



THE FRENCH NATIONAL RESEARCH INFRASTRUCTURE FOR BIOLOGICAL IMAGING

ACTIVITY REPORT

2011-2013









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1 Introduction

France-BioImaging is the laureate, in the field of Biological Imaging, of a national initiative to support the access to innovative research in France to a wide scientific, medical and industrial community. Full understanding of life processes relies on the development of new technologies enabling the multiscale observation and quantification of biological systems. At the frontier between molecular and cell biology, biophysics and engineering, mathematics and bioinformatics, France-BioImaging gathers, in a coordinated infrastructure unique in France, several outstanding cellular imaging centers supported by laboratories for state-of the art R&D with the aim to cover recent advances in microscopy, spectroscopy, probe engineering and signal processing. This provides quantitative measurements, computational analysis and an integrative understanding of a wide range of cellular and tissular activities, in simple biological models up to small animals, in normal and pathological situations.

The ultimate goal of France-BioImaging is to give faster access to advanced imaging techniques and methods to a wide scientific community and to participate to socioeconomical development through industrial partnerships and innovations.

BioImaging is a priority in the road map of the French research development strategy (SNRI, "Stratégie Nationale de Recherche et Innovation"). Among BioImaging technologies, Cellular/Biological Imaging, at the heart of France-BioImaging, has been selected as a priority by the French National Center for Scientific Research (CNRS). France-BioImaging's main tutor is thus the CNRS, while it is also supported by all the main other scientific institutions and universities - Inria, INSERM, Institut Curie, Institut Pasteur, Ecole Normale Supérieure, Ecole Polytechnique, Université Aix-Marseille, Bordeaux, Montpellier 1 & 2, Paris Descartes, Paris Diderot, Paris Sud).

France-BioImaging further represents the national structuration that will be the link between French resources in biological imaging and the "Euro-BioImaging" project in the roadmap of ESFRI European Strategy Forum on Research Infrastructures.

FBI was successful in the first call of AAP 2010 "National Infrastructure in Biology and Health" in the frame of " Investments for the Future". A difficulty rose from unexpected financial rules (specific for AAP 2010). The whole budget (26 M€) had to be divided between each node (5 geographical nodes and 1 transnational node) at the beginning. The initial transversal node, BioImage Informatics - Image Processing and Data Manipulation (IPDM), was built on a scientific basis and gathered several hosting institutions (CNRS, Inria, Institut Curie) headed by Institut Pasteur. Because each hosting institution of the different nodes got its attributed funds without the possibility of subcontracting to other partners, the transversal node would not have been able to work. Thus, all financial annexes had to be modified and a novel hosting institution (Inria-Rennes) had to be created, delaying to April 2012 the signature of the conventions between the French National Research Agency (ANR) and the different hosting institutions. Due to these administrative and financial issues, funds were available only in July 2012, although the official starting date was November 2011.

Without waiting for the funds to be available and the Project Manager to be recruited (delayed to October 2012 because of administrative issues), FBI had defined early its overall governance at the strategic and operational levels. Finally, the infrastructure was officially launched in November 2012 with the organization of FBI Kick-Off meeting.

Governance of FBI is based on four main committees: the **Executive Board** (composed of the node coordinators and the national coordinator), the **National Advisory Committee**, the **Scientific Advisory Board** and the **National User Committee**, allowing a sustainable and long-term management of the infrastructure (see page 8). In addition to the many tasks covered daily by the national Coordination (constituted by the National Coordinator and the Project Manager), the Executive Board, helped by the Project Manager (consultative), stands every month to drive the project and take decisions. The strength of FBI is to put efforts together to overcome technological barriers persisting at different levels in Biological Imaging. Different solutions for each challenge are proposed among different nodes, justifying an organization in shared technological and methodological Work Packages (WPs) (see page 29). Node representatives are joined together around a particular WP theme to form the corresponding Working Group (WG) where technical and scientific exchanges are performed. Other WPs are organized to tackle other



aspects of biological imaging such as, high throughput acquisition & high-content analysis, probe development, optomanipulation & optogenetics and image processing and data manipulation (WP2, WP3 and WP4 respectively). In addition, France-BioImaging aims to coordinate strong technology transfer and training, matched by the ability to provide quality assurance to external users, giving rise to WP5 – Training, Dissemination & Technological transfer (see page 30).

PRESENTATION OF THE COMMUNITY

French Cellular/Biological imaging core-facilities have been organized through the IBiSA program (Infrastructures in Biology, Health and Agronomy). However, a strong need for further organization appeared - in particular through better links of R&D programs - to answer the requests for large-scale Research Infrastructures and to the National Strategy For Research and Innovation. Consequently, FBI arised from a tight connection between state-of-the-art R&D teams and IBiSA core facilities at the regional level, integrated in a national infrastructure.



Figure 1 : Criteria originating in the foundation of France-BioImaging

The partnership built on research expertise of French institutions in fundamental and biomedical research - CNRS, INSERM, INRA, Inria, Institut Curie, Institut Pasteur and Universities - will deliver state of the art technological developments, a comprehensive scientific cooperation, training and mentoring.

As shown in Figure 2, France-BioImaging is composed of 5 geographical nodes - Paris Centre, Ile de France Sud, Bordeaux, Montpellier and Marseille - and 1 transversal node – BioImage Informatics / IPDM (Image Processiong and Data Manipulation). The ten imaging IBiSA-labeled facilities and their associated R&D teams are described in **Chapter 2**.

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Figure 2: France-BioImaging general infrastructure

Numbers of persons in FBI perimeter (facilities and R&D labs): Paris Centre (ImagoPole included) - **141**; Bordeaux - **78**; Marseille - **35**; Montpellier - **27**; IPDM (Pasteur+Inria+Curie+Montpellier) - **28**; IdF Sud - **24**. (see detailed PERIMETERS in **Appendix 1**)

It is important to note that France-Bioimaging as it is represented by these different nodes is only the "nucleus" of the entire capabilities of the French community, concerned by advanced imaging techniques for life sciences. As a result, the present consortium has the crucial task to keep gathering relevant initiatives in the field. Since other centers with strong training, service offering and R&D capacities are likely to reach in the near future the maturity level to participate to the core of France-Bioimaging, questions are arising concerning the long-term perimeter of the infrastructure. Importantly, node partners or teams inside the nodes could be removed and replaced by others in the future. To ensure such a turn over process, we will need to propose strong, accurate and recurrent evaluation processes, based on 1) peer and international review, 2) user satisfactory process and 3) we also understand that we need to match institutional requirements in terms of societal and economical impacts.

France-BioImaging is at the heart of the National Strategy For Research and Innovation priorities in cancer, neurodegenerative diseases, increase in biotechnology potentials, nutrition through our plant cell programs, nanotechnologies and nanobiotechnologies through our program on new nanoprobes for imaging. Furthermore, France-BioImaging is a highly pluridisciplinary project with participants in Biology, Physics, Chemistry, Mathematics, Computer science and engineering.

It was also clear from the AAP "National Infrastructure in Biology and Health" that a national infrastructure should have a European echo. Since its very beginning, France-BioImaging has been strongly involved in the preparatory phase of the ESFRI project, EuroBioImaging (see Chapter **6**-**General achievements, strength/weakness and perspectives**, for a more precise description of France BioImaging participation to EuBI).



2 Organization 2-1 Governance

France-BioImaging is directed by a **national coordinator**, Maïté Coppey-Moisan, elected in June 2012 by the-**node coordinators** (see committees below). A **project manager**, Séverine Fantapie, was recruited in October 2012 for a period of five years by the national coordinator.

The national coordinator and the project manager constitute the **National Coordination** of France-BioImaging.

Besides the National Coordination, the governance of France-BioImaging is based on **four main committees** allowing a sustainable and long-term management of the infrastructure:

- Executive Board (EB);
- National Advisory Committee (NAC) ;
- Scientific Advisory Board (SAB);
- National User Committee (NUC).

Executive Board

The **Executive Board (EB)** comprises the **nodes coordinators and the national coordinator**. To achieve a regular follow-up, EB stands monthly using videoconference.

- Maïté Coppey, National Coordinator co-representative of Paris Centre;
- Daniel Choquet, representative of Bordeaux ;
- Pierre-François Lenne, representative of Marseille ;
- Jean-Christophe Olivo-Marin / Charles Kervrann, co-representatives of BioImage Informatics IPDM (Image Processing and Data Manipulation) trans-node ;
- Nadine Peyriéras, representative of Ile de France Sud ;
- Marcelo Nollmann, representative of Montpellier ;
- Jean Salamero, co-representative of Paris Centre.

National Advisory Committee

The NAC is composed of **legal members** constituting the **Executive Board** and **five advisory members** :

- FBI project manager : Séverine Fantapie ;
- 2 representatives of the National User Committee (NUC): Lydia Danglot, INSERM (NUC coordinator) and Marc Tramier, RTmfm (« Multi-dimensional Fluorescence Photonic Microscopy » Technological Network);
- 1 industrial representative: **Bertrand Vieillerobe**, CNOP (*National Optics-Photonics Committee*);
- 1 institutional representative : **Daniel Boujard**, CNRS.

Missions of the *National Advisory Committee* are to help and advise the Executive Board relative to the perimeter and investments of France-BioImaging actions at the strategic and operational levels. NAC is helped in its decisions by the external *Scientific Advisory Board*. Decision agreements are taken by the legal members upon consensus, the national coordinator makes the final decision in case of disagreement.

The NAC stands three times a year. The national coordinator proposes an outline which can be modified after recommendations or suggestions from any NAC member.



Scientific Advisory Board

- Enrico Gratton (President), Professor of Biomedical Engineering and Physics, University of California, Spectroscopy / Biological Imaging ;
- John Briggs, Group leader, EMBL (Heidelberg), Cryo-Tomo Electron Microscopy/ Assembly of macrocomplexes;
- Francesco Pavone, Professor, University of Florence, Biophotonics Microscopy / Neurology;
- **Rainer Pepperkok**, Team leader, Head of Advanced Light Microscopy Core Facility, EMBL (Heidelberg), High Throughput Screening / Membrane Traffic ;
- **Robert Singer**, Professor, Albert Einstein College of Medicine (NY), Single Molecule Imaging / Molecular & Cellular Biology ;
- **Michael Unser**, Professor and Director of Biomedical Imaging Group, EPFL, BioImage Informatics.

The SAB is composed of six members from the scientific international community external to FBI. It gives recommendations and suggestions on the scientific perimeter and technological developments carried out by FBI, but also on the efficiency and pertinence of technological transfer from R&D teams to the Imaging Core Facilities open to users.

The SAB will examine the results and projects of the infrastructure every 2 years to evaluate its progresses, strengths and weaknesses, and to propose new avenues of development and investigation.

National User Committee

• Representatives of user committees from nodes facilities :

Bordeaux : Emmanuelle Bayer / substitute : Frédéric Saltel ;

Ile de France Sud : Renaud Legouis / substitute : Laurent Combettes;

Marseille : Bernard Charroux / substitute : Hugues Lelouard;

Montpellier : Marion Peter / substitute : Thierry Cheutin;

<u>Paris Centre :</u> Yohanns Bellaiche and Lydia Danglot / substitutes: Philippe Chavrier and Saaid Saffieddine.

Representatives of French microscopy networks;

<u>GdR2588 ("Microscopy</u> and Imaging of Life" National Research Group): **Bernard Ducommun** / substitute : Laurent Héliot ;

<u>RCCM</u> (Network of Microscopy Common Centers for Electron Microscopy) : **Brigitte Gaillard-Martinie** / substitute : **Béatrice Burdin** ;

<u>RTmfm</u> (« *Multi-dimensional Fluorescence Photonic Microscopy*" Technological Network<u>)</u>: **Marc Tramier** / substitute : **Frédéric Brau**.

• Industrial representative : Jean-Baptiste Galey (L'Oréal).

Lydia Danglot was elected coordinator of the committee (30/05/2013).

The NUC is composed of ten representative members from local user committees, industry and French microscopy networks. The latters allow FBI to keep contact with the overall national community involved in the development of bioimaging instruments and methods in R&D labs and core facilities. It stands three times a year, shortly before the National Advisory Committee meetings in order to add user recommendations and suggestions to the NAC outline meeting.







Figure 3: Schematic presentation of France-BioImaging governance



2-2 Nodes

Paris Centre

The Paris-Centre node gathers biological imaging resources in the center of Paris, associating **4 imaging facilities** and **8 R&D teams**. The node Paris-Centre has the objective to make new developments and implementations of state of the art imaging systems, chemical probes and image processing for single molecules localization, dynamics and interactions in live cells, tissues and organisms and to perform cell functional analysis by using opto-manipulation. Paris Centre node was deeply involved in the "proof of concept" study led by the EurobioImaging ESFRI project.



ImagoSeine / IJM

ImagoSeine imaging facility gathers advanced light microscopy, cytometry and electronic microscopy activities, installed on 450 m² at IJM. Rooms for cells and tissues manipulations are provided to external users. ImagoSeine has been assessed and found to meet the requirements of ISO 9001 by AFNOR Certification. ImagoSeine brings together technologies and expertise in these fields, thanks to the 7 permanent and 2 contractual engineers. The originality of ImagoSeine-IJM is the close collaboration between the imaging facility which have a long experience of making available standard approaches but also new developments to the biological community and a research team involved in the development of state of the art functional imaging technologies (FLIM-FRET; FCCS) and more recently superresolution and non-linear microscopy. The ultimate goal is to provide access and training to these emerging techniques and methods for the realization of competitive biological projects.



PICT/Institut Curie

The PICT-IBiSA imaging facility is spread over 4 different buildings, at the closest to the different scientific departments of the Institute (cell/dev. biology and cancer, signalization and cancer research, biophysics). 25 engineers and researchers run PICT. It covers a broad spectrum of imaging modalities from advanced light imaging to High Resolution EM, including, functional or in vivo imaging and innovative approaches in High space-time Resolution light microscopy. More than 40 setups are in open access. PICT associated teams gather expertise in all these domains. They are engaged in particular, in the development of multi-scale and multi-modal approaches. PICT and associated teams are collaborating with IPDM-Inria Node and Institut Curie-IT Team, with the aim to develop "BioImage Informatics" for big data set management, generated by HCS, 3D-EM, Full-CLEM and in depth imaging. Most research topics are related to cancer research, from molecular processes in tumor cell differentiation and progression up to mathematic modelling of tumor behavior in its physiological environment.

ImaChem/IBENS

The Imachem imaging platform provides advanced light and electronic microscopy techniques to IBENS researchers and external users. Imachem is operated by 5 engineers. The main originality of IMACHEM is its ability to undertake innovative technical developments in optical microscopy and to make them available to all users. The first expertise of the platform is super-resolution microscopy, with the development of 3D-PALM using adaptive optic methods. It can perform ultra-structural imaging and single-particle tracking in 3D with a few tens of nanometers of spatial resolution. The second expertise is ultrafast two-photon microscopy for in vivo functional recordings with a temporal resolution in the msec range. A two-photon microscope using acousto-optic scanners for 2D scanning was first designed and installed in the platform. A new system providing ultrafast 3D scanning is currently under development. Additionally, electron microscopy using high-pressure freezing will be developed for correlative light and EM imaging.

ImagoPole/Pasteur

ImagoPole (Pole de dynamique moléculaire et fonctionnelle, Institut Pasteur, www.imagopole.org) is a research technologies platform comprising three technology "platform" groups (around thirty research engineers) and a translational research center (CIH; centre d'immunologie humaine). We develop and apply optical, ultrastructural, and cytometry imaging methods to the study of host-pathogen interactions at a molecular, cellular, tissue and whole organism level, notably using analysis of spatial features and temporal dynamics in situ. We use fluorescence and bioluminescence based high-content imaging techniques (including flow-cytometry, and intravital imaging), and ultrastructural technologies including transmission and scanning electron microscopy. Along these lines we have three main areas of interest, and funded projects running: 1) development and application of new molecular probes, and cell/tissue preparation methods; 2) development of novel imaging modalities, and/or biological models/paradigms, and 3) development of image analysis, database & visualization solutions. Multiple access modalities include: assisted access full service; autonomous access; and scientific collaboration.



Physico-Chemistry-Institut Curie: PCC team (M. Dahan)

The team research is focused on developing and applying advanced single molecule imaging tools for cell biology. To this end, the group combines novel optical, computational and labelling methods in order to localize, track, or count individual molecules in their cellular context. It owns several home-built microscopy set-ups, including a multifocus microscope for 3D imaging, a set-up combining FCS and single molecule, two TIRF single molecule microscopes, and a system combining single molecule detection, micropatterning and optogenetic manipulation. The lab is equipped with all the equipments (incubators, hood,...) required for live cell imaging and sample preparation.

University Paris-Descartes: Neurophotonic laboratory (V. Emiliani)

This site, comprises two R&D teams: the Wave front engineering microscopy group (V. Emiliani) and the Optical probing and biophysics of astrocytes-neuron communication (M. Oheim) dedicated to the development and use of advanced opical method in the field of neurobiology. the Emiliani group has pioneered new scanless illumination techniques for patterned photoactivation and imaging based on shaping of optical wave fronts, techniques including computer generated holography, generalized phase contrast, and temporal focusing. They also have set up a STED microscope for super resolution. The Oheim group has been developping evanescent-wave based techniques for near-membrane spectroscopy and imaging single-organelle dynamics using TIRF and supercritical angle fluorescence (SAF). They also built a multi-colour TIRF-SIM superresolution microscope offering isotropic 100-nm resolution over a large field-of-view.

ENS Chemistry (L. Julien)

ENS Chemistry facility gathers instruments devoted to the characterization and purification of optical probes and actuators by means of various spectrometries (UV-Vis absorption, fluorescence emission) and chromatographies (capillary electrophoresis, HPLC analytical or preparative), installed on 100 m² at ENS Chimie. Access is provided to external users with technical and conceptual assistance from the 8 permanent members involved in FBI. The originality of ENS Chimie is the close collaboration between the characterization facility making available established approaches to the biological community and a research team involved in the development of state of the art chemical technologies for the optical control and reading out of living systems. The ultimate goal is to provide access and training to these emerging techniques and methods for the realization of competitive biological projects.



Indicators 2012-2013

		Number of		Utilization infrast	Utilization rate of the infrastructure		Number of publications (FBI cited)		Number of patents (FBI cited)	
	Year	users	trained on- site	by users	by FBI members for developments or maintenance	from FBI members	from users	from FBI members	from users	
ImagoSeine	2012	166	88	75,0%	18,1%	/	/	/	/	
/ IJM	2013	202	115	85,0%	11,8%	1 accepted	0	0	0	
BICT / Curio	2012	360	70 (16-17 hours/user)	> 100%	20,0%	/	/	/	/	
PICI / Curie	2013	450	97 (14-16 hours/user)	> 100%	35,0%	3 pub./acc.	0	1	0	
TRENS	2012	55	18	104,0%	50,0%	/	/	/	/	
IDENS	2013	60	20	98,0%	100,0%	0	0	0	0	
ImagoPole /	2012	250	250	99,0%	<1%	/	/	/	/	
Pasteur	2013	250	490	99,0%	<1%	4 pub./acc.	1 accepted	0	0	
Curie	2012	/	/	/	/	/	/	/	/	
Physics	2013	10	4	100,0%	75,0%	0	0	0	0	
ENS	2012	1	20	20,0%	100,0%	/	/	/	/	
Chemistry	2013	1	1	20,0%	100,0%	1 accepted	1 accepted	0	0	
Paris	2012	/	/	/	/	/	/	/	/	
Descartes	2013	13	/	12,5%	77,0%	5 pub./acc.	0	0	0	



Bordeaux

The Bordeaux node is centered on the Bordeaux Imaging Center core facility that provides service training and R&D in photonic and electronic imaging, surrounded by 9 R&D teams. The strength of the Bordeaux node lie in the strong integration of physics at the optics School, imaging, chemistry and neurobiology at IINS and plant science at LBM. Bordeaux is a flagship center for super resolution imaging, both using photonics and electronic microscopies, applied to Neuroscience and Plant biology.



The Aquitaine initiative for high resolution Imaging

BIC

The BIC (Bordeaux Imaging Center) offers resources in photonic and electronic imaging, mainly in life, health and plant sciences. It is a core facility identified at the national level as IBISA that gathers 12 highly skilled engineers. It has the ISO9001 label. The different components of the BIC are: PHOTONIC imaging, ELECTRONIC imaging, PLANT imaging. The Bordeaux Imaging Center offers access to the most advanced bio-imaging techniques for fixed and live cell imaging such as video-microscopy, confocal microscopy, multiphoton microscopy, transmission electron microscopy and scanning electron microscopy. The BIC provides a unique set of high-end equipment for super-resolution microscopy such as STED confocal microscopy, FRAP video-microscopy, lifetime imaging FLIM for the measurement of molecular interactions. We also provide access to equipment for sample preparation such as ultra-microtoms, high pressure freeze (HPF) and we can host live samples.

Sibarita team

The "Quantitative Imaging of the Cell" team is a R&D team composed of engineers and researchers coming from various disciplines (microscopy, image processing, image visualization and micro-fluidics). Together, they aim to develop novel imaging techniques to better understand the living cell activity at high spatial and temporal resolutions, in a high throughput context. The team works in close collaboration with industrial partners (Roper Scientific, Imagine Optics, Nikon, Physik Instrumente, Cytoo, and Molecular Devices). Three main research area are investigated:

-Novel instruments for high-resolution microscopy of living samples, focusing on the development of new instruments for Single Molecule Tracking by Photo-Activation Localization Microscopy (SPT-PALM), Local Photoperturbation Microscopy (FRAP/PA), 3D imaging of thick biological specimens (Multi-photon Imaging) and Structure Illumination microscopy (SIM, Compress Sensing).

-Analytical tools for object segmentation, tracking and visualization using CPU and GPU.

-High Content Screening Microscopy to quantify the dynamics of active proteins within living cells, using super-resolution microscopy and micropatterning/microfluidics to control cell geometry and their local chemical environment.



Thoumine/Giannone team

Our aim is to understand the role of adhesion proteins and the actin cytoskeleton in the assembly and turnover of multi-molecular complexes at cell-cell and cell-extracellular matrix contacts. To this aim, we are using a combination of bio-mimetic physico-chemical assays to establish spatiallycontrolled and molecularly-specific adhesive contacts, and high resolution microscopy imaging to probe in real time the dynamics of these multi-protein complexes.

We are developing four specific axes:

1.Assembly of macromolecular synaptic complexes triggered by neurexin/neuroligin adhesion

2.Adhesion and actin dynamics in growth cone steering and dendritic spine shape

3.Integrin-dependent adhesion and actin dynamics in migrating cells

4.New imaging methods to probe ligand binding and receptors dynamics in membranes

Choquet team

We have a transdisciplinary approach to study the interplay between the organizational dynamics of the molecular components of glutamatergic synapses and synaptic transmission. We demonstrated that a) trafficking of neuronal molecules such as glutamate receptors is highly dynamic, b) regulations of protein-protein interactions play key roles in the control of this trafficking at different steps, including lateral diffusion, endo and exocytosis, c) modulation of glutamate receptor trafficking has a profound impact on synaptic transmission, including on both short and long term post-synaptic plasticity. By combining chemistry, superresolution imaging and physiology, we aim to unravel the dynamics and physical-chemistry of the macro-molecular complexes of the synapse, the nano-scale organization and dynamics of synaptic proteins and membrane trafficking, and the impact of the dynamic of synapse organization on synaptic physiology. Results obtained in these three axes are constantly integrated to provide a global view of glutamatergic synapse physiology, from nano-scale interactions to function.

Naegerl team

The advent of fluorescence microscopy beyond the diffraction limit has opened up huge experimental opportunities to directly image and resolve key physiological signaling events inside single synapses in intact brain tissue, a possibility which was considered a pipedream until recently. Our group is invested in harnessing these exciting technological developments to study synapses in their natural habitat and under realistic conditions, aiming to better understand higher brain function and disorders in terms of the underlying synaptic mechanisms. To this end, we are applying novel superresolution microscopy approaches (STED microscopy), giving us a much more complete and refined view of the dynamic behavior and plasticity of neuronal synapses and their interactions with glia cells inside living brain slices. This approach is complemented by a combination of 2-photon imaging & photoactivation and patch-clamp electrophysiology aided by tools from molecular genetics.

Groc team

While early intrinsic factors shape initial neuronal contacts, most fine-scale network wiring is driven by environmental factors and experience. A great challenge for our comprehension of brain development is to identify how different environmentally-driven modulators control the dynamic maturation of neuronal connections and circuit assemblies. The project of the team is to understand how neurotransmitter systems dialogue in the developing brain in order to shape functional networks. We focus our attention on the molecular physiology of glutamatergic (e.g. NMDA-dependent signaling) and dopaminergic loop and the role of such cross-talk in the developmental encoding of learning and novelty. These fundamental issues will be tackled using a challenging and original set of approaches, including in depth imaging with new probes, gaining insight into the dynamic cross-talk between receptors (e.g. single molecule approach, ensemble measurement, and biochemistry), the synaptic and network physiology (e.g. in vivo electrophysiology, opto-genetic), and rodent models of early life challenge (e.g. schizophrenia, stress).



Landry team

Chronic pain relies on maladaptive plasticity that induces neuronal sensitization in dorsal spinal networks. The aim of our project is to shed light on basic mechanisms responsible for cellular, and network dysfunctions in the dorsal spinal cord of rodent models of neuropathic pain. Within FBI, we investigate how GABAB inhibition of calcium-dependent intrinsic properties of dorsal horn neurons is hampered in neuropathic conditions by the association of the receptor with various partner proteins. Those interacting proteins impair GABAB inhibition through specific, distinct molecular mechanisms. To this aim we develop an extensive set of approaches for Correlative Light Electron Microscopy.

Lounis team

The Nanophotonics group activities focus on two themes: nanophysics and biophotonics. The common denominator of these work is the detection and analysis of properties of individual objects of nanometric sizes. The first axis concerns the study of photophysical properties of nano-objects to optimize their use as original light sources or optical nanoprobes for biological applications. The second axis concerns the development of original spectroscopy approaches to study the properties of these systems under various conditions of temperature (ambient or cryogenic) and environments (solid or liquid solutions or in biological systems). An important outcome of this work is the application of single molecules techniques and superresolution microscopies to address important biological question in collaboration with biologists notably with IINS. In FBI, the aim is to provide our innovative techniques to users of this national infrastructure.

		Number of	Number of	Utilization rate of the infrastructure		Number of publications (FBI cited)		Number of patents (FBI cited)	
	Year	users	trained on- site	by users	by FBI members for developments or maintenance	from FBI members	from users	from FBI members	from users
BIC	2012	360	251	60,0%	10,0%	0	0	0	0
ыс	2013	362	266	60,0%	15,0%	0	0	0	0
	2012	2	4	10,0%	50,0%	0	0	0	0
LFZN	2013	2	5	10,0%	50,0%	3 pub./acc.	0	0	0
	2012	12	17	10,0%	50,0%	0	0	0	0
1103	2013	14	20	10,0%	50,0%	2	0	0	0

Indicators 2012-2013



Montpellier

The Montpellier node is composed of a tight collaboration between research and development teams and microscopy research platforms. The main objective of the Montpellier node is to develop and implement and diffuse to the community state-of-the-art optical microscopy systems. Specifically, our emphasis is on fluctuation and super-resolution microscopies, high-throughput high-content microscopies, and live opto-genetic approaches. The Montpellier node has been selected as a 'proof of concept' Eurobioimaging site.

The node is formed by a microscopy development and research facility (MARS), two microscopy facilities (MRI and iPAM), and several research and development groups. Several active and synergistic collaborations exist between these different entities in which R&D groups contribute their expertise in optical/instrument development and platforms contribute their savoir-faire in user service and in general logistics.



MARS

The MARS research facility is a collaboration between two research teams (led by E. Margeat and M. Nollmann, respectively) at the Centre for Structural Biochemistry (CBS) and MRI (see below). MARS aims at offering to the local, national and international scientific community access to a selection of advanced optical microscopy technologies developed in house at the CBS and generally not commercially available. In particular, MARS specializes in super-resolution (2-color PALM/STORM, SIM), and fluorescence fluctuation (scanning FCS, FCCS, N&B, RICS) microscopies. MARS developments are performed by the groups of E. Margeat and M. Nollmann, and the user projects are performed by two research engineers (C. Clerte and JB Fiche). Access to the facility is evaluated on a project-by-project basis. The overall strategy of MARS is to select very exciting projects that require methodological developments and that are highly promising. MARS has been selected as a "proof of concept" Eurobioimaging site.



IGF

IPAM-IGF (Scientific leader, P. Mollard; Tech. leader, C. Lafont) dedicated to cellular in vivo imaging techniques developed new microscopes and tools. Our latest development allows 2-photon cellular in vivo microscopy with long range objectives (Mitutoyo, wd: 2cm, x20 magnification, NIR transmission) readily applicable to imaging of deep tissues structures (metabolic brain, pancreatic islets from animal models of diabetes). A new in vivo 2 photon microscope will be developed soon in the frame of MARS/FBI project (combination with an OPO, BNIF-CPER application). Access to IPAM-IGF equipment is based on project selection (see http://www.ipam.cnrs.fr/, IPAM platform is under ISO9001 certification (June 2014)). IPAM-IGF is also an international member of the National Biophotonics and Imaging Platform Ireland (NBIPI, http://www.nbipireland.ie/), the Irish representative of the ESFRI Euro-BioImaging project.

MRI

Montpellier RIO Imaging (MRI) is a distributed imaging facility present on six sites in Montpellier (<u>www.mri.cnrs.fr</u>). MRI is labeled IBiSA and certified ISO9001-2008 LQRA. It has a staff of 24 engineers and is directed by E. Bertrand (CNRS, IGMM). MRI manages numerous microscopes and analysis workstations (34 and 14), and especially microscopes for long term or short live experiments. MRI offers a complete set of state-of-the-art technologies, from single molecule to small organism imaging. The platform offers and develops 3D-SIM, SPIM, FCS/FCCS, CLEM and 2-photons microscopies, and also develops a new service of High Content Screening, with a specific emphasis on gene expression analysis by smFISH techniques. MRI organizes regular training sessions with theoretical presentations and practical sessions about advanced light microscopy and image analysis. Once trained, a user can freely access microscopes on a pay-per-use basis. For the screening facility, the access is evaluated on a project-by-project basis.

							Number of		Utilization rate of the infrastructure		Number of publications (FBI cited)		Number of patents (FBI cited)	
	Year	Number of users	persons trained on- site	by users	by FBI members for developments or maintenance	from FBI members	from users	from FBI members	from users					
	2012	4	2	20,0%	50,0%	0	0	0	0					
MARS-CBS	2013	10	2	40,0%	20,0%	1	0	0	0					
MARS-IPAM-	2012	5	2	20,0%	50,0%	0	0	0	0					
IGF	2013	8	3	50,0%	25,0%	0	0	0	0					
MRI-CRBM	2012	104	114	29,0%	30,0%	0	0	0	0					
	2013	143	135	42,0%	20,0%	0	0	0	0					

Indicators 2012-2013



Marseille

The Marseille node gathers two institutes in life sciences and one in photonics, which aim at fostering new technologies and photonic innovation for cell imaging. Founded on strong scientific and technological collaborations and dynamic industrial partnerships, the Marseille node offers state-of-the-art imaging methods to a large community of users, while maintaining its leading position in specific R&D areas. Our technological objectives are the development of imaging systems for dynamic and high resolution measurements in living organisms for developmental biology and immunology.



PICSL Plateforme d'Imagerie Commune du Site de Luminy – Shared Imaging Platform of Luminy Campus

PICsI has been founded in 2002 in the context of a partnership between CIML and IBDM. This technological facility has for main objective to give access to forefront technologies in cellular imaging to 45 research groups (500 scientists). The facility is located in the two institutes on the same campus and offers a variety of state-of-the art imaging systems (multiphoton, confocal imaging, electron microscopy). In addition, during the past years, specific R&D projects have been launched in research teams of the institutes, which now benefit to an increasing number of scientists. First built as collaborative projects between teams (e.g. H. Rigneault/D. Marguet, PF Lenne/T Lecuit), they have proven to be of general interest for a large community at local, national and international levels. Bringing these new technologies to a shared infrastructure in Marseilles has significantly enlarged the service offered to users.



IBDM

The Developmental Biology Institute of Marseille (IBDM) is an international and interdisciplinary research institute oriented towards developmental biology and pathologies. The research activity is at the crossroads of development, neurobiology, cell biology, biophysics and genetics. The general theme of IBDM is to understand how the instructions encoded in the genome are translated to build structures (cells, tissues, organs) that perform specific functions, and how these processes are regulated and integrated in the whole organism. There are links between developmental biology and diseases such as cancer, neurodegenerative and genetic diseases. One of the priorities of IBDM is to foster interdisciplinarity through the integration of new and original approaches that create conceptual and technical interfaces. At IBDM, **Lenne team** aims at determining how (1) mechanical and (2) physical interactions are organized at cell surfaces *in vivo* and (3) how these interactions are processed to produce cell and tissue responses. To tackle these questions, we focus on two aspects of tissue morphogenesis, namely cell polarization and force transmission in fields of cells. We are using *Drosophila* and *C. Elegans* as systems to address questions (1-2) and question (3), respectively. The originality of our project relies in the integration of both physics and experimental biology to study quantitatively tissue morphogenesis.

CIML

Founded in 1976, the Centre d'Immunologie de Marseille Luminy (CIML) is a research institute internationally renowned in its discipline. From worm to man, from molecule to the whole organism, from physiology to pathology, the CIML addresses, over numerous models and scales, all fields of contemporary immunology: the genesis of different cell populations, their patterns of differentiation and activation, their implication in cancer, infectious and inflammatory diseases and the mechanisms of cell death. At CIML, **Marguet team** aims at understanding the role of membrane lateral dynamics and organization in T lymphocyte signaling, by analyzing the molecular interaction/association events at high spatial-temporal resolutions. A special emphasize is made at examination of the molecular dynamics in the plasma membrane to initiate and to integrate extracellular stimuli. In this context, Marguet team develops analytical methods based on the combination of single molecular sensitive detection approaches such as fluorescence correlation spectroscopy (FCS) and derivatives, of single particle tracking with optical tools allowing to manipulate the biological samples such as dynamic holographic optical tweezers.

Fresnel Institut (MOSAIC Group)

The Mosaic group of the Fresnel Institute headed by H. Rigneault has been involved for almost a decade in developing dedicated optical instruments for biological imaging. Among other, the team has developed together with CIML the "FCS diffusion law" approach in Fluorescence Correlation Spectroscopy that has been successfully applied to the cell membrane. More recently single particle tracking using multiple targets have proved to be powerful to distinguish confinement zone at the cell membrane and Holographic Optical Tweezers shows potential application into TCR/MHC control. Phase control for micro-manipulation and imaging is an active field of research at Mosaic. Since 2002, the Mosaic group has been involved in coherent Raman microscopy and nonlinear imaging and was the first in France to build and develop a CARS microscope. One of the group world recognized expertise is in polarization resolved fluorescence and nonlinear microscopy that has proved to be able to retrieve molecular order in cell and tissue imaging. The group is now also involved in the development of nonlinear imaging using endoscopes using innovative microstructured optical fibers. Another active field of research is fluorescence enhancement at the nanoscale using metallic nano-antenna that have the ability to perform dynamic analysis on time and spatial scales unreachable with far field optics.



Indicators 2012-2013

			Number of	Utilization infrast	Utilization rate of the infrastructure		Number of publications (FBI cited)		Number of patents (FBI cited)	
	Year	users*	trained on- site	by users	by FBI members for developments or maintenance	from FBI members	from users	from FBI members	from users	
	2012	99	75	46,0%	30,0%	/	/	/	/	
IDDM	2013	101	82	50,0%	25,0%	2	0	0	0	
CIMI	2012	93	25	46,0%	35,0%	/	/	/	/	
CIME	2013	84	39	40,0%	27,0%	1	1	0	0	
Erospol	2012	2	2	3,5%	96,0%	/	/	/	/	
riesnei	2013	4	4	10,0%	90,0%	6	0	2	0	



Ile de France Sud

IdFsud gathers an important task force in biology, physics, applied mathematics and complex systems modelling. These disciplines synergize to develop innovative methodologies and tools in microscopy imaging and image analysis for applications in developmental biology, cell biology, plant biology, physiopathology and neurosciences. IdF Sud gathers three core partners with internationally acknowledged expertise: <u>BioEmergences</u> (CNRS-IBiSA), <u>Imagif</u> (CNRS-IBiSA) and <u>Polytechnique LOB</u> (Lab for optics & biosciences, CNRS – INSERM).

