PROPOSITION D'ARC INRIA 2007-2008

DynaMIT
Dynamic Modeling for Intracellular Transport in nD Imaging

Abstract. In this proposal, we plan to define new methods dedicated to the analysis of nD microscopy data and to the modeling of molecular and macromolecular mechanisms at the cell level. In combination with the amount of information provided by *high-throughput nD microscopy* and automated image analysis methods (object segmentation, tracking, ...), we aim at designing computational and statistical models to understand membrane trafficking and, more precisely to better elucidate the role of Rab family proteins inside their multiprotein complexes. Our main objective is then to provide computational methods and mathematical models to automatically extract, organize and model dynamic information, present in temporal series of images obtained in multi-dimensional (nD) microscopy. This represents a real scientific challenge in applied mathematics, computer science and biology.

Nevertheless and beyond, we believe that our studies will help to describe and better define the dynamic architecture of multi-protein complexes involved in the time and space regulation of many essential molecular mechanisms of the living, such as, the dynamic organization of the cytoskeleton, cytokinesis, nuclear envelop biogenesis, cell adhesion, ...

The goals of this proposal fit in the INRIA priority challenge
"Modeling Living Structures and Mechanisms".

CONSORTIUM

[Partner 1]	INSTITUT CURIE (UMR 144 CNRS) Paris Compartimentation et Dynamique Cellulaires Équipe Mécanismes Moléculaires du Transport Intracellulaire: *Bruno Goud* (DR CNRS, leader) Compartimentation et Dynamique Cellulaires Groupe Imagerie Cellulaire et Tissulaire: *Jean Salamero* (DR CNRS, leader), *Jean-Baptiste Sibarita* (IR CNRS) with the participation of *Cyril Favard* (IPBS, Toulouse).
[Partner 2]	INRIA Rennes Projet VISTA - VIsion Spatio-Temporelle et Apprentissage: *Patrick Bouthemy* (DR INRIA, leader) *Charles Kervrann* (CR INRA, mis à disposition INRIA)
[Partner 3]	INRA Jouy-en-Josas Unité MIA: Mathématiques et Informatique Appliquées: *Alain Trubuil* (IR INRA, leader)
[Partner 4]	EPFL Lausanne BIG - Biomedical Imaging Group: *Michael Unser* (Professor, leader)

1 Introduction

Systemic biology. Since the beginning of this century and thanks to the development of advanced imaging technologies and probes, we can observe the molecular dynamics and interactions in live cells at both the microscopic and the nanoscopic scales. Dynamic imaging in microscopy is then exploited as an investigation tool to understand the function of particular biomolecules of interest. However, the mechanisms of life are commonly driven by multimolecular interactions and most of these interactions take place within "molecular machines" resulting of the assembly of multiple macromolecular species. Such "machines" are submitted to a continuous "building-destruction" process. They insure key steps of the biology of the cell and their dysfunctioning necessarily induces pathological situations. 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) while being often maintained outside a thermodynamic equilibrium. 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. Then, a dynamic, quantitative and integrated description of molecular interactions within macromolecular complexes at the cellular level appears today essential for the global understanding of live mechanisms. This global approach also related to the emergence of *systemic biology*, which can be defined as part of the natural extension of the genomic era, will be necessary to identify the processes resulting in pathological situations (cancer, degenerative diseases), as well as to validate future therapeutic agents.

Rab protein family for membrane transport. The present project will focus the Rab protein family involved in the membrane transport. This family of small GTPases (more than 60 in human), plays multiple roles via their association to internal membranes. Each member of this family exists under different dynamic states in the cell: they exchange continuously between the cytosol and the target membrane of one specific organelle, the donor compartment. They are associated to transport intermediates which are routed along the cytoskeleton networks. Finally, they may associate, most probably transitory, to their membrane of destination, the acceptor compartment. Their functions are regulated by a cycle of GTP hydrolysis as well as by their molecular interaction with a large number of partners, in the context of multiprotein complexes. The analysis of the assembly of these complexes, the dynamics of their assembly and the role of Rab proteins inside those complexes are fundamental for the understanding of the molecular mechanisms responsible for membrane transport and for the maintenance of the cell integrity. Moreover, the interconnectivity and the spatial organization of such protein systems support the basic cellular functions and must be considered in the context of the global architecture of the biological unit: the cell. Then, cellular functions must be thought as an integration of molecular activities in space and time. This constitutes one of the challenges of the modern biology and it becomes imperative to develop new approaches and to reinforce the technologies that are necessary for the measurement of the dynamics of those biochemical processes in "intact" cells. Today, it is one of the main concerns at the Institut Curie and more particularly in the team "Mécanismes Moléculaires du Transport Intracellulaire" at the UMR 144-CNRS.

