P. Hellier: coordination of DocScience “Médecine et Informatique”, 5 conferences in high school (around 350 students), member of INRIA INTERSTICES committee.

8.3. Participation in seminars, invitations, awards

- Members of the team have presented the published papers at conferences. The reader can refer to the bibliography Section to obtain the complete list. We list below all other talks given at seminars and meetings:
 - Charles Kervrann: Invited talk (“Analysis of intracellular trafficking in nD imaging at the single cell level”), INRA GIS AGRALE meeting, Dijon, November 2010; talk (“Neighbourhood-wise multiscale decision fusion for redundancy detection in TIRF microscopy image pairs”) at the GdR Ondes-ISIS CNRS meeting, ESPCI Paris, March 2010.
- Scientific visits and seminars: Bruno Cernushi-Frias (University of Buenos Aires / 1 week, March 2010); Nir Sochen (University of Tel Aviv / 3 days, July 2010), Grégoire Pau (University of Heidelberg / 2 days, January 2010); Christoph Schnörr (University of Heidelberg / 3 days, June 2010).

9. Bibliography

Major publications by the team in recent years

- [1] 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.

- [2] 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.
- [3] J. BOULANGER, C. KERVRANN, P. BOUTHEMY, P. ELBAU, J.-B. SIBARITA, J. SALAMERO. *Patch-based non-local functional for denoising fluorescence microscopy image sequences*, in "IEEE Transactions on Medical Imaging", 2010, vol. 29, n^o 2, p. 442–454.
- [4] P. COUPÉ, P. HELLIER, C. KERVRANN, C. BARILLOT. *NonLocal Means-based speckle filtering for ultrasound images*, in "IEEE Transactions on Image Processing", 2009, vol. 18, n^o 10, p. 2221–2229.
- [5] P. HELLIER, C. BARILLOT. *Coupling dense and landmark-based approaches for non rigid registration*, in "IEEE Transactions on Medical Imaging", 2003, vol. 22, n^o 2, p. 217–227.
- [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

Doctoral Dissertations and Habilitation Theses

- [8] T. CRIVELLI. *Mixed-state Markov models for image motion analysis*, Université Rennes 1, Mention Traitement du Signal et Télécommunication, 2010.
- [9] P. HELLIER. *Image processing methods for 3D intraoperative ultrasound*, université de Rennes 1, Mention Informatique, June 2010, Habilitation à Diriger des Recherches, <http://tel.archives-ouvertes.fr/tel-00536133/en>.
- [10] C. KERVRANN. *Modèles statistiques pour l'analyse d'images, algorithmes et applications en imagerie bio-cellulaire et moléculaire*, université de Rennes 1, Mention Traitement du Signal et Télécommunications, June 2010, Habilitation à Diriger des Recherches, <http://tel.archives-ouvertes.fr/tel-00541298/fr>.
- [11] T. PÉCOT. *Modélisation et estimation du trafic intracellulaire par tomographie de réseaux et microscopie de fluorescence*, Université Rennes 1, Mention Traitement du Signal et Télécommunication, 2010, <http://tel.archives-ouvertes.fr/tel-00541304/fr/>.

Articles in International Peer-Reviewed Journal

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