The imaging facilities of IdFsud are developed and coordinated by: Nadine Peyrieras (INAF, CNRS, Gif-sur-Yvette), Béatrice Satiat-Jeunemaitre (ISV, CNRS, Gif sur Yvette), Emmanuel Beaurepaire (Ecole Polytechnique, CNRS, Palaiseau).

The activity of the Ile-de-France Sud node (IdFsud) consists in developing novel optical biological imaging methods, in giving access to a broad range of imaging approaches to a large community, and in providing adequate training in light microscopy and CLEM. The 3 partners are complementary in their R & D approaches and their involvement in FBI WPs is summarized in the scheme below. BioEmergences (N. Peyriéras) coordinates the node. Béatrice Satiat Jeunemaitre co-coordinates the node and WP1c. Emmanuel Beaurepaire co-coordinates WP1d. Between parentheses are the personal that left the project unexpectedly. Our contribution to WP1a was compromised because of that for this Phase 1 of the project.

IDFsud



FBI 2011-2013

coordination

(Left)



BioEmergences

The BioEmergences platform (UPS in 2014) develops, applies to specific case studies, and offers as services, original methodologies and tools for the multi-scale phenomenological and theoretical reconstruction of the development of model organisms, animal or vegetal. The work is organized along the lines of an applied epistemology that we defined in the context of the Institute for Complex Systems which is part of the national and international roadmap established by the Complex Systems community http://roadmaps.csregistry.org/. We developed a workflow going from photonic microscopy for *in vivo* and *in toto* imaging of developing organisms to the automated reconstruction of 3D+time data, analysis of digital specimens and modeling of morphogenetic processes. In this context provide collaborative services that most of the time, correspond to new challenging applications (WP1b, WP1d, WP2, WP3). FBI funding is used to develop a so-called artificial assistant based on a SPIM or MLMS imaging concept with real time image processing and feedback on the imaging scheme. All our activity involves image processing (WP4). This is reflected by the "indicateurs table" where we gathered our activity "IDFsud" and "IPDM". We expect to provide the community with original webservices to access the BioEmergences reconstruction workflow and grid computing.

ImaGif

The cell biology pole of Imagif (www.imagif.cnrs.fr) is localized on the CNRS campus of Gif sur Yvette, in a new building dedicated to platform activity. This IBiSA platform provides efficient access to high quality services and state of the art technologies. It is open to the whole academic scientific community and to industrial partners. The management and development of this pole is under the responsibility of the team "Dynamics of cell compartmentation" (group leader B. Satiat-Jeunemaitre, Institut des Sciences du Végétal, Gif sur Yvette). It uses cell biology approaches and multiscale imaging (cytometry, bio-imaging and electron microscopy) to explore the cell. The development of new protocols and the mastering of update imaging approaches are part of the R & D objectives of the platform. Those are then transferred to platform services and disseminated through numerous training and teaching events, and opened to the whole scientific community. The cell biology pole of Imagif activities contributes to the working groups "super resolution", "Probe development" and "CLEM" within the France-BioImaging consortium.

LOB

Ecole Polytechnique is an internationally attractive institution combining research, teaching and innovation. Laboratory for Optics and Biosciences (LOB) is affiliated with the French Research institutions Inserm and CNRS, and Ecole Polytechnique. The Laboratory employs researchers with expertise in optics, molecular and cellular biology with the aim to explore new concepts and methods. Two LOB research teams are involved in optical imaging developments co-funded by France BioImaging. Access to instruments is currently provided on a collaborative basis and will be extended within the timeframe of the project.

* "Advanced Microscopies and Tissue Physiology" (E. Beaurepaire, M.-C. Schanne-Klein, W. Supatto, G. Gallot, M. Joffre et al). LOB "advanced microscopies" pole is a leading group in nonlinear microscopy of live tissues and small organisms, and develops pioneering approaches based on multimodal multiphoton imaging (multicolor 2PEF, SHG, THG, FWM, CARS), polarimetry, light-sheet illumination (SPIM), photomanipulation, wavefront control, pulse shaping.

* "Nanoemitters and Single Molecule Tracking" (A. Alexandrou, C. Bouzigues, et al). LOB "nanoemitters" team develops innovative assays based on non-blinking lanthanide-ion doped nanoprobes and microfluidics devices to study signaling processes in live cells.



Indicators 2012-2013

	Number of		Number of		rate of the ructure	Number of publications (FBI cited)		Number of patents (FBI cited)	
	Year	users	trained on- site	by users	by FBI members for developments or maintenance	from FBI members	from users	from FBI members	from users
BioEmergen	2012	20	15	60%	40%	/	/	/	/
ces	2013	23	15	60%	40%	1	0	0	0
ImaGif	2012	235	84	70%	30%	/	/	/	/
Imagin	2013	270	134	70%	30%	0	0	0	0
LOR	2012	/	/	30%	70%	/	/	/	/
LUB	2013	/	/	30%	70%	4 pub./acc.	0	0	0



BioImage Informatics-IPDM

The objective of the IPDM node is to create a general framework and a complete and integrated image analysis and IT (Information Technology) solution to address a number of challenges in biological imaging and microscopy, as well as setting up a high performance grid-computing infrastructure dedicated to massive computational and data storage demands. The FBI-IPDM node proposes different IT frameworks to deal with the data flow from the different imaging nodes. FBI-IPDM node is thus transverse, by definition.



Institut Pasteur

The scientific project of the BioImage Analysis (BIA) unit is to develop image analysis and computer vision tools for the processing and quantification of multi-channel temporal 3D sequences in biological microscopy. The topics are centered about the development of new algorithms for multi-particle tracking, deformable models, mathematical imaging and spatial distribution analysis. The group has produced powerful tools for spot detection and counting in real-time imaging of virus and genes, movement and shape analysis in 3D+t microscopy and cell growth analysis. These methods and algorithms have now been regrouped under the open-source and free platform Icy (http://icy.bioimageanalysis.org), which provides a comprehensive framework for extended reproducible research in bioimage informatics. They have been instrumental for the successful achievement of a large number of collaborative biological projects.



Inria-Serpico

The Serpico team provides computational methods and mathematical models to automatically extract, organize and model information present in temporal series of images as they are obtained in multidimensional light and cryo-electron microscopy. In the field of membrane traffic, Serpico addresses the following themes in close collaboration with Curie Institute: image superresolution and image denoising to preserve cell integrity (photo-toxicity vs exposure time), information extraction from images and videos in multidimensional microscopy for molecular interaction analysis, spatiotemporal modeling of molecular species and multi-scale architectures, computational simulation and modeling of membrane transport at different scales. In collaboration with UMR 144 and PICT at Institut Curie, the members of Serpico participate in several joint projects (PhD and post-doc supervision, industrial contracts...). They have proposed user-friendly algorithms for processing 3D or 4D data. Other projects are related to instrumentation in microscopy including computational aspects (SERPICO@Mobyle web service) and data management (CID iManage) on the reconstruction and enhancement of images related to sub-diffraction light microscopy and multimodal approaches.

Institut Curie

Institut Curie is active in image databases and management. The PICT imaging facility is engaged since 2011 with the Strand Life Sci. company in the development of the CID (Curie Image Database)/iManage (supported by Paris-Centre Node). The CID is linked to the "Curie Image Data Center" (2x 100Tb Storage equipment and cluster for image processing and analysis). Since December 2013 CID is open to all FBI users of the PICT, under demand and common rules of imaging platforms (web client). iManage is the commercial version (with licensing), offering support to labs, to install and adapt CID on their own microscopy, at their own sites. Plugins to access the CID from Icy (Institut Pasteur) are developed and published on the central repository of Icy. An interface to interoperate with the servers at Institut Curie is under development. Integration of software developed in collaboration with Inria-Serpico (http://mobyle-serpico.rennes.inria.fr/) such as ND-SAFIR, Hullkground are now integrated in the CID for automated treatment. Institut Curie, specifically with Serpico's Team@Inria-Rennes, also develops new algorithm solutions for dynamic events detection and classification, sub-diffraction light microscopy and CLEM approaches.

Team Montpellier-IPDM-MRI & MARS

The team is working on a data and image management software. Its main purpose is the management of the data created by the resources of the facility that are driven by a computer (for example microscopes and flow cytometers). All data acquired on the machines of the facility can be uploaded into WIDE. They can then easily be managed on the website, using any computer that has an internet connection. MARS is working on improving a local storage solution and computing for the user's data



Indicators 2012-2013

	Number o	Number of	Number of	Utilization infrast	Utilization rate of the infrastructure		Number of publications (FBI cited)		Number of patents (FBI cited)	
	Year	users	trained on- site	by users	by FBI members for developments or maintenance	from FBI members	from users	from FBI members	from users	
Dactour	2012	300 active versions of Icy	20	N/A	N/A	1	/	/	/	
Pasteur	2013	1200 active versions of Icy	120	N/A	N/A	2 pub./acc.	50 articles citing Icy	0	0	
Inria	2012	N/A	N/A	N/A	N/A	/	/	/	/	
Inna	2013	Mobyle@SERP ICO: *	/	/	/	2 accepted	0	0	0	
Institut	2012	/	/	/	/	/	/	/	/	
Curie	2013	45 projects on CiD-iManage	20	N/A	N/A	2 accepted	0	0	0	
WIDE	2012	11	7	N/A	0	0	0	0	0	
WIDE	2013	34	14	N/A	0	0	0	0	0	

* 25 computation hours / 150 International- 450 national tests + 229 software download



2-3 Work Packages

France-BioImaging operational level relies on five Work Packages (WPs) described below. They are trans-nodes composed of experts in the corresponding technological domains. Their aims are to define the policy of FBI technological developments and to solve eventual barriers in sharing methods and techniques or in the setting up of user access to emerging technologies.



Figure 3: France-BioImaging Work Packages

WP 1 "Instrumentation & Methods" is divided in four sub-groups due to the numerous axes covered by the domain. Names of the persons in charge of each Working Group (WG) are indicated in red.

WP 1 - Instrumentation and Methods. Optical methods have emerged recently for the visualization of single molecules and biological structures at multiscale (nano, micro and mesoscopic) levels, their dynamics and interactions. Moreover, the integration of different scale imaging is certainly at the forefront of cell biology imaging because this provides structural information (obtained with electron microscopy) of the microenvironment of the macromolecules visualized in living cells by fluorescence microscopy. New technological and methodological developments are proposed to overcome technological barriers in the field of super resolution and single molecule tacking (WP 1a), functional imaging (WP 1b), Correlative Light and Electron Microscopy (CLEM) and Super resolution (SuperCLEM / Full-CLEM) (WP 1c) and new contrast & in depth imaging (WP 1d).

WP 2 - High Throughput & High Content Screening. After years of transcriptomics approaches aiming at defining signature and targets, which have been quite frustrating despite prestigious publications, functional validation on a large scale appears a required step to annotate genes forming the signature. Large-scale imaging screening has been a major challenge due to the logistics of imaging and lack of automated image acquisition and analysis software. During the last years, technological advances in automated cell manipulation, microscopy and software, together with an ever-growing pool of commercially available collections of genetic tools (knock-out, over-



expression, histone point mutations, fluorescent-tagged, etc.) have allowed the development of high throughput functional genomics and high-content imaging systems that exploit the power of genetics in a systematic way.

WP 3 - Probe development, Optomanipulation & Optogenetics. Methods for remote control of cell signaling have seen explosive growth. In addition to caged compounds and reversibly caged photochromic ligands, efforts have led to the engineering of light-sensitivity into proteins by the attachment of photoswitched tethered ligands that turn the function of the protein on and off in response to light. Associated with optical methods of excitation beam shaping, these approaches to the control of protein function provide an outstanding alternative to standard methods of controlling proteins through genetic or pharmacological means by providing the control of protein function at high temporal and spatial resolutions.

WP 4 - BioImage Informatics / Image Processing & Data Manipulation. Bioimage Informatics address the questions related to the computerized analysis and handling of image data. New Imaging technologies as they are presented above are very greedy in terms of image processing and data management. New methodological approaches to extract information from massive amounts of image data are definitively required. If not developed concomitantly, the lack of accurate methods in this field can become the real bottleneck of innovative bioimaging approaches. Several lines of research and development can be delineated: 1. image processing and analysis solutions for bioimaging data quantification and modeling; 2. intelligent image data archival and retrieval; 3. high performance computing infrastructures dedicated to massive computational demands. Because these tasks require the gathering of expertise from each Nodes, WP4 is supported by the only transversal node of FBI (BioImage Informatics/IPDM node).

WP 5 - Dissemination, Training and Technological transfer. Considering **Dissemination & Training** a lot has already been achieved over the years, through technological and research networks (RTmfm, "*Mission pour l'Interdisciplinarité CNRS"*, GDR-MIV 2588) and research organizations (CNRS & INSERM trainings) at the <u>national</u>, <u>nodes and inter-nodes levels</u>. Extension and support for existing Core Facility management tutoring (metrology, quality etc.) is now becoming mandatory. Specific trainings remain to be implemented such as tutoring in "Managing image data". Since advanced technologies are obviously growing fast in imaging, new types of interdisciplinary training actions with strong theoritical and practical parts have to be organized. They will concern PhD candidates, research fellows, engineers and permanent researchers at the national and European levels. In order to foster **Technological transfer inside FBI**, a better coordination/facilitation of inter-nodes collaboration programs, proof of concept studies, technological transfer and exchanges between industrials and academics has to be built.

All WGs stand at least once a year and report their results and strategy to the *Executive Board* and to the *National Advisory Committee* during France-BioImaging annual meeting. (See their distribution in **Appendix 2**)



3 General overview 3-1 Financial statement

France-BioImaging is funded through two different phases, the Investment phase (running up to September 2017) and the Functioning phase (running up to december 2019). All the funds were attributed at the beginning of the project to the different node institutions as shown below:

	Bartnor	Total Euroding	Investment phase	Functioning phase
	Partner	Total Funding	01/11/2011-30/09/ 2017	01/11/2011-31/12/ 2019
Partner 1 (Coordinator)	Paris Centre + Coordination	7 275 293€	6 131 293€	1 144 000€
Partner 2	Marseille	3 809 560€	3 212 600€	596 960€
Partner 3	Montpellier	3 007 313€	2 414 513€	592 800€
Partner 4	Bordeaux	5 495 800€	4 830 040€	665 760€
Partner 5	ldF Sud	3 481 880€	2 887 000€	594 880€
Partner 6	Institut Pasteur	2 323 674€	1 982 554€	341 120€
Partner 7	Inria	606 480€	542 000€	64 480€
TOTAL		26 000 000€	22 000 000€	4 000 000€

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Extracted funds for		Investment phase	Functioning phase
Coordination from Paris Centre node	Total Funding	01/11/2011-30/09/ 2017	01/11/2011-31/12/ 2019
Paris Centre	6 148 993€	5 368 993€	780 000€
Coordination	1 126 300€	762 300€	364 000€

Figure 4: Distribution of France-BioImaging funds.

NB: For administrative reasons, the financial partner "Institut Pasteur" covers the expenses of the IPDM team of J.C. Olivo-Marin and the ImagoPole imaging facility (S. Shorte) both located at Institut Pasteur. The "Inria" partner had to be created to cover the expenses of the IPDM team located at Inria-Rennes (C. Kervrann). In figures and tables dealing with financial aspects, ImagoPole facility and these two IPDM teams are grouped in an "Inria Pasteur" financial node.

22 M€ in total of the "Investment" phase - ending in September 2017 - are devoted to the development of new instruments, probes and/or methods, information technologies (data storage solutions and grid computing) and bioimage informatics (Equipment funds). In some cases, renewal of old microscopy systems is required to keep outstanding level of core facilities. Funds are also allocated to the recruitment of engineers and research fellows to perform these developments and/or to support users (Human Ressources funds). In addition, costs to sustain development, maintenance and construction on the facilities, but also expenses to attend meetings are also covered by the Investment phase (Running costs funds). Eventually, five years of Project Manager and one year of webmaster salaries are provisioned through this phase.

Besides, only 390 k€ of the Investment phase (running costs) are allocated to the national Coordination for supporting large-scale actions in training, communication, and dissemination in Biological Imaging as well as for covering the expenses of this vital organ of the infrastructure. As shown in **Figure 12 (Chapter 5-3 Financial perspectives of the National Coordination)**, this amount appears largely underestimated to meet the goals of the French driving forces in Biological Imaging at the international scale.



Summary of investments

As shown in Figure 5, in 18 months (July 2012 - Dec 2013), 54% of Equipment funds have been invested, with heterogeneity among the nodes.

	FBI funds	Equipment	Human ressources	Running Costs
al I.	Intended	0€	372 300 €	390 000 €
ation	Invested	0€	71 673€	90 287 €
Ň	% invested	/	19%	23%
ntre	Intended	3 793 333 €	885 660 €	650 000 €
s Cei	Invested	2 583 862 €	271 110 €	173 488 €
Pari	% invested	68%	31%	27%
xn	Intended	3 462 000 €	924 384 €	443 656 €
rdea	Invested	1 125 774 €	114 438 €	0€
Bo	% invested	33%	12%	0%
lier	Intended	1 726 333 €	243 180 €	445 000 €
Itpel	Invested	929 434 €	91 558 €	148 350 €
Moi	% invested	54%	38%	33%
le	Intended	2 003 000 €	774 600 €	435 000 €
arsei	Invested	1 086 814 €	220 037 €	103 553 €
PM	% invested	54%	28%	24%
p	Intended	1 870 000 €	658 000 €	359 000 €
dF Su	Invested	876 279 €	298 461 €	193 690 €
Ί	% invested	37%	45%	54%
-	Intended	1 376 454 €	1 025 850 €	122 250 €
IPDM	Invested	1 095 845 €	263 441 €	36 316 €
	% invested	80%	26%	30%
Invested G	LOBAL	54%	28%	27%



Figure 5: Percentage of funds invested per node (2012-2013).

Investment phase. NB: ImagoPole is associated to the financial "Inria Pasteur" node.

Equipment investments arise at the beginning of the project and explain a mean percentage of 54% of investment for the whole project. Nevertheless, heterogeneity is seen between nodes which is explained by the large diversity of equipments, commercial or home made set-ups, light or electron microscopy or computing systems. The means of 28 % of funds invested for Human resources and Runing costs correspond likely to the 2 years over the 5 years of planned fundin.

A complete statement of the Investment phase is detailed in Appendix 3.



3-2 Human ressources involved in the nodes

Several engineers have been recruited mainly to setup novel microscopy systems or to develop new algorithms. Their input in the project is critical for state of the art technologies to be open quickly to the user community. The added value brought by FBI in terms of human resources is summarized in Figure 6.



Figure 6: Funding sources for FBI members. Human resources involved in FBI project are expressed in human-month. Contribution of persons financed by FBI appears in red. The effective number of persons involved in FBI projects is indicated per node (right side). NB: ImagoPole is included in the financial node "Inria Pasteur" and not in Paris Centre.

Interestingly, it appears that the contribution of non-permanent positions (in red, funded by FBI and orange, funded by other sources) is high. Their implication is, however, essential for the project.



3-3 Scientific and socio-economic production

Although the past year and half was mainly spent to order and develop new technologies, some important results (technical and/or scientific) have already been published and patents deposited. The high impact factors of numerous publications highlight the strong input of FBI project in addition to the scientific quality of the teams and facilities.

So far, **36 distinct publications acknowledged France-BioImaging**. Two articles are common between Paris Centre and IPDM nodes.

PARIS CENTRE (16)

ImagoSeine / IJM (1 accepted)

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Patents :

2 patents have been deposited by members of Institut Fresnel, Marseille:

- Berto P., Andresen E., **Rigneault H**. (26/03/2013). Number 1300694. The invention describes an original modulation scheme in stimulated Raman microscopy to improve the sensitivity by two fold and decrease the noise level.
- Bon P., Wenger J., **Monneret S.** (13/11/2013). Number 1361065. The invention describes a phase imaging scheme to detect single nanoparticles at the focus of a laser beam with nanometer accuracy.

3 other patents are currently under review: two from CIML, Marseille and one from Institut Pasteur, Paris.

Creation of a Start-Up

June 2013 Creation of the Start Up Company CRYOCAPCELL by Xavier Heiligenstein research fellow at Institut Curie, Paris. The start up originated from the innovative creation of a device for CLEM: the CryoCapsule.

September-November 2013 CRYOCAPCELL and its founder were awarded two prices for innovation : "Prix Creation Innovante CREACC 2013" and "Lauréat Tremplin Entreprise 2013".



3-3 Technological overview

Main technologies and methods available on/or developed in FBI facilities and teams are summarized in **Figure 7.**

Distribution of Technologies Paris Centre 10 48% 30% IdF Sud 60% WP 2 **8%** WP 1d 8% WP 1c GENERAL 8% ALM National 31% 44% Bordeaux WP 1b 56% 15% WP 1a 17% 14% 22% 22 53% 36% Marseille Montpellier

Figure 7a: Distribution of techniques and methods. *In percentage.*

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Figure 7b: Distribution of techniques and methods

Number of instruments. Percentage of opening is added on top of the bars.

General ALM: General Advanced Light Microscopy (Microscopy (Spinning disk, mono and twophoton confocal microscopy, wide field deconvolution microscopy, TIRF microscopy, electron microscopy & cytometry)

Work Package 1 "Instrumentation & Methods"

WP 1a: Super Resolution & Single Molecule Tracking (STED, PALM, STORM, SIM, ...)

WP 1b: Functional Imaging (FRAP, FRET, FLIM, Fluorescence fluctuations based spectroscopy,...)

WP 1c: CLEM & Super CLEM (Electronn Microscopy & sample preparation devices)

WP 1d: New Contrast & In-Depth Imaging (Non linear microscopy, CARS, Light Sheet Microscopy,...)

Work Package 2 "High Throughput & High Content Screening"



3-4 Added value of France-BioImaging in terms of instruments available to the Community

The added value of FBI funds in terms of instruments acquired is displayed per node in **Figure 8**.









Figure 8 : Added value of FBI in terms of instruments available to the Community.

The type of instruments are classified by their membership to WPs, WP 1a : Super resolution & Single Molecule Tracking, WP 1b: Functional Imaging; WP 1c: CLEM; WP 1d: New Contrast & In-Depth Imaging. Blue : Number of instruments not funded by FBI ; Red : Number of instruments funded by FBI.

WP2 - High content screening (also known as high content analysis - HCA) and high throughput screening - displays a hybrid situation, since it covers by high end instrumentation for both sample manipulation (large robotic handling platforms) and imaging (automated microscopes, considered as part of **ALM instruments**) on the one hand and **IT-systems**/Software developments for analysis and storage of data on the other hand. Of note, some local nodes have the intention to acquire commercial setups, others have the ambition to integrate innovative imaging approaches and most will invest in storage/cluster systems for heavy data handling. WP2 is thus organically linked to **WP4** - **BioImage informatics-IPDM**. Consequently, the added value of FBI investments in this context is spread over different WPs and depend on local nodes requirements.

WP3 aims at establishing state of the art chemical technologies for optical control and reading out of living systems associated with new optical methods of excitation beam shaping. Thus, the read out of the investments is more difficult to display because this WP is involved in different types of activities (chemical, biological and microscopy methods). The investment in terms of instruments is shared with WP 1b (with FRAP and photactivation systems) and WP 1d (with excitation beam shaping techniques). Investment in terms of probes development and methods of remote control of cell signaling is seen mainly in the publications (see **Appendix 4**).



WP4 aims at coordinating developpement efforts and software initiatives to store, manage and process image data. The objective is to limit redundancy and integrate software developpements by sharing tools and IT methodologies. Interoperability between distributed storage solutions and implementation of meta-data indexing are crucial and discussed in the workpackage. Several storage and computing solutions are experimented in several groups and IPDM node will recommend the appropriate storage and computing solution for each geographical node. One main achievement is to propose APIs which allow inter-connection of hardware facilities and software platforms.



Investments in Information Technologies Biolmage Informatics / IPDM

Figure 9 : Added value of FBI in terms of information technologies available to the Community. *In blue: Total FBI funds intended; in red: FBI funds invested in 2012-2013; in green: invested funds from other sources.*



Opening of instruments: the fundamental aim of FBI is to improve access of the scientific community to cellular imaging instruments and methods. Hence, the setups and methodologies developed or acquired through FBI must become available to the community through a transparent access process. User access and instrument opening is thus a constant concern within FBI.

Interestingly, the number of new instruments (corresponding to WP1) funded by FBI that are open to the community will increase up to 2017 as shown below in **Figure 10**:



Figure 10: Opening rate of FBI funded intruments (corresponding to WP1). *ImagoPole facility is merged with Paris centre.*

A complete list of instruments available on FBI facilities and R&D labs is detailed in **Appendix 5**.

3-5 Added value of France-BioImaging in terms of Dissemination

In 18 months, France-BioImaging has already supported major events in Biological Imaging:

- FBI specials such as FBI Kick-Off meeting, MiFoBio 2012 and FBI-AT 2013;
- external events: ELMI 2013, EMBO practical course on Super Resolution and "Assises du RTmfm".

http://france-bioimaging.org/category/fbi-events

A web portal has been opened on our website to apply for such supports: http://france-bioimaging.org/application-support/

These activities are linked to WP 5 and financially supported by the national Coordination. For more information, see **Chapter 4 - WP5 achievements** and **Appendix 6**.



4 Scientific and Technological Achievements

WP 1a – Super Resolution and Single Molecule Tracking

- Context and presentation

Optical microscopy has been transformed by the recent development of "superresolution microscopy" or "nanoscopy" techniques, which permit imaging of living cellular structures and molecular dynamics with a resolution approaching a few nanometers. These techniques are either based on the localization of single molecules (PALM/STORM/uPAINT) or engineering of the optical transfer function (OTF) / point spread function (PSF) of the microscope in wide-field or laser-scanning microscopy (SIM/STED).

- Methodological developments and complementarity between the sites

The primary aim of this working group is R&D on superresolution microscopy techniques, which we seek to transfer to the academic community and industrial partners through technology transfer agreements. To accelerate these efforts, the constituent members of the working group meet and discuss regularly to harness synergies and avoid redundancies.

Major efforts currently are underway to improve the following key aspects of superresolution microscopy: optical performance and instrumentation (time resolution, color contrast, depth penetration, z-axis resolution etc), fluorescence labeling (probe specificity, sensitivity, biocompatibility etc), as well as image processing and analysis (computational imaging).

Other important areas of development are technology integration to combine these novel techniques with other advanced experimental approaches (e.g. two-photon photo-perturbation, electrophysiology etc) and application development to broaden the scope and usefulness of the techniques for more complex and physiologically relevant biological samples.

The various members of WG1a have strong and complementary track records and scientific expertise to match the broad spectrum of technological challenges. Working collaboratively, also with commercial companies, current efforts specifically are focused on:

- (i) extending these superresolution microscopy to 3D imaging using several approaches like multi-plane imaging, astigmatism, double helices point spread functions
- (ii) allowing such approaches to be performed in intracellular environments and in thick samples by using single plane illumination strategies, multi Gaussian beam, nearinfrared imaging
- (iii) enhancing color contrast and spectral flexibility for PALM, STORM or uPAINT
- (iv) combining different imaging modalities, in particular single molecule and/or superresolution approaches like PALM/STED, STORM/PALM, uPAINT/PALM, PALM/AFM, PHI/Electron microscopy, DIC widefield-STED, PALM/OT
- (v) allowing long-term (> minutes) single molecule tracking by gold nanoparticle and carbon nanotube imaging
- Scientific projects conducted: see Chapter 7 – Detailed Activity report of the WPs per node



WP 1b - Functional Imaging

- Context and presentation:

The working group 1b aims at conceiving, implementing, developing and applying advanced functional imaging methodologies to investigate biomolecular function, interactions and dynamics in complex environments, spanning from "in vitro" systems, to bacteria, eukaryotic cells, and living organisms. We focus on photonic tools that allow to measure molecular diffusion and dynamics (FCS, FRAP, FLIP, SPT, BRET...), molecular interactions (FCCS, FRET, FLIM), and we develop advanced data analysis and modelling methods.

- Methodological developments and complementarity between the sites

The measurement of molecular diffusion and interactions requires the ability to extract spectroscopic and spatial information from the signal (being most of the time fluorescence emission) generated by the biological sample under study. From a spectroscopic point of view, this includes the measurement of signal fluctuations, excited-state lifetimes, polarization, photochromism, or FRET. The spatial information comes from the ability to localize accurately the position of the emitters, follow them through time, and map spatially the molecular interactions. It can also rely on the ability to generate non-fluorescent signal (photothermal imaging, nonlinear contrast, etc...). Finally, it is important to be able to induce a perturbation on the system under study, and this is achieved through nanomanipulation, or modifications of the imaging substrate.

Toward these goals, each of the nodes participates by providing classical imaging methodologies, including FCS, FCCS, ICS, spFRET, FLIM, FRAP or SPT (that is developed as well in WG1a "Superresolution"). In addition, we have implemented some advanced methodologies on our optical setups (commercial systems or home-made microscopes) that include : spot-variation FCS (svFCS), smFRET in cells using uPAINT, scanning FCS and derived methods (RICS, N&B, ...), alternating laser excitation FCCS, 3D Single particle tracking, lifetime filtered FCCS, Fast-FLIM using multiphotonmultispot imaging (TrimScope).

Finally, new techniques have been invented in our laboratories and are currently under implementation to offer them to the community, such as: FCS coupled with single plane illumination (SPIM-FCS), dual-color bioluminescence/BRET imaging coupled with 2-photon excitation microscopy or SPIM, fast confocal polarization imaging, photothermal microscopy, or holographic optical tweezers for cell manipulation.

Thus, altogether, we cover a broad range of the most advanced functional imaging technologies. It is important however to ensure that highly specialized and trained research staff is present to implement and use them correctly. In this context, each node appears specialized in a fraction of these techniques, and can serve as support for the other research groups when needed. For example, the Paris Centre node is recognized for FLIM-FRET technologies, lifetime filtered FCS and FCCS, or FLIP. The Paris Sud node is implementing a novel setup fusing bioluminescence with 2-photon/SPIM microscopy. The Bordeaux node has implemented photothermal imaging and uPAINT. Montpellier has a strong expertise in 2-photon scanning FCS, FCCS, and N&B, in single-pair FRET, and in vivo imaging. Finally Marseille has introduced svFCS and SPIM-FCS, and develops polarization and 2-photon nonlinear imaging.

We also develop full software packages of plug-ins for popular imaging programs, in order to perform advanced data modelling and analysis procedures. Most of them have been freely distributed to the community. On example is the MTT (multiple-target tracing) software developed in Marseille for particle tracking at high densities that is downloaded more than 2-4 times / month on average.

Concerning the biologicals systems under study, the same type of organization is implemented, where all nodes are able to handle classical biological samples such as cultured eukaryotic cells, but the various nodes have their specificity in terms of biological samples and research topics, that altogether constitute a solid complementarity:

Paris Centre has a strong expertise in eukaryotic cell biology, and relies on research groups specialized in adhesion proteins, nuclear factors, epigenetics, signal transduction and signaling, and biosensors.



- The Montpellier node, through its interactions with a structural biology center, has a specific focus on the measurement of biomolecular structure, interaction and dynamics on purified "in vitro" samples. In addition, the node has a local expertise in imaging of prokaryotic cells (*E. coli*, *B. subtilis*). It is backed by strong research groups studying the regulation of gene expression and epigenetics.
- In Marseille, the node encompasses two Research institutes focusing on immunology and development biology, and specializes its research activities at cellular level in membrane biology and cytoskeleton and on whole organisms (mainly on *C. elegans*, *D. melanogaster* and *M. musculus*).
- Finally, Bordeaux has an internationally recognized expertise in brain imaging and neurobiology.
- Scientific projects conducted: see Chapter 7 – Detailed Activity report of the WPs per node



WP 1c - CLEM & SuperCLEM

- Context and presentation:

The working group WG1c "*Correlative Light and Electron Microscopy (CLEM) and Super Resolution (SuperCLEM)*" aims at developing innovative equipments and methods to achieve a workflow of integrated observations from data obtained on cells and tissues by light and electron microscopy, so called CLEM or SuperCLEM when super resolution is used or Full CLEM when high space-time resolution is used at the light microscopy level. These methods are applied to - Study host-pathogen interactions -understand the dynamics and ultrastructure of highly differentiated cells or subcellular organelles, in health or pathological conditions (cancer, lysosomal and neurodegenerative diseases, phytopathology). The goal by using these systems as models is then to extend to additional applications to the all community.

- Methodological developments and complementarity between the sites

Three Institutes were initially declared as pilots to develop, give access and disseminate new methodologies and technologies regarding correlative microscopy approaches, namely in the **Paris Centre (Curie and Pasteur institutes)** and **Bordeaux nodes**. Strong complementarities between these Laboratories are obvious in their biomedical field of applications (Cancer Research, Host Pathogens interactions and neurosciences). In all three institutes, methodological and technological approaches are different, some favoring full instrumental integration (Pasteur) while other develop simple tools (Curie) and use more Bioimage Informatics (Curie and Bordeaux) to solve similar problems. These cores laboratories by providing expertise and equipment access are now conducting collaborative projects with FBI partners. In addition, the development of correlative microscopy approaches is a today objective for numerous laboratories part of the France-BioImaging network, which are actually now all represented in the CLEM working group. The success of the CLEM days organized in 2012 and 2013 by the Node Paris-Centre (130 participants, and the efforts to tame the community to CLEM practices performed by the node Ile de France Sud (workshops within annual courses, workshop in FBI-AT 2013) to CLEM approaches is a right indicator of the dissemination task proposed by this working group

- Technological & Scientific projects conducted

Institut Curie: Application of the CryoCapsule to the CLEM workflow and automating CLEM steps. The CryoCapsuledevice simplifies the multiple sample preparation steps separating the live cell fluorescence imaging from high-resolution electron microscopy (by Xavier Heiligenstein, post-doctoral fellow, Institut Curie). Collaboration with the newly created start-up company CryoCapCell that designed an automated High Pressure Freezer connected to a high-end fluorescence light microscope for correlative experiments within less than two seconds. The production will be conducted within the coming year by CRYOCAPCELL Company. Transfer of the CryoCapsule technology to **Institut-Pasteur** is ongoing.

Intracellular trafficking intermediates involved in cargo transport in highly pigmented cells. Study that aims at the identification at the ultrastructural level of very small (50-60 nm) Clathrin/GGA coated structures that can only be visualized by live cell imaging during very fast exposure time (30ms). This pilot study highlights the difficulty of « immobilizing » and "pinpoint" highly dynamic events across the different scales of observation. This difficulty can be overcome using newly developed tools that allow a faster fixation of the cells.

Improve the image registration (rigid, non-rigid) protocols during image processing/analyses. A simple macro using ImageJ has been written from existing image registration programs to align precisely various types of light and electron microscopy images for CLEM studies. The macro was developed together between members of the PICT-IBISA at Curie and the Necker light microscopy platform. The macro is now in several laboratories inside and outside FBI (EquipeRaposo&Equipe Perez, Institut Curie CNRS UMR144; Plateau d'imagerie electronique du LBME-CNRS, Toulouse; Plateau Imagerie Hopital Necker Enfants Malades....). New image registration algorithms would have to be developed and tested when High Resolution for light microscopy will be integrated in the Full-CLEM.

Development of in depth High-Resolution Microscopy for the Full-CLEM project 1) by TIRFM Incidence Angle scanning 3D reconstruction (In collaboration with the Roper Sci. Company). New approach enabling fast multi-wavelength azimuthal averaging and incidence angles scanning, in order to computationally reconstruct four-dimensional (4D) images.50 nm axial resolution over 800 nm in depth, away from the cell membrane, is achieved. This imaging modality was applied to obtain structural and dynamical informations on actin architectures and to follow the coordination of multiple actors in membrane exocytosis-recycling in single cell, at fast rate (35 frames/ seconds)



and in 3D, with high axial resolution (in collaboration with L. Blanchoin's Team iRSTV, Grenoble). 2) by fast 3D High-Res SIM. We have worked (Jerome Boulanger, CR, CNRS) with Nikon and **IPDM-Inria node** on a 3D-SIM reconstruction algorithm workflow that significantly improves both the lateral and the temporal resolution of Structure Illumination Microscopy (HR-SIM).