Time-lapse fluorescence microscopy. This last decade, advances in XFP-tagging and time-lapse microscopy have revolutionized intra-cellular and molecular biology and have opened new avenues to study the dynamics and the function of multiple proteins. The nD images are constituted by a large number of dimensions (two or three spatial dimensions and one temporal dimension for each spectral channel considered and corresponding often to only one particular biomolecular 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). Consequently, this corpus becomes particularly

difficult to qualitatively and quantitatively interpret manually; most commercial image analysis tools for automatically extracting information are rather limited and/or require a large amount of user interactions. Moreover, data-sets that could be exploitable are insufficiently standardized, making them difficult to compare. 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 treatments (e.g. image processing) which are limiting factors in front of the data flux, and to favor the organization and the interpretation of the information associated to this data corpus.

Image processing for video-microscopy. Image sequence analysis in video-microscopy for life sciences has not been investigated by the past by computer vision groups, but now has gained importance since molecular biology is presently having a profound impact on the way research is being conducted in medicine. One can see the constitution of teams (Intelligent Bioinformatics Systems group (Heidelberg, Germany), Laboratory for Computational Cell Biology - The Scripps Research Institute (La Jolla, California)) concerned by the acquisition, treatment and analysis of dynamic image data. Moreover, networks at the European level [EAMnet for European Advanced Microscopy Network (since 2003) ; ELMI for European Light Microscopy Initiative(since 2001) ; French/German Initiative for Biophotonics (since 2005)] but also at the national level [GDR 2588 "Microscopie du Vivant" (since 2002) ; RT "Microscopie Fonctionnelle et Multidimensionnelle" (since 2004)] attempt to gather the research efforts made in this multidisciplinary context. Most of the partners of the present proposal are active members of these networks. This motivates our present research effort which is to develop novel approaches based on recent techniques in computer vision and signal processing but also in modeling and mathematical biology to extract quantitative measurements from nD data related to intracellular dynamics in biology. The current techniques (particle filtering-based tracking methods, ...), are not able to process complex data corresponding to interactions between several dozens of moving objects with variable velocities. In addition, analytical models of movements are poorly adapted for such complex situations. For those reasons, new developments and other parsimonious models should be envisaged. They also rely on the adaptation of acquisition tools, the increase of post acquisition treatment robustness and efficiency aiming at improving the image resolution and contrast.

2 Description of the project

At the origin of this proposal, the "Groupe Imagerie Cellulaire et Tissulaire" (Institut Curie - UMR 144/CNRS), the INRA MIA Unit and the VISTA team have initiated a pilot study on a minimal biological model of intracellular vesicular movement. This collaborative work was engaged end of 2004 and was financially supported by the ACI-IMPBIO program (1 PhD grant (MIA Unit), see `http://www.irisa.fr/vista//ftp/ckervran/ACI-IMPBIO/MODYNCELL5D.html`). Main objectives of this preliminary study were to accurately process 2D+t and 3D+t data-sets of microscopy images in order to extract statistics of oriented movements related to the dynamics of two proteins (Rab6A and Rab6A') which are mainly located at the cytosolic face of the Golgi apparatus at the steady state. A novel 3D kymographic analysis was developed to study the movement of Rab6A and A'-GFP tagged objects along well defined trajectories [Racine, 2006; Racine, accepted], [Sibarita, 2006]. The statistical analysis of extracted descriptors/trajectories enlightened an unexpected heterogeneity of behaviors of vesicles [Sachse, in revision]. The INRA MIA Unit proposed a high -level representation of vesicle dynamics based temporal signal modeling and graph theory [Béchar, 2006]. The VISTA teams has also developed a spatio-temporal filtering method for significantly increase the signal-to-noise ratio (SNR) in noisy fluorescence microscopic image sequences where small particles have to be tracked over time. Tracks can be then better estimated and object motion can be reliably computed in restored image sequences [Boulanger, 2005; Boulanger, 2006].