Both **High space-time Resolution light microscopy** approaches could be now tested for integration in the high-end fluorescence light microscope connected to the **automated High Pressure Freezer** mentioned above, allowing an accurate follow up multi-scale study of biological samples, defining the instrumental aspect of our **Full-CLEM project**.

Paris Sud Set up of a working flow to identify the ultrastructural support of fluorescent signal in eukaryotic cells (ImaCLEM1, ImaCLEM2).

The aim of the first project conducted on Imagif platform (**ImaCLEM1**) was to adapt the use of bi-functional commercial probes (quantum dots, nanofluorogold and fluorogold) to the most commonly used plant experimental system (BY-2 cells, Medicago, Arabidopsis), taking into account the needs to shorten electron microscope heavy protocols and to avoid the use of radioactive contrasting agent increasing contrasts (Carpentier et al., 2012; Barois et al., in preparation). In parallel, a working flow to perform CLEM approaches based on correlation between the fluorescent signals observed on ice or resin section and its ultra structural support has been established for plants and c.elegans(**ImaCLEM2**) (respectively Le Bars et al. 2013; Manil-Segalen et al., 2013).

Bordeaux Imaging Center

Development of CLEM on brain slices expressing endogenous GABAB-GFP to study the changes of GABAB receptor distribution upon neurochemical treatments. We want to establish the identification of the area of interest under both light microscopy and electron microscopy. Means employed are brain slices, chemical/cryo fixations, STED, pre-embedding and post-embedding immune-detection.

Development of "In-Resin Fluorescence (IRF)". This technique allows keeping intrinsic or immunolabeled fluorescence in cell and tissues sample in order to make correlative microscopy. First assays on *C. elegans* with TRITC functionalized nanoparticules cryofixed using Leica EM-HPM100 device have been performed. We have determined that the fluorescence of TRITC functionalized nanoparticles can be kept in our conditions.

CLEM approaches are also under way in plant models for investigating the ultrastructure modifications of cell compartments during virus propagation processes in various plant tissues. To target specifically the front of infection with light microscopy (LM), virus tagged with fluorescent proteins are used. The aim of this project consists firstly in the development of methods to maintain fluorescence during all steps of the fixation processes of those specific plant tissues until the embedding step.

Institut Pasteur:

Previously, we developed an original method to visualize specific cytoskeleton rearrangement, during pathogen infection using Correlative Light and Scanning electron microscopy (CL-SEM) at room temperature, (Adeline Mallet and Anna Sartori, Institut Pasteur, *S. Mostowy et al. Cell Host & Microbe, 433, 8 - 2010.*). However, specific and breakable structures (Tunneling NanoTubes in collaboration with Chiara Zurzolo, Institut Pasteur, and viral biofilm in collaboration with Marie-Isabelle Thoulouze, Institut Pasteur) are sensitive to classical electron microscopy sample preparation and we are now developing **Correlative Light and Scanning Electron Microscopy (CL-SEM) using cryo conditions** allowing to visualize samples in cryo fluorescence (cryo box developed by Anna Sartori) and in cryo scanning electron microscopy to avoid artifacts and image samples in their native state.

Development of registration method for Super-CLEM (combination of PALM/STORM imaging and SEM, in collaboration with Mickeal Lelek from Christophe Zimmer's group, Institut Pasteur and Carl Zeiss company). Based on Matlab network we developed a specific algorithm to improve alignment between super-resolution light microscopy and scanning electron microscopy data.

Workflow development of "Shuttle and Find" module, in collaboration with Carl Zeiss company. This approach allows to automate correlative sampling from Super Resolution Light microscopy (ELYRA system) and Scanning Electron Microscopy (Auriga microscope) based on a customized sample holder compatible with the stage mount of both systems. New workflows are being developed to improve the quality of both imaging modality.

PTR 424: The cytoskeleton organization is supporting IL7-signal transduction regulating CD4 human T-cells homeostasis. Transient microtubules anchor membrane receptors induced by IL7 to nucleus and drive molecular motors transporting signaling molecules from receptor to nucleus where they initiate proliferation cell programs. SIM, STORM/PALM are required to image



and detail the compartmentalized signaling system in the 200nm-thick cytoplasm and SEM/TEM to integrate these components within the fine cell structure.

PWJPR: Radial microtubules are induced by activated IL7-receptor, and bridge transiently the receptors to nucleopore to support the carriage of signaling molecules by molecular motors. We aim imaging and discovering the structural and functional links between IL7-receptors, microtubules and nuclear pores by large volume ultrastructural 3D-imaging of human T-cells using FIB-SEM and figure out the 3D-organization of traffic along these MTOC-independent microtubules unclustered all around the cell, tightly regulating the nucleus import of lage signaling molecules.

Other related ongoing scientific projects within FBI or not:

Recycling Endosome Tubule Morphogenesis from Sorting Endosomes Requires the Kinesin Motor KIF13A C. Delevoye et al. (Institut Curie) published in 2014

Identification of structures enriched on melanocyte/keratinocyte contact (C. Delevoye et F. Marsens, Institut Curie)

Characterization of the aggregated nature of an overexpressed protein used in a breast cancer diagnosis assay, G. Millot, X. Heiligenstein, A. Nicolas, G. Raposo (Institut Curie Paris).

Investigate the process of exocytosis of synaptic vesicles upon a quick stimulation of agonist using the CyroCapsule, S Horellou, S. Marty (Ecole Normale Superieure, Paris), X. Heiligenstein, G. Raposo (Institut Curie, Paris).

Ultrastructural analysis of actin/septin bundles by cryo-tomography (A. Bertin; D. Lévy, Institut Curie UMR 168, G. Koenderink (FOM-Amolf, Amsterdam)

Structural analysis of Myosin 1b (E. Coudrier UMR 144, P. Bassereau, UMR 168, D. Levy, UMR 168, Institut Curie)

CLEM identification of the large granules that accumulate in B lymphocytes from Chediak Higashi patients to pinpoint and analyze their ultratsructure, G. Menashe, G de Saint Basile (Necker, Paris), X Heiligenstein, G. Raposo (Institut Curie)

Dynamics studies on focal adhesion. J. Salamero, F. Waharte (Institut Curie) P. Hersen (Laboratoire Matières et Systèmes Complexes, Univ. Paris-Diderot)& Michael Sheetz (Mechanobiology Institute, Singapore).

Tracking gene expression at high spatiotemporal resolution in living cells. J. Salamero, J. Boulanger (Insitut Curie) C. Dargemont (Institut Jacques Monod), P. Hersen (Laboratoire Matières et Systèmes Complexes, Univ. Paris-Diderot)

Spatiotemporal plasticity of the Nuclear Pore Complex functions, at a single living cell and single NPC level.J. Salamero, F. Waharte (Institut Curie) Michael P. Rout (Rockefeller University) NY, Rick Wozniak (Univ Alberta) CA,USA.Biology of autophagosomes along C.elegans development (R.Legouis, Gif sur Yvette) and plant development (B. Satiat-Jeunemaitre, Gif sur Yvette)

Disinhibition processes in neuropathic chronic pain (M. Landry coordinator, IINS and E. Gontier, BIC electron imaging facility).

Use of electron tomography for the study of the Endocannabinoid System (G. Marsicano coordinator, Institut Magendie and E. Gontier, BIC electron imaging facility).

Ultramicro-anatomy of glia-neuron interactions (S. Oliet coordinator, IINS and E. Gontier, BIC electron imaging facility).

Trans-Golgi Network domains in plant tissues revealed by STED microscopy (Y. Boutté, P. Moreau, LBM and M. Mondin, C. Poujol, BIC photonic imaging facility).

Ultrastructural organization of plasmodemata in plant cells (W. Nicolas, E. Bayer, S. Mongrand, LBM and L. Brocard, BIC plant imaging facility)

- Scientific projects conducted: see Chapter 7 – Detailed Activity report of the WPs per node



WP 1d - New Contrast and In-Depth Imaging

- Context and présentation :

The working group W1d "New contrasts and in-depth imaging" aims at developing innovative photonics tools to perform in depth imaging in microscopy and endoscopy. Two axes are explored, the first one focuses on new contrast generation that do not rely on conventional refractive, absorption or fluorescence contrast mechanisms; the second aims at developing new methodologies and instruments that can extend the imaging depth of conventional microscope.

- Methodological developments and complementarity between the sites

The group is very ahead of any available commercial systems and therefore relies on the physical core expertise of seminal Laboratories working in the field such as **the Institut Fresnel (Marseille)**, **Laboratory for Optics and Biosciences (Palaiseau)**, **Neurophotonics Laboratory (Paris)**, **Institut Jacques Monod (Paris)**, **Institut de Biologie de l'Ecole Normale Supérieure (Paris)** and **Photonics, Digital and Nanosciences Laboratory (Bordeaux)**. These core Laboratories are conducting many collaborative projects with FBI partners all over France and ensure the accessibility and dissemination of the new methodologies and technologies developed within this working group.

- Technological & Scientific projects conducted: see Chapter 7 – Detailed Activity report of the WPs per node

WP 2 - High Throughput & High Content Screening

- Context and presentation :

High Content Screening (HCS) provides us with a method to systematically study phenotypes of cells or organisms in varying conditions, such as drug exposure or gene silencing. HCS is the method of choice when the biological question at hand involves a large number (up to hundreds of thousands) of experiments performed under ideally identical conditions. HCS therefore relies on a very high degree of automation regarding sample preparation and microscopy and typically requires specialized and particularly robust methods for automatic image and data analysis and dedicated tools for data handling and visualization.

Methodological developments and complementarity between the sites

In our workgroup, we have 5 facilities with an activity in HCS (either planned or already in place). Two facilities have a long-standing experience in HCS and propose this service to external users since years (BioPhenics at the Institut Curie and ImagoPole at the Institut Pasteur). Three facilities propose to develop their HCS activities and to automate and to scale up new and innovative imaging technologies to become accessible in screening mode. The BioEmergences-IMAGIF facility proposes to scale up 4D imaging of developing embryos (multiphoton, SPIM of developing zebrafish, ascidian or sea urchin) to become applicable in screening mode. This strategy is the last step of a three steps drug screening scheme, where 3D+time imaging and cell lineage reconstruction is reserved to potential hits. The facility MRI-Montpellier aims at extending their screening activities, started in 2005, and focuses on automating advanced microscopy based techniques like single molecule FISH (smFISH), FCS and FCCS to make them available in screening mode. The **IINS-Bordeaux** facility aims at capitalizing on its expertise in single-molecule imaging to develop a pipeline dedicated on medium to high-throughput imaging using PALM or uPAINT. In addition to the facilities, we have workgroup members who develop new methods dedicated to HCS data analysis (working at Institut Curie and Institut Pasteur).

We have therefore a good mix of experienced centers, which have already run many projects, leaders in certain imaging technologies they propose to scale up and several bioimage informatics specialists who develop new methods to take best advantage of these new data and who provide the link to the IPDM node.

- Technological & Scientific projects conducted: see Chapter 7 – Detailed Activity report of the WPs per node



WP 3 - Probe Development, Optomanipulation & Optogenetics

- Context and presentation

Methods for remote control of cell signaling have seen explosive growth. In addition to caged compounds and reversibly caged photochromic ligands, efforts have led to the engineering of light-sensitivity into proteins by the attachment of photoswitchable chromophores that turn the function of the protein on and off in response to light. WP3 aims at establishing state of the art chemical technologies for the optical control and reading out of living systems associated with new optical methods of excitation beam shaping.

- Methodological developments and complementarity between the sites

Different types of probes in association with different techniques are developed:

- (i) photochemical actuators (ENS Chimie),
- (ii) (ii) functionalized gold nanoparticles and near-infrared nanoprobes for deep tissues single molecule imaging (LP2N Bordeaux),
- (iii) (iii) photo-control of protein-protein interations (IIN Bordeaux),
- (iv) (iv) optogenetics toolboxes as well as structured illumination techniques optimized for optogenetics (PCC group at Institut Curie and Neurophotonic laboratory, Paris-Centre)
- Scientific projects conducted: see Chapter 7 – Detailed Activity report of the WPs per node

WP 4 - BioImage Informatics - IPDM

- Context and presentation :

The working group WG4 Bioimage Informatics (previously "Image Processing and Data Management") aims at addressing the questions related to the computerized analysis and handling of bioimage data. For this, it develops several lines of research and development: 1. image processing and analysis solutions for bioimaging data quantification and modeling; 2. intelligent image data archival and retrieval; 3. high performance grid-computing infrastructures dedicated to massive computational demands.

Methodological developments and complementarity between the sites

Since the inception of FBI, the WG4 has focused mostly on the first two axes: development of integrated software packages (Icy, Bioemergences) adapted to the large variety of biological imaging modalities found in the FBI network and development of data management systems (Curie Image Database CID-iManage, WIDE software (Web Images and Data Environment), BioEmergences database), but preliminary work on high-end computing has also been done (Mobyle@Serpico, OpenMOle-BioEmergences). The WG4 relies on the joint expertise and work of laboratories from five institutions (Institut Pasteur, Institut Curie, Inria-Rennes, CNRS-Gif/Yvette, MRI-Montpellier). These core laboratories have proposed solutions that are becoming widely used by the community not only within the FBI network, but also, and more remarkably, in Europe and the USA.

- Methodological & Scientific projects conducted

A sub group focusing on image analysis and modeling was created in 2012. Its main activities have been:

• Development of the software Icy (http://icy.bioimageanalysis.org), a community software platform that proposes a comprehensive framework that strives to bridge the gap between the life science, bio-imaging and image processing communities. Icy is a fully integrated framework designed from the ground-up using modern concepts in programming and ergonomics, taking end-users and developers into equal consideration



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through a community-oriented paradigm. This has been achieved via two key concepts: 1) a plug-in-oriented Java-based software that is adapted to the needs of biological imaging and simplifies the development of new plug-ins; 2) a community web-site to publish, share and manage algorithms and protocols in a straightforward manner, fostering interaction and collaborations between researchers from all backgrounds. One highlight of the project are protocols, which are a graphical front-end that implement software development by enabling end-users to design image processing pipelines in a graphical manner, without any programming knowledge.

Web-based demonstrator without any installation and configuration of image analysis software at Inria-Serpico (Mobyle@SERPICO :http://mobyleserpico.rennes.inria.fr/.

It comprises the following modules:

HotSpotDetection: Robust detection of fluorescence accumulation over time in 0 video-microscopy

Hullkground: Separation of moving and non moving part in an image sequence 0

CRFMovingSpotDetection: Detection of moving spots/vesicles and background 0 subtraction using Conditional Random Fields

KLTracker: Tracking of image-based features in an image sequence 0

Motion2D: Estimation of a 2D parametric motion model and image registration 0

ND-SAFIR: Denoising of N-Dimensional images 0

OpticalFlow: Computing of optical flow and non-parametric motion from a 2D image 0 pair

OpticalFlowStack: Computing of optical flow between each image pair of an image 0 sequence

Several software packages have been compiled for the main operating systems (Linux, MacOS, Windows). We have developed ImageJ plug-in Java versions of the following algorithms: ND-SAFIR, Hullkground, Motion2D, HotSpotDetection, OpticalFlow. Additional Icy plug-in Java versions will be developed using JNI (Java Native Interface) wrappers in 2014.

BioEmergences submitted end of 2013 the publication of the BioEmergences workflow concept for 3D+time imaging of developing model organisms, going from the microscope to the custom made interactive visualisation software Mov-IT. The concept includes a graphic user interface for experiment description on a standard datasheet linked to a relational database for both raw data, processed images and data analysis, that can all be displayed through Mov-IT. Mov-IT is the first tool for in silico embryology with digital specimens reconstructed from 3D+time imaging data. The image processing workflow launched directly from the experiment datasheet on the computation grid EGI includes custom made filtering, segmentation and tracking algorithms and has been made modular and versatile enough to include any desirable processing and analysis algorithm. BioEmergences adapted with FBI funding the OpenMOLE worklow engine http://www.openmole.org/ for biological applications and contributed with a more general scope to the development of OpenMOLE web services. Upon publication, the BioEmergences workflow will be publicly available (http://www.bioemergences.eu)

A sub group focusing on image databases and management was created in 2012. Its main activities have been:

Following the meetings of the sub-group, it was agreed that two projects would be developed on parallel: one from Montpellier called WIDE which is an open source project, and the other one by the company Strand Avadis in collaboration with Institut Curie CID iManage, which will be a commercial solution offering support and open access.

Two main objectives were identified for 2013-2014: the first is to provide a programming interface which will allow accessing any image database, providing that the database software has developed a wrapper library of access making the bridge between this generalized interface and its own interface. This interface will allow to make gueries in several image data bases from the same software, and to allow an easy development of other programs using a database, such as image processing software or electronic lab book, and therefore simplifying the interoperability between software platforms. The second one is to write a document evaluating objectively the difference between available



software, in order to help facilities making their own choice and having a list of elements to consider.

• A first version of a set of plugins to access the Curie image database from Icy have been developed and published on the central repository of Icy. An interface to interoperate with the servers of Institut Curie is under development.

WP 5 - Training, Dissemination & Technological transfer

Objectives

1) Dissemination & Training

-National Level (node and inter-node)

a) Extension and Support for existing Core Facility management tutoring: metrology, quality, purchasing, etc....

b) Co-organization of existing or specific organization of conferences, training and education activities

c) Propositions for FBI specific trainings to implement

-European Level

a) Maintain involvement of WP 5 members in the Euro-BioImaging WP dedicated to Training and in ELMI organization.

b) Insure France-BioImaging presence and visibility in international meetings related to biological imaging.

c) Participation (or coordination) in European consortium for training programs in H2020.

2) Technology transfer

Make propositions to coordinate/facilitate inter-nodes collaboration program, proof-of-concept studies, technological transfer and exchanges between industrials and academics.

Constitution and organization of WP 5: (see Appendix 2)

All FBI nodes and most sites are represented as well as the national networks RTmfm (technological network CNRS) and GDR MIV-2588 (National Research Group Microscopy and Imaging of Life, CNRS). FBI Coordination is also involved and manages funding of transverse WP 5 activities. More focused dissemination and training activities are managed by local nodes or eventually between nodes.

Supervision: Christel Poujol (Bordeaux), Laurent Heliot (GDR-MIV 2588/associated Paris Centre) & Jean Salamero (Paris Centre)

- General achievements (2012-2013) : see Chapter 7 – Detailed Activity report of the WPs per node and Appendix 7.



5 Perspectives 5-1 Scientific perspectives of the WPs

WP 1a - Super Resolution and Single Molecule Tracking

PROJECTS AND COMMON PERSPECTIVES AT 1 AND 5 YEARS

It is now clear that each super-resolution imaging modality comes with its own benefits and limitations in terms of resolution, sensitivity, acquisition speed,.... Thus, in the coming years, a major goal will be to combine the different imaging techniques (PALM/STORM, STED, SIM,...). Next, we will aim at pushing further the spatial and temporal resolution that can be achieved in superresolution microscopy and extending its applicability beyond cultured cells. To this end, we envision that progress in hardware will be necessary but not sufficient. The development of bright probes (either organic or genetically encoded) will be essential as well as the implementation of advanced computational tools that allow rapid processing of vast amount of data (in particular for PALM/STORM images), or to infer biological information from high-resolution images or from highdensity single molecule data (obtained for instance with sptPALM or uPAINT). Finally, we will aim at complementing super-resolution microscopy and single molecule tracking techniques with tools to manipulate and perturb biological samples, such as electrophysiology, optogenetics or AFM. Thereby, it will be possible to measure, at the nanoscale and the single cell level, the biological response to quantified electrical, signalization or mechanical stimuli. Importantly, meeting the goals of our WG will imply active exchanges and frequent meetings with other WGs within France BioImaging.

Detailed Projects per node:
see Chapter 8 – Detailed scientific perspectives of the WPs per node

WP 1b - Functional Imaging

PROJECTS AND COMMON PERSPECTIVES AT 1 AND 5 YEARS

Within the frame of the FBI objectives, WG1b aims at implementing tools and actions in order to appropriately answer the requests of users on biomolecular interactions and dynamics in complex environments. Currently, we have identified the following points and will propose during the next years appropriate actions:

- 1 year perspective:
- To make an inventory of the analytical tools developed by FBI members. We aim at creating a database of the algorithms and utilities developed by the partners of WG1b inorder to share them between platforms and to provide the most appropriate analytical tools to users.
- To develop standards to calibrate the experimental setups and make possible a metrology of specific setups over the time and between experimental systems. Such standards will be used to define experimentally the limits of what is quantitatively measurable, in fluorescence fluctuation experiments for example.
- To organize actions of dissemination of the know-how between WG1b's partners in order to extend the local service and expertise of the platforms on different sites. In addition, we will continue to organize international events such as the EMBO practical Course on advanced microscopies organized in Montpellier in 2013.
- To organize subgroup meetings on specific aspects relevant to WG1b themebut open to the whole community. For the next year, we will plan meetings on the FCS-related methods for the quantification of molecular interactions, on the best practice for fluorescence lifetime measurements, and on standardization & metrology.
 5 year perspective:



Each node will pursue its efforts to improve the sensitivity and extend the applications of dedicated setups. A special effort will be done on FCS-related methods (svFCCS, SPIM-FCCS, scF(L)C(C)S, *in vivo*- and endoscopic-FCS), on merging bioluminescence with 2-photon/SPIM, on polarimetry with multicolor fast polar imaging as well as on new techniques such as the ones based on photothermal effects or on the use of photonic crystals.

- Detailed Projects per node: see Chapter 8 – Detailed scientific perspectives of the WPs per node

WP 1c - CLEM & SuperCLEM

PROJECTS AND COMMON PERSPECTIVES AT 1 AND 5 YEARS

A number of innovative equipment and methods aimed at providing a workflow of integrated observations from data obtained on cells by light and electron microscopy have been already developed within the first active years of FBI. Technological transfer (sample manipulation devices, and automated systems for fast multi-scale transition) and exchange of new methodologies (preparation of samples, image processing and reconstruction methods) are going on and required for the overall FBI project. Most of those developments will be soon, if not already, transferred on the FBI Core Facilities. While progresses have been made in integrating high resolution light microscopy in the CLEM workflow, Super-CLEM development will be one of the main tasks of the very next years. At longer term, CLEM approaches will need to achieve a workflow of integrated observations from data obtained by light and electron microscopy, on tissues and even in live animals or on model organisms, always at the best space-time resolution achievable. There are more technologies to integrate in the future, such as SBF or FIB-SEM. That could give rise to a "Meta-CLEM" approach, the little sister of the Full-CLEM concept. In this respect, WG1b will approach the members of the WG1d (In Depth Imaging and new constrasts) who have expertise in Non Linear Optics and Light Sheet microscopy. Because this will inevitably lead to the bottleneck of managing mass of images and their accurate reconstruction and visualization, interaction with BioImage Inforatics-Image Processing and Data Management group (WG4) is mandatory.

- Detailed Projects per node: see Chapter 8 – Detailed scientific perspectives of the WPs per node

WP 1d - New Contrast and In-Depth Imaging

PROJECTS AND COMMON PERSPECTIVES AT 1 AND 5 YEARS

<u>1 YEAR PERSPECTIVES</u>:

New contrast generation:

Extending nonlinear imaging modalities to polarization resolved schemes Exploring phase imaging for cell and tissues investigation Performing cell membrane diffusion analysis with FCS enhanced by nano-antennas Improving schemes for simultaneous multi-contrast imaging

Extending the imaging depth:

Improving the wavefront control in scattering media for deep tissue imaging Developing ultrafast 3D scanning in non-linear microscopy Developing holographic endoscopes Developing nonlinear imaging endoscopes



Relation with industry:

Finalization of the commercial prototype (3i) for 2P holographic photoactivation Performing active molecule penetration in skin with L'Oreal partner

Inter-node forthcoming collaborations:

- Marseille (IF) – Paris (Paris V): holographic endoscopes

5 YEAR PERSPECTIVES:

Long term perspective is difficult to plan because the WG1d activity is tightly related to technological developments. 'New contrasts' and 'in-depth imaging' are eager users of laser technology and opto-electronic components. For sure, the on-going revolution in the field of fiber based lasers will contribute to the field, also quite awaited is the new deformable mirrors technologies that are emerging today (segmented mirrors with many actuators) and new technology for liquid crystal based devices. New detectors (such as SPAD arrays) and fast electronics shall be key players as well.

New contrast generation shall certainly take advantages of the ultra-short (<10fs) laser pulse control at the focus of a microscope. Most is also awaited from phase retrieval algorithms that can retrieve light emitters patterns by measuring their far field only.

Extending the imaging depth should also come with close loop algorithm that re-optimize the wavefront for each new locations. Most is also expected from endoscope to take over and push the depth limit further in.

WP 1d will work along those directions always looking from 'what's new' to apply it to bio-imaging.

- Detailed Projects per node: see Chapter 8 – Detailed scientific perspectives of the WPs per node

WP 2 - High Throughput & High Content Screening

PROJECTS AND COMMON PERSPECTIVES AT 1 AND 5 YEARS

In our opinion, the strength of FBI in the field of HCS is the variety in expertise at different sites that clearly exceeds the expertise typically available at a single node and will therefore on the long run improve the quality of the offered service. The main objective of this workgroup is therefore to reach a level of integration that makes best use of the expertise such that the final user can actually benefit from it.

There are two major points we would like to address, one concerning the user-side, i.e. measures to improve and to structure the visibility of the available service and the other - more challenging – concerning the internal internode functioning of our work group. In order to achieve this, we propose the following concrete actions: (i) to present the various facilities in better integrated and unified manner to improve the visibility of the palette of offered services; (ii) to develop tools that will facilitate sharing of resources and experiences (from reagents to software scripts); (iii) to identify common problems and provide solutions applicable at the different sites; (iv) to initiate collaborative projects based on the specific experience of each node. The actions (ii)-(iv) concern the inner functioning of our work group. We would like to highlight the importance of point (iv), as we strongly believe that concrete projects in common will really boost the collaborations and set the basis for integration.

Our long-term goals are (i) to integrate as much as possible the different facilities and to maximize the benefits from mutualisation, while keeping the specificity of each site; (ii) develop tools for annotation, data sharing and management in collaboration with BI-IPDM WG (e.g. work on phenotypic ontologies and data formats) (iii) gain national and international visibility by providing a single entry point to several facilities and organizing courses and meetings. These aims will need to be accompanied by the development of few high-impact collaborative projects and technologies setting the baseline and providing pilots for the integrated service we are willing to offer.

- Detailed Projects per node: see Chapter 8 – Detailed scientific perspectives of the WPs per node



WP 3 - Probe Development, Optogenetics & Optomanipulation

PROJECTS AND COMMON PERSPECTIVES AT 1 AND 5 YEARS

Three actions have been planned for 2014: (i) The implementation of a forum on the FBI web site about identifying, characterizing, or using optical probes or photochemical actuators. The WP3 supervisors will forward questions from users to the best national expert(s), who will post an answer (or access to instrumental platforms); (ii) The installation of a committee of expert chemists over the whole French territory that will act as advisers/trainers for users; (iii) The collection of information to build a list of instruments and know-how available in the laboratories of the expert chemists belonging to the preceding committee. This delocalized facility will allow us to be more reactive for local users while benefiting from a national platform.

On a longer term, three other types of actions are presently under consideration: (i) Organization of a Summer School. The purpose would be to gather designers and users of optical probes and photochemical actuators; (ii) Organization of an INSERM workshop. Following our initiative in 2012 which gathered more than one hundred of attendees, we would like to organize a training (including practical activities) oriented toward biologists who do not presently exert in Optogenetics; (iii) Making short movies aiming at training users without experience in optical probes and photochemical actuators (to be put on the FBI Web site).

- Detailed Projects per node: see Chapter 8 – Detailed scientific perspectives of the WPs per node

WP 4 - BioImage Informatics - IPDM

PROJECTS AND COMMON PERSPECTIVES AT 1 AND 5 YEARS

The IPDM node proposes solutions for a large variety of biological questions and imaging modalities. The common perspectives at 1 year are to:

- Install an image database (CID) iManage on a user pilot node;
- Install a storage and grid/cloud computing facility on a user pilot node;
- Integrate several algorithms and image processing algorithms from several IPDM groups on the Icy platform;
- Install the web service Mobyle@Serpico on different FBI nodes using Mobyle-Net.

The major global goals at 5 years are to develop:

- an integrated workflow for launching algorithmic pipelines on the computation grid or local computer clusters for high throughput processing of image data sets;
- the development of standardized evaluation metrics for assessing the quality, precision and accuracy of a processing workflow;
- a data management solution to accommodate the storage, query and annotation of large amounts of image data and metadata;
- creation of a common API for interoperability of the different management systems;
- a resource management system to allow remote reservation, payment and image management from internal or distant sites by all the users of the infrastructures.

More specifically, we will pursue the following developments during the next 5 years:

- automation of acquisition processes and analysis / design of automated multi-channel acquisition systems in HTS and high throughput microscopy (HTM) ;
- exploitation and analysis of multi-parametric data (high speed) / Archiving and indexing of data / algorithmic and statistic of high speed data ;
- development of multi-scale (spatio-temporal) image databases, analysis, structuring and integrating these data into mathematical models to generate predictive models iteratively ;
- design and manipulation of models to better understanding the observed system (living or physical) as a complex system.



WP 5 - Training, Dissemination & Technological transfer

PROJECTS AND COMMON PERSPECTIVES AT 1 AND 5 YEARS

Education, Training and Dissemination

Dissemination through meetings and conferences on focused technical and methodological aspects is more accurately organized by other WPs or at the nodes level. National (or international) training and dissemination actions are directly supported by FBI national Coordination (see already supported actions in **Appendix 6**).

However, a common and regular scientific France-BioImaging meeting should be planned and open at the international level, in the near future.

Proposition for FBI specific training to implement:

-Among trainings for Imaging Facility staff members, WP 5 proposes to launch the first edition of "Managing image data on Imaging core Facilities" at the end of 2014 / beginning of 2015. This Workshop / Training session will be worn by Paris-Centre and BioImage Informatics-IPDM nodes. Instituts Curie and Pasteur are volunteers to organize it, involving specialists from all other nodes. This training could be organized in the frame of training / workshops INSERM / CNRS / Aviesan for 20 to 30 FBI facility managers.

-MiFoBio, national **Bi-annual summer** school in biophotonic and cell imaging, will be permanently supported by FBI. **FBI-Advanced Training**, a bi-annual workshop organized in several FBI nodes, will be open at the European Level: some modules should be driven by other European teams. A call for Next FBI-AT sessions (2015, 2017) should be open. **Montpellier, Bordeaux & Paris-Centre Node** expressed their intention to organize a FBI-AT session, in the future.

-Proposal in the frame of the <u>MSCA European Training Network (ETN-H2020)</u>. It could specifically target imaging platform managers+ exchange of people at the European level. Target : 2015 session. Start the consortium building.

- Bordeaux has very recently been selected to host the "European NeuroScience School Center", a perennial training center supported by FENS and IBRO. Training in BioImaging will be at the heart of this school and performed in collaboration with FBI.

-In the frame of WP 13 of Euro-BioImaging, reinforce presence and activity of FBI.

Definition of an educational system to be established for biomedical imaging; A strategy for a pan-European educational portfolio based on biomedical imaging infrastructures)

University curriculum of biomedical imaging training Proposal of a consensus curriculum for a European Master Degree on biomedical imaging.

Technological transfer

FBI could facilitate collaboration program, proof of concept studies, tech. transfer and exchange between Industrials and Academics.

-By extending access to premises and IT facility (see Chapter 7- Detailed Activity report of the WPs per node)

-By **Fast help** in development or integration of « ready to go » (Proof of Concept is done) Tech. or Meth. (Example : Through funding of M2, Engineer School training.

-By **Short Term** development or integration of «ready to go» (Proof Of Concept is done) Tech. or Meth. Through small and adapted funding of equipment (typically new detector, illumination source, optical modules....), to put pre-competitive photonic components and systems in the hands of researchers and students.

-By **Fast Providing of means** (missions) for travelling, short term journey (a week), fellow exchanges between Labs/Advanced Microscopy Facilities (AMF), Labs/Labs, or AMF/AMF.

-Those last items will require a small but dedicated budget from FBI (10-30 k€ / year)



5-2 Financial statement of the Investment phase

	FBI funds	Equipment	Human ressources	Running Costs
National Coord.	Intended	/	300 627€	299 713€
	Preferred distribution	/	348 627 €	251 713 €
Paris Centre	Intended	1 209 471 €	614 550 €	476 512€
	Preferred distribution	1 028 192 €	787 000 €	485 341 €
Bordeaux	Intended	2 336 226 €	809 946 €	443 656 €
	Preferred distribution	2 336 277 €	809 946 €	443 606 €
Montpellier	Intended	796 899€	151 622€	296 650 €
	Preferred distribution	796 899€	151 622€	296 650 €
Marseille	Intended	916 186€	554 563€	331 447 €
	Preferred distribution	917 000 €	549 999 €	331 447 €
IdF Sud	Intended	993 720 €	359 540 €	165 310€
	Preferred distribution	810 626 €	449 412 €	258 531 €
MDAI	Intended	280 609€	762 409 €	85 934 €
	Preferred distribution	280 609€	762 409 €	85 934 €

Figure 11 : FBI funds available from January 2014 to September 2017 and eventual preferred distribution (Investment phase)

The initial distribution (between Equipments, Human resources and Running Costs) of FBI funds was decided in 2010 (at the submission of the project). Four years later, it appears that an actualized distribution is needed, especially for home made systems that require, to day, more human resources than anticipated.



5-3 Financial perspectives of the National Coordination

HUMAN RESSOURCES (permanent/contractual)									
Intended	Intended 300 627 €								
Preferred	348 627 €								
distribution		FBI contracts		Non FBI contracts					
	Name	Type of contract - Period of recruitment	Invested (01/11/11 - 31/12/13)	Name	Organization - Type of contract	Period and % of involvement on FBI projects			
National Coordinator				COPPEY Maïté	INSERM - DR	01/11/11 - 31/08/14 (retirement) - 80%			
Project Manager (5 years)	FANTAPIE Séverine	IR 01/10/12 - 30/09/14	252 627 €						
Webmaster (4 years at 70%)	GALLOIS Juliette	IE 06/01/14 - 05/01/15	96 000 €						
RUNNING COSTS									
Intended		299 713 €							
Preferred distribution		251 713 €							
General running costs & Communication media				43 000 €					
Missions		Visit of facilities, national and international conferences etc.		40 000 €					
		Annual meeting		160 000 €					
		Executive Board meetings - monthly throughout the		7 200 €					
		National Advisory Committee		8 000 €					
FBI m	eetings	meetings - 3 times a year National User Committee meetings -		10,000,0					
		3 times a year		16 000 €					
		Scientific Advisory Board - every two years		40 000 €					
		Audit - Once		50 000 €					
Support to "FBI specials" events*		MiFoBio / FBI-AT - every 2 years alternatively + "exclusive" national events - 20 000€ per year		80 000 €					
Support to external events*		Online applications - 2 sessions per year - 20 000€ per year		80 000 €					
Support to WPs		Support to WP5 - Training, Dissemination & Technological Transfer - 15 000€ per year		60 000 €					
Participation to Provision - 20 000€ per year EuroBioImaging Provision - 20 000€ per year		80 000 €							
Total Runing Costs (estimates) up to 09/2017				664 200 €					
Missing				-412 487 €					
Functioning Phase added (see below)				350 000 €					
TOTAL Investment & Functioning Phases (Running Costs only) up to 09/2017				-62 487 €					

Investment phase (01/01/14 - 30/09/17)

Figure 12 : Statement of the Investment phase and estimated costs for the national Coordination for 2014-2017.