In this proposal, we have extended the consortium to involve another team ("Mécanismes Moléculaires du Transport Intracellulaire" (B. Goud, leader)) at the Institut Curie, and the Biomedical Imaging Group from EPFL Lausanne. Our main objective is to go further in the modeling of molecular processes for membrane transport. For that purpose, the involvement of new biologist scientists (B. Goud's team) and other bio-mathematicians from the INRA MIA Unit (A. Trubuil, leader) is then needed. Moreover, recent advances in time-lapse fluorescence microscopy imaging have thrown open new challenges in image process-

ing. They will be mainly addressed by the VISTA team and the Biomedical Imaging Group in collaboration with the other partners.

2.1 Biological context and motivations

Previous results. Like many of the "machine members" involved in membrane transport, Rab6 GTPases display highly complex dynamics, difficult to analyze such as bi-directional movements with a strong balance in the the plus-end direction along given microtubules, high frequencies of vesicles moving on the same track, high variations in speed among vesicles and a number of particular events such as, relatively stable structures outside the Golgi apparatus, some of them disappearing during acquisition times [Sachse, in revision]. The molecular mechanisms responsible for this complexity partly depend on the identification of the motor proteins that mediate transport of Rab6 vesicles along cytoskeleton [Echard, 2000; Fontijn, 2001; Matanis, 2002; Short, 2002]. This transport is known to be, at least, microtubule-dependent [White,, 1999]. [Partner 1] hypothesized that different dynein and kinesin motors would be responsible, at least, for the bi-directional movement and the heterogeneity in the speed distribution of Rab6 vesicles.

In order to identify which molecular motors are involved, [Parter 1] performed a systematic screen to inactivate each dynein and kinesin molecules using RNA interference. For each inactivated molecular motor, [Partners 1] started to observe (by 3D+t video-microscopy) the dynamics of GFP-Rab6 vesicles in Drosophila S2 cells. These Drosophila S2 cells were chosen for four reasons:

1. this system is simple as compared to the mammalian cells (1 Rab6 isoform in drosophila versus 4 in mammals, and 25 kinesins in drosophila versus 45 in mammals);
2. RNA interference in Drosophila cells is easy to perform and highly efficient;
3. we have an available and validated RNA interference library [Echard, 2004].

One first and important finding is that the movement of Rab6 vesicles is never totally abrogated after inactivation of individual motors. However, visual inspection of the image series clearly indicates strong qualitative differences and reveals that at least four different kinesins are involved in Rab6 vesicle movements: the minus-end-directed kinesin Ncd and the plus-end-directed kinesins Klp53D, CG17461 and Pavarotti. Depending on which kinesin was inhibited, vesicles accumulated either in the cell periphery or in the Golgi area and vesicle speeds seemed differently affected.

Expected results. In this project, we will use those previous results to inactivate the corresponding orthologous human kinesins (by RNA interference or with dominant negative constructs) and investigate the consequences on GFP-Rab6A/A' movement in HeLa cells. We started to validate this analysis and our preliminary results confirmed that: 1/ Pavarotti counterpart in Human is Rabkinesin-6, a plus-end-directed kinesin that binds specifically to Rab6A [Echard, 1998]; 2/ KIF1C/Klp53d and KIF-C3/Ncd are localized to the Golgi apparatus in human cells.

We now need to get a quantitative and automated analysis of the Rab6 behavior (size, number, motion, ...) after individual inactivation of the identified motors in Drosophila and human cells, using the diverse descriptors given by the image analysis methods developed by partners and that are foreseen in this project. In a first time we plan to assign a specific motor to different categories of Rab6 vesicles, which could be classified by speed distributions, orientation or other features. However, due to possible interactions or cooperation between molecular motors we plan, in a second step, to use mathematical models on data obtained from cells in which the dynamic of Rab6 was bio-molecularly perturbed. This will allow us to gain in understanding of the molecular mechanisms beyond the complexity in Rab6 movement. Future multi-disciplinary perspectives of the project will concern:

- the influence of cell shape on Rab6 vesicle orientated movements by constraining the cell polarity on adhesive micro-patterns successfully developed in the M. Bornens's team (UMR144, Institut Curie) in collaboration with [Partner 1] (see [Théry, 2005]).
- the analysis of the Rab6 dynamics with respect to microtubule plus-end growth to determine the dynamic reorganization of the microtubule network with time.