Functioning Phase (01/01/12 - 31/12/19)

Institutions fees deduced (4%)

	Amount distributed per node (net of management fees)	Person months involved in FBI projects	Amount per person months involved in FBI projects	Number of persons involved in FBI projects	Amount per person involved on FBI projects
Coordination	350 000 €				
Paris Centre (-ImagoPole)	750 000 €	473	1 586€	103	7 282 €
Marseille	574 000 €	311	1 846 €	31	18 516 €
Montpellier	570 000 €	120	4 750 €	25	22 800 €
Bordeaux	640 000 €	608	1 053 €	38	16 842 €
ldF Sud	572 000 €	144	3 972 €	23	24 870 €
Pasteur+Inria +ImagoPole	390 000 €	122	3 197 €	21	18 571 €

Figure 13 : Distribution of funds from the Functioning phase to the nodes and national Coordination.

The distribution to the nodes of funds from the Functioning phase (4 000 k€ in total over 8 years) was decided at the submission of the project. As for the Investment phase, the funds for the national Coordination were allocated to Paris Centre node. An amount of 350 k€ was decided (voted) by the Executive Board, leaving 750 k€ for facilities and R&D labs of Paris Centre. The total estimated costs of the national Coordination represent 664,2 k€ (up to 2017 only). It appears that the total funds dedicated to this vital organ of the infrastructure, from the Investment and Functioning phases, are not sufficient to cover the estimated expenses (at least 60 k€ are missing according to **Figure 12**). In addition, as shown in **Figure 13** the amount of funds allocated to the nodes from the Functioning phase per person-month - or per person involved in FBI projects - is much lower for Paris Centre than for the other nodes.

Importanly, tranversal WPs activities were not supported by specific allocated funds, unless for WP 5 for which the Executive Board has voted a budget of 15 k \in per year from the national Coordination funds (see **Figure 12**). It was recently voted that nodes provide 10 k \in per year (from the Functioning phase) for allowing activities from the WGs. As a result, a total amount of 280 k \in are put aside for projects of the WPs for the period of 2014-2017. WGs have been affiliated to specific nodes as follow:

WP 1a: Bordeaux;

WP 1b: Montpellier;

WP 1c: IdF Sud;

WP 1d: Marseille;

WP3 & WP2: Paris Centre;

WP4: Inria-Pasteur (IPDM);

WP5 is supported by the national Coordination.

They have to describe their project to the Executive Board to get its green light prior to address their needs to their affiliated node.



6- General achievements, strength/weakness and perspectives

An operational and large-scale infrastructure in construction

Establishing an innovative organization: A lot has been accomplished between November 2011 and the "Kick off Meeting" in November 2012. Let's quote, official conventions and contracts between the leading institution and most stakeholders, funding distribution between nodes and coordination, definition of the governance bodies, recruitment of a Project Manager, design and opening of the website, setting up of the various transversal thematic working groups and user committees, significant implication in the EuroBioImaging ESFRI project and participation in training and dissemination activities in France and in Europe.

Operational structuring and enlivening of the infrastructure: From the recruitment of the project manager to date, the national Coordination has been responsible for the operational construction and animation of the infrastructure by bearing its management on scientific, administrative and financial aspects. Inventories of equipments, human ressources and activities of FBI members, collaborators & partners; implementation of FBI entities and rules of governance and support; design of communication tools; building and animation of France-BioImaging *Community* on our website; attendance to major events as *University Meets Business*, Paris 2013 (poster & flyers); ELMI 2013, Arcachon (poster); EuroBioImaging Workshop on Training, Prague 2013 (oral presentation; together with representatives of WG5), CLEM Days, Paris 2013 (flyers & kakemono); "Assises du RTmfm" (Multi-dimensionnal Fluorescence Photonic Microscopy Technological Network), Villefranche sur mer 2013 (oral presentation), EuroBioImaging stakeholders meetings, Vienna & Heidelberg etc. are a few examples of the missions accomplished by this vital organ of France-BioImaging.

Progress in technology accessibility: Although the core facilities and teams composing FBI were obviously already active prior creation of FBI, one may consider that FBI operation only started once a first set of new technologies were acquired through the FBI funds, either directly purchased from providers or developed in the R&D labs or on core facilities and sometimes through industrial partnerships. That was the main achievement during year 2013 in all FBI-Nodes. A large majority of these technologies are related to microscopy setups, other to Information Technology (IT) and innovative methodologies in Bioimage Informatics, some to biochemical and chemical characterization and synthesis. In many cases, equipment is already accessible to a broad research community, thanks to the efficiency of the 10 Imaging Core Facilities, members of the FBI consortium.

Preliminary and encouraging results: R&D Teams were also productive as assessed by a number of already published or accepted "FBI" articles in 2013 (see indicators Table), describing or exploiting innovative imaging approaches. Interestingly, some of these papers are clearly the result of collaborative work between FBI members from different sites, indicating that FBI is also successful in promoting exchange of knowledge and expertise that will create in the future, new research projects on bioimaging. These indicators, among others (patents, copyrights, contractual industrial partnerships), could not be delivered without the recruitment of researchers and engineers in both R&D labs and core facilities, also made possible through the FBI funding.

Promoting active WGs as an operational task force: Importantly, this last year was largely occupied by the structuration and meetings of the WGs which, besides the "FBI Nodes", constitute the other "task force" of FBI. WGs address the specific aims defined by the WPs (see WP achievements and projects). WGs are composed of Nodes members but also of external or associated members. We believe this is an important sign as it demonstrates the ability of FBI to be open to emerging or improving new sites or labs in the field. FBI has indeed the ambition to promote a bottom-up extension of the French Bioimaging community as well as to train users of the infrastructure to frontier technologies and methodologies as they become available.

Coordinating training: Training has been ongoing in individual FBI facilities, but an additional level of integration has started to be constructed. Fast technological transfer from R&D labs and



teams to imaging core facilities will be one of the strongest tools in this matter. We also need to consider "Training the Trainers". These are FBI members who are actively teaching and training young researchers, through education and institutional training or by training people on sites, on systems and use of sophisticated software. Fortunately, FBI is strongly associated with national and international "Technological and Research Networks" that already developed a number of innovative training actions in the past. FBI already started to reinforce their activity and will implement new education and training programs which are actually missing in the field, at the National and European levels (see WP5).

Positioning in Europe: In the last decade, members of FBI, either as individual research teams or as sites, have been strongly involved in international projects related to microscopy in general - bioimaging, biophotonics or image processing in particular. Some have participated to European Education or Teaching networks since their very starts (EAMnet, ELMI, EMBO's workshops, courses..). France, through FBI, has been heavily involved since 2010 in the preparatory phase of the ESFRI EuroBioImaging (EuBI). Of important note, France – and FBI – is the first country in Europe to have financed BioImaging through a national initiative. FBI was represented at EuBI Steering Committee, at the Biological Imaging Group and co-leads its "Generalized Advanced Light Microscopy" WG. Paris-Centre, Bordeaux and Montpellier Nodes participated to the "Proof of Concept Study" in spring-summer 2012, showing the feasibility and restrains of hosting transnational projects on Imaging Core facilities.

France BioImaging, a multi-sited node in the EuroBioImaging ESFRI project: France-Bioimaging has been ranked as "Highly recommended" by an Independent Evaluation Board (IEB) of experts (www.eurobioimaging.eu/content-page/independent-evaluation-board) as to become a future "EuroBioImaging" node (see **Appendix 8**). However, at this stage (Letters of Intent were sent in April-May 2013), the IEB of EuBI raised important and accurate comments, which we have carefully started to take into account. Importantly, IEB members expressed their questioning on the FBI added value for its diverse elements, as compared to their own capacity to independently carrying out their local missions. We believe we have actually started to address these issues and to gather strong arguments, through our WP achievements, some common papers between node members, and through our strong and common activities in dissemination, training and education. Moreover, examination of innovative technology distribution among FBI local nodes (see Chapter 3-3 Technological overview) shows intrinsic complementarities and local specializations. For example, Bordeaux is heavily involved in "Super Resolution" developments (WP 1a) whereas Montpellier is more focused on "High Content & High Throughput Screening" (WP 2) and "Functional Imaging" (WP 1b). Marseille puts also efforts in this latter activity as well as in "New contrast & In-Depth Imaging" (WP 1d). Even Paris Centre node, which has the broadest R&D and core facility services and activities, invested particulalrly in "CLEM & SuperCLEM" (WP 1c). Internodes and inter-WPs future projects should reinforce FBI achievements, for the mutual benefit of the overall community of FBI and its users.

IEB members pointed out a possible over-management (multitude of meetings and management bodies), which might overshadow Science. Although we tried to involve as many actors of the French BioImaging community, our executive governance (Executive Board and national Coordination) is restricted to nine people, including only two persons to sustain the national Coordination (recently helped for Communication by a part-time webmaster), which is obviously insufficient. Only those 9 persons (among more than 300 participating in the project throughout the country) meet monthly, most often through video conference. At the national level, impact is rather marginal in terms of scientific progresses. Other internal governance bodies (National Advisory Committee and National User Committee) gather twice or three times a year.

IEB members also raised some doubts on how the consortium will exactly improve the level of imaging in France and in the European Union, apart from those already involved in it. Now that the groundwork of FBI has been established, this is clearly one of our main and immediate objectives. As it will be addressed in the following chapter "**FBI in the future?**" we are looking for advice for establishing accurate procedures of "entry-exit" to our consortium. Nevertheless, as already explained, WGs and all FBI training and education activities are open to a wide community.

Interestingly, **in the EU context**, France and Finland were the only two countries to propose an integrated "multi-sited" node model. Somehow in contrast to the IEB, we believe that it shows the maturity of the French project as compared to other European countries that still have to develop their national coordination in this domain. FBI is also at the forefront of the EuBI project, in terms of educational and training programs, as it has been recently evidenced in a EuBI workshop organized in Prague last autumn. In conclusion, participation of France to



EuroBioImaging is a strategic strength for FBI and the clear organic link that connects FBI and EuBI should be for their mutual benefit.

Funding and positioning in the ecosystem: France-BioImaging constitution and funding has already been a lever for further gathering of resources. This is clear at the local-node level (region, cities...), also in the context of FBI associated "Investments for the future" ANR programs (Labex, Idex...) and finally in successful proposals to charities and other funding agencies (FRM, Inca, Canceropoles....). Although it is too early to make statistics and give numbers, we have the strong feeling that the FBI program reinforces partnerships with industrials. While FBI members incorporated the infrastructure with their own partners, a number of new projects and programs were really starting in the FBI frame, at the technological level as well as for promoting and dissemination of methodological tools and "savoir-faire". Start-up companies have been created in the first 2 years of FBI and PhD candidates, research fellows or Engineers, trained by FBI R&D labs and teams, have already been hired by high-tech companies and/or FBI industrial users.

FBI in the future ?

Although we can be proud of what has already been accomplished, we are fully aware that at this stage of France-BioImaging progress and construction, there are still many unresolved issues, bottlenecks and weaknesses. In this respect, we seek advice and we ask for suggestions from our Scientific Advisory Board and Institutional partners.

Which support beyond 2020 ? - As the future "National Infrastructure for Cellular BioImaging", the missions of FBI are founded on the triptych "Service-Development-Training". While all 10 core facilities that compose FBI are built under this scheme, FBI as an entity still needs to progress towards integration on these different aspects. Beyond setting up the infrastructure itself, the first 2 years of FBI have been mainly devoted to the development/implementation of new systems. Lying in front of us is improving integrated user-access through a transparent service offering -"offre de service" - as well as multi-level training schemes. We will be helped in this process by the expertise of the individual facilities on the one hand and the studies performed at the European level on the other hand. Part of our questioning is related to support from Institutions to secure or not the future of the Infrastructure. FBI contract and program as they are stand until 2019. However, it is not fully clear yet which decisions and priorities will be taken by institutional bodies in terms of funding and human resources. At the local-node level - and in priority on imaging core facilities - long-term or permanent positions are required to insure sustainability of the highly requested expertise acquired in the frame of FBI. Among the 10 imaging Facilities which are constitutive members of FBI, the largest ones have existed for more than ten years, in the frame of RIO and IBiSA previous programs. Those local infrastructures would not have been able to constitute the France-BioImaging backbone, without expert staff employed on long-term contracts. Stability and expected growth of FBI will require a clear engagement and solutions from partner institutional bodies.

Which legal entity, governance and staff? If one considers "France-BioImaging" as the establishment of a genuine "National Infrastructure in Life and Medical Science", at least a limited number of FBI bodies should be secured. In particular, the national Coordination remains very precarious and needs insurance in terms of human resources and reinforcement in communication and management tools. While FBI is actually running, we are still missing a truly legal framework, causing many difficulties on a daily basis and threatening this vital organ of the infrastructure. The national Coordination actually relies on the national Coordinator, Maïté Coppey, who might retire next September and on the Project Manager, Séverine Fantapie, whose contract ends in September too. The question of the sustainability of the national Coordination is specifically asked regarding the contract of the Project Manager. No more than five years of project management have been provisioned - until September 2017 with the end of the "Investment" phase (like all other positions funded by France-BioImaging), which is obviously inconsistent with a long-term viability of the infrastructure. Moreover, in the absence of any structure dealing with the financial management of the national Coordination - which covers more than 300 FBI members without counting the committees of governance and partners - this task has been carried out at the operational level by the project manager. In order to ensure the high stakes of a national project whose one of its aims is to point out the competitiveness and attractiveness of France in the field of Biological Imaging, the national Coordination needs long-term security and adapted means. The consortium



agreement, currently being written by CNRS, should take carefully into account all parties of the FBI infrastructure and address the question of a potential independent legal status for this national entity.

Which scientific priorities, challenges and "cutting-edge" technology in the future? For the time being, we can only make assumptions of what will be the next challenges in bio-imaging. A number of our objectives and projects have been already described in the report. As examples among many others, we hope to make progress in the following areas: i/ isotropic and live High Resolution photonic approaches, especially for imaging in deep tissues; ii/ quantification methods in functional imaging ; iii/ hybrid combination of imaging modalities, including integration in CLEM and HCS such as FCCS in HCS, or for "*in vivo*" multimodal, manipulation and observation, approaches. As a result, only two years after the constitution of WGs and a bit more than one year of operational work inside the WPs, it becomes clear that transversal activities between WGs (Inter-WGs work) are now mandatory. The next question is to define a strategy to make the right decisions for research and users.

Which technologies for transfer ? Which and how innovative technologies and methodologies, developed by R&D teams, should be transferred to imaging core facilities for a broad use by the research community remains to be accurately addressed. Although there are good examples to be followed inside FBI, we are lacking an established pipeline for this process. Our National Advisory and User Committees will have an important role in order to define the best directions. Industrial partnerships are also compulsory in this matter. Our inventory of local and common actions toward such activities throughout FBI, gives us some guidelines. However and again, a general strategy to delineate what FBI offers and what mutual accomplishments have to be reached remains to be defined.

Which data management technology and staff? Another important issue is the robust sharing of data. FBI is strongly involved in providing tools for new image processing and image data basis. However, the need in this domain for the FBI perimeter is growing fast and has probably been under-estimated. All nodes and several technological WGs, pointed out important IT issues to be more developed: design of standardized evaluation metrics for assessing the quality, precision and accuracy of a processing workflow; ii/ development of data management solutions to accommodate the storage, query and annotation of large amounts of image data sets and metadata; iii/ design of a common API for interoperability of different management systems. Finally, these activities will provide IT tools for investigation in biology. It is now commonly admitted that the constitution of spatio-temporal, multi-scale and multimodal image databases is the starting point in computational cell biology. Analysis, structuring and integrating these data into mathematical models will generate predictive models whose design and manipulation will allow iteratively a better understanding of the observed systems. By chance the "BioImage Informatics/IPDM" Node has been thought as a transverse node and a WP (WP4) at the same time in order to transmit suggestions and information sustained to all other Nodes. Should new Inter WGs projects be launched in this purpose? How could we improve the funding and secure the required human resources in the area of IT domain? Is it one of the main challenges of FBI in the future ? Which decisions must be taken in priority for the future operation of FBI ? Ideas and advice from the SAB are expected.

FBI as Part of a "National Infrastructure in Biology and Health" network- Interaction and synergy with other national infrastructures has also been one of our aims. Our three "natural" partners are France-LifeImaging (medical imaging - FLI), Frisbi (structural biology), and the diverse laureates of the "Investments for the Future" program in "Bioinformatics". Regular contacts have been ongoing in the last years with FLI thanks to our co-participation to EuBI. However, we have to realize that the models of the two infrastructures are inherently different due to the nature of the technologies involved and the largely separate user pool they address. Punctual collaborations are ongoing in several nodes between the two infrastructures FBI and FLI, but it should be clearly stated that they cannot merge in a single infrastructure. While being at an earlier stage, collaboration with "Frisbi" as well as joint meetings, at least at the level of governance, has been envisaged many times and should be organized. Indeed, there are technological and scientific "bridges" between the two communities. High resolution Electron microscopy is one example. Lack of time on both sides, is the only reason why we did not get any further yet.



Which strategy to make FBI a dynamic infrastructure? Building "France-BioImaging" has been our common and constant worry in this first two years. Engagement of both the national Coordination and the Executive Board but also of members at the Node level and energy of WGs were important and successful. While many aspects of the overall project remain to be clarified, time has come to envisage the evolution of the infrastructure and the future entry of new members in the consortium. Several expert R&D teams as well as reliable national Imaging Facilities have probably been left during the project submission three years ago. To reach new objectives and face new challenges, the participation of new teams and labs is required. Meanwhile, among current FBI members, some groups may disperse or dissolve; their scientific orientation or environment may change, and even imaging Core Facilities may not fulfill standards in terms of service or technology over the years. If FBI is expected to last over this decade, we certainly need an objective evaluation process at multiple levels (innovation, scientific, service, employment, education and other impacts...). We also clearly need "Entry-Exit" procedures at the Node, Facilities and R&D labs. Our internal governance bodies (National Coordination, Executive board, National Advisory Committee) cannot actually propose such procedures and are not suitable to make decisions, easily. We expect our SAB to propose guidelines in this issue.

Which communication tools for Facilities and Users ? Communication tools for common work and dissemination have been proposed and provided from the very beginning of FBI (website, groups' pages, posters, inventories...). The national Coordination is deeply engaged in their continuity and improvement (recent hiring of a webmaster) and some tasks are ongoing (definition and communication of the FBI service offering). It will be also the responsibility of FBI members and users to make the best use of them. A first version of a resource management system to allow remote reservation and payment from internal or distant sites by all the users of the infrastructure has been set and should be enriched in the future. Other ways to improve communication could be suggested as well.



7- Detailed Activity report of the WPs per node

WP 1a - Super Resolution and Single Molecules Tracking

MARSEILLE

CIML

<u>FBI support</u>: Equipment: 59 900 + 100 000 €, Functioning: Supervision & development D. Marguet (CNRS, DR)

Recruited personnel: V. Rouger (IE: 01/07/2012 31/06/2013),

Other personnel: A. Velayandon (INSERM, IE CDD 09/13-02/14); C. Billaudeau (CNRS, CIML, research fellow, 11/2011-04/2013), S. Brustlein (Inserm SANOFI, CR), M. Fallet (CNRS, IE, CIML), S. Mailfert (CNRS, IR, CIML),

<u>Objectives of the project</u>: We currently develop the super-resolution approaches to decipher quantitative analysis of the distribution of components in native membrane of primary immune cells. Challenges come from the need to get numbers and precise location of receptor and adaptator molecules.

Work undertaken:

- Design and implementation a super-resolution set-up for dSTORM imaging (done).

- Implement new methodological approaches to fixe cells and label them with fluorescently labelled probes (work under progress).

- Develop and implement dedicated algorithm to analyse data from super-resolution imaging (work under progress).

<u>Important results</u>: Our main efforts have been focus on setting proper conditions of sample fixations, developing specifically designed fluorescent probes for dSTORM labelling applications. Part of these works has been successfully realized.

Algorithm. This work, done in collaboration with Nicolas Bertaux at the Fresnel Institute, aim at investigating the noise introduced by the electron multiplication process within the electronmultiplying charge-coupled device (EMCCD) camera. We have derived a signal-dependent Gamma noise model, consistent with experimental data. This new realistic model has a simple and efficient estimator whose performance reaching at best the Cramèr-Rao Lower Bound (CRLB).

<u>Opening to the community</u>: Experimental set-up will be open soon. Algorithm will be freely available for academic researchers

<u>Running scientific projects</u> (tile & collaborator name):

1C- THe Immune function of Natural Killer cells) / coord. Eric VIVIER, coll. D. MARGUET

2C - nanoDIGICODE - Deciphering the molecular dynamics & organization of the Ras signaling node in the control of the analog/digital signal processing / coord. D. MARGUET, coll.H. RIGNEAULT 4C - iSa - Systems Biology of Immunoreceptor Signaling in Allergic Inflammation / Coord. B. MALISSEN, coll. D. MARGUET

Relation with industry: Nikon & Imagine Optics

Additional grant obtained: Funding agency: FRM, ANRs, INFORM Labex. Total amount: 100 000€

Published FBI publications:

Rabaoui et al Statistical EM-CCD-based camera modeling, manuscript in preparation

Communications and Training courses:

PhD program LABEX INFORM - theoretical and practical courses - 2014 January 13/15
EMBO practical courses - Super-resolution and single molecule microscopies in living cells - 2013, May 13/19
PhD program CIML - theoretical and practical courses - 2013, May 27/29

- PhD program CIML - theoretical and practical courses – 2013, May 27/29



IBDM

FBI support : Equipments: 39 025,09 (PALM) + 312 870,68 (STED) €

Supervision & development PF Lenne; (RICHARD Fabrice, MATTHEWS Cédric (user support, PICSL) <u>Recruited personnel</u>: TRUONG-QUANG Binh-An (dvt) (IR: 01/09/13 - 31/12/13); SOLEILHET Davy 50% (IE: 01/01/12 - 31/12/12)

Other personnel: TRUONG-QUANG Binh-An (dvt, PhD candidate then ATER 01/11/11-31/08/13)

<u>Objectives of the project</u>: Development and implementation of PALM/STORM setup for tissue imaging

Work undertaken:

We have developed a PALM/STORM setup in TIRF/Oblique illumination mode with astigmatic detection for axial localization. It is used for PALM imaging in tissues in whole organisms (Drosophila and C. elegans).

We are currently developing a PALM setup combining light sheet for optical sectioning and high NA for single molecule detection. It is setup on an upright microscope.

<u>Important results</u>: In vivo imaging of adhesion complexes in a whole organism.

<u>Opening to the community</u>: 3 groups at IBDM are currently using this setup and one external user (IST, Austria).

<u>Running scientific projects</u> (tile & collaborator name):

11 Polarisation and binary cell fate decisions in the neuronal system (V. Bertrand IBDM)

2I New physical approaches to dissect the principles of apical junction supramolecular organization in cancer epithelial cells (A. Le Bivic, IBDM)

3I Rôle de Crumbs dans le remodelage des jonctions adhérentes de l'épithélium de l'aile de drosophile (A. Le Bivic, IBDM)

4I Dissection moléculaire des mécanismes assurant l'architecture et la plasticité d'un épithélium (T. Lecuit, IBDM)

5I The Biomechanics of Epithelial cell and Tissue morphogenesis (T. Lecuit, IBDM)

6I In vivo mechanics during tissue morphogenesis (PF Lenne, IBDM)

7I Mesoscopic origins of cell behaviours during tissue morphogenesis: mechanics and biochemical signaling (PF Lenne, IBDM)

8I Expression et fonction des VEGFRs (Vascular Endothelial Growth Factor Receptors) dans la biologie des cellules neurales du SNC: aspects développementaux et physiologiques; potentielles utilisations pour la réparation tissulaire (F. Mann, IBDM)

9I Rgulation of anti-bacterian response in respiratory and gut epithelia of Drosophila melanogaster (J Royet, IBDM)

<u>Relation with industry</u>: Nikon & Imagine Optics

<u>Additional grant obtained:</u> Funding agency: FRM (PALM); LABEX INFORM (STED). Total amount 64488,53 € + 250 000 €

Published FBI publications:

Truong Quang, B.-A., Mani, M., Markova, O., Lecuit, T., and Lenne, P.-F. (2013). Principles of e-cadherin supramolecular organization in vivo. **Current biology**: CB 23, 2197–2207.

Communications and Training courses: see WP 5



MONTPELLIER

CBS

<u>FBI support</u>: Equipment: 35 070 € Supervision & development Marcelo Nollmann; (user support) <u>Recruited personnel</u>: Mutin (IE: 03/2012-11/2013), <u>Other personnel</u>: JB Fiche, IR, CBS.

Objectives of the project:

Development of super-resolution microscopy methods to study the architecture and dynamics of proteins within cells. In particular, we focus on extending PALM/STORM towards 3D and 2-colors and on combining PALM/STORM with other methods such as SIM. We specialize in studies involving nuclear organization.

Work undertaken:

We built a second home-made PALM/STORM microscope based on our previous prototype and with improved stability (low drift).

Implemented hardware and software to perform 2-color STORM with \sim 3-5 nm correction between channels. Manuscript in preparation.

Implemented full acquisition and analysis suite to perform fully automated PALM acquisition and analysis. High-throughput setup in development. Manuscript in preparation.

Developed method to perform sequential PALM imaging in a micro-fluidics device, method paper published. Setup used for a second paper in press.

Implemented whole-cell 3D-PALM based on adaptive optics. Setup fully functional and being used for several biological projects.

Important results:

<u>Super-resolution imaging of bacteria in a micro-fluidics device.</u> Cattoni* DI, Fiche* JB, Valeri A, Mignot T, Nöllmann M. **PLoS ONE 2013.**

Recruitment, Assembly, and Molecular Architecture of the SpoIIIE DNA Pump Revealed by Superresolution Microscopy. Fiche JB, Cattoni DI, Diekmann N, Langerak JM, Clerte C, Royer CA, Margeat E, Doan T, Nöllmann M. **PLoS Biol. 2013.**

Opening to the community:

The PALM/STORM setup built at the MARS research platform is open to the community but projects need to be submitted and validated by the MARS scientific board.

Running scientific projects (tile & collaborator name):

(1) Structural and Spatial organization of bacterial division elements in biomimetric media: analysis by super-resolution and advanced microscopy, German Rivas

(2) Meiotic Recombination in Mice, De massy, IGH, CNRS Montpellier

(7) Analysis of the super-resolution distribution of Polycomb proteins by PALM and STORM, Giacomo Cavalli, IGH, CNRS Montpellier

(10) In Vivo organization and Dynamics of the nucleoprotein complex assembled on the bacterial centromer at the single molecule level, JY Bouet, Laboratoire de microbiologie et genetique moleculaires, CNRS Toulouse

(12) Architecture of M. Xanthus adventurous gliding machine, Tam Mignot, LCB, CNRS Marseille

<u>Relation with industry</u>: Industrial collaboration with two companies: Image Optics and Andor.

Additional grant obtained: Funding agency ANR, INSERM, HFSP, ERC starting grant. 95 000 €

Published FBI publications:

Diego I. Cattoni, Shreyasi Thakur, Cedric Godefroy, Antoine Le Gall, Josephine Lai-Kee-Him, Pierre-Emmanuel Milhiet, Patrick Bron and Marcelo Nollmann. Structure and DNA-binding properties of the Bacillus subtilis SpoIIIE DNA translocase revealed by single-molecule and electron microscopies. **Nucleic Acid Research** 2013.

<u>Communications and Training courses</u>: EMBO Practical Course on Super-resolution and advanced microscopies in living cells, held in Montpellier in May 2013. See **Appendix 6.** Dissemination courses given locally through BioCampus, June 2012.



BORDEAUX

IINS/BIC

<u>FBI support</u> : Equipments: 274 892 + 73 434 + 334 110 €. Functioning: 30 920 € Supervision & development: Valentin Nägerl (R&D, IINS); Christel POUJOL (User Support, CNRS-BIC UMS 3420)

<u>Recruited personnel</u>: LENZ Martin (R&D, IR 01/08/2012 au 31/07/2014) <u>Other personnel</u>: Philippe LEGROS (R&D, CNRS-BIC UMS 3420); IINS: Eric HOSY (R&D, CR CNRS), Jennifer PETERSEN (R&D), Matthieu SAINLOS (R&D, CR CNRS), Marouen BOUAZZI (R&D, IE CNRS), Adel KECHKAR (R&D, PhD candidate), Florian LEVET (R&D, IR INSERM), Jean-Baptiste SIBARITA (R&D IR CNRS), Vincent STUDER (R&D CR CNRS), Gregory GIANNONE (R&D CR CNRS), Olivier ROSSIER (R&D CR CNRS), Olivier THOUMINE (R&D DR CNRS), Valentin NAGERL (R&D PR UB), Jan TONNESEN (R&D PhD candidate), Marc LANDRY (R&D PR UB), Julie ANGIBAUD (R&D IE UB2), Lasani Wijetunge (R&D, research fellow)

Objectives of the project:

Bordeaux flagship projects revolve around unraveling the structure function of neurons and plant cells using a combination of super resolution imaging methods and various cell biology, physiology and chemistry approaches. Bordeaux R&D teams are deeply involved in developing a variety of superresolution instruments that are then transferred to the users through the BIC. These involve STED, PALM, STORM, uPAINT. Of particular note, Bordeaux has a leadership in unravelling synapse structure and function.

Work undertaken:

We developed single and two-photon excitation STED microscopy in brain slices.

We are developing a microscope platform to carry out STED and PALM/uPAINT measurements in parallel.

We are developing a new instrument capable to perform in depth 3D single-molecule-based superresolution microscopy. It relies on a patented innovative solution to perform SPIM using a single high NA objective. Aberration-free in depth imaging of single-molecule will be performed using adaptive optics.

We've installed the Ground State Depletion (GSD) imaging instrument from Leica that has been recently upgraded to 3D super resolution capability.

We are developing a new microscope set-up that combines the capability to perform 2 photon uncaging, PALM and electrophysiological recordings (This will allow to monitor simultaneously the organization of receptors together with synaptic transmission.

Important results :

We have developed nanoscale STED imaging in acute brain slices and *in vivo* in two colors (e.g. GFP/YFP).

Several research projects are ongoing on the GSD instrument, regarding the nanoscale organization of synapses and the organization of the axon initial segment. A first paper has been published using in part this instrument and demonstrating the nanoscale organization of AMPA receptors.

We have achieved STED imaging of dendritic spines and single particle tracking of membranebound fluorescent proteins (PAmCherry).

Opening to the community :

Several SuperRes Instruments are fully opened to the community (PALM/GSD/STED) while others under development (PALM/STED; SuperResPhysio) will be partly opened to the community in 2015.

Running scientific projects:

3D SuperRes (5): Development of 3D-CLEM to correlate photonic microscopy with electron tomography. Leader E. Gontier (BIC), Collaborators D. Choquet (IINS); B. Lounis (LP2N)

InDepthMultiphoton (6): multiphoton microscopy to investigate functional activity of neurons in intact brains by calcium imaging. Leader S. Marais (BIC), Collaborators D. Choquet (IINS)

STED-PALM (7):Combinging STED with Spt-PALM imaging for applications in neurobiology. Leaders Naegerl/Sibarita (IINS). Collaborators D. Choquet (IINS); O Thoumine (IINS)

SCREENDYN (8): Developing a highThrouhput Screening instrument on mobility using fast sptPALM: Leaders Sibarita/Choquet (IINS).



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INDEPTH (9): Developing a new modality for Single Molecule detection inside tissues. Leader Sibarita (IINS)

SuperResPhysio (10): Combining super resolution microscopy with electrophysiology and uncaging. Leader Choquet (IINS)

PhACS (13): Photothermal Microscopy. Leaders Lounis/Cognet (LP2N)

Parallel STED (14): Developing a new modality for STED with over 100 donuts. Leader Lounis (LP2N).

Low temperature super-resolution imaging (16) Lounis/Tamarat (LP2N)

<u>Relation with industry</u>: Collaborative projects (grants obtained) are ongoing with Imagine Optics/Leica MicroSystems/Nikon

Additional grant obtained: Funding agency : Conseil Régional d'Aquitaine 2013-2014 420 K€; LaBex BRAIN 2012-2013 180 K€; Fondation Recherche Médicale 2013 100 K€. Total amount: 480 000 € + 180 000 € + 60 000 €

Published FBI publications:

B. Yang, F. Przybilla, M. Mestre, J.-B.Trebbia, B. Lounis. Massive Parallelization of STED Nanoscopy Using Optical Lattices. **Optics Express.** Accepted 2014.

Communications and Training courses: see WP5



LP2N

FBI support : salary for Pierre Vermeulen (research engineer)

Supervision & development: Brahim LOUNIS (R&D PR, LP2N) & Laurent COGNET (R&D DR CNRS, LP2N)

<u>Recruited personnel</u>: VERMEULEN Pierre (R&D, 12/11/2012-11/11/2013) <u>Other personnel</u>: C. Leduc (CR CNRS, LP2N), P. TAMARAT (R&D, PR UB), JB. TREBBIA (R&D CR CNRS), Z. Gao (research fellow, LP2N), S. Si (research fellow, LP2N), E. Shibu (research fellow, LP2N), P. Winckler (research fellow, LP2N), A. Godin (research fellow, LP2N)

Objectives of the project:

Our objectives are to improve super-localization and super-resolution microscopy techniques on several aspects by developing (i) a photothermal microscope prototype to track single gold nanoparticles, (ii) correlative microscopy between photothermal microscopy and electron microscopy, (iii) low temperature super-resolution microscopy to reach true nanometer resolutions (iv) parallelized STED microscopy (v) FRET based super-resolution by uPAINT to study interacting molecules and (vi) developing single molecule tracking methods in the near-infrared.

Work undertaken:

We developed Photothermal Heterodyne Imaging (PHI) with optimized functional gold nanoprobes. We are now developing a PHI prototype. We are also developing correlative microscopy between PHI and electron microscopy

We developed a new single molecule based super-resolution without photoactivation called uPAINT. A two-color uPAINT was also developed and by combining single molecule FRET and super-resolution imaging, it was possible to study receptor dimers at high resolution.

We are developing a wide-field STED microscope based on optical lattices.

We are developing near-infrared single molecule tracking based on carbon nanotube imaging.

We are developing low temperature single molecule detection to achieve true nanometer resolution on bio-samples.

Important results:

We have conducted intracellular single molecule tracking by gold labeling.

We have developed and demonstrated super-resolution of live cell endogenous activated receptors. We have developed and demonstrated the first combination of super-resolution imaging and FRET. As a demonstration, we studied dimerized receptors in live cell.

We have developed a highly parallelized STED approach based on fast camera detectors and optical lattices containing more than 100 doughnuts.

<u>Opening to the community</u>: Photothermal microscopy will be opened to the community in 2014. All other developed modalities will be opened through scientific collaborations

Running scientific projects:

(a) User Friendly Photothermal Microscopy Prototype for User Plateform, Lounis/Cognet (LP2N)

(b) Parallel STED, Lounis/Trebbia (LP2N)

(c) SUPERCLEM: Correlative microscopy - Photothermal vs Electron microscopy, Lounis (LP2N)

(d) SmFRET-uPAINT Single molecule FRET+UPAINT, Cognet/Lounis (LP2N)

(e) Low temperature super-resolution imaging, Lounis/Tamarat (LP2N),

(f) Near infrared microscopes for single molecule tracking, Cognet/Lounis (LP2N), Groc (IINS)

Relation with industry: Leica Microsystems: photothermal microscope

<u>Additional grant obtained</u>: Funding agency : ANR PNANO Excitube 2008-2012; Conseil Régional d'Aquitaine 2009-2013. Total amount 30 000 € + 60 000 €

Published FBI publications:

B. Yang, F. Przybilla, M. Mestre, J.-B.Trebbia, B. Lounis. Massive Parallelization of STED Nanoscopy Using Optical Lattices. **Optics Express.** Accepted 2014.

Pascale Winckler, Lydia Lartigue, Gregory Giannone, Francesca De Giorgi, François Ichas, Jean-Baptiste Sibarita, Brahim Lounis & Laurent Cognet. Identification and super-resolution imaging of ligand-activated receptor dimers in live cells. **Scientific reports**. 2013.

Communications and Training courses: see WP5


PARIS CENTRE

IJM/IMAGOSEINE: SUPER-RESOLUTION PALM-STORM-SIM

FBI support : Equipments: 506 146€

Supervision & development Orestis Faklaris; France Lam (user support)

<u>Recruited personnel</u>: Olivier Blanc (IR: 09/2013-10/2015), Karen Uriot (IE: 09/2012-10/2012) <u>Other personnel</u>: Vincent Contremoulins (IE CNRS), Karen Uriot (PhD candidate 11/2012-10/2015), Claire Lovo (IE – master2, 02/2013-08/2013)

<u>Objectives of the project</u>: Development of superresolution microscopy in order to study macromolecular organization and dynamics beyond the optical resolution scale. These techniques will be adapted to different biological samples and will be available to the scientific community through ImagoSeine facility.

Work undertaken:

We have purchased a super-resolution PALM/STORM/SIM commercial microscope (ELYRA, Zeiss). We made the microscope operational, and got familiarized with the PALM/STORM/SIM techniques, in terms of sample preparation, data acquisition and data treatment. During this validation phase, we applied these techniques at biological research subjects, varying from cellular dynamics and signalisation, nuclear pore structure to membrane traffic and neuronal morphogenesis. We already have had the first results (accepted publication).

In addition, a developmental project is in progress: a home-made 3D PALM/STORM microscope, for live cell multi-color imaging. The setup will dispose 4 laser lines, with high power for efficient dSTORM imaging.

<u>Important results</u>: We deciphered the structure of the PML nuclear bodies by using SIM microscopy (in press).

Opening to the community:

The commercial system is already open to the community (since 05/2013) and it will be more available from 03/2014, date when the confocal head (LSM780) will be separated from the super-resolution module. The home made system will be available at the end of 2014.

<u>Running scientific projects</u> (tile & collaborator name):

(1) SNARE proteins organization during endo and exocytosis in neurons. L. Danglot & T. Galli (IJM), Paris

(2) Structure of PML nuclear bodies, Valerie Lallane (Univ. Paris 7)

(3) Netrin-1 receptors recruitment and dynamics in living cells. K. Uriot, P. Girard & M. Coppey-Moisan (IJM), Paris

(4) Test for the importance of the conformational changes of the Iga/Igb tail in the initiation of BCR signalling. Jianying YANG (Max Planck Institute), Freiburg

<u>Relation with industry</u>: Zeiss demonstration site in France for 3D dSTORM imaging

<u>Additional grant obtained</u>: Funding agency IBiSA, Univ-Paris-Diderot, Zeiss fellowship of 1 PhD candidate and bench fees. Total amount: $150 + 299 + 80 = 529 \text{ k} \in$

Published FBI publications:

Sahin, U., O. Ferhi, M. Jeanne, S. Benhenda, C. Berthier, F. Jollivet, M. Niwa-Kawakita, O. Faklaris, N. Setterblad, H. de The and V. Lallemand-Breitenbach (2014). "Oxidative stress-induced assembly of PML nuclear bodies controls sumoylation of partner proteins." **Journal of Cell Biology**, in press.



LABORATOIRE PHYSICO-CHIMIE CURIE – PCC Group

FBI support : Equipment: 95 667 € €, Functioning: 58 324 € Supervision & development Maxime DAHAN (DR CNRS)

Recruited personnel: none

Other personnel: NORMANNO Davide (set-up development, research fellow CNRS-PCC, 09/12-09/15), BASSAM Hajj (set-up development, research fellow, CNRS-PCC, 04/13-12/14), EL BEHEIRY Mohamed (software development, PhD candidate, 09/12-09/15)

<u>Objectives of the project</u>: We are developing tools for single molecule imaging and manipulation in living cells. We especially focus on the acquisition, processing and visualization of 3D single molecule data

Work undertaken:

We are developing a multifocus microscope for fast, ultrasensitive, 3D multicolor imaging. This microscope incorporates a set of diffractive elements in the emission pathway in order to acquire in parallel nine planes within a sample, and thereby reconstruct cellular volumes. We are implementing PALM-STORM techniques for 3D super-resolution imaging in whole cells. We are developing a set of computational tools to reconstruct and visualize 3D data. We are combining high-density single molecule techniques (uPaint, sptPALM) with computational inference schemes to determine the diffusivity and energy landscapes of membrane proteins at the cell surface.

Important results:

We are able to track individual molecules over unprecedented depth (~5 microns) in living cells, with two spectral channels. We are also able to acquire PALM/STORM data in two colors over large volumes. We have developed ViSP, a software for the visualization of 3D super-resolution PALM/STORM data. The program is freely available for the scientific community (http://umr168.curie.fr/en/researchgroups/locco/software/).

Together with J.B. Masson at the Institut Pasteur, we have developed a mapping technique that provides a description of the cellular diffusive and energy landscapes based on high-density imaging

<u>Opening to the community</u>: the multifocus microscope is accessible for selected projects

<u>Running scientific projects (tile & collaborator name):</u>

Mapping the nanoenvironment in living cells using high-density single molecule imaging, with J.B. Masson (Pasteur)

Analysis of the organization of the neuronal membrane, with A. Triller (IBENS)

3D Organization and dynamics of telomeres in the yeast nucleus, with A. Taddei (Institut Curie) 3D dynamics of TALE proteins, with J. Lopes (MNHM)

Relation with industry: Nikon & Imagine Optics

Additional grant obtained: none for the moment

Published FBI publications: publications under submission



IBENS

FBI support : Equipment: 105 662 €, Running cost: 5 755 € Supervision & development DARZAC (CR1 CNRS) / SPECHT (CR1 INSERM);

Recruited personnel (name and duration):

Other personnel (name, statut and lab): Ignacio Izzedin (Research fellow 01/2012-03/2014); Xavier Marques (IE CDD 01/2012-12/2016),

<u>Objectives of the project</u>: Adaptive optics (AO) is a powerful technique borrowed from astronomy that, by means of a deformable mirror, allows us to modify the wave-front of a detected lightemitting signal. We recently adapted the use of AO for three-dimensional localization of SMs within the depth of focus of the detection plane. AO can be used not only to induce a controlled degree of astigmatism to the point-spread function of the detected molecules, but also to correct the aberrations that degrade the optical signal and improve the signal-to-noise ratio (SNR). Thus, it can be used to achieve 3D SM based super-resolution imaging. Another powerful application in 3D super-resolution microscopy is the homogenization of the distribution profile of SM detections along the optical axis. The latter is especially relevant in the context of the study the stoichiometry and the spatial organization of chromatin and nuclear factors, avoiding experimental artefacts due to a spatially uneven detection probability.

Work undertaken:

-Live-cell super-resolution to study structural dynamics

-Dual color PALM / STORM

-Live-cell super-resolution to study macromolecular assembly dynamics

-Use of adaptive optics to increase photon budget and PSF engineering for 3D super-resolution -spt-PALM in the nucleus of living cells

-Development of algorithms for 3D FISH quantification

-Development of approaches for quantification of molecule numbers

-Development of probabilistic methods to access energy of molecular interactions

-Implementation of adaptative optic for 3D detection

Important results:

The "Functional Imaging of Transcription" group (Darzacq lab) is focused on understanding transcription and transcription regulation in mammalian cells. It has developed a novel approach for three-dimensional localization of single molecules using adaptive optics.

The "Cell Biology of Synapes" group (Triller's lab) has implemented and duplicated for common use the setup designed in Darzacq lab. More precisely, a 52-actuator deformable mirror is used to both correct aberrations and induce two-dimensional astigmatism in the point-spread-function. A z - localization precision of 40 nm for fluorescent proteins and 20 nm for fluorescent dyes, over an axial depth of ~800 nm can be achieved. The capabilities of this approach has been demonstrated for three-dimensional high-resolution microscopy with super resolution images of actin filaments in fixed cells and single-molecule tracking of quantum-dot labelled trans-membrane proteins in live HeLa cells. PALM microscopy was used for the quantification of the absolute number of molecule. Furthermore, high-density single-molecule imaging together with Bayesian inference allowed the mapping of the energy and diffusion landscapes of membrane proteins diffusing at the neuronal cell surface.

Running scientific projects (tile & collaborator name):

-Localization-based super-resolution (PALM/STORM) set-up combined with local activation/imaging configuration (local activation, pattern activation/imaging, combined FRAP/FLIP with PALM/STORM)

Relation with industry: Nikon France and Imagine Optic

Published FBI publications: one in preparation



UNIVERSITY PARIS DESCARTES

FBI support: Equipment: 42 800€

Supervision & development: Valentina Emiliani (DR2, CNRS)

Other personnel: Marc Guillon (MCU Univ. Paris Descartes), Marcel Lauterbach (Research fellow, Marie-Curie Grant), Martin Oheim, (DR2, CNRS), Maia Brunstein (Research fellow, INSERM)

<u>Objectives of the project</u>: The wave front engineering microscopy group has developed a superresolution STED microscope in the frame of a collaboration with the ENP (Ecole de Neuroscience Paris) network. This microscope allows addressing biological processes occurring at scales ~45nm in two labelling channels. The STED microscope has been implemented with a phase contrast channel for detection of non-labeled structures and recently with an holographic system for high resolution photoactivation.

The Oheim group has built a whole-field super-resolution microscope for imaging in several colour channels dynamic interactions between organelles in live cells over a large field-of-view. They also combined evanescence in excitation (TIRF) and emission (supercritical angle fluorescence, SAF) for quantitative imaging of the near-membrane space.

Work undertaken:

-Prospection for new labelling techniques of living samples -Combination of holographic photolysis and STED microscopy

Important results:

-Improvement of a STED microscope with a quantitative and highly-sensitive phase contrast channel in widefield and confocal mode

- Development of a SIM-TIRF superresolution microscope for imaging dynamic processes in live, cultured cells.

Running scientific projects:

- PRR and Lamp1 mapping by STED microscopy, M. Guillon (MCU, Univ. Paris Descartes), G.Nguyen (College de France) and M. Groszer (Institut du Fer à Moulin)

- *Exocytotic mechanisms in cochlea, Saaid Safieddine* (CR1, CNRS, Institut Pasteur), Jacques Boutet de Monvel (Institut Pasteur)

Relation with industry:

- Patent on combination of SPC and STED, EP12305509 (Guillon, Lauterbach, Emiliani)

- Transfer agreement with Scientifica (Oheim)

Additional grant obtained: Funding agency: Era Net & ENP. Total amount: 550 000 €

FBI Publications

- Maia Brunstein, Kai Wicker, Rainer Heintzmann, and Martin Oheim, *Full-field dual-color 100-nm* super-resolution imaging reveals organization and dynamics of mitochondrial and ER networks, **Optics Express**, (2013) 21(22): 26162-73.

- Maia Brunstein, Maxime Teremetz, Christophe Tourain, and Martin Oheim, *Combined evanescent-wave excitation and supercritical-angle fluorescence detection improves optical sectioning*, http://arxiv.org/abs/1302.1615

- Dongdong Li, Cendra Agulhon, Elke Schmidt, Martin Oheim and Nicole Ropert, *New tools for investigating astrocyte-to-neuron communication*, **Front Cell Neurosci**. (2013) 7: 193.

- Maia Brunstein, Maxime Teremetz, Karine Hérault, Christophe Tourain, and Martin Oheim, *Eliminating unwanted far-field excitation in objective-type TIRF. Part I. Identifying sources of non-evanescent excitation light*, **Biophys J**, (2014) 106(5): 1020-1032.

- Maia Brunstein, Karine Hérault, and Martin Oheim, *Eliminating unwanted far-field excitation in objective-type TIRF.* Part II. *Combined evanescent-wave excitation and supercritical-angle fluorescence detection improves optical sectioning*, **Biophys J**, (2014) 106(5): 1044-1056



WP 1b - Functional Imaging

MARSEILLE

CIML

FBI support : Equipments: 319 000 + 11 000 €

Supervision & development D. Marguet ; (name user support)

<u>Recruited personnel</u>: A Loman (IR: 03/13-02/17), V. Rouger (50%) (IE: 01/07/12 31/06/13) <u>Other personnel</u>: A. Velayandon (INSERM, IE CDD 09/13-02/14); S. Brustlein (INSERM, SANOFI CDD); C. Billaudeau (CNRS, IR CDD leave on 05/13); S. Mailfert (CNRS, IR 50% on R&D and 50% on platform activity); M. Fallet (CNRS, IE, 50% on R&D and 50% on platform activity)

Objectives of the project:

We aim at understanding how cells respond in a decisive manner (digital) to a graded (analog) input of increasing amounts of receptor stimulation. Experimental approaches are based on biophotonics in order to describe the dynamics of the spatiotemporal organization of molecular complexes that take place at the plasma membrane in primary immune cells.

Work undertaken:

- Design and implementation of spot variation FCS and related approaches at the theoretical and experimental level with extension to the measurements association/dissociation processes in living cells.

- Design and implementation of combined holographic optical tweezers set-up on a spinning disk confocal microscope for signaling experiments induced cell-cell contact.

- Design and implementation of polarization resolved microscopy for the analysis of molecular orientation of receptors in cell membrane (in collaboration with S. BRASSELET at Institut Fresnel). -Design and implementation of multimodal NLO microscope for multicolor 2P imaging and morphological intrinsic signatures for intravital imaging.

Important results:

- Analysis of molecular exchange at the plasma membrane for the lipid-anchored RAS proteins.

- Quantitative analysis of the infection of *Caenorhabditis elegans* by *Drechmeria coniospora* at different stages of worm development by HOT and confocal imaging.

- Analysis of the molecular orientation of proteins of the T cell receptor in living cells.

- Imaging cellular tracking in lymph nodes during immune responses.

Opening to the community (2 lines):

- svFCS set-up : open to the community after training by the CIML platform engineers

- HOT-Confocal imaging: open to the community in collaborative manner

- Intravital NLO imaging: open to the community with supervision by the CIML platform engineers

Running scientific projects (tile & collaborator name):

1C- THe Immune function of Natural Killer cells) / coord. E. Vivier, coll. D. Marguet

2C - nanoDIGICODE - Deciphering the molecular dynamics & organization of the Ras signaling node in the control of the analog/digital signal processing / coord. D. Marguet, coll. H. Rigneault

3C - ReceptOrient - Observing the T cell receptor (TCR) activation in real time: monitoring the intracellular domain orientation dynamics of the receptor complex in live cells with polarized time-resolved fluorescence imaging / coord. HT HE, coll. D. Marguet & H. Rigneault

4C - iSa - Systems Biology of Immunoreceptor Signaling in Allergic Inflammation / Coord. B. Malissen, coll. D. Marguet

<u>Relation with industry</u>: Partnership with ISS for future developments on the ALBA system. Sanofi / Transversal mission on microscopy

<u>Additional grant obtained</u>: Funding agency: INSERM, ERC (partner), ANRs (coordination & partners). Total amount: 450 000 €

Published FBI publications:

- Salles et al PLOS Comput Biol (2013) 9:e1003245

- Ghigo et al **J Exp Med** (2013) 210:1657-64
- Rouger et al **Biophys J** (2014) in revision



FRANCE-BIOIMAGING

Communications and Training courses:

- PhD program LABEX INFORM - theoretical and practical courses on fluorescence microscopy - 2014 Jan 13/15

- PhD program CIML - Theoretical and practical courses on fluorescence microscopy– 2013, May 27/29

- EMBO practical courses – Super-resolution and single molecule microscopies in living cells – 2013, May 13/19

- Inserm Training – Intravital NLO imaging on small animals – 2013 Feb 4/8

- Inserm Training - Cytométrie en flux : du photon à la cellule - 2013, Nov. 19/22

- Inserm Training – De la vidéo-microscopie à la microscopie confocale, concepts, pratiques et contrôle qualité – 2013, Oct. 28/30

IBDM

FBI support : Equipment: 26 217,41 € Supervision & development : PF Lenne

<u>Recruited personnel</u>: CAPOULADE Jérémie (Dvt, 50% IR: 01/05/12 - 30/04/13), SOLEILHET Davy 50% (IE: 01/01/12 - 31/12/12)

Other personnel: DETAILLEUR Brice (Mecanics, AI, PICSL), CHARDES Claire (Dvt, IE IBDML)

<u>Objectives of the project</u>: Our main interest is to understand how biochemical and mechanical signals are received and are processed during tissue morphogenesis, with a special focus on cell-cell contacts.

Work undertaken:

We have developed a light sheet microscope combined with FCS, which would allow single color FCS measurements in whole optically sectioned planes of an organism (C. elegans or Drosophila). We have used the setup for in toto imaging of C. elegans (see publication).

<u>Important results</u>: We propose a simple implementation for in toto imaging of C. Elegans (see publication)

Opening to the community: Open to 2 groups at IBDM

<u>Running scientific projects</u> (tile & collaborator name):

11 Polarisation and binary cell fate decisions in the neuronal system (V. Bertrand IBDM)

2I New physical approaches to dissect the principles of apical junction supramolecular organization in cancer epithelial cells (A. Le Bivic, IBDM)

5I The Biomechanics of Epithelial cell and Tissue morphogenesis (T. Lecuit, IBDM)

Published FBI publications:

Chardès, C., Ménélec, P., Bertrand, V., and Lenne, P.-F. Setting-up a simple light sheet microscope for in toto imaging of C. elegans development. **Journal of visualized experiments**. In press



INSTITUT FRESNEL

FBI support : Equipments: 40 000€ Supervision & development: S. Brasselet (CNRS, DR)

Recruited personnel: none

Other personnel: Cesar Valades Cruz (PhD, Erasmus Mundus), Haitham Ahmed (PhD, Erasmus Mundus), Patrick Ferrand (MCF AMU), Hervé Rigneault (DR CNRS)

Objectives of the project:

- We developed polarization resolved fluorescence microscopy for molecular and orientational imaging in cell membranes and tissues.

Work undertaken:

- We have demonstrated lipid orientation in living cell membrane using fluorescent dye inserted into the cell bilayers lipid leaflet (Kress et al, BioPhys J 2013).

- We have implemented a fast fluorescence polarization imaging microscope based on spinning disk technology (Wanget et al, Rev. Sci. Inst 2012)

Important results:

- We demonstrated morphological changes at the sub-diffraction scale in labeled COS-7 cell membranes whose cytoskeleton is perturbed. Molecular orientational order is also seen to be affected by cholesterol depletion, reflecting the strong interplay between lipid-packing regions and their nearby cytoskeleton.

Opening to the community:

- Fast polarization imaging microscope has been transferred to the `Centre d'Immunologie de Marseille Luminy' (CIML) and is now open to collaboration.

<u>Running scientific projects</u> (tile & collaborator name):

- nanoDIGICODE - Deciphering the molecular dynamics & organization of the Ras signaling node in the control of the analog/digital signal processing / coord. D. Marguet, coll. S. Brasselet / H. Rigneault

- ReceptOrient - Observing the T cell receptor (TCR) activation in real time: monitoring the intracellular domain orientation dynamics of the receptor complex in live cells with polarized time-resolved fluorescence imaging / coord. HT HE, coll. D. Marguet & S. Brasselet / H. Rigneault - Molecular order of actin filament in furrow canals of drosophila embryos (S. Brasselet IF, T. Lecuit

- Molecular order of actin filament in furrow canals of drosophila embryos (S. Brasselet IF, T. Lee IBDML)

<u>Additional grant obtained</u>: Funding agency: ANR (100 000€)

Published FBI publications:

- A. Kress, X. Wang, H. Ranchon, J. Savatier, H. Rigneault, P. Ferrand, S. Brasselet, "Mapping the local organization of cell membranes using excitation-polarization-resolved confocal fluorescence microscopy", **Biophys. J.** 105, 127-136 (2013).

- X. Wang, A Kress, S. Brasselet, P. Ferrand, 'High frame-rate fluorescence confocal angle-resolved linear dichroism microscopy', **Rev Scientic Instrument** 84, 053708 (2013)



MONTPELLIER

CBS/MARS/MRI

FBI support : Equipments: 443 000€ . Functioning : 45 000€

Supervision & development name : Emmanuel Margeat ; (Caroline Clerte : user support)

Recruited personnel :

Other personnel: C. Clerte (IR INSERM, 50% R&D, 50% Platform); JB Fiche (IR INSERM, 50% R&D, 50% Platform); V. Georget (IR, 100% Platform); S. De Rossi (IE, 100% Platform)

Objectives of the project:

The Montpellier node is the result of collaboration between three existing IBiSA structures:

- The Biophysics Platform at the has been active in the development and implementation of state of the art instrumentation for probing bio-molecular interactions, dynamics and localization, both in vitro, and in live cells at the single molecule level.

- The in vivo imaging facility at IGF is dedicated to the development of molecular functional imaging techniques in mice models for translational research.

- The MRI platform serves as the major imaging resource for the biological community of Montpellier (>1000 researchers), and it indeed provides cell biologists with access to both standard and high-end commercial microscopes

Work undertaken:

Purchase (with FBI funds) and building of a 2-photon microscope dedicated to fluorescence fluctuation microscopies (FCS, FCCS, N&B, RICS). This setup is available to external users through the MARS platform.

Design and implementation of a Pulsed Interleaved Excitation microscope based on a supercontinuum source, for single molecule FRET and FC(C)S.

Important results:

- Quantification of the estrogen receptor / corepressor interaction in live cells by FCCS, N&B

- Measurement of the structural dynamics of antiterminator RNA hairpins, and the metabotropic glutamate receptors, using single molecule FRET and filtered FCCS

- Measurement of transcriptional noise in Bacillus Subtilis by N&B

- Quantitative measurement of the GKAP-DLC2 interaction in living neurons using crossN&B

Opening to the community: All microscopy systems are open through collaborations

<u>Running scientific projects</u> (tile & collaborator name):

(2) Analysis of nuclear pre-ribosomal stalk complexes in yeast by Fluorescence micro-microscopy techniques; CSIC, Madrid, Instituto de química física Rocasolano Dep Química Física Biologica

(3) Dynamic remodeling of scafforld interactions in dendritic spines controls synaptic transmission; IGF, CNRS Montpellier

(6) Analysis of G protein-coupled receptor oligomerization in native tissues-Importance of monomer/oligomerization equilibrium; IGF, CNRS Montpellier

(8) Probabilistic differentiation in the Bacterial Heat-shock response, Dajkovic, Faculté de Médecine, Paris

(9) Detection of GFP-DspA in the yeast S. cerevisae, Barny, INRA paris

<u>Relation with industry</u>: Collaboration with Roper Scientific

Additional grant obtained: Funding agency : ANR, Gis IBISA

<u>Communications and Training courses</u>: EMBO Practical Course « Superresolution and single molecules microscopies « (CBS, Montpellier) ; Atelier Biocampus : Nouvelles microscopies photoniques : Superrésolution (CBS, Montpellier) ; MRI Atelier Biocampus : Les bases du logiciel image J (MRI, Montpellier) ; MRI "Atelier Biocampus : Microscopie à épi-fluorescence et microscopie confocale: de la base à la pratique" (MRI, Montpellier) ; MRI Atelier: Immunocytologie in situ hybridization, plant anatomy (INRA-CIRAD) ; MARS-CBS : Cours UPSTREAM : Fluctuation Correlation Spectroscopy (CBS, Montpellier), See also WP5



BORDEAUX

LP2N - Institut d'Optique Graduate School

FBI support : salary for Pierre Vermeulen (Research Engineer) Supervision & development: Brahim Lounis and Laurent Cognet

<u>Recruited personnel</u>: Pierre Vermeulen – 2 years (FBI) Other personnel: Antoine Godin (Research fellow LP2N)

Objectives of the project:

Our objectives are to achieve functional imaging in super-resolution microscopy techniques and single molecule related techniques. In particular, using uPAINT, we aim at demonstrating and developing the first FRET based super-resolution microscope in order to study interacting molecules. We also wish to provide to the community a correlation based photothermal microscope to study diffusing nanoparticles.

<u>Scientific projects</u> (tile & collaborator name): (a) User Friendly Photothermal Microscopy Prototype for User Plateform, Lounis/Cognet (LP2N) (b) SmFRET-uPAINT Single molecule FRET+UPAINT, Cognet/Lounis (LP2N)



PARIS CENTRE

IJM/IMAGOSEINE

FBI support: Equipment: 23096€

Supervision & development Nicolas Audugé (IR CNRS)

Recruited personnel: Philippe Bun (IR, 03-2013), ZengZhen Liu (IE, 10-2013/12-2013) Other personnel: Nicolas Borghi (CR CNRS)

Objectives of the project:

We are interested in the molecular mechanisms of mechanotransduction. Specifically, we use and develop functional imaging techniques (FLIM-FRET, FCS/FCCS, ICS) and biosensors to monitor mobilities, interactions and mechanical tensions of proteins involved in signaling pathways downstream of mechanical extracellular cues, from the cell membrane, down to within the nucleus.

Work undertaken:

We have implemented a system that performs FCS, bleed-through-free FCCS (FLCS) and FLIM/FLIM-FRET (Microtime 200) to increase the detection sensitivity, spatial resolution and range of excitable fluorescent probes. Briefly, we achieve sub-wavelength confinement of the excitation volume at the surface of a coverslip nanopatterned with dielectric photonic crystals (coll. with A. Levenson and A. Giacomotti) excited with a pulsed supercontinum (Fianium) tunable laser. Laser wavelength fine selection induces resonant interaction with the dielectric crystal that results in a strong evanescent excitation field, thereby providing smaller excitation volume and increased signal-to-noise ratio. The effect is comparable to TIRF excitation without the requirement of oblique illumination. We have de-coupled an Elyra system from a Zeiss LSM 780 confocal microscope to dedicate the confocal microscope to ICS applications (RICS, STICS, TICS).

Important results:

We have applied FCS, FLCS, FRAP and FLIM-FRET to characterize the mobilities and interactions of proteins of Focal Adhesions in stem cells in response to substrate rigidity. We have applied STICS to investigate vesicle trafficking (coll. T. Galli).

Opening to the community:

FCS/FCCS system (Microtime 200): FCS, bleedthrough-free FCCS (FLCS), FLIM/FRET FRET-FLIM system (TriMScope/Picostar): FLIM/FRET, anisotropy, 2PE and multifocal to perform fast FLIM imaging. LSM710 Confocor Zeiss: FCS, FCCS, FRET (spectral mode). LSM780 Zeiss and SP5 Leica: FRET (spectral mode), ICS. Leica DMI6000B: FRET (cubes: widefield). Spinning Disk: photoactivation/FRAP.

Running scientific projects (title & collaborator name): Focal Adhesion mobilities and interactions. Z. Liu, N. Audugé, N. Borghi, M. Coppey-Moisan (IJM). Vesicle trafficking dynamics. P. Bun, coll. A. Burgos, T. Galli (IJM). Photonic crystals for enhanced FCS/FCCS excitation. N. Audugé, coll. A. Levenson and A. Giacometti (LPN, CNRS UPR20) Analysis of supramolecular organization and function of GPI-anchored proteins. N. Audugé, coll. C. Zurzolo and S. Lebreton (Institut Pasteur, Paris) Live-cell imaging of cytoskeleton regulators activity during Shigella invasion. N. Audugé, coll. G. Tran Van Nhieu (Collège de France, Paris) Interactions between nuclear pore proteins. N. Audugé, coll. V. Doye (IJM) Interactions between mitotic proteins in drosophila. N. Audugé, coll. K. Katsani (Democritus University of Thrace, Greece), R. Karess (IJM) Live cell imaging of B cell antigen receptor signaling cascade. N. Audugé, coll. J. Yang (Max-Planck-Institute of Immunbiology, Freiburg).

<u>Relation with industry</u>: Collaborative development with **ERROL** (C. Hubert) on *Enhanced* FCS/FCCS FLIM-FRET.

Additional grant obtained: Funding agency: CNano IdF, FRM. Total amount: 400000€



INSTITUT CURIE: Physico-Chimie Curie – PCC Group

FBI support : Equipments: $0 \in$; Fonctioning: $0 \in$ Supervision & development : Maxime Dahan (DR, CNRS), Davide Normanno (Research fellow, Institut Curie)

Recruited personnel: None Other personnel: None

Objectives of the project:

We are interested in assessing the role of nuclear factors mobility, genome organization, and DNA topology and their interplay in orchestrating gene expression plasticity. To this end, we will mostly use micro-manipulation and advanced optical tools to directly visualize and quantify dynamical processes and biochemical reactions *in situ* in living cells, at the single-molecule level.

Work undertaken:

Conception and setting up of an integrated microscopy platform to perform single-molecule imaging and two-photon FCS (Fluorescence Correlation Spectroscopy) and FLIP (Fluorescence Loss In Photo-bleaching) measurements in single cells. Development of quantitative tools to measure nonspecific interactions between individual proteins and DNA. Development of quantitative tools to measure *in situ* association rate constants in individual cells.

Important results:

Demonstration of the presence of long-lasting non-functional non-specific interactions between DNA-binding proteins and non-cognate DNA sites. Direct measurement of the association rate constant of a DNA-binding protein in live cells and demonstration of the weak role of chromatin organization in the association rate of DNA-binding proteins to their specific binding sites.

Opening to the community:

Custom-built microscope equipped with temperature control and micro-injector for: a) Single-particle-tracking and PALM/dSTORM (UV, yellow, and red lasers), with the possibility of using an astigmatic lens for 3D imaging in TIRF/HILO illumination; b) Two-photon FCS/FLIP (Ti:Sa tunable laser). Open to the community through scientific collaborations.

<u>Running scientific projects</u> (tile & collaborator name):

Study of chromatin remodeling factors – B. Burman and T. Misteli (Center for Cancer Research, National Cancer Institute, NIH, Bethesda, MD - USA)

Study of nonspecific interactions of TALE proteins – J. Lopes (Muséum National d'Histoire Naturelle, CNRS UMR 7196 / INSERM U565, Paris)

Study of chromatin accessibility at the X inactivation center – L. Giorgetti, T. Pollex, and E. Heard (Institut Curie, CNRS UMR 3215 / INSERM U934, Paris)

Relation with industry: None

Additional grant obtained: None

Published FBI publications:

Normanno D., Boudarene L., Dugast-Darzacq C., Chen J., Richter C., Proux F., Bénichou O., Voituriez R., Darzacq X., et Dahan M., "Probing the target search of individual DNA-binding proteins in mammalian cells". Submitted (2014)



INSTITUT PASTEUR / IMAGOPOLE

FBI support : Equipments: None

Supervision & development Spencer Shorte; Joe Dragavon (user support)

<u>Recruited personnel</u>: Joe Dragavon (1/1/2013 – 28/2/2014) <u>Other personnel</u>:

Objectives of the project:

By applying cutting edge scientific research and imaging technologies, we aim to study the structural molecular dynamics of biological processes and their appropriation by infectious diseases that involve bacteria, parasites, and viruses.

Work undertaken:

We have developed multiple advanced imaging modalities capable of providing fundamental insight to the interactions of invading organisms and their cellular hosts at the molecular scale. In this domain we have developed a fluorescence lifetime imaging microscope with spinning disc confocal (FLIM-SD). By combining these two technologies we are able to determine the 3-dimensional fluorescence lifetime distribution of a given probe within living cells while traditionally visualizing multiple fluorophores that provide complimentary structural and/or biophysical information of the host and the pathogen.

Important results:

To date, we have shown a rapid and significant decrease in the global oxygen concentration within macrophage during exposure to pathogenic bacteria. Further, we have shown the localization of lipids within neuron and fibroblast networks after chemical stimulation of actin modification.

Opening to the community:

The FLIM-SD has been open to the community since October 2013.

<u>Running scientific projects</u> (tile & collaborator name):

3D mapping of the internal oxygen concentration of single cells during bacterial invasion, Ellen Arena (Philippe Sansonetti).

The determination of Avanti polar lipid accumulation upon actin modification in neuron and fibroblast networks, Soraya Victoria and Stéphanie Lebreton (Chiarra Surzolo).

Relation with industry:

The FLIM-SD is a developmental project that is running in collaboration with Andor Technologies (Northern Ireland) and Lambert Instruments (the Netherlands).

Additional grant obtained:

Funding agency: Institut Pasteur, Pasteur Foundation, Nichols Foundation, Institut Carnot, ANR, Region Île de France, Conny-Maeva Charitable Foundation. Total amount: 300,000.00€

<u>Published FBI publications</u>: None, though at least one is in preparation pending final experimental results



WP 1c - CLEM & SuperCLEM

BORDEAUX

BIC / IINS / LBM

FBI support: Equipment: 40 269 €, Functioning: 23 544 € (Lucie GEAY salary) Supervision & development: Landry Marc, Prof. Univ. Bordeaux, IINS/BIC ; Moreau Patrick, DR-CNRS, UMR5200/BIC ; Gontier Etienne, IR Univ. Bordeaux, BIC; Brocard Lysiane, IR Univ. Bordeaux, BIC.

<u>Recruited personnel</u>: GEAY Lucie (User support, ITA , 15/10/2013-14/8/2014) Other personnel: personnel from BIC and some people from IINS and LBM involved.

Objectives of the project:

Correlative Light Electron Microscopy (CLEM) in various experimental models in the domains of *neurosciences and plant sciences*:

-Development of "In-Resin Fluorescence" approaches (IRF).

-Development of Super-CLEM to correlate high resolution photonic microscopy with EM.

-Development of 3D-CLEM to correlate photonic microscopy with electron tomography.

Scientific projects:

Regarding the R&D activities, based on proposed development projects, the work that has been carried out was as follows:

At first, our work consisted in developing the method of Neuronal Culture on Photo-etched coverslips suitable for the Correlation with TEM. We have performed the 3D CLEM after cryo-fixation project as follows:

In anticipation of the 200kV TEM acquisition that will allow to perform tomography studies, the electron imaging facility of the BIC was able to develop one protocol for high pressure freezing on neuronal culture and 2 Freeze-substitution protocols, one dedicated to morphological study and another for immune-detection studies. **These methods are now available in routine as a service to users.** The acquisition of tomography softwares has just been achieved in order to implement the important Tomography analysis which is under progress in the BIC plant imaging and electron imaging facilities.

Intra-node collaborations:

1/ Collaboration with Landry's Team in IINS: the objective is to study the changes of GABAB receptor distribution with correlative microscopy to explore possible disinhibition mechanisms in chronic pain conditions. The technical objective is to establish the correlation in neuronal cultures endogenously expressing recombinant GABAB-GFP by using squared glass support.

2/ Collaboration between the BIC plant imaging facility and electron imaging facility for the development of "In-Resin Fluorescence" approaches (IRF). The aim of the project is to keep fluorescence on biological samples. For that, the BIC plant facility tests plant samples containing YFP or m-cherry fluorochromes, with chemical fixation or HPF. The electronic facility tests animal tissue samples containing GFP or rhodamine, also with chemical fixation or HPF. Regarding chemical fixation, we are focusing our developments on protocols using lowicryl resin embedding. 3/ Collaboration with Moreau's Team in LBM and the BIC photonic imaging facility: STED on plant tissue samples has been succeeded.

Communications and Training courses:

Etienne Gontier has participated in 2012 to the France-BioImaging Kick-Off meeting held at the Institut Curie, Paris. Lysiane Brocard has participated in 2013 to the national workshop on CLEM, Institut Curie and Institut Pasteur (October 21st- 22nd) supported by GDR 2588 and FBI.
 Marc Landry is also member of the Training and dissemination WP5.

Additional grant obtained/required:

Complementary financial support will be purchased from the next CPER program 2015-2020, National Research Organisms and Region Aquitaine.



PARIS CENTRE

INSTITUT CURIE: Full-CLEM project

FBI support : Equipment: 770 000€

Supervision & development Graça Raposo, Daniel Levy, Jean Salamero; (ROMAO Maryse, IR, IC user support)

Recruited personnel:

<u>Other personnel</u>: BERGAM Ptissam (User support and R&D, CNRS IE), HURBAIN IIse (User support and R&D, IR, CNRS), HEILIGENSTEIN Xavier (Setup dvt CLEM, research fellow CNRS), BERTIN Aurélie (R&D, CR CNRS), Jerome BOULANGER (Computing Scientist, R&D,CR CNRS), Perrine PAUL-GILLOTEAUX (User support and R&D, CNRS IR)

Objectives of the project:

Understand the dynamics and ultrastructure of highly differentiated cells or subcellular organelles, in health or pathological conditions (cancer, lysosomal and neurodegenerative diseases, physiopathology), at various scales.