Investigation in time-lapse fluorescence microscopy. Multi-modal microscopic techniques that will be used in this project are all based on *wide-field videomicroscopy*. This is the fastest acquisition principle, compatible with fast processes like Rab vesicles displacement. With the new highly sensitive detectors, is it possible to monitor living cells in 3D+t at up to 30 frames per second during minutes.

From now, our biological models can also be "photonicaly" perturbed and analyzed by multi-modal laser-assisted microscopic techniques. In particular, 4D imaging associated to either discrete photonic perturbation modes (FRAP/4D, PA-4D, Chromophor (or Fluorophor) Assisted Laser Inactivation/4D) or with other observation modes (TIRF/4D with or without FRAP) give access to another order of dynamics in cell biology. Some of these methods, often recently developed, are currently under evaluation while some others have been already implemented by [Partner 1]. As some of those imaging techniques will be available during the time course of the project, data will be integrated progressively in the model(s) developed to describe or predict intracellular vesicle movements. The acquisition of new biological image sequences is a requirement in the framework since we plan to generate a nD data library in the future (beyond the scope of this proposal). The acquisition and processing of new image sequences will continuously improve our models. This will constitute one of the driving forces of our bi-directional "modeling-experimentation" strategy between the activities of the different partners. It is worth noting that our proposal is consistent with the emerging international (ELMI ; F/G Initiative for Biophotonics) and national (GDR 2588 "Microscopie du Vivant"; RT "Microscopie Fonctionnelle et Multidimensionnelle") research activities dedicated to the development of new methods of observation and analysis in living cells and to which we actively participate.

2.2 New approaches and methods for dynamic image analysis

The already developed methods are yet limited and, in a new approach we will address both the methodological and computational issues involved in object detection and multiple object tracking in order to better quantify motion in cell biology. The image analysis techniques to be developed are very challenging since no existing methodology can be easily adapted for simultaneous reliable tracking several hundred spots with variable velocities and for generating information on partial or complete trajectories. In addition, new image analysis methods adapted to "photo-perturbations" applied to biological samples, must developed. Finally, the detection and tracking results will be validated by computer simulations as well as by comparisons with real biological image data corresponding to membrane trafficking involving the movement of small transport vesicles moving along polymers (microtubules-microfilaments) from donor to acceptor compartments within the living cell.

Previous results and state-of-the-art. Tracking methods that estimate the state trajectories of objects as they change over time (e.g. Kalman filter [Bar-Shalom, 1988]) 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. solve the difficult data association problem [Perez, 2004]. 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 [Perez, 2004] or matching techniques [Genovesio, 2004; Racine, 2006; Racine, accepted; Smal, 2006] is problematic when tracking several hundreds of similar objects.

New methods for tracking in video-microscopy New developments are then necessary to improve these tracking methods to be adapted to nD imaging in microscopy. We propose several approaches:

- Specifically, we propose to work on two fronts. First, we will refine current particle detection/enhancement schemes. In particular, [Partner 4] will design new multi-dimensional polyharmonic spline wavelet bases that have the distinctive property of being quasi-isotropic and nearly shift-invariant. The idea here is to use a non-separable construction with a single Laplacian-like wavelet, instead of the 2n-1 distinct wavelets that are obtained using the traditional separable approaches. The scheme should work with

arbitrary dilation matrices both in 2D and 3D, which would constitute a significant extension of a previous 2D construction for the quincunx lattice [Van De Ville, 2005]. This wavelet transform will allow us to simultaneously perform signal denoising and particle enhancement/detection. The fact that the wavelets are Laplacian-like is highly desirable for background suppression; this choice can also be justified on theoretical grounds (optimal pre-whitened matched filter) because many biological images have a 1/f like spectral decay and particle have a Gaussian-like (PSF induced) shape.
Second, we will feed the enhanced feature map obtained by wavelet analysis into a particle tracking algorithm that will be designed to operate globally; i.e., it will extract all trajectories at once using the full information available (past + future). The grand challenge will be to tackle the detection and linking of trajectories simultaneously, as [Partner 4] has managed to do previously for the case of single particle using dynamic programming [Sage, 2005]. We will also investigate a parametric approach where the time-trajectories will be described by spline curves. The optimal trajectories will be determined by using a global optimization process, which is akin to snakes, except that the parameterization is along the t-dimension. The advantage of such a scheme (that can be used to refine the results of a previous algorithm) is that it can localize particles with sub-pixel accuracy. There also remains the possibility of using our knowledge of the optical image formation process to achieve super-resolution localization, similar to the model-based approach presented in [Aguet, 2005]. The underlying working hypothesis of the time-linking approach is that the movement from one time frame to the next is small; this can be ensured by performing faster data acquisition with a corresponding decrease in intensity to avoid photobleaching.