Work undertaken:

Optimization of sample preparations for correlation between light and transmission electron microscopy (Institut Curie) This work has permitted the establishment of a start-up in Septembernovember 2013, CRYOCAPCELL (see important results)

Towards the development of multiscale observations for improving the identification of highly dynamic structures (Institut Curie). A pilot study on intracellular trafficking intermediates involved in cargo transport in highly pigmented cells.

Improve the image registration (rigid, non-rigid) protocols during image processing/analyses (Institut Curie). A simple macro to align precisely various types of light and electron microscopy images for CLEM studies. Developed together with members of the PICT-IBISA and the Necker light microscopy platform

Development of in depth High-Resolution Microscopy for the Full-CLEM project 1) by TIRFM Incidence Angle scanning 3D reconstruction. 2) by fast 3D High-Res SIM. Both approaches could be now tested for integration in the high-end fluorescence light microscope connected to the automated High Pressure Freezer mentioned above, allowing an accurate follow up multi-scale study of biological samples.

More interactions and exchanges are planned with Image Processing Node IPDM-Inria for image analysis for CLEM.

Important results:

June 2013: **Creation of the Start Up Company CRYOCAPCELL** by Xavier Heiligenstein research fellow at Institut Curie. The start up originated from the innovative creation of a device for CLEM: the CryoCapsule.

September-November 2013: CRYOCAPCELL and its founder were **awarded two prices for innovation** : "Prix Creation Innovante CREACC 2013" and "Lauréat Tremplin Entreprise 2013".

Opening to the community:

The first version of the HR-SIM microscope and the two EM setup are open since 2013. The High end Light Microscope connected to HPF HPM100 and the Theta Scanning TIRF microscope are dedicated to R&D.The fast HR-SIM microscope (second version) is still under development.

<u>Running scientific projects</u> (tile & collaborator name):

with the group of Alain Nicolas/Gael Millot Institut Curie Paris, we have performed CLEM on yeast cells to characterize the aggregated nature of an overexpressed protein used in a breast cancer diagnosis assay.

with the group of Geneviève de Saint Basile we have performed CLEM on B lymphocytes from Chediak Higashi patients to pinpoint and analyze the ultrastructure of the large granules that accumulate in these cells

with the group of Dr Serge Marty Ecole Normale Supérieure Paris we have used the CryoCapsule to investigate the process of exocytosis of synaptic vesicles upon a fast stimulation of neurotransmitter release. In this context special CryoCapsules are being developed to culture brain slices directly on the membrane.

with the colleagues at Institut Pasteur we are in the course of transferring the CryoCapsule technology to their HPM010 to adapt to their specific SEM workflow.



with the group of Renaud Legouis (CGM, Gif sur Yvette), we develop CLEM in order to understand the biology of autophagosomes along *c.elegans* development

with the group of J.M. Hermel (INAF, Gif sur Yvette), we provide expertise to develop CLEM for studying brain of growing zebrafish

with the group of L. Blanchoin (iRTSV, CNRS/CEA/INRA/UJF, Grenoble) we have used Theta-Scan TIRFM for the study of cytoskeleton dynamics and architecture at high axial resolution, both in vitro and in vivo.

Relation with industry:

Nikon : Development of programs for automated acquisition on sections and specific microscope to combine with HPM3. New software workflow for 3D HR-Structured Illumination Microscopy reconstruction.

CRYOCAPCELL : Production of different types of cryocapsules (Institut Curie-ENSAM)

L'Oreal and Clarins: Research and development studies exploiting imaging methods including CLEM(Institut Curie)

Roper Scientific : 3D high-Resolution Theta-Scan Microscopy for Full CLEM project.

<u>Additional grant obtained</u>: Funding agency : Labex CellTissPhyBio, Institut Curie, FRM, Industrial Partnership. Total amount: 824 847 €

Published FBI publications:

Xavier Heiligenstein, Jérôme Heiligenstein, Cédric Delevoye, Ilse Hurbain, Sabine Bardin, Perrine Paul-Gilloteaux, Lucie Sengmanivong, Gilles Régnier, Jean Salamero, Claude Antony, Graca Raposo. *The CryoCapsule: Simplifying correlative light to electron microscopy*. **Traffic** 2014 (accepted)

Communications and Training courses: See also WP5

Practical CLEM workshop at the MIFOBIO 2012 Summer School (Institut Curie with Y. Schwabb & W. Kukulski, EMBL, C. Genoud, FMI, Basel).

National workshop on CLEM, by the Paris Centre Node (Institut Curie and Institut Pasteur) supported by the GDR-MIV 2588, several sponsors and in partnership with France-BioImaging. The meeting was held at Institut Pasteur on October 21st- 22nd 2013.

In 2012 and 2013 Instituts Curie and Pasteur organized 1 week of teaching on electron microscopy and CLEM for students following the "Engineer of Platforms" Master 2 of University Paris Diderot.



INSTITUT PASTEUR: Full CLEM project

<u>FBI support</u> : Equipment: Auriga-SEM/Elyra-SR, 1,050,000€ Functioning: 0€; Salary 65,000€ Supervision: S Shorte & Anna SARTORI;

Development & user support: Audrey SALLES (Super-Res), Perrine BOMME

(SEM/FIB/CLEM/cryoCLEM), Adeline MALLET (SEM/FIB/CLEM/cryoCLEM), Emmanuelle PERRET, (HSQPE) Nathalie AULNER (HSQPE), Marie-Christone PREVOST (PFMU Resp), Anne DANCKAERT (Analyses) Pascal ROUX (Super-Res) Joe DRAGAVON (User Support)

<u>Recruited personnel</u>: Laure Wasniewski (1 year, Licence student); Joe Dragavon (FBI Engineer) <u>Other personnel</u>: Anne-Marie BALAZUC (Resp. Quality), Christiane PACAUD (Admin/Secretarial), Thierry ROSE (permanent researcher)

Objectives of the project:

Development of correlative methods mainly dedicated to Scanning Electron Microscopy (SEM) studies (CL-SEM). Ongoing project correlating SEM/TEM with FL-super resolution techniques (Structured Illumination Microscopy), PALM (PhotoActivated Localization Microscope) and STORM (Stochastic Optical Reconstruction Microscopy) with SEM. Future studies consist on correlation between light and STEM (Scanning and Transmission Electron microscopy) and FIB (Focused Ion Beam) column.

Work undertaken :

-Development of Correlative Light and Scanning Electron Microscopy (CL-SEM) approaches for cells imaging at room temperature and in cryo condition.

-Development of Super-Resolution Correlative Light and Scanning Electron Microscopy by combination with PALM/STORM and SIM imaging methods.

-Development of semi automated registration methods based on fluorescent beads, in collaboration with Institut Pasteur group (C Zimmer)

-Implementation of automated correlation solution provided by Carl Zeiss Company, named "Shuttle and Find". This method allows us to correlate automatically data from High Resolution Light Microscope (Elyra) to Scanning Electron Micsocope (Auriga)

-We are developing a workflow to correlate SIM (Elyra) and SEM (Auriga) imaging.

Opening to the community:

Both imaging systems, Elyra (Super Resolution Light Microscope, Carl Zeiss) and Auriga (SEM, Carl Zeiss) are open to the FBI community, the French national life sciences community, and the larger European community.

Scientific projects (title & collaborator name):

- We have conducted several projects using CL-SEM approaches mainly based on cellular context: 1) with the group of Dr. Thierry Rose, Institut Pasteur, we study the IL7-signal transduction supported by the cytoskeleton in primary CD4 T-cells using SIM/SEM and STORM/SEM approaches. 2)with the group of Pr. Pascale Cossart, Institut Pasteur, we have performed CL-SEM approaches to characterize septin cages around Shigella flexneri during cell infection. (S. Mostowy et al., Cell Host & Microbe, 433, 8 - 2010), 3) with the group of Jost Eninga, Institut Pasteur, we studied of the entry of Salmonella Typhimurium into Hela cells.

-We have conducted CL-SEM in cryo condition to characterize really specific and breakable structures as Tunelling Nanotubes (TNT) involved into prion spread with the group of Pr. Chiara Zurzulo, Institut Pasteur and viral biofilm with the group of Marie-Isabelle Thoulouze.

-We demonstrated the feasibility of High Resolution CLEM by combination of SIM and SEM data, on ongoing project based on actin network of amiba studies with the group of Pr. Nancy Guillen, Institut Pasteur.



WP 1d - New Contrast and In-Depth Imaging

MARSEILLE

IBDML

FBI support : Equipments: 56 682,36 €

Supervision & development PF Lenne; Claire Chardès

<u>Recruited personnel</u>: CAPOULADE Jérémie (Dvt, 50%, IR 01/05/2012 - 30/04/2013), BLANC Olivier (Dvt, IR 01/06/2013 - 31/07/2013)

Other personnel: DETAILLEUR Brice (mechanics, PICSL), CHARDES Claire (Dvt, IBDM)

Objectives of the project: Providing a versatile fast imaging system for developmental biology

Work undertaken:

We have developed a light sheet microscope with different modes of illumination: simple light sheet produced by a cylindrical lens, scanned Gaussian beams or scanned Bessel beams.

Important results:

The setup is functional and we are characterizing its performances on different model systems (*Drosophila* and *C. elegans*).

<u>Opening to the community</u>: This set-up is already used by 3 teams in the institute.

<u>Running scientific projects</u> (tile & collaborator name):

New physical approaches to dissect the principles of apical junction supramolecular organization in cancer epithelial cells (A. Le Bivic, IBDM)

The Biomechanics of Epithelial cell and Tissue morphogenesis (T. Lecuit, IBDM)

In vivo mechanics during tissue morphogenesis (PF Lenne, IBDM)

Polarisation and binary cell fate decisions in the neuronal system (V. Bertrand IBDM)

INSTITUT FRESNEL

FBI support : Equipments: 211118€ Supervision & development: H Rigneault (DR1-IF)

Recruited personnel: Julien Savatier (IR, 01/12/2012 - 30/11/2013)

Other personnel: Petru Guenuche (Research fellow-IF), Jérome Wenger (CR1-IF), Sophie Brasselet (DR2-IF), Julien Duboisset (MCF AMU-IF), Esben Andesen (Research fellow-IF), Cesar Valades (PhD-IF), Patrick Ferrand (MCF AMU-IF), Serge Monneret (CR1-IF)

<u>Objectives of the project</u>: Our objective is to develop advanced optical tools for bio-molecular detection in cells and tissus that are not commercially available. Our aim is to transfer these new tools to imaging facility platforms associate to FBI.

Work undertaken:

- We develop coherent Raman microscopy (CARS/SRS) as label free, chemically specific imaging modality for cell and tissue imaging.

- We develop polarization resolved nonlinear microscopy for molecular and orientational imaging in cell membranes and tissues.

- We develop quantitative phase imaging microscopy.

- We develop ultra-sentive molecular detection in FCS using nanoscale metal nano-antennas
- We develop miniature lensless endoscope for in depth imaging

Important results:

- We have demonstrated label free drug imaging in mice and human skin using CARS/SRS and implemented a new scheme that permits background free SRS.

- We have developed and demonstrated a multimodal polarization resolved microscope including fluorescence, SHG and FWM to retrieve molecular orientational information.

- We have shown that quantitative phase imaging can retrieve the dry cell mass during the cellular division cycle and that 3D refractive index tomography is possible.



FRANCE-BIOIMAGING

- We have demonstrated a best ever fluorescence enhancement using a new concept of antennain-a-box concept (fluorescence enhanced by a factor of 1000).

- We have demonstrated for the first time a two-photon lensless endoscope.

Opening to the community:

The fluorescence polarization resolved spinning disk based system developed at the Institut Fresnel has been transferred in the framework of FBI to CIML in Sept 2013. Institut Fresnel is also conducting collaborative project with IBDML.

<u>Running scientific projects</u> (tile & collaborator name):

- Molecular order: receptor trans-membrane proteins, lipid probes (S. Brasselet, D Marguet CIML)
- Mol. order of actin filament in furrow canals of Drosophila embrios (S. Brasselet, T. Lecuit IBDM)
- Molecular lipd probe engineering for near infrared detection imaging (Olivier Maury, ENS Lyon)
- Molecular order in spinal cord myelin (S. Brasselet, F Debardieux INT)
- Multimodal NLO imaging of melanoma (H. Rigneault, D Marguet CIML)
- Imaging drug penetration in skin (H. Rigneault, L'Oreal & Galderma)
- Deep brain imaging (H. Rigneault, R Cossart INMED)

Relation with industry:

L'Oréal: In depth drug penetration in skin investigated by coherent Raman microscopy. Galderma: skin investigation using nonlinear microscopy

Published FBI publications:

1- D. Punj; J. de Torres; H. Rigneault; J. Wenger, 'Gold nanoparticles for enhanced single molecule fluorescence analysis at micromolar concentration', **Opt Exp** 21, 27338-27343 (2013)

2- E. Andresen, G. Bouwmans, S. Monneret, H. Rigneault, "Two-photon lensless endoscope", **Opt. Expr.** 21, 20713-20721 (2013)

3- A. Kress, X. Wang, H. Ranchon, J. Savatier, H. Rigneault, P. Ferrand, S. Brasselet, "Mapping the local organization of cell membranes using excitation-polarization-resolved confocal fluorescence microscopy", **Biophys. J.** 105, 127-136 (2013).

4- D. Punj, M. Mivelle, S. B. Moparthi, T. S. van Zanten, H. Rigneault, N. F. van Hulst, M. F. Garcia-Parajo, J. Wenger, "A plasmonic 'antenna-in-box' platform for enhanced single-molecule analysis at micromolar concentrations" **Nature Nanotechnology** 8, 512-516 (2013)

5- X. Wang, A Kress, S. Brasselet, P. Ferrand, 'High frame-rate fluorescence confocal angleresolved linear dichroism microscopy', **Rev Scientic Instrument** 84, 053708 (2013)

6- E. R. Andresen, G. Bouwmans, S. Monneret, and H. Rigneault, 'Toward endoscopes with no distal optics: video-rate scanning microscopy through a fiber bundle', **Optics Letters** 38, 609-611 (2013)

Communications and Training courses:

- IF co-organized a national event "Autour des ondes" (Around waves) at the Fresnel Institute on Fev 15th -16th 2013. This event gathered 50 researchers from all over France to present their latest development related to in-depth imaging in biological and scattering media.

- IF co-organized a winter school on "Micro Coherent Raman Imaging" in the Physics center of Les Houches Feb 23rd – 28th 2014 together with EU Cost MicroCoR. This school is opened to all PhD, Post Doc, Engineers and Researchers working or entering the field. Tutorial will be given by experts whereas attendees will be able to present their latest work through informal short talks. 70 attendees are expected from all over Europe.

http://os.tnw.utwente.nl/cost/index.php?option=com_content&view=article&id=20&Itemid=221

Patents:

Berto P., Andresen E., <u>Rigneault H</u>. (26/03/2013). Number 1300694. The invention describes an original modulation scheme in stimulated Raman microscopy to improve the sensitivity by two fold and decrease the noise level.

Bon P., Wenger J., <u>Monneret S.</u> (13/11/2013). Number 1361065. The invention describes a phase imaging scheme to detect single nanoparticles at the focus of a laser beam with nanometer accuracy.

BORDEAUX



LP2N / PHOTONIC, DIGITAL & NANOSCIENCE LABORATORY

FBI support : Equipments: € ; Functioning: €

Supervision & development: Brahim Lounis, PRCE LP2N

Recruited personnel: Pierre Vermeulen – 2 years (FBI)

Other personnel: Laurent Cognet (DR2, LP2N)

Objectives of the project:

 Develop a versatile photothermal microscopy machine, which allows efficient detection of nanometer-size gold nanoparticles via their absorption and using the photothermal contrast
 install this "user-friendly apparatus" on any microscopy platform.

Work undertaken:

- we build a module that can be adapted on any fluorescence microscope.

- This module comprises light sources, detection and electronic systems, and a scanning system built around a galvanometric mirrors device.

- The light beams are delivered by two diode-lasers and specific home-built electronics (including a fast detector and a lock-in amplifier) is under development and characterization.

- Elaboration of an acquisition software.

The assemblage of the different parts of the photothermal module will be realized in the next months. We plan to have a complete system, installed on the BIC (Bordeaux Imaging Center) in the next few months.

Opening to the community:

Photonics, Digital and Nanosciences Lab will transfer its newly developed photothermal microscope to the Bordeaux Imaging Center (BIC)

Running scientific projects (tile & collaborator name):

(a) SUPERCLEM: Correlative microscopy - Photothermal vs Electron microscopy, Lounis (LP2N)

(b) Parallel STED, Lounis/Trebbia (LP2N)

(c) Near Infrared probes, Lounis/cognet (LP2N), Choquet/Sainlos (IINS)

(d) SmFRET-uPAINT Single molecule FRET+UPAINT, Cognet/Lounis (LP2N)

- (e) Low temperature super-resolution imaging, Lounis/Tamarat (LP2N),
- (f) Near infrared microscopes for single molecule tracking, Cognet/Lounis (LP2N), Groc (IINS)

Relation with industry:

Amplitude systemes: development of new laser sources Leica Microsystems: photothermal microscope



IdF SUD

ECOLE POLYTECHNIQUE-LOB

FBI support: Equipment (spent): 104 073 € Supervision & development Emmanuel Beaurepaire

<u>Recruited personnel</u>: C.Stringari (method dvts, research fellow, 01/01/14 - 01/06/14) <u>Other personnel</u>: LOB CNRS UMR7645: MC. Schanne-Klein (dvt, DR), W. Supatto (dvt, CR), P. Mahou (dvt, PhD), M. Zimmerley (dvt, research fellow), G. Latour (dvt, research fellow), X. Solinas

(dvt, IE), JM. Sintes (dvt, IE)

<u>Objectives of the project</u>: - Developing advanced optical methods for tissue imaging, by combining several multiphoton contrasts, and improving imaging depth.

- Opening these methods to the community by simplifying them and implementing applicationdedicated setups.

Work undertaken:

- We pursue a theoretical and study of the multiscale non-linear response of collagenous and lipidic organized molecular assemblies, and of the use of these signals for bioimaging.

- We develop robust methods for multicolor two-photon imaging and simultaneous 2PEF-THG-SHG imaging.

- We opened these capabilities (polarimetric and multimodal multiphoton imaging) to external collaborators.

- We develop methods for wavefront shaping in multiphoton microscopy.

Important results:

- We have implemented a microscope allowing polarization-resolved imaging of biological tissues submitted to mechanical traction

- We have shown that SHG signals can be used to detect structural abnormalities in corneas from diabetic rats.

- We have discovered that polarization-resolved THG is sensitive to molecular order in multilamellar lipid assemblies (such as in in skin stratum corneum), and we have modelled this response.

- We have developed efficient multiphoton imaging of live brainbow tissue.

Opening to the community:

FBI-cofunded setups have been opened to external users through collaborations (see below), leading to publications. These projects drive the ongoing developments.

<u>Running scientific projects</u> (title & collaborator name):

(LOB1) Multiphoton THG-2PEF imaging of engineered adipose tissue - I.Georgakoudi, Tufts University, Boston, USA.

(LOB2) SHG imaging of human and rat corneas - F. Behar-Cohen & JL. Bourges, Centre de Recherches des Cordeliers, Hôtel-Dieu Hospital, Paris.

(LOB3) Multicolor 2PEF imaging of novel brainbow constructs - J. Livet, Institut de la Vision, Paris & X.Morin, IBENS, Paris.

(LOB4) Multiphoton imaging of human brain samples - G. Latour & F. Pain, IMNC, (Orsay) (LOB5) Multiphoton imaging of bone - D. Débarre & A. Gourrier, LIPhy (Grenoble)

Relation with industry: L'Oréal: confidential study.

Additional grant obtained: Région IDF interDIM: 370 000 €. Total amount spent: 35 420 €

Published FBI publications:

M.Zimmerley, P.Mahou, D.Débarre, MC.Schanne-Klein, E.Beaurepaire. Phys.Rev.X 2013.

T.Chang, M.Zimmerley, KP.Quinn, I.Lamarre-Jouenne, DL.Kaplan, E.Beaurepaire, I.Georgakoudi. **Biomaterials 2013.**

L.Kowalczuk, G.Latour, JL.Bourges, M.Savoldelli, JC.Jeanny, K.Plamann, MC.Schanne-Klein, F. Behar-Cohen. **Trans.Vis.Sci.Tech 2013.**

K.Loulier, R.Barry, P.Mahou, Y.LeFranc, W.Supatto, K.S.Matho, S.Ieng, S.Fouquet, E.Dupin, R.Benosman, A.Chédotal, E.Beaurepaire, X.Morin, J.Livet. **Neuron 2014.**



PARIS CENTRE

NEUROPHOTONIC LABORATORY / Univ PARIS DESCARTES

FBI support : Equipments: 143 538 €

Supervision & development: V. Emiliani (Wave front engineering microscopy group; DR CNRS)

<u>Recruited personnel</u>: R. Conti (combination of 1P holographic light patterning & electrophysiology, IR 04-13/12-13); V. Zampini (2P holographic light patterning in vivo & in vitro, IR 10-13/12-13) <u>Other personnel</u>: Eirini PAPAGIAKOUMOU (in vivo and in vitro 2P holography, CR INSERM), Christophe TOURIN (electrical circuits, AJT), Vincent DESARS (software developement 1P and 2P holography, IR); Marc GUILLON (1P and 2P holography, MCU); Emiliano Ronzitti (video rate Hilo Microscopy; 2P in vivo imaging and photoactivation, research fellow); Emmanuelle Chaigneau (2P in vivo imaging and electrophysiology, research fellow).

Objectives of the project:

Development of 1P and 2P optical microscopes for patterned photoactivation in vitro and in vivo.

Work undertaken:

- -Improvement of the performance of holographic light patterning for use with thick samples
- -Development of new algorithms for improved intensity homogeneity and zero order removal in computer generated holography
- -Combination of 2P light patterning with 2P imaging, for applications in vitro and in vivo
- Design and optimization of a commercial prototype for 1P and 2P holographic light patterning and imaging to be commercialized by the company 3i.

Important results:

-The propagation of wave front shaped beams deep in scattering tissue has been characterized; we demonstrated that the combination of lateral light shaping with the technique of temporal focussing permits the transmission of holographic beam at depth > 500mm with minimal distortion in the original shape and axial resolution (*Nature Photonics 2013*).

-A first prototype for 1P holographic photoactivation from 3i has been installed at Paris Descartes and implemented with electrophysiology and high speed HiLo microscopy.

-Two new optical systems combining 2P holographic photoactivation and imaging are under construction for application in vivo and in vitro.

-A holographic fiberscope for 1P patterned optogenetics has been demonstrated and used for optogenetics and calcium imaging in freely moving mice (paper under review).

- A new optical scheme for zero order removal in computer generated holography has been designed and tested: the design has been submitted for a patent; (paper is in preparation).

Opening to the community:

The *Wave front engineering microscopy group* has opened the system for 1P light patterning and electrophysiology to external collaboraiotn, precisely is conducting a collaborative projects with - Stephane Dieudonne (IBENS-ENS); (1P patterned optogenetics by fast scanning of low numerical aperture beams) and Gilles Fortin (Gif-sur Yvette) Holographic Dissection of Neural Circuits.

<u>Running scientific projects</u> (tile & collaborator name):

(1) Myelin gene expression in oligodendrcoytes by 1P photoactivation; V. Emiliani and C. Massaad (Univ. Parisdescartes)

(2) Dissecting the neurogenic hypothesis in neurovascular coupling with holography and optogenetics; V. Emiliani and B. Cauli (UPMC)

(3) In vivo mapping of functional synaptic circuits in mouse visual cortex by two- photon patterned optogenetic; V. Emiliani and L. Graham (Univ. Descartes)

(4) Elementary Properties of Individual Synaptic Contacts of the SNC Studied with 1P holographic photolysis; V. Emiliani and F. Trigo (Univ Parisdescartes)

(5) video rate HiLo microscopy for in vivo calcium imaging; V. Emiliani and C. Wyart (ICM)

<u>Relation with industry</u>: 3i - Intelligent Imaging Innovations, Inc.: 1P holographic photoactivation by means of wavefront shaping. Transfer agreement ongoing.

Additional grant obtained: Funding agency: FRC & Ville de Paris. Total amount: 340 000 €



ENS/IBENS

FBI support: Equipment: 250 560 €, Functioning: 13 965 €
Supervision & development: L. Bourdieu (DR CNRS), S. Dieudonné (DR INSERM); B. Mathieu (IE INSERM, dvt & user support)

<u>Recruited personnel</u>: Walther AKEMANN (IR, 02/05/2013 - 01/05/2016) <u>Other personnel</u>: Jean-François Léger (CR, CNRS, IBENS), Cathie Ventalon (CR, CNRS, IBENS)

Objectives of the project:

A major limit of two-photon fluorescence microscopy (TPFM) is its time resolution, due to the inertia of the traditional deflectors (galvanometers). Our laboratory has developed a TPFM based on a 2D scanner that uses acousto-optic deflectors (AOD) to obtain ms temporal resolution. This first system is installed at the IBENS imaging platform. The FBI project aims at developing a new AOD-based scanning system for addressing in 3D a predefined set of points at high frame rate.

Work undertaken:

To perform an ultrafast scan in 3D, various systems have been proposed in the recent years based on a lensing effect in an AOD driven by an acoustic wave with a frequency gradient. However, the decoupling of the scanning effects in the focal plane and along the optical axis of the microscope requires the non-optimal use of 4 AODs under conditions away from the Bragg incidence, which limits the performance of existing systems. We have designed a completely new and simple solution to this problem (patent pending). For this, we have acquired through the FBI program a regenerative amplifier and an optical parametric amplifier. A research engineer (Walther Akemann) was recruited. He is currently achieving the demonstration of the optical performance of the proposed solution (publication in preparation) and sets up a new microscope at the IBENS platform incorporating this innovation.

Important results:

Our main result is the first demonstration of the possibility to use an AOD as a one-dimensional wavefront shaper by using our new innovative method, which is based on a regenerative amplifier. As a consequence, the optimal 3D scanning of a set of points is possible at frame rates of several kHz, by applying simple spherical curvature to the wavefront (defocus aberration).

Opening to the community:

IBENS will open its TPFM with 3D scanning capability at kHz refresh rate to the users of the IBENS imaging plateform (IBENS researchers and external users) by the end of this work.

<u>Running scientific projects</u> (tile & collaborator name):

- Wavefront shaping with an AOD (W. Akemann)
- Construction of an ultrafast 3D two-photon microscope using AODs (W. Akemann)

<u>Relation with industry</u>: A&A opto-electronic provides us AOD with custom size, bandwidth and diffraction performances. We have also a tight collaboration with Imagine Optics to implement adaptive correction to improve depth penetration of scanning microscopes.

Communications and Training courses: See also WP5

- ENP spring school "Optical Imaging and Electrophysiological Methods in Neuroscience" at IBENS, www.biomedicale.univ-paris5.fr/physcerv/ENP.pdf

- Organization and participation to the MIFOBIO school: B. Matthieu, X. Darzacq. www.mifobio.fr
- Single Cell Physiology EMBO-FPGG-ICAM workshop, July 2012, www.singlecellphysiology.com.
 Optic Courses :
- Introduction à l'optique. L3 Biologie, ENS, (30h). L. Bourdieu.
 - Imagerie microscopique. M1, Biologie, ENS, (5h). S. Dieudonné.
- Imageries à balayage laser. M2 de Biophysique, Univ. Paris Diderot, (2h). L. Bourdieu.
- Two-photon microscopy introduction and new evolution. M2 BME/BIN, ESPCI ParisTech, (3h). L. Bourdieu and S. Dieudonné.
- Imagerie fonctionnelle des neurones corticaux in vivo, M2 Biologie, ENS, (2h). L. Bourdieu.
 Experimental Biophysics (20h). M2 Physique, ENS. L. Bourdieu and V. Croquette.
- Introduction to optical microscopy (12h) in the course "biophysics", M2 IPB (Physics Biology Interface), University Paris Diderot. JF. Léger.

- Introduction to optical microscopy (6h), M2 Neurosciences, ENS and UPMC. JF. Léger.



IJM/IMAGOSEINE

FBI support : 173 063 €

Supervision & development; Nicolas Tissot (IE); Aude Jobart-Malfait (IE-INSERM); Xavier Baudin (IE-CNRS)

<u>Recruited personnel</u>: Nicolas Tissot (04/2012-04/2015) <u>Other personnel</u>: Orestis Faklaris (IR-CNRS); Philippe Girard (MCU-P5-IJM)

Objectives of the project:

The project aims at having a multimodal multiphoton microscope in order to perform non-linear imaging as 2PEF, SHG, THG and photomanipulation (ablation, activation, conversion) in thick samples.

Work undertaken:

We are setting up in the context of FBI, a high sensitive multimodal nonlinear optical microscope. The goal is to combine 2PEF, SHG, THG and photomanipulation on the same system with a high temporal resolution. It is an upright microscope, with resonant/standard scanners for imaging with high sensitive detectors (5 GaAsP), and a third 2PE scan head to perform photomanipulation. It is being coupled with MaiTai DeepSee (one output beam 680-1040nm) and Insight DeepSee (2 output beams: 1040nm and 680-1300nm) lasers (SpectraPhysics).

In addition a Hamamatsu CMOS camera was setup on a Light Sheet Microscope to improve the acquisition speed.

Important results:

-We have developed a system and characterized all the parameters to photoablate microtubules keeping the drosophila oocyte alive.

-We have established the pre-requisite to optimize photomanipulation and the imagery simultaneously.

Opening to the community:

The High sensitive multimodal nonlinear microscope developed at the Institut Jacques Monod is part of Imagoseine imaging facility, and is open to a wide community of users.

<u>Running scientific projects</u> (tile & collaborator name):

-Membrane dynamics and vesicular traffic (T. Galli)

-Migration of transient populations of neurons (A. Pierani)

-Relationship between nucleus and microtubules arrays in drosophila oocyte (A.Guichet)

-Development of mouse embryo: how identical cells come to adopt different identities (J. Collignon)

-Mechanics of cell adhesion and mechanosensitivity at cell-cell junctions (B. Ladoux)

-Tissue mechanics: create blood vessel and force propagation in 3D active tissue (C. Wilhelm)

Relation with industry : Lavision Biotech, Spectra Physics

Additional grant obtained: Funding agency: ANR PF & FRM. Total amount: 120 000 € & 320 000 €



INSTITUT CURIE (PICT-IBISA/UMR144):

FBI support: Equipments: 120 000 € (CID-iManage, Image Data Base management system, also used for other FBI projects) + small material (optics and samples): 30 000€ Supervision & development: J. Salamero (DR1); P. Chavrier (DR1; F. Waharte (IR2)

<u>Recruited personnel</u>: Marie Irondelle (IE-Pic BIM UMR144/PICT, 31/12/2012 - 31/12/2014) <u>Other personnel</u>: Sylvie YU (IE-PICT/Nikon) (01/10/2013)

<u>Objectives of the project</u>: Propose a multi-scale NLO microscopy platform for intra-vital studies (live small rodents). Our aim is to offer it as a service on the open PICT-IBiSA core facility before the end of 2014 for a broad diversity of projects. Our scientific environment primarily addresses tumor models of cell migration intravasation and matrix degradation in xenografts (man/mouse or rat), coordination between extravasation and angiogenesis, in vivo cellular immunology topics in immune-deficient mice models and processes of the melanogenesis, in vivo.

Work undertaken:

This work started middle of 2013, once the overall budget was acquired:

- Installation of Microscope MP/IR multi-modal Nikon A1RMP +piezo Z, 4 detectors NDD PMT GaAsP- Auto-alignement.

- Development of large field of view Macroscopy based on AZC2 Confocal Macroscope (from organs to subcellular): proof of principle and optimization of a prototype AZC2-MP with 3 NDD PMT GaAsP, new Macro objectives (from Nikon) and coupling with Adaptive Optics (AZC2MP-AO).

- Both systems are interfaced with a unique Insight Deepsee pulsed Laser (near IR, usable for TPEF/SHG/THG) with dual output: 1 tunable from 680 to 1300 nm (~ 1700 mW peak) + 1 fixed at 1040 nm (500 mW)

- Tests with A1RMP on explants, spheroids, organoids for TPEF and SHG (on adipocytes in mammary glands).

Important results:

- Imaging MT1MMP-mCherry (endosomal) and collegen fibers by SHG in injected tumor cells in explants with A1RMP.

- Matrigel-embedded Tomato-GFP/pVillin-CreERT2 organoid; Long-term imaging in organoid (13 hours, 1 stack 55 frames every 10 min). Development of Registration-3D reconstruction, new algorithms.

- In depth imaging of DCIS-YFP injected tumor cells in mammary glands (with SHG)

- TPEF imaging with AZC2-MP macroscope (5X objective, variable zoom from 2x to 8x) in Mammary gland.

<u>Opening to the community</u>: 21 projects from teams in Paris-Centre Node, IGR, IUH St Louis, Institut Curie on small animals are planned for the next 2 years. Opening should start between summer and end of 2014 in spaces reorganized and dedicated for imaging, microsurgery and cell culture experimentation, linked to the animal house (quarantine).

Running scientific projects (tile & collaborator name):

-Remodeling of the extracellular matrix on human samples of tumors biopsies (Anne Vincent-Salomon (MD-PhD, Département d'Anatomopathologie for the Pic-BIM at Institut Curie)

-Crypt cell migration in intestine organoids (D. Vignjevic, UMR 144)

-3D imaging of labeled tumor cells in mammary gland and role of Metallo-Proteases (P. Chavrier) -Cell migration in primary tumors and angiogenesis (AM. Lennon, U 932 INSERM)

-Study of pre-clinical models of T-cell acute lymphoblastic leukemia (T-ALL) in immunodeficient mice. (J. Soulier, U944 INSERM)

<u>Relation with industry</u>: Nikon and Optique Peter: coupling DeepSee pulsed laser with AZC2 Macro confocal system; Imagine Optics: Coupling AO to Macro AZC2-MP; Strand Life Sciences: development of the CiD-iManage Image database (contractual partnership, IP sharing)

<u>Additional grant obtained</u>: "CaNOLI project" Canceropole IdF DIM-Cancer; FRM Grands Equipements; Inca: MP microscopy in cancer research; Inca: Image database CID: sharing and processing very large data sets. Total budget: 2.8 M€

Communications and Training courses: Planned: Workshop on AZC2MP-AO in MiFoBio 2014



WP 2 - High Throughput & High Content Screening

PARIS CENTRE

PICT-BIOPHENICS

FBI support: Equipment 0 €

Supervision & development Elaine DEL NERY

Recruited personnel: Dmitri Voitsekhovitch (CDD 12 months)

<u>Other personnel</u>: Aurianne Lescure (IR), Sarah Tessier (IE), Elodie Anthony (IE) and Elton Rexhepaj (Research fellow)

Objectives of the project:

The main activity of the BioPhenics Cell-Based High-Content Screening (HCS) facility is to support researchers in assay development and optimization for HCS and to perform high content screening with chemical or siRNA libraries. BioPhenics operates with a philosophy of collaboration, rather than fee-for-service. The facility, which has existed for over 6 years, has enabled screening projects for different research groups in Paris, most of them located in cancer centers, aiming to benefit from the platform capabilities for identifying both the genes and pathways that mediate disease states and novel compounds that modulate these pathways. Two persons were recruited in 2013: Elton Rexhepah, in charge of the bioinformatics developments as well as the development of new Matlab-based pipelines for the image analysis process and Dmitri Voitsekhovitch, an informatician who is in charge of the development of an image viewer application that we expect to be useful for the HCS community.

<u>Work undertaken</u>: The screening activity has been fully opened to the scientific community around and during the past two years the platform has contributed in many projects concerning human pathologies as well as basic science.

Cancers with specific sub-categories

Cell death, cancer, autophagy DNA-repair cancer-related Cancer, metastasis and invasion Cancer related signal transduction Homeostasis of the tumor niche

Primary cilium in physiology and pathology Kidney hereditary diseases Basic cellular functioning

Opening to the community:

BioPhenics launches the call 2013/2014 "High-Throughput Cell Biology for Cancer Research: from screening to applications" supported by the Paris Alliance of Cancer Research Institutes - PACRI. This call aims to support research groups throughout Ile-de-France working on cancer-related cell models for target validation or/and drug repositioning. Research groups will receive financial and technological support in their needs of image-based High Content and High Throughput Screening of chemical and siRNA libraries.

An important element for open access service is also the presentation of the provided service. For this, the Biophenics website is available at www.biophenics.net

<u>Running scientific projects</u> (tile & collaborator name):

DEVELOPMENT OF A SCREENING SYSTEM TO IDENTIFY MODULATORS OF HER-FAMILY PROTEIN EXPRESSION AT THE PLASMA. The RUSH concept. Franck Perez, DR2, CNRS UMR144, Institut Curie

SYSTEMS BIOLOGY OF EWING'S TUMOR: HIGH-THROUGHPUT PHENOTYPING AND MATHEMATICAL MODELING. Olivier DELATTRE, MD, PhD U830 Inserm "Génétique et Biologie des Cancers" Institut Curie Centre de Recherche, 26 rue d'Ulm, Paris

HIGH THROUGHPUT SCREENING OF THE JAK/STAT SIGNALING PATHWAY ACTIVATED BY TYPE AND TYPE II INTERFERONS. Christophe LAMAZE, UMR 144, Institut Curie

TOWARDS NEW THERAPEUTIC STRATEGIES FOR HEREDITARY STEROID-RESISTANT NEPHROTIC SYNDROME. Géraldine MOLLET, Inserm U983, Hôpital Necker-Enfants Malades, Université Paris Descartes, 75015 Paris



RANCE-BIOIMAGING

IDENTIFICATION OF VESICULAR TRAFFICKING PATHWAYS INVOLVED IN CILIOGENESIS. Alexandre BENMERAH - INSERM U1016, ²Institut Cochin, CNRS UMR 8104, Université Paris Descartes ; 27, rue du Faubourg St Jacques, 75014 Paris

IDENTIFICATION OF SYNTHETIC LETHAL INTERACTION WITH ACTIVATING B-CATENIN MUTATIONS IN HEPATOMAS CELL LINES. Christine PERRET, Institut Cochin, Département Endocrinologie Métabolisme et Cancer, 24, rue du Faubourg Saint-Jacques 75014

IDENTIFICATION AND CHARACTERIZATION OF PROTEINS INVOLVED IN THE FUNCTIONING OF THE ENDOCYTIC PATHWAY. Philippe BENAROCH, INSERM U932, Institut Curie, Paris

FOR THE CREATION OF A HIGH CONTENT SCREENING (HCS) PLATFORM FOR MEDIUM AND LARGE-SCALE FUNCTIONAL GENOMICS AT THE INSTITUT CURIE. Kristine SCHAUER and Bruno GOUD, UMR 144, Institut Curie, Paris

COMPOUND SCREEN IN CELLULAR MODELS DEPLETED FOR NEPHROCYSTIN PROTEINS. Sophie SAUNIER, Inserm U983 Hôpital Necker-Enfants Malades, Université Paris Descartes, 75015 Paris

SEARCH FOR NEW ASSOCIATIONS AND NEW THERAPEUTIC TARGET IN CELLULAR MODELS OF PROSTATE CANCER. Dr. Anne CHAUCHEREAU & Karim FIZAZI - Prostate cancer group, INSERM unit 981, Institut Gustave Roussy, Villejuif.

INSTITUT CURIE/CENTRE FOR COMPUTATIONAL BIOLOGY (CBIO)

FBI support: Equipment 0 €

Supervision & development: Thomas Walter

<u>Recruited personnel</u> : <u>Other personnel</u>: Alice Schoenauer Sebag

Objectives of the project:

Thomas Walter, a senior scientist at the Centre for Computational Biology (CBIO, Jean-Philippe Vert) which is part of the mixed research unit U900 "Bioinformatics and Computational Systems Biology of Cancer" (Institut Curie, INSERM, Mines ParisTech, Emmanuel Barillot), leads a small research team on BioImage Informatics which is mostly concerned with the development of new methods for High Content Screening data analysis, combining image analysis, machine learning and data mining. Thomas Walter has been working on several large-scale screens in the past and is a co-developer of the open-source software CellCognition (cellcognition.org).

Work undertaken:

In 2013, the team has been working on automatic methods for tracking, cell trajectory analysis and unsupervised clustering of cellular phenotypes in HCS data. In addition, the team has started to work on histopathological image analysis, segmentation and classification of bright field images of yeast cells, and Bayesian Network inference for HCS image data.

In 2014, the team intends to work with the Bertrand group and the Perez group on the analysis of subcellular localization patterns.

Opening to the community:

The software CellCognition is already an open source software project and has been applied at different institutes for the analysis of HCS data.

<u>Running scientific projects</u> (tile & collaborator name): Systems Microscopy (main collaborators: FIMM Helsinki, EMBL Heidelberg, IMBA Vienna)

Relation with industry: none

Additional grant obtained: Funding agency: FP7. Total amount: 200.000 Euros

Communications and Training courses: see WP5

Training in HCS data analysis at (1) the FBIAT 2013 course, (2) EMBO course on High Content Screening



INSTITUT PASTEUR/IMAGOPOLE/BIOIMAGE ANALYSIS UNIT

FBI support : 0€

Supervision & development Spencer Shorte & Nathalie Aulner; (Nathalie Aulner : user support)

Recruited personnel: Not applicable

Other personnel: A. Danckaert (IR, Institut Pasteur), A. Dufour (CR Institut Pasteur)

Objectives of the project:

The Imagopole has been developing several High Content Projects over the last 4 years. The key instrumentation for this part of our activity is an automated spinning confocal microscope equipped with a sample loader robotic arm located in a BSL2 + environment allowing notably the acquisition on live pathogen materials. Since last year, the addition of two automated pipetting stations in BSL2 and BSL2+ environment thanks to a FRM grant facilitates user access to high throughput microscopy notably on life samples.

Work undertaken:

Over the last two years, we have implemented several types of assays, from simple High Content Analysis to targeted siRNA screen to identification of pathogen genes and morphofunctional analysis and characterization of primary cells.

In addition to the work performed at the facility, it is worth noting that Alexandre Dufour is also actively participating in the Work group. Alexandre Dufour is a staff scientist at the group "Bioimage Analysis Unit" (headed by Jean-Christophe Olivo-Marin), leading a research team on cell deformation and motility. He is interested in BioImage Informatics in general and in new tools for High Content Screening data analysis in particular. He is also a co-developer of Icy and involved in the IPDM.

Opening to the community:

All instrumentation and services are fully operational and open to the FBI community. We also offer expert assistance and training in the different phases of the project from assay development to image and data analysis and management, with bridges to the WP4.

<u>Running scientific projects</u> (tile & collaborator name):

- Numeration of lipid droplets and autophagosomes in multiple cellular models (N. Dupont, INSERM, La Pitié Salpétrière)
- siRNA screen for host factors affecting salmonella entry and cytoplasm release (J. Fredlund, Institut Pasteur)
- Legionella mutants library screens (P. Escoll-Guerrero, Institut Pasteur)
- morphofunctional analysis and characterization of a primary skin fibroblasts biobank in the framework of the "Milieu Intérieur" (B. David-Watine, Institut Pasteur)
- Phenotypical screens of anti-leishmanial compounds (E. Prina, Institut Pasteur)
- Screens for anti microbian peptides (B. Sperandio, Institut Pasteur)

Additional grant obtained: Funding agency : FRM (2011-2013). Total amount: 392 280 €

Published FBI publications:

- Neutral lipid stores and lipase PNPLA5 contribute to autophagosome biogenesis. N. Dupont, S. Chauhan, J. Arko-Mensah, E. F. Castillo, A. Masedunskas, R. Weigert, P. Codogno, H. Robenek, T. Proikas-Cezanne and V. Deretic. **Current Biology** (in press).
- The 4 Notch receptors play distinct and antagonistic roles in the proliferation and hepatocytic differentiation of liver progenitors. Ortica S, Tarantino N, Aulner N, Israël A, Gupta-Rossi N. FASEB J. 2014 Feb;28(2):603-14.
- 3. Pharmacological assessment defines the Leishmania donovani casein kinase 1 as a drug target and reveals important functions in parasite viability and intracellular infection : Rachidi N, Taly JF, Durieu E, Leclercq O, Aulner N, Prina E, Pescher P, Notredame C, Meijer L, Späth GF. . Antimicrob Agents Chemother. 2013 Dec 23.

Communications and Training courses:

Trainings

01/03/12 & 21/02/13-UPMC : Master « *Outils Technologiques de la Recherche en Pharmacologie »* (resp. P. Michel) « Intérêt des imageurs pour le screening de molécules à potentiel thérapeutique» 20/02/13 & 11/02/14: The University of Dublin/Trinity College, Irland: Master High Content Screening "Concept in Microscopy" (resp. A. Davies)



Communications

1. High Content Analysis, San Francisco, Etats Unis, 07-11 Janvier 2013 – présentation affichée: « High Content Analysis for the study of infectious diseases at the Institut Pasteur»

2. EuCAI founding meeting, Dublin, Irlande, 18-19 Février 2013 – Présentation orale (Conjointe avec Spencer Shorte) – The Imagopole at the Institut Pasteur: role, mission, ...

3.Drug Discovery Automation : Track "High Content Screening & Cell Based Assays", Hambourg, Allemagne 6&7 Juin 2013 - présentation orale : "Imagopole - Leveraging the Power of Automation and HCA on Infectious Disease Models for Basic Science, Drug Screening and Personalized Medicine."

4. Swiss Image-based screening 2013, Lausanne, Suisse, 10&11 Juin 2013 – présentation affichée: « High Content Analysis for the study of infectious diseases at the Institut Pasteur»



ILE de FRANCE SUD

BIOEMERGENCE/IMAGIF

FBI support: 0 € Supervision & development (& user support): Nadine Peyriéras

Recruited personnel: None

Other personnel: Sylvia Bruneau (IE, INRA Amagen), Louise Duloquin (CDD Sympactis, prestataire pour BioEmergences), Nadine Peyriéras (DR CNRS N&D), Raphaël Rodriguez (CR CNRS, ICSN), Beatrice Satiat-Jeunemaitre (DR CNRS IMAGIF)

Objectives of the project:

Multistep phenotypic screening with non-mammalian and non-vertebrate model organisms The two IBiSA platforms of Ile de France sud launched in 2013 in collaboration with the ICSN (Gif sur Yvette), the development of an original drug screening methodology. The aim of the project is to develop and implement a workflow of 3D+time image data acquisition and computation analysis for high content phenotypic screening of developing embryos.

Work undertaken:

The proof of concept of the BioEmergences PharmaTox screening workflow is established in collaboration with the Institute of Chemistry Natural Substances http://www.icsn.cnrs-gif.fr/. The ICSN, known for the development of the Taxol as an anticancer drug, is in great need of the complete renewal of its drug screening strategies based so far on the assessment of cell survival in cell cultures. Phenotypic drug screening on developing embryos in the context of the 3Rs principles is expected to readily lead to the identification of potential hits in signaling pathways conserved throughout evolution including in human. We standardize, systematize and robotize a screening chain from the observation of macroscopic phenotypes (step 1), the potential perturbation of chosen gene expression patterns in transgenic lines (step2) to the observation of cell behaviors by 2-photon microscopy and automated processing of 3D+time image data and the quantification of cell displacements, shape and fate changes (step3). The project will deliver its protocols and a database gathering raw and processed data for further evaluation, comparison and modeling, leading to a new paradigm in fundamental and applied biological investigations.

Important results:

We validated a protocol for our phenotypic screening (step 1) with a series of drugs commercially available and supposed to interfere with the Shh pathway. We confirm that the observation of phenotypic defects in 24 hpf zebrafish embryos is a powerful tool to identify in one step the useful concentration of the drug and the phenocopy of known mutant phenotypes.

Opening to the community: Cannot happen before we realize and publish our pilot screen.

<u>Running scientific projects</u> (tile & collaborator name):

Project ID: BioEM1: Multistep phenotypic screening with non-mammalian and non-vertebrate model organisms

<u>Relation with industry</u>: The pilot screen will be used to advertise the strategy. Our partner Sympactis is interested in our strategies and is ready to propose our platform to industrial partners.

Additional grant obtained: none

Published FBI publications: None on the subject

<u>Communications</u>

Congrès de la société française de toxico pharmacologie cellulaire, Chatenay Malabry Juin 2013. Connaissances et modélisation intégrée en toxicologue et ecotoxicology", Univ. Paris Descartes, Avril 2013



MONTPELLIER

MRI

FBI support : Equipments: 454 704 € (liquid handling robot; Cellomics upgrade; Spinning disk) Functioning: 105 857 €

Supervision & development; Virginie Georget and Edouard Bertrand

<u>Recruited personnel</u>: Julien Bellis (IR, 02/2013-02/2015); Cédric Hassen Khodja <u>Other personnel</u>: HASSEN-KHODJA (Bioinformatics, CDD IE, MRI -CRBM); V. GEORGET (IR CNRS, MRI-CRBM); LIONNETON (IR CNRS, MGC)

Objectives of the project:

The screening facility at the CRBM, Montpellier, part of the MRI facility, was created in 2005 but expanded considerably in 2013 with the acquisition of a robot for automatic pipetting and specific families of siRNA libraries. This will complement the Cellomics automated microscope already present at the MRI facility to perform automatic acquisitions. The facility will therefore offer screening for cellular phenotypes following down-regulation of genes expression by siRNA. Two persons were recruited in 2013: Julien Bellis, in charge of the wet lab part of projects as well as image analysis and Cédric Hassen Khodja, a bio-informatician who is responsible for performing the normalisation steps, quality metrics evaluation and hit identification on the performed screens.

Work undertaken:

Three approaches are currently under development: (i) a service of "intelligent acquisition" where the analysis of a low resolution image can trigger several acquisitions at higher resolution, for instance to screen for protein interactions using FCCS or FLIM techniques. The intelligent acquisition approach is being tested using the Matrix Screener module on a CLSM SP5 and using an Andor spinning-disk acquired at the end of 2013 and co-financed by FBI. (ii) a protein interactions screening project, which will use the matrix screener together with a CAM ImageJ module and the SMD FCS/FLIM modules from Leica. This will be done in close collaboration with the MARS facility. (iii) a service performing high-throughput smFISH (single molecule FISH), which is the method of choice to analyze gene expression at the level of single cells, single molecule, and with spatial resolution of mRNA localization. To this end, a protocol for smFISH was recently adapted to be performed at high throughput on the robot. This is part of an ambitious project aiming at providing high-throughput smFISH as a readout for screening purpose, enabling exact cell-by-cell quantification of gene expression and analysis of intra-cellular mRNA localization. This project is based on the expertise of research groups working with MRI and it will open new perspectives for high-throughput microscopy.

Important results:

-Validation of a robotized protocol for smFISH.

-Development of low-cost smFISH probes

-Successful pilot smFISH screen with 200 genes; identificatin of new localized mRNAs.

Opening to the community:

The screening service will be fully opened to the community around the first trimester of 2014. Four in vitro screens have been performed so far and two cellular screens are being developed.

<u>Running scientific projects</u> (tile & collaborator name):

(14) screening of chemicals on CDK-cyclin activity reported by fluorescent sensor (in vitro assay)-May Morris Montpellier

(15) screening of siRNA family of genes on cell cycle assay-Bénédicte Delaval CRBM Montpellier

(16) screening of genes by smFISH - Edouard Bertrand IGMM Montpellier

(17) screening of mutants on cell infection by Coxellia- CPBS Montpellier

Additional grant obtained: Funding agency FRM._Total amount: 300 k€



WP 3 – Probe Development, Optomanipulation & Optogenetics

PARIS CENTRE

ENS Chemistry: Optical actuators and reading out systems for living systems

FBI support : 135 000€ ; Functioning: 48 750 € (from 2012 up to 2017)

Supervision & development: Isabelle Aujard, Thomas Le Saux, Emmanuelle Marie-Begue (user support)

Recruited personnel: Not applicable

Other personnel: Isabelle Aujard (IR ENS), Arnaud Gautier (MC ENS), Zoher Gueroui (CR CNRS), Ludovic Jullien (PR UPMC), Thomas Le Saux (MC UPMC), Emmanuelle Marie-Begue (CR CNRS), Sylvie Maurin (AJT ENS), Christophe Tribet (DR CNRS).

<u>Objectives of the project</u>: To make available to the biological community established and state of the art chemical technologies for the optical control and reading out of living systems. The ultimate goal is to provide access and training to these emerging techniques and methods for the realization of competitive biological projects

Work undertaken:

The 135 000€ budget allocated to the Work Package 3 has been made available in 2012. After comparative evaluations of instruments from several providers, this budget has been used by the end of 2012 and in 2013 to establish an analytical and semi-preparative platform aiming at characterizing optical probes and photochemical actuators. More specifically, the Pole of Biophysical Chemistry of the ENS Chemistry Department gives now access to an HPLC setup equipped with various columns (inverse polar and exclusion) and UV detection, and to a capillary electrophoresis equipped with Laser-induced Fluorescence detection (at three wavelengths). Added to already available UV-Vis spectrometers and a fluorimeter as well as various types of microscopes, the Pole of Biophysical Chemistry now provides a state-of-art instrumental platform to fully characterize optical probes and photochemical actuators.

In addition, several projects linked to this platform are in progress: (i) Development of various technologies aiming at photo-controlling the activation or the degradation of proteins; (ii) Development of technologies to photorelease proteins from microcapsules; (iii) Development of fluorescence turn-on strategies for labelling proteins and RNA.

Important results:

Three important papers will be soon submitted respectively dealing with (i) photodegradation of proteins; (ii) highly selective imaging of photoswitchable fluorescent proteins; (iii) a new fluorescence turn-on strategy to label proteins.

Opening to the community:

The characterization platform is already open to the community (since 09/2013) by appointment with the "local contact" supervisors.

<u>Running scientific projects</u> (title & collaborator name):

(1) Photocontrol of protein degradation by means of caged compounds. A. Gautier et al (ENS Chimie), Paris.

(2) Highly selective imaging of photoswitchable fluorescent proteins. A. Gautier, L. Jullien et T. Le Saux (ENS Chimie), Paris; collaboration with A. Espagne (ENS Chimie) and V. Croquette (ENS Physics).

(3) Characterization of photoactivatable self-immolative spacers for biological applications. F. Schmidt (Curie Institute, Paris) and L. Jullien (ENS Chimie), Paris.

<u>Relation with industry</u>: Not yet applicable (but contacts with an important cosmetic company)

<u>Additional grant obtained</u>: Funding agency: ANR, CNano Ile-de-France, FEBS Grant, FRM, PSL grant. Total amount: $> 500\ 000 \in$ over the last two years.

Published FBI publications: At least four to be published in 2014



LABORATOIRE PHYSICO-CHIMIE CURIE – PCC Group: Optogenetic control of intracellular

signaling

FBI support: not funded

Supervision & development: Mathieu Coppey (CR CNRS)

Recruited personnel: not applicable

<u>Other personnel</u>: Maxime DAHAN (DR CNRS), Marouen Bouazzi (DMD software development, Postdoc, CNRS-PCC), Amanda Remorino (Application to cell polarity, research fellow, EMBO-PCC), Simon De Beco (Application to cell division, research fellow, CNRS-PCC), Léo Valon (Application to cell migration, PhD candidate, PCC).

<u>Objectives of the project</u>: Our project is to set up optogenetics toolboxes to activate intracellular signaling with a spatial and temporal control (μ m, seconds). We are implementing genetically encoded molecular actuators based on light-triggered dimerization as well as structured illumination techniques optimized for optogenetics.

Work undertaken:

We developed genetically encoded proteins to control RhoGTPases signaling (RhoA, Rac1, Cdc42) with a subcellular resolution (up to 4.5μ m) and a temporal resolution in the range of tens of seconds.

We used fluorescent imaging and data processing to measure quantitatively spatiotemporal inputoutput relationships in signal transduction.

We developed an optomechanical device based on micro mirrors chips to achieve a parallelized structured illumination on standard microscopes.

We started an optogenetic project on the control of oriented cell division for which we want to orient the mitotic spindle by recruiting locally cortical determinant of cell division orientation.

Important results:

We were able to induce several modes of cell migration in non-migratory mammalian cells by maintaining a local activation of RhoGTPase signaling.

We produced an optogenetic activable RhoA actuator.

We measured various effector as well as actin and myosin spatiotemporal time courses following an optogenetic perturbation (a manuscript will be submitted soon).

<u>Running scientific projects</u> (tile & collaborator name):

Light Induced Cell Organization and Polarity, Mathieu Coppey, Maxime Dahan.

Investigating oriented cell divisions from a molecular to a systems-level using optogenetics and micropatterning, Xavier Morin (CR CNRS, IBENS), Mathieu Coppey, Maxime Dahan

Additional grant obtained: Funding agency ANR. Total amount 450k€



BORDEAUX

Interdisciplinary Institute for Neuroscience

FBI support: not funded

Supervision & development: Matthieu Sainlos (CR CNRS)

Recruited personnel: not applicable

Other personnel: Isabel Gauthereau (AI, IINS), Dolors Grillo-Bosh (Research fellow, IINS)

Objectives of the project:

Our projects involve on the one hand developing new biomolecules to modulate with an optical control protein-protein interactions and on the other generating new probes for imaging purposes.

Work undertaken:

We have developed phototriggered competing ligands against the PDZ domain-mediated interactions involved in synaptic retention of glutamate receptors. Photocontroled modulation of interactions was achieved by designing peptide or protein-based tools that incorporate photolabile or photoswitchable groups Photocontrol has been validated in vitro and is now being implemented to live cell. In parallel we have developed new protein-based probes that are characterized by a minimal size, monovalency and precise control over the fluorescent dye labeling.

Important results:

We were able to design and validate in vitro a strategy to cage ligands of specific PDZ domainmediated interactions.

We have developed and validated monovalent streptavidin-based probes to monitor biotinylated membrane proteins in confined cellular environments.

<u>Running scientific projects</u> (tile & collaborator name):

(1) Photocontrol of PDZ domain-mediated interactions, Matthieu Sainlos (IINS)

(2) Monomeric streptavidin as a probes to monitor membrane proteines in confined cellular environments, Matthieu Sainlos (IINS), Olivier Thoumine (IINS)

Additional grant obtained: Funding agency: ANR, region. Total amount: 580 k€

Published FBI publications: One publication to come on monomeric streptavidin



LP2N - Institut d'Optique Graduate School

FBI support: salary for Pierre Vermeulen (Research Engineer)

Supervision & development: Brahim Lounis and Laurent Cognet

<u>Recruited personnel</u>: Pierre Vermeulen – 2 years (FBI) <u>Other personnel</u>: Cécile Leduc (CR CNRS, LP2N), Zhenghong Gao (research fellow, LP2N), Satyabrata Si (research fellow, LP2N), Edakkattuparambil Shibu (research fellow, LP2N)

Objectives of the project:

Our projects concern the development new probes for single molecule tracking in complex environments: non-bleaching probes based on functionalized gold nanoparticles and near-infrared nanoprobes for deep tissue single molecule tracking.

Work undertaken:

We have developed non-bleaching functional gold spherical nanoparticles (5nm) probes for single particle tracking on live cells by photothermal microscopy

We are developing near-infrared single molecule probes based on gold nanorods

We are developing near-infrared single molecule probes based on short carbon nanotube

We are developing fully organic near-infrared luminescent single molecule probes

Important results: We have conducted membrane and intracellular single molecule tracking by gold labeling using our newly developed functional gold spherical nanoparticles (5nm) probes based on nanobodies.We have developed fully organic near-infrared luminescent nanoparticles that are biocompatible and detectable at the single particle level in live cells (in press in Adv. Mat.).

Opening to the community: Protocols to produce new probes are available on request. Newly developed probes can be available on a collaboration basis.

Running scientific projects (tile & collaborator name):

(1) NanoSynDyn - new gold nanoprobes, Brahim Lounis (LP2N) & Daniel Choquet (IINS)

(2) Ultrashort carbon nanotubes, Laurent Cognet (LP2N), Laurent Groc (IINS) Brahim Lounis (LP2N)

(3) NanoTubesInBrain, Laurent Cognet (LP2N), Laurent Groc (IINS) Mireille Blanchard-Desce (external collaborator (ISM)

Additional grant obtained: ERC NanoSynDyn, ANR UshortNT, Interlabex NanoTubesInBrain – Univ. Bordeaux

Published FBI publications:

Emilie Genin, Zhenghong Gao, Juan A. Varela, Jonathan Daniel, Talia Bsaibess, Isabelle Gosse, Laurent Groc, Laurent Cognet and Mireille Blanchard-Desce. "Hyper-bright" Near-Infrared Emitting Fluorescent Organic Nanoparticles for Single Particle Tracking. Advanced Materials. 2013



WP 4 - BioImage Informatics - IPDM

PASTEUR/CURIE/INRIA-RENNES/BIOEMERGENCES/MRI-MONTPELLIER

FBI support: Equipments: 45 536 € (Inria) + 182 866 € (BioEmergences) +100 000 (Institut Curie-Paris Centre)

Supervision & development Jean-Christophe Olivo-Marin & Charles Kervrann

<u>Recruited personnel</u>: Several IPDM-FBI engineers have been recruited in 2012-2014 to design and build the Information Technology (IT) infrastructure:

Icy software (Institut Pasteur): Stéphane Dallongeville, software engineer, 36 months CDD-OD since 01/10/2012: development of core functionalities and functions. Thomas Provoost, software engineer, 12 months CDD (01/11/2012-31/10/2013): development of core functions and plugins.

Mobyle@SERPICO and grid-computing infrastructure (Inria Rennes): Tina Rakotoarivelo (since 01/12/2011) and Thierry Pécot (since 15/09/2013) participate to the dissemination, distribution, implementation on a grid computing architecture (200 core server / 200 TeraByte storage server) and interaction with the Curie Image database CID_iManage (Institut Curie data center) of user-friendly image processing algorithms

BioEmergences (IDFsud): Mathieu Hermann (2012-2013) developing PROMAT for the automated mathematical formalization of models, Julien Delile (2013), developing the Mecagen platform for the mecanogenetic modeling of morphogenesis, Barbara Rizzi (2012) development of filtering, cell nucleus detection, PDEs based membrane segmentation algorithms.

WIDE software (MRI-Montpellier): Alexandre Granier, software engineer, 38 months CDD since 01/07/2010 : development of web functionalities. Philippe Estival, software engineer, 3 months: audit and advice in technology.

<u>Other personnel</u> : Lecorgne Tristan (IT Engineer, Inria-Rennes, from October 2011 to October 2013): Mobyle@SERPICO and grid-computing infrastructure.

<u>Objectives of the project</u>: The major goals are to develop 1/ an integrated and cutting-edge image processing software platform to harbor next generation algorithms for image processing; 2/ an integrated workflow for launching algorithmic pipelines on the computation grid or local computer clusters for high throughput processing of image data sets; 3/ a data management solution to accommodate the storage, query and annotation of large amounts of image data and metadata.

Work undertaken:

1/ A sub group focusing on image analysis and modeling was created in 2012:

- Development of the software Icy (http://icy.bioimageanalysis.org), a fully integrated framework designed from the ground-up using modern concepts in programming and ergonomics.

- Web-based demonstrator without any installation and configuration of image analysis software at Inria-Serpico : Mobyle@SERPICO :http://mobyle-serpico.rennes.inria.fr/.

- BioEmergences developped the Mov-IT workflow concept for 3D+time imaging of developing model organisms and the OpenMOLE worklow engine www.openmole.org/ for biological applications.

2/ A sub group focusing on image databases and management was created in 2012:

- Development of the Montpellier academic open source WIDE project and the Institut Curie CID iManage in collaboration with the Strand Avadis Company.

- Study of programming interface for image database access and image queries

- Guildeline document for software evaluation to help facilities.

- A first version of a set of plugins to access the Curie image database from Icy have been developed and published on the central repository of Icy.

Important results:

- Development of two image database management solutions: WIDE open-source) and CID iManage (commercial solution open to the community@Institut Curie)

- Development of 3 image processing user interfaces: Icy, Mobyle@Serpico (web service), OpenMole

- Development of complementary storage and computing architectures: cloud and cluster grid.



FRANCE-BIOIMAGING

<u>Opening to the community</u>: All the software resulting from the activities of the WG4 are made available to the community at large either as open-source programs or as executable codes. In all cases, end users have full usability of the programs and can report back to the developers to ask for improvements, bug correction and evolution.

Running scientific projects:

Inria: Membrane traffic analysis (J. Salamero, Institut Curie UMR 144 CNRS), diffusion and directed motion analysis (J. Pecreaux, IGDR Rennes), Biofilms and bacteria dynamics analysis (A. Trubuil, INRA Jouy-en-Josas), ...

Institut Pasteur: actin dynamics (N. Guillen, IP), clathrin-independent endocytosis (N. Sauvonnet, IP), TNT dynamics (C. Zurzolo, IP)

Relation with industry:

Inria: 2 PhD grants in image processing are supported by the Quaero Program (www.quaoro.org) and Innopsys Company (www.innopsys.org)

Additional grant obtained:

Funding agencies: Région IDF interDIM (BioEmergences), Rennes Métropole (Inria). Total amount: 150 000 € (BioEmergences) + 40 000 € (Inria)

Published FBI publications:

Fabrice de Chaumont, Stéphane Dallongeville, Nicolas Chenouard, Nicolas Hervé, Sorin Pop, Thomas Provoost, Vannary Meas-Yedid, Praveen Pankajakshan, Timothée Lecomte, Yoann Le Montagner, Thibault Lagache, Alexandre Dufour & Jean-Christophe Olivo-Marin. Icy: an open bioimage informatics platform for extended reproducible research. **Nature Methods**. 2012.

Thibault Lagache, Gabriel Lang, Nathalie Sauvonnet, Jean-Christophe Olivo-Marin. Analysis of the Spatial Organization of Molecules with Robust Statistics. **Plos One**. 2013.

Nicolas Chenouard, Ihor Smal, Fabrice de Chaumont, Martin Maška, Ivo F Sbalzarini, Yuanhao Gong, Janick Cardinale, Craig Carthel, Stefano Coraluppi, Mark Winter, Andrew R Cohen, William J Godinez, Karl Roh, Yannis Kalaidzidis, Liang Liang, James Duncan, Hongying Shen, Yingke Xu, Klas E G Magnusson, Joakim Jaldén, Helen M Blau, Perrine Paul-Gilloteaux, Philippe Roudot, Charles Kervrann, François Waharte, Jean-Yves Tinevez, Spencer L Shorte, Joost Willemse, Katherine Celler, Gilles P van Wezel, Han-Wei Dan, Yuh-Show Tsai, Carlos Ortiz de Solórzano, Jean-Christophe Olivo-Marin & Erik Meijering. Objective comparison of particle tracking methods. **Nature Methods**. 2014.

Thierry Pécot, Jerome Boulanger, Charles Kervrann, Patrick Bouthemy, Jean Salamero. Estimation of the flow of particles within a partition of the image domain in fluorescence video-microscopy. **ISBI**. 2014

Communications and Training courses: see WP5

Co-organisation of European symposium on Bioimage analysis (EuBias) in Barcelona: Icy was invited among other open source software, as well as OpenMole-BioEmergences. Strand Avadis (partner of Institut Curie for the image database) sponsored the event and gave a talk on open access to the iManage solution (7-11 Oct 2013). WIDE was presented during a poster session.

Co-organisation with GDR 2588 Microscopie fonctionnelle du vivant of the one day meeting "Bioimage Informatics" at Institut Curie (8-9 July 2013)

Icy and Curie Image database CID_iManage were presented at Elmi 2013, during a meeting of the WP11 Data Storage and Analysis by the Eurobioimaging work package leaders as a solution available for the whole European network.

WIDE was presented at the User & Developer ImageJ Conference (24-26 october 2012) and during a meeting of "Microscopie Photonique de Fluorescence Multidimensionnelle" (RTMFM) conference in Villefranche-sur-Mer (14-15 November 2013).

Training organization with CNRS (FBI Advanced Training: "BioImage Informatics" & "Reconstruction of multilevel dynamics"): 1 week training to 8 CNRS staff (PhD students, Post-docs, engineers, scientists) on analysis methods for the dynamics in biology, in collaboration


between Institut Curie and Inria Rennes (also 20 hour training course of Master 2 IRIV University of Strasbourg). Participants were introduced to Icy and Mobyle@SERPICO.

Training with Inserm: 3 full days training on Icy and ImageJ co-organised by L. Danglois (Institut Jacques Monod) and A. Dufour (Institut Pasteur) (9-11 Dec 2013).

Training with CNRS: 3 full days training on image processing methods for dynamics analysis in cell biology organized by P. Paul-Gilloteaux and F. Waharte (Institut Curie) in collaboration with Inria-Rennes (P. Bouthemy) (4-6 Dec 2013, Institut Jacques Monod).

Icy presentation and training at the Conference Imaging the Cell, Strasbourg, 28-30 August 2013.

Kick-off meeting of Curie Image Data Centre: this meeting was the occasion to announce that Curie was now able to propose image data base, storage and computational power to all members of FBI using the microscopy image facility either for acquisition or processing. Organic link between Icy and CID-iManage were evoked during the meeting (Use of the API) (13 Nov 2013).



WP 5 - Training, Dissemination & Technological transfer

Work undertaken :

-Support for existing dissemination and training activities, in connection with research organizations (CNRS, INSERM training, Institut Curie, institut Pasteur, universities etc.) most often by the two previously described national networks (RTmfm and GDR MIV). "Do not reinvent the wheel!", but support and select specific training actions accordingly to criteria defined by FBI: in 2013: "Assises des Plateformes", "CLEM days" at Institut Pasteur... -MiFoBio 2012 summer school, (with CNRS, Inserm, Aviesan, RTmfm and GDR MIV-2588)

-MiFoBio 2012 summer school, (with CNRS, Inserm, Aviesan, RTmfm and GDR MIV-2588) Gathered 330 academic (for 420 demands) and 80 industrial attendees, among them, 15-20% are associated to the "France BioImaging" infrastructure. FBI members are co-oroganizers or members of the Scientific Committee. This School aims to develop the grip of advanced bio-photonic approaches by a combination of basic/advanced courses and hands-on workshops exploiting a set of acquisition systems and analysis tools of biological images. MiFoBio is currently winning a European notoriety (www.mifobio.fr)

- France-BioImaging-Advanced Trainning (FBI-AT, 2013; Gif sur Yvette, IdF-Sud Node with the participation of IPDM-, Bordeaux- and Paris-Centre Nodes). FBI-AT aim is to foster researchers' ability to tackle focused biological studies with up to date imaging approaches. For a full week, small groups of teachers (4-6) and students (less than 8), concentrated on particular biological or biomedical issues (6 to 8 distinct modules).

- ELMI (European Light Microscopy Initiaitive) meeting, 2013.Co-organized by PICT-IBiSA Institut Curie, members of Paris-Centre and BIC IINS, members of Bordeaux-Node. ELMI is both a conference on biological imaging and innovative biophotonic technologies and a workshop (30 hands on sessions; 370 participants)

-Involvement in the "FBI Consortium Agreement" writing, for "IP and exploitation, publications/ confidentiality/ communications".

Important activities :

-listing of all training (internal and external, for users or "trainers"), education (M1,M2, PhD program...) and dissemination activities in all FBI-Nodes (see **Appendix 6 and 7**)

Relation with industry:

-Start a list of temporary disposal of premises in FBI imaging facilities and labs, for industrial partnership and tech transfer (see achievements/site). Participation to the "PIPAME Medical Imaging" report.

ACHIEVEMENTS PER NODES/SITES (2012-2013)

Education, Training, Dissemination and Technology transfer activities at the Node/site level during the starting phase (2012-2013) of "France-BioImaging", were not all supported financially by FBI funding, especially for Education and some Training activities that are described below by each nodes/sites. However, most of these activities are related to the FBI perimeter. They define the actual activities of FBI members in the frame of WP5.