– An alternative approach to object tracking explained above, is based on the concept of Network Tomography (NT) [Vardi, 1996], a new field which we believe will benefit greatly from the wealth of statistical theory and algorithms [Castro, 2004]. In this approach, a dynamic scene formed by moving particles along a dense set of microtubules, is modeled as a network of interconnected regions of interest. In such a network, each node represents a sub-cellular location selected by a biologist or expert. A connection between two nodes is called a path and each path consists of one or more unidirectional or bidirectional links, that is physical links (microtubules) connected by intermediate routers. Broadly speaking, network inference involves estimating network performance parameters based on traffic measurements at a limited subset of the nodes. In traffic intensity estimation, the measurements consist of counts of objects that pass through nodes in the network. Based on these measurements, the goal is to estimate how particles traffic originated from a source node to a destination node along a path which generally passes through several nodes. In this approach, it is not necessary to track the moving objects, we just need to determine when an object reaches a node, something that is generally easier than estimating a continuous trajectory. The measurements are usually the number of vesicles successfully detected at each destination region receiver or the path time of the vesicle between the source and each destination. The inherent randomness in both link-level and path-level measurements motivates the adoption of a statistical framework.

The second approach simplifies 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. On the other hand, it does not allow us anymore to estimate an object state, which can be also desirable. These two approaches are probably complementary and will validated on real data as explained in the previous section.

2.3 New modeling approaches for the identification of effects of molecular motors on dynamics

The central problem addressed by this project concerns the roles played by different molecular motors in Rab6 dynamics and a rich set of data (mostly image sequences in video-microscopy) will support the analysis. The data to be considered are twofold: a/ data related to a wild type golden standard (no motor inactivated); b/ data related to perturbed situations (at least one motor inactivated). Taking into account the possible dependences between motors, an experimental design will result in many sets of dynamic acquisitions of Rab6A/A' traffic. Moreover, data in relation with the cytoskeleton will also be considered.

Previous results. Preliminary inactivation experiments of some molecular motors have already been made and as some inactivations results in a complete disorganization of the traffic, others seem to result in more subtle changes in dynamics. Basic tools exploiting only first level descriptors of dynamics like speed or directionality obtained by image analysis methods (deconvolution [Sibarita, 2002], image denoising [Boulanger, 2005], photobleaching correction and extraction of at least parts of trajectories of moving vesicules [Racine, 2006; Racine, accepted] [Bechar, 2006]), are not powerful enough to identify the contribution of a particular molecular motor to the global dynamic. These results There are interactions between the effects of different motors and this should be addressed in a statistical framework taking into account the different sources of variability and errors.

Probabilistic and statistical framework for modeling. We think general probabilistic models of interaction have to be considered first, and a particular statistical approach must be emphasized here: the experimental design methodology based on fractional factorial designs [Gauchi, 2006,]. This methodology proposed by [Partner 3] provides very useful tools for analyzing experimental (or computer) phenomena notably characterized by interactions between many factors. This analysis is optimal in a certain sense and can lead to a very reasonable number of experiments. For example, if we wish to study all the second-order interactions between 40 factors (the molecular motors here) it is necessary to achieve a set of only 2048 computer experiments. In this situation the effects of the 780 interactions will be individually quantified. For a first approximative approach, only 128 computer experiments are necessary for analyzing the main 40 effects (of the molecular motors) on one hand, and the interaction clusters on the other hand.

Moreover, in a further step, an original proposal for modeling variability is to consider the dataset in its globality and model the dynamics as a statistical mixture of dependent dynamics to be identified from the data. The spirit is the same as for well-known mixture models in statistics, except that in the present context an observation is not just a single value, but a multidimensional signal corresponding either to a possibly censored vesicle trajectory either to a temporal signal observed in particular localizations. However, in opposition to the usual mixture model, data (inactivation of motors) are available to learn the different components of the mixture and we hope this will make the problem tractable. Original machine learning techniques combined with image analysis-based tracking results will be then developed.

3 Conclusion

Our ambition is to develop mathematical models that will robustly describe the molecular mechanisms of membrane transport within the cells. We are convinced that this will be necessary to solve the spatio-temporal relationships between cargo vesicular membranes, molecular machines responsible for intracellular trafficking, molecular motors and cytoskeleton elements. This multidisciplinary proposal requires knowledge in genetics, cell biology, optics and microscopy, image processing, statistical analysis and modeling. All these competencies are gathered in our consortium. Accordingly, the two-year postdoc position is available for one (or two) motivated PhD student(s) in applied mathematics, image processing, computer science, biophysics. Our intention is that the student(s) will stay for a few months at the different labs to participate to the developments in the project.

The results of this proposal will be described in reports and journal and conference papers. In addition, image analysis methodologies described above will be necessarily achieved through algorithms and integrated as computed tools. Moreover, the Institut Curie has already developed a pilot software with the goal to considerably increase the computation (e.g deconvolution) speed by distributing over the network the computation of individual stacks to remote computer [Sibarita, 2005]. This can be extended to other image processing tasks (denoising, tracking, ...). For future work, there is clearly a need for well documented data storage in data bases including raw images, image results, acquisition protocols, image processing parameters and the high level information like segmented objects, extracted trajectories, statistics, etc. All those data are stored into a file server accessible via the network at any time with the standard network file exchange (like FTP or network neighborhood). Standardized file architecture is defined to allow the user to found out all its data easily. Depending on its needs, the user could copy images onto its local computer and perform a personal analysis on its data independently to the file server. The user could also ask for standardized processes like deconvolution, noise filtering or dynamic analysis.

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J Cell Biol. 147:743-60, 1999.

4 Profile and role of partners

[Partner 1] INSTITUT CURIE (UMR 144 CNRS) Paris
Compartimentation et Dynamique Cellulaires:
– Équipe Mécanismes Moléculaires du Transport Intracellulaire
http://www.curie.fr/recherche/themes/detail_equipe.cfm/lang/_fr/id_equipe/24.htm
– Groupe Imagerie Cellulaire et Tissulaire:
http://www.curie.fr/recherche/themes/detail_equipe.cfm/lang/_fr/id_equipe/296.htm

Study of the regulation of vesicular transport and membrane traffic in eukaryotic cells. Expertise in instrumentation and microscopy data collection, and in image processing:

- **Evaluation of the biological relevance of the observation and results;**
- **Study of microscopy modes, and molecular and cell biology models.**

[Partner 2] INRIA Rennes
Projet VISTA - VIsion Spatio-Temporelle et Apprentissage
http://www.irisa.fr/vista/

Methods for image sequence processing, fundamental and probabilistic aspects of motion estimation and tracking, application-oriented projects in collaboration with researchers in physics and biology:

- **Development of image analysis methods and stochastic methods ("Network Tomography") for trafficking analysis in time-lapse microscopy.**

[Partner 3] INRA Jouy-en-Josas
Unité MIA: Mathématiques et Informatique Appliquées
http://www.inra.fr/mia/unites/MIA_jouy.htm

Fundamental and applied statistics for epidemiology, interaction between genotype, environment and crop management, food risk analysis, gene expression analysis:

- **Expertise in experimental design methodology;**
- **Statistical methods for identification of effects of molecular motors on dynamics.**

[Partner 4] EPFL Lausanne
BIG - Biomedical Imaging Group
http://bigwww.epfl.ch/

Development of new algorithms and mathematical tools for the advanced processing of medical and biological images (fundamental and mathematical aspects of imaging, application-oriented projects in collaboration with researchers in medicine and biology) and contributions to the theory and practice of splines in image processing, as well as for its pioneering role in the introduction of wavelets in bio-imaging applications:

- **Development of stochastic temporal filtering ("particle filtering") methods and detection methods for object tracking in time-lapse microscopy.**

5 Justification for resources

- One two-year post-doctoral position[1] : 2 x 40 Keuros
- PCs: 5 Keuros
- Meetings and participation to conferences: 15 Keuros

6 Budget of involved INRIA projects

- Vista Team: 400 Keuros

[1] Les 2 années de post-doc seront éventuellement partagées par deux étudiants qui seront amenés à effectuer des séjours de plusieurs mois dans plusieurs laboratoires au cours du projet.