PARIS-CENTRE

ENS/IBENS

FBI support : functioning and mission Supervision : Laurent Bourdieu

Education:

Master 1, 2 & PhD program (ED), participation (courses, lectures and practical sessions): «Introduction to optics », Licence 3 Biologie, ENS ; « Imagerie microscopique » Master 1, Biologie, ENS ; « Imageries à balayage laser » Master 2 Biophysique, U. Paris Diderot ; « Two-photon microscopy : introduction and new evolution » Master 2 BME/BIN, ESPCI ParisTech ; « Imagerie fonctionnelle des neurones corticaux in vivo » Master 2 Biologie, ENS ; « Experimental Biophysics » Master 2 Physique, ENS ; « Introduction to optical microscopy », Master 2 IPB (Physics – Biology Interface), University Paris Diderot ; « Introduction to optical microscopy » Master 2 Neurosciences, ENS and UPMC Paris 6.

Dissemination (in 2012-2013):

IBENS ENP spring school Optical Imaging and Electrophysiological Methods in Neuroscience (IBENS, Paris) (summer school every year); IBENS Organization, participation to the MIFOBIO school (every 2 years).

ENS Chemistry

FBI support : functioning and mission Supervision : Ludovic Julien

Education:

Master 1, 2 & PhD program (ED), participation (courses, lectures and practical sessions) : Chemistry Master M2 ; Chimie "Physico-Chimie du Vivant", ENS-UPMC Paris 6

Institutional training:

Atelier Inserm 217 on Photocontrol and optogenetic of biological systems and functions (INSERM, Bordeaux & Paris)

Dissemination (in 2012-2013):

EMBO-ICAM-FPGG summer school on Single Cell Physiology; Organization, lectures and practical courses (Paris)

Paris Descartes-LNNM

FBI support : functioning and mission Supervision : Valentina Emiliani

Education:

Master 1, 2 & PhD program (ED), participation (courses, lectures and practical sessions) : LNNM/ IJM Licence "Sciences Biomedicales" (Univ Paris Descartes); LNNM Master "Physique Médicale et du Vivant"(Univ Paris Descartes); LNNM Master: "Advanced optical methods for neuroscience" (Monabiphot Master course, Univ Paris Descartes); LNNM Master "Bio engineering and Innovation in Neuroscience" (BIN), (ESPCI, Paris); Paris Descartes, PACES - First year medical studies (Univ Paris Descartes) ; Paris Descartes Medecine-Sciences MD PhD excellence programme (Univ. Paris Descartes)

<u>Dissemination (in 2012-2013)</u>: LNNM ENP spring school 'Optical Imaging and electrophysiological methods in Neuroscience (summer school every year); LNNM Imaging structure and function of the nervous system', Cold Spring Harbor, New York, USA (summer school, every year); LNNM EMBO practical course: Two photon imaging of brain circuit function Zurich, Switzerland (Aug. 2013); LNNM FENS-IBRO school "Imaging Neural Function" in Lausanne/Geneva Switzerland (Aug. 2012).

<u>Technology transfer & Relation with industry:</u> Transfer Agreement signed with Intelligent imaging innovation (3i) for the development of a commercial 1P and 2P holographic microscope.

Temporary disposal of premises and IT facility for industrial partners in Paris Descartes LNNM: -20 m2 optical rooms with air conditioning, optical bench and computers for prototype development of the 1P and 2P holographic photostimulation microscope (3i).



INSTITUT PASTEUR

FBI support : functioning and mission Supervision : Spencer L. Shorte

Education:

Masters :participation (courses, lectures and practical sessions) : Master Biologie Cellulaire Physiologie et Pathologie (BCPP) (Univ Paris Diderot-UPMC) ; Master de Sciences et Technologies, Mention Biologie Moléculaire et Cellulaire, Spécialité Microbiologie, Parcours Parasitologie-Mycologie (UPMC) ; Pharmacologie Clinique et Expérimentale, UE "Outils technologiques de la recherche en pharmacologie" (UPMC) ; Master des Sciences et Technologies, Introduction to high Content Screening (UPMC) ; Master "High Content Screening", Trinity College, in Dubin Ireland ; Imagerie Biomédicale (Paris Descartes, UPMC), imagerie biologique (École Nationale Supérieure de Techniques Avancées (ENSTA) ; Dresden Biotech PhD program (Dresden International PhD Program-DIPP)

Institutional Continuous Training:

-Training on Icy and Fiji with IJM-Paris centre (INSERM),

-Training in ultrastructural microscopy (Institut Pasteur, Imagopole/PFMU)

-Training in optical imaging (Institut Pasteur, Imagopole/PFID)

-Training in image analysis & biostatistics (Institut Pasteur, Imagopole/PFID)

-Imaging Infection, Advances in Live Cell and Tissue Imaging and High Content Microscopy (VRTC Summer School on Advanced Microscopy 2013, Gent, Belgium)

On site Internal Continuous Training

Training on software and on systems (IMAGOPOLE/ Plateforme d'Imagerie Dynamique PFID) per year: 570 h for 168 persons, Training on systems (IMAGOPOLE/ Plateforme de Microscopie Ultrastructurale, PFMU) per year 200h for 15 persons. Icy: a community software platform (Institut Pasteur-IPDM); 110 h (for 184 persons on site + 80 external people).

<u>Dissemination (in 2012-2013)</u>: ELMI 2012, Leuven, Belgium; 1st and 2nd CLEM days, with GDR MIV 2588/FBI (Institut Curie, Institut Pasteur); EMC 2012 (Manchester, UK)

Technology transfer & Relation with industry:

BioAxial (Paris, France): collaboration on the development of a novel super-resolution technique with low phototoxic impact.

Building collaborative partnership with Carl Zeiss Jena around correlative light-microscopy technologies (SEM Auriga, Elyra SR).

Andor (UK), Spectral Applied Research (Canada), Lambert Instruments (Holland): Fluorescent Lifetime Imaging resolved in 3D.

MEMI-OP: A Carnot-Fraunhofer project for the development of optogenetics applications based on specific micro-mirror devices. With: Fraunhofer Institute Dresden (IPMS, Germany), InVision (Austria).

<u>Temporary disposal of premises and IT facility for industrial partners:</u> BioAxial: Initial validation of a novel super-resolution technique against existing ones. Sanofi: Cellular analysis. Clarins: histo-patho analysis of wide-area tissue arrays.



INSTITUT CURIE

FBI support: functioning and mission. Supervision : all PICT-IBiSA members and associated Teams concerned/Inst Carnot "Curie-Cancer" for Private-Public partnership

Education :

Masters :participation (courses, lectures and practical sessions), Microscopie pour la biologie(Lille II), "Bio. Cell. Physiologie et Pathologies (BCPP)"(UPMC-Paris Diderot), Signalisation Cellulaire, Neurosciences (Paris XI), GPBIDM (Univ Poitiers), R&D Biotech (UPMC), « En Biofísica » (Univ Madrid), Bioinfo et Biostatistiques (Paris XI), Biol. Tech. (Ecole Centrale-Paris). ED (PhD courses) : B3MI Technique d'études des intéractions protéine-protéine. (Paris Diderot)

Institutional Continuous Training :

Introduction to image processing under ImageJ (INSERM, Univ Paris Diderot, CNRS); Tasks automation under ImageJ: 3 days training (Bordeaux, Gif-sur-Yvette); Java programming for ImageJ: 3 days training (Gif-sur-Yvette); Metamorph (PICT with BIC, INSERM); Workshop on Image processing (Bordeaux, Gif-sur-Yvette); Formation Microscopie confocale (Institut Curie,CNRS, INSERM, IJM); Traitement du signal: filtrage spatial et fréquentiel (CNRS-Université Paris Diderot); Atelier de tomographie électronique (SFm) ; Initiation to the electronic microscopy and associated workshops (Institut Curie); Journées Imagerie et Analyse 3D (IMO,UPMC); «Microscopie electronique », COURS DE BIOCHIMIE DES PROTEINES (Institut Pasteur); La tomographie électronique: théorie et pratique (CNRS)

With Inria Rennes

- France-BioImaging Advanced Training 2013, "BioImage Informatics & Reconstruction of multilevel dynamics" module (CNRS, GDR2588, FBI) (Gif sur Yvette)

- Comprendre les différentes méthodes d'analyse de la dynamique en biologie et leurs paramètres (CNRS, Univ. Paris Diderot)

On site Internal Continous Training

-theoritical courses: 40 h/50 persons per year; Training on software (Amira/MIA sous Metamorph/ Cell Profiler/Imanage/Fiji) 58h/25 pers; Training on systems (1500h for 450 users)

Dissemination (in 2013):

-*ELMI 2013* PICT Curie with Bordeaux Node (BIC-IINS), Arcachon. -*Bio image analysis* course 1(EMBL, Heidelberg)-CID-iManage presentation, *Bio image analysis course2* (in EUBIAS-Barcelona) -*Conference Imaging the Cell* (SBCF, Strasbourg) -"*1st BioImage Informatics Days* GDR 2588/MIV", *Kick off meeting CID-iManage* : Curie Image Data Base presentation, Institut Curie - *CLEM Days*, GDR-MIV 2588/FBI, Institut Curie with Institut Pasteur, Institut Pasteur.

Technology transfer & Relation with industry:

-Contractual partnership with Nikon B.V. for the Nikon Imaging Center -Collaboration with Imagine Optics: Integration of AO in Spinning disk confocal microscopy.

With Institut Carnot "Curie-Cancer":

-Co-dev and IP Agreement with Strand Life Sci. for "CiD-iManage" Image data base. Dev. with Roper Scientific of 4D-high resolution microscopy (contractual partnership) -Software licence transfer to Nikon Corp. for fast HR-SIM reconstruction and development of new algorithms (with IPDM-Inria Node)-Co-development of the AZC2-MPAO (NLO adaptive optics in macro-confocal) with Nikon Corp (NDA), Imagine optics, Optic Peter. –Dev. tools for CLEM, Support for the creation of the CryoCapCell company.-Demonstrator and consultancy for Fluigent S.A. microfluidic systems. Public type of collaboration (FP7 Nexpresso)-NDA contracts: L'Oreal (2), Clarins, Servier, Novartis, Sanofi, as Users.

<u>Temporary disposal of premises and IT facility for industrial partners on PICT-IBiSA at Institut</u> <u>Curie.</u>

Location: PICT-IBiSA 12th Rue Lhomond, Paris:

-2x 15m2 optical rooms (occlusion, confidentiality through electronic keys) with optical benches, computers, desk, fluids, for prototype development and/or validation.

-2x inverted microscopy set ups in a 10m2 equipped optical room for small equipment validation (camera, new objectives, lasers (continuous), microfluidic control systems,...).

- "Big Image Data" management system "*Curie Image Database*" & "*Image Data Center* "(300 Tbytes, storage and cluster; scalable), Web client, dev. and upload of analysis tools (Image J, ICY, Cell Profiler, other software library, ND-SAFIR, MIA...) through Application Programing Interface.



IJM/IMAGOSEINE

FBI support: functioning and mission. Supervision : all ImagoSeine members and associated Teams concerned

Education :

Participation (courses, lectures and practical sessions), Licence professionnelle en "Biophotonique" (University Paris Diderot VII), Masters : "Physiologie et Pathologies (BCPP)"(University Paris Diderot VII). Magistère Européen de "Génétique" (Universities Paris V & Paris VII,). Master "Physique Médicale et du Vivant" (University ParisDescartes). Master "BioMedical Engineering" (University ParisDescartes).

Institutional Continuous Training :

-Formation Microscopie confocale (Institut Curie / CNRS)

-Formation Microscopie plein champ et confocale (INSERM/Institut Curie / IJM)

-Maitriser imageJ pour le traitement d'images en microscopie de fluorescence (University Paris Diderot VII / CNRS)

-workshop : Experimental approaches in mechanotransduction: from molecules to tissues and pathology (Inserm)

-Image Analysis with ImageJ (CNRS – University Paris Diderot VII)

On site Internal Continous Training:

-Training on Icy and Fiji with Institut Pasteur-Paris centre (INSERM),

-Internal Training on ImageJ 40h 20 users

-Training on software (Amira/Imaris/Fiji) 350h for 35 users

-Training on systems (400h for 103 new users)

Dissemination: Organization, participation to the MIFOBIO school (2012).

Technology transfer & Relation with industry:

- collaborative partnership with Carl Zeiss Jena on super-resolution microscopy development (ELYRA). ImagoSeine is demonstration center for 3D dSTORM imaging of Zeiss France. PhD Zeiss fellowship 2012-2015 for PALM/STORM imaging.

Temporary disposal of premises and IT facility for industrial partners:

- All equipment of the Plateforme is fully open to industrial partners.

- a 10m² room with optical bench is available for demonstrations or short period collaborative experiments



IdF SUD

ImaGif/ BioEmergences/LOB

FBI support : functioning and mission Supervision : Béatrice Satiat Jeunemaitre, Nadine Peyrieras

Education :

Masters : participation (courses, lectures and practical sessions) : Imagif Master pro, BCPP specialite ingénierie de plate-forme. Imagif Master Pro "Génétique et Gestion de la Biodiversité" (Univ Paris Diderot) ; Imagif Master "Biologie Santé" (UPMC) ; ImaGif Master 2 « Génome, Cellules, Développement, Évolution » (with Paris Centre Institut Curie, Univ. Paris XI) ; Imagif Master « Sciences du Végétal »(Univ. Paris Sud) ; Imagif Cours international (with Paris Centre Institut Curie, Univ. Paris XI) ; BioEmergences : Master 1 UE Imagerie » (Univ. Paris Sud) ; ImagifMaster 1, Biologie Santé, Unité d'Enseignement transversale » (Univ. Paris Sud)

Institutional Continuous Training :

Imagif: annual course : «Atelier de Microscopie Electronique en Transmission pour la Biologie», (Formation Permanente & CNRS Formation Entreprises) ; Imagif : annual course (with contribution of BioEmergences) Atelier de microscopie confocale (CNRS Formation Permanente et CNRS Formation Entreprises) ; Imagif = Atelier de cytometrie (CNRS Formation Entreprises) ; LOB «Microscopie Non-linéaire en Sciences du Vivant » (formation CNRS).

BioEmergences-Roscoff Imagerie de l'embryogenèse d'organismes modèles marins, (Roscoff) (one month)

On site Internal Continous Training:

Imagif onsite training in photonic microscopy, 300 hours for 65 users ; Imagif onsite training by specific fields in photonic microscopy, 100 hours for 25 users ;Imagif onsite training in electron microscopy, 200 hours for 40 users ; Imagif onsite training in cytometry 15 hours for 120 users. BioEmergences training on site and on setups MLSM 30 hours for 20 users; BioEmergences distant & onsite training for the use of the BioEmergences workflow 600 hours for 30 users. Imagif = Atelier de cryo-microscopie électronique en film minces (Société Française de microscopie Gif/Orsay ;

Dissemination

Imagif Congrès National de la Société Française des microscopies/symposia Biosensors; Environmental microscopy (Nantes); Imagif Physique/Biologie Interface (PSB 2014- Gif-sur-Yvette). Kick-off meeting of BIG : superresolution approaches (Lidex project): Meeting of Lhermit (Labex)/presentation of the cell biology pole of Imagif; Meeting SPS (Labex Science Plant Saclay)/presentation of the cell biology pole of Imagif; annual meeting IFR 87.

BioEmergences/Imagif Morphogenesis in living systems, MLS'13 (Gif-sur-Yvette); BioEmergences / ImaGif / GdR 2588 / France-BioImaging FBI-Advanced Training (Gif-sur-Yvette).

Technology transfer & Relation with industry:

Contractual Partnership with Leica; contractual Partnership with Nikon; Organisation of a Leica scientific forum (Sept 2013), Leica user meeting (Dec 2013)

<u>Temporary disposal of premises and IT facility for industrial partners:</u> The 300 m² of the cell biology pole of Imagif harboring the whole of imaging equipment of Imagif and some equipment of BioEmergences are fully open to industrial partners. Platform engineers were involved in the treatment of 10 industrials research projects.



MONTPELLIER

MRI, MARS-CBS, IPAM

FBI support: functioning and mission Supervision: Vicky Diakou, Marcello Nollman

Education:

Masters: participation (courses, lectures and practical sessions):Master 1 Practicals on microscopy, (MARS-CBS, Univ Montpellier);Master2 :Practicals on PALM microscopy (MARS-CBS, Univ Montpellier);MRI : Master "Biologie cellulaire"(Ecole Pratique de Hautes Etudes-EPHE);MRI Master "BIOTIN" (Université Montpellier 1);MRI Master "Plant cell imaging" (Université Montpellier 2)

Institutional Continuous Training:

MARS- CBS Atelier Biocampus : Nouvelles microscopies photoniques : Superrésolution (CBS, Montpellier) ; MRI Atelier Biocampus : Les bases du logiciel image J (MRI, Montpellier) ; MRI"Atelier Biocampus : Microscopie à épi-fluorescence et microscopie confocale: de la base à la pratique" (MRI, Montpellier) ; MRI Atelier: Immunocytologie in situ hybridization, plant anatomy (INRA-CIRAD) ; IPAMAtelier Biocampus : Analyse du vivant par imagerie-Quantifications (INSERM-IPAM).

On site Internal Continous Training : Trainnig on software and systems. MRI : 1000-1300h for 350-425 persons

Dissemination (in 2012-2013):

MARS- CBS"EMBO Practical Course: Superresolution and single moleculemicroscopies in living cells" (Montpellier)MARS-CBSUser & Developer ImageJ Conference (Montpellier)

Technology transfer & Relation with industry:

MRI: Training of scientists and engineers from private companies (Vilmorin, Nestlé, Coca-Cola France). Sharing knowledge technologies with Technical Institut (Institut de la Vigne et du Vin, Institut Français du Caoutchouc).

MARS-CBS: Partenariat avec Imagine Optics and ANDOR Technology.

<u>Temporary disposal of premises and IT facility for industrial partners:</u> MRI: Development of new optical setup for vertical plant imaging under controlled microclimatic environment in collaboration with Carl Zeiss Company. Location: MRI-PHIV, avenue Agropolis, CIRAD, Montpellier, France



MARSEILLE

IBDML / CIML / INSTITUT FRESNEL

FBI support : functioning and mission

Supervision : Cedric Matthews, Pierre François Lenne

Education: IBDML : Master & PhD programs : participation (courses, lectures and practical sessions) : ISAB (Imagerie et Systèmes Appliqués en Biologie) ; CIML PhD program (one mandatory for new PhD students, one common with the INFORM Labex and the university); IBDML PhD program (commun labex inform et école doctorale AMU) Fresnel: Erasmus Mundus « Europhotonics » - Master program Erasmus Mundus « Europhotonics » - Doctorate program Institutional Continuous Training: CIML "Cytométrie en flux : du photon à la cellule : théorie et pratique" (CIML/CIPHE/PRECYM) ; CIML / IBDML « De la video-microscopie à la microscopie confocale » (INSERM) ; CIML Inserm Training on intravital NLO imaging, 2013 IBDML Le traitement d'images sous ImageJ (INSERM); IBDML automatisation du traitement d'images sous ImageJ (INSERM) ; IBDML Le traitement d'images sous ImageJ (CNRS); IBDML Plateforme, mutualisation d'instruments scientifiques : création et développement d'un service (CNRS); Institut Fresnel Autour du contrôle des ondes pour les applications en biologie (Institut Fresnel) ; Institut Fresnel Coherent Raman spectroscopy and microscopy (les Houches); IBDML Action nationale de formation CNRS Metrologie des systèmes d'imagerie (CNRS). On site Internal Continous Training: CIML Pratical courses on image processing imageJ (internal); CIML Theory et Pratical courses on widefield & confocal microscopy (internal); CIML Training on Fluorescence Correlation Spectroscopy (internal): CIML Training on Spinning disk microscopy on living samples (internal); CIML total trainings: 90 users for 220 hours IBDMLcours imageJ pour débutant (internal) + practical ; IBDML cours theory and pratical, microscopy (internal). Training on systems : 80-140 hours for 150 persons. Dissemination (in 2013):

CIML Theoretical course during the EMBO practical courses - Super-resolution and single molecule microscopies in living cells - 2013, May 13/19 - Montpellier

Fresnel J. de Torres, P. Ghenuche, E. Grinenval, M. Brennan, G. Baffou, H. Rigneault, J. Wenger, "Nano-antennes plasmoniques pour la biophotonique" Photoniques 65, 37-41 (2013).

IBDM Methodological partnership with Sfax-Tunisia Biotechnological center for developing an imaging facility (Pr. Hafedh Belghith)

Technology transfer & Relation with industry:

CIML Development of a multimodal contrast imaging on a commercial multiphoton microscope (SP5-MP) in close collaboration with LEICA (paper in preparation)

CIML Development of analysis software in MATLAB dedicated to cell tracking and calcium imaging (Methods for Automated and Accurate Analysis of Cell Signals: MAAACS)

IBDM Contractual partnership with Nikon B.V. & Nikon France for the Nikon Application Center (IBDM)

Temporary disposal of premises and IT facility for industrial partners: CIML: Slide scanner, 5 days, 10m² Fresnel: Industrial contract with L'Oreal: 'percutaneous aborption of skin active compounds '

Industrial contract with Galderma: 'Drug imaging in tissus'



BORDEAUX

BIC/IINS

FBI support : functioning and mission

Supervision : Christel Poujol (with all BIC members), Daniel Choquet

Education:

Courses, lectures and/or practical sessions Master Biologie-Santé spécialité BioImagerie Master Neuroscience Synapse Summer school

Institutional Continuous Training:

-Microscopie à épi fluorescence et microscopie confocale : des bases à la pratique (INSERM, CNRS) -Vidéomicroscopie et microscopie confocale : des bases à la pratique : du tissu à la molécule unique (INSERM, CNRS)

-Initiation Traitement, acquisition et analyse d'images avec Metamorph (INSERM, CNRS) -Les bases du Logiciel Image J (INSERM, CNRS)

-Aller plus loin dans l'analyse d'image à l'aide du logiciel ImageJ (INSERM, CNRS)

-Logiciel Metamorph : macros et fonctions avancées (INSERM, CNRS)

-Introduction à la mesure de la mobilité de protéines par FRAP et SPT: Bases théoriques, pratique et analyse (INSERM, CNRS)

-Techniques d'immuno-marquage pour la photonique et l'électronique (INSERM, CNRS)

On site Internal Continous Training (260 pers/ 800 h/an)

Training on systems: from electron microscopy to macroscopy.

Training on sample preparation for electron microscopy

Training on sample preparation for electron microscopy or photonic microscopy

Dissemination (in 2013):

ELMI 2013 Bordeaux Node (BIC-IINS) with Paris Centre Node (PICT-Curie). Arcachon (450 pers) Neurophotonics in Bordeaux (150 pers)

Technology transfer & Relation with industry:

Technological transfer with Molecular Devices on superresolution Collaboration with Leica Microsystems on GSD microscopy and u-paint microscopy Collaboration with Leica Microsystems on the development of a photothermal microscope Collaboration with Imagine Optic on deep imaging Collaboration with Nikon on superresolution

Patents:

1- Galland R, Viasnoff V, Levecq X, Studer V, Sibarita JB (2013) Système et méthode de microscopie par éclairage par la tranche. Imagine Optic, CNRS, Univ. Bordeaux Segalen, Patent pending FR1358226, France.

2- Gianluca G, Galland R, Studer V, Sibarita JB, Viasnoff V (2013) Device for single-objective light-sheet microscopy. NU. Singapore, CNRS, Univ. Bordeaux Segalen, Patent pending GB1315248.3, UK.

3- Sibarita JB (2012) *Method and apparatus for single-particle localization using wavelet analysis CNRS, Univ. Bordeaux Segalen, Patent* 12166450.2, *Europe/US*



Late contributions of Ile de France Sud node

WP 1a - Super Resolution and Single Molecule Tracking LOB and IMAGIF:

<u>FBI support</u>: for the lanthanide-ion doped nanoparticles project setup2 (see IDFsud LOB) and IMAGIF setup6 Equipments: for the lanthanide-ion doped nanoparticles project setup2 (see IDFsud LOB) and IMAGIF setup6 Functioning: see IDFsud resources

<u>Supervision</u> & development : Antigoni Alexandrou (LOB), Béatrice Satiat-Jeunemaitre (IMAGIF) Recruited personnel (name and duration): M. Temagoult (2 mois)

<u>Other personnel (name, status and lab)</u>: L. Besse (CDD IMAGIF), R. Lebars (CDD IMAGIF), M. Temagoult (CDD IMAGIF), B. Satiat Jeunemaitre (DR ISV, IMAGIF), C. Bouzigues (MC, LOB), A. Alexandrou (DR, LOB)

Objectives of the project (5 lines):

- LOB (Antigoni Alexandrou): develops non-blinking single-molecule labels and sensors based on lanthanide-ion doped nanoparticles and use them for studying toxin-cell interaction and toxin receptor confinement in the cell membrane, and hydrogen peroxide production in signaling processes, respectively. This project fits both with WP1a and WP3 objectives

- IMAGIF: installation of state of the art PALM/STORM setup in the IMAGIF platform.

Work undertaken (10 lines):

- We used peptidic toxins (family of oligomerizing and pore forming toxins) labeled by Eu-doped nanoparticles to investigate their interaction with cells: binding to receptors on the cell membrane and receptor motion inside membrane microdomains. Novel single-molecule trajectory analysis techniques based on Bayesian inferences can extract the force field felt by the receptor. These toxins can thus also be viewed as tools for investigating the membrane organization.

- IMAGIF: installation of state of the art PALM/STORM setup in the IMAGIF platform.

Important results (5 lines):

- LOB: 1) We probed membrane protein interactions with their lipid raft environment using singlemolecule tracking andBayesian inference analysis. 2) We observed the confinement potential of bacterial pore-forming toxin receptors inside rafts with nonblinking Eu(3+)-doped oxide nanoparticles.

Opening to the community (2 lines):

- LOB: opening of setup2 to the community in march 2014

- iMAGIF: march 2014

Running scientific projects (title & collaborator name):

- **LOB6** Production of reactive oxygen species (ROS) in the context of glomerulonephrite. Collaboration PL Tharaux PARCC, HEGP, Paris.

- IMA1, 2, 3, 4

Relation with industry (3 lines):

Additional grant obtained:

- LOB6 and setup2 180 k€ DIM NanoK Region Ile de France, 35 k€ RTRA Triangle de la Physique
 - IMAGIF setup 6 CGM/CNRS, Labex SPS

Publications (FBI not mentioned as funding has not been mobilized yet on these projects):



S. Türkcan, J.-B. Masson, A. Alexandrou *A Bayesian inference scheme to extract diffusivity and potential fields from confined single-molecule trajectories*Biophys. J. **102**, 2288-2298 (2012) Supp. Info.
S. Türkcan, D. Casanova, J.-B. Masson, G. Mialon, M. R. Popoff, T. Gacoin, J.-P. Boilot, A. Alexandrou *Observing the confinement potential of bacterial pore-forming toxin receptors inside rafts with non-blinking Eu³⁺-doped oxide nanoparticles*Biophys. J. **102**, 2299-2308 (2012) Featured article Supp. Info.
Communications and Training courses:

Patents:

WP 1b - Functional Imaging

- Paris Sud is strongly involved in developing novel biosensors for biological force and calcium measurements with strong and internationally recognized expertise in *in vivo* embryo imaging. Its power resides in multiscale dynamics studies realized during animal morphogenesis.

BIOEMERGENCES:

<u>FBI support</u> :Equipments: see WP3 Supervision & development; Nadine PEYRIÉRAS, Adil BAKAYAN (user support)

Recruited personnel (name and duration): Other personnel (name, statut and lab): Adil BAKAYAN (Postdoc, 100% R&D, CNRS)

Objectives of the project (5 lines):

The project is aimed at engineering and characterizing new biosensors for measuring and monitoring biomechanical force across AJs in zebrafish embryo. It is as well complemented by developing a novel sensor for ROCK activation and subsequent actomyosin contraction, responsible for force generation inside the cell. In parallel, our goal as well is to develop and implement new imaging setups by merging bioluminescence and 2-photon or SPIM technology.

Work undertaken (10 lines):

-We are currently developing and implementing advanced imaging modality based on 2-photon and SPIM (in near future) with adaptation for bioluminescence use.

-Construction of the bioluminescence setup is nearly to be finished by summer 2014. By merging these two imaging technologies, we will be able to image bioluminescence signal from a selected imaging plan, for improved spatial information and resolution.

- we are also embarking on construction of these novel force biosensors that are will be implementing FRET and BRET modalities.

Important results (5 lines):

So far we have successfully used 2-photon and confocal microscopy to follow with single cell resolution cell behaviours in time and space (3-D) using fluorescence and also SHG/THG; for cell lineage tracking, gene expression patterns, calcium signalling, and all during in vivo embryonic development mainly in zebrafish and sea urchins.

Opening to the community (2 lines):

2-photon and confocal microscopes for imaging embryonic development using fluorescent probes in Bioemergences platform.



Running scientific projects (tile & collaborator name):

Construction of novel force biosensors (Adil BAKAYAN)

Contruction of bioluminescence-based microscopy imaging system (Adil BAKAYAN, Julien DUMONT) Implmenting and combining technologies from bioluminescence and 2-photon systems, as well as SPIM (Adil BAKAYAN, Julien DUMONT, Gaelle RECHER)

Relation with industry (3 lines):

Additional grant obtained: see WP3 Published FBI publications: check WP3 Communications and Training courses: see WP3 Patents:

WP 1c – CLEM & SuperCLEM

ILE de FRANCE SUD IMAGIF

FBI support : NO FBI Support

Supervision & development : Béatrice Satiat-Jeunemaitre ; (Claire Boulogne IR CNRS et Cynthia Gillet AI CNRS for user support).

Recruited personnel (name and duration): No personnel recruited on FBI

Other personnel (name, status and lab):

Remi Leborgne, (CDD IE CNRS, Imagif 6 months); Jessica Marion AI CNRS, (B. Satiat-Jeunemaitre group Gif sur Yvette); C. Boulogne IR CNRS Cell Biology Pôle Imagif).

Objectives of the project (5 lines max):

Propose a working flow protocol to identify the ultrastructural support of fluorescent signal in eukaryotic cells by correlated light and electron microscopy.

Work undertaken:

Two complementary approaches are developed:

- use of bi-functional commercial probes (quantum dots, nanofluorogold and fluorogold) to correlate signals between Light and electron data taking into account the needs to shorten electron microscope heavy protocols and to avoid the use of radioactive contrasting agent increasing contrasts (Carpentier et al., 2012; Barois et al., in preparation). (project IMA10) - In parallel, a working flow to perform CLEM approaches based on correlation between the fluorescent signals observed on ice or resin section and its ultrastructural support has been established for plants and c.elegans (respectively Le Bars et al. 2013; Manil-Segalen et al., 2013).(Project IMA11)

Important results:

- optimization of CLEM protocols based on quantum dots in plant cells, using silver enhancement (Barois et al., communications and paper in preparation)

- identification of LC3 labeled structures shows that LC3 is acting downstrzam of GABARAP to degrade autophagosomes and interact with VPS39 (Manil-Ségalen et al., 2014. Developmental cell 28, 43-55

- Atg5 labels early autophagosme formation forming on the ER surface (Lebars et al. Nature communication in revision process).

Opening to the community :



- all the set-ups and a lab dedicated to material preparation are opened to the community along the chart of Imagif, IBiSA and the norm ISO 9001.

Running Scientific projects :

- developments of protocols to increase the conservation of fluorescent signals in resin embedded material (M. Bianchi, Boulogne C.); Tokuyasu method and CLEM in plant cells (Thomine team, B. Satiat-Jeunemaitre team) and medaka cells (Joly team). (Project IMA11)

Additional grant obtained/required:

The project benefited from IBiSAfundings (equipment); 70 000 No additional grant is required.

<u>Publications</u> : Manil-Ségalen M., Lefebvre C., Jenzer C., Trichet M., Boulogne C., Satiat-Jeunemaitre B., Legouis R. (2014). Developmental cell28, 43-55 Lebars R., Marion J., Leborgne R., Satiat-Jeunemaitre B., Bianchi M. (2014). Nature Communication, in revision process.

<u>Communications and training course</u>: - workshops on correlative microscopy in the annual course"Atelier de Microscopie confocale" and Atelier de Microscopie electronique pour la biologie cellulaire" (Imagif, cours CNTS Formation permanete et Formation entreprises; -workshop during the national course FBI-AT, December 2013 at Gif sur Yvette; Comunication (poster) IFR Congress 2013 ; participation to « CLEM days » 2012-2013

WP 3 - Probe Development, Optomanipulation & Optogenetics

BioEmergences

FBI support: see setup 5 in IDFsud

Supervision & development: Nadine Peyriéras (DR, MDAM & BioEmergences), Adil Bakayan (Post doc, MDAM & BioEmergences)

Recruited FBI personnel (name and duration): None on FBI funding for probes development

Other personnel (name, statut and lab): Monique Frain (CR1 CNRS MDAM), Adil Bakayan (Post doc, MDAM & BioEmergences), Adeline Boyreau (IE CDD CNRS, MDAM & BioEmergences)

Objectives of the project (5 lines):

- Development of sensors for the quantification of biological activities in developing embryos: 1) calcium fluxes, 2) strains and stresses

- Development of staining strategies for mosaic nucleus and membrane staining for lineage tree and tissue morphogenesis reconstruction from live imaging of developing model organisms

- Screening of chemical dyes for membrane and nucleus staining in emerging animal models (collaboration S. Bruneau, AMAGEN Gif sur Yvette)

Work undertaken (10 lines):

- Directed mutagenesis to obtain variants of tdtomato-aequorin fusion for improved sensing of calcium fluxes in cells and developing embryos based on BRET.

- Design of a force sensor based on a-catenin and comparing FRET vs BRET for reporting the activity

- Design of new rainbow type constructs for splitting stained nuclei populations between different channels

Important results (5 lines):



FRANCE-BIOIMAGING

- MDAM/BioEmergences we are currently validating new protein variants derived from a tdtomatoaequorin fusion for improved sensing of calcium fluxes in cells and developing embryos based on BRET.

Opening to the community (2 lines): Sensors made available upon publication

Running scientific projects (tile & collaborator name): **BioEM2** Reconstruction and modeling of zebrafish gastrulation: BRET and FRET based sensors for the *in vivo* quantification of biological processes **BioEM4** Reconstruction and modeling of zebrafish hindbrain segmentation **BioEM11** Reconstruction and modeling of Ascidian development **BioEM12** Marine organisms' Embryome project **BioEM13** Reconstruction and modeling of Dogfish gastrulation

Relation with industry (3 lines): None yet Additional grant obtained: Funding agency: zf-Health EC project Total amount : 150k€ Currently applying for additional funding (ANR) Published FBI publications: Authors Journal Communications and Training courses: Nothing specific yet with these results Patents:

Imagif

<u>FBI support</u>: Equipment (spent): see IDFsud setup 8 Functioning (salary) Supervision & development: Béatrice Satiat-Jeunemaitre (DR CNRS) & Spencer Brown (DR CNRS)

Recruited personnel (name and duration): Sébastien Bellow (15-10-2012/14-06-2014)

<u>Other personnel</u> (name, statut and lab): Spencer Brown (DR CNRS, ISV), Beatrice Satiat-Jeunemaitre (DR CNRS, ISV), Jim Dompierre (IR CNRS, Imagif), Marie Noëlle Soler (IE CNRS, Imagif).

Objectives of the project (5 lines max):

- provide robust methods for proper spectral analysis methods for tissue and cell imaging

- optimize the use and compatibility of fluorescent probes through flow cytometry analyses and light confocal microscopy

Important results

- The use of the click EdU reaction has been rendered compatible with GFP imaging

- protocols for pulse chase of proteins in cellulo using photoconvertible proteins

Opening to the community

FBI-cofunded setup has been opened to external users as a main service of the platforms. The engineer in charge of the set-up has been en essential user support in all the training actions and in the support of two main research projects conducted by non-FBI members.

Running scientific project:

(Ima5) Ubiquitinilation receptors/endocytosis. G. Vert, ISV, Gif sur Yvette (Ima6) Biosensors : The CLOPs probe in plant cells. A. Di Angeli & S. Thomine, ISV, Gif sur Yvette



FRANCE-BIOIMAGING

(Ima7) Combination of organelles sorting, imaging and close contacts interorganelles. Functional relations with endoreduplication processes. Chevalier, INRA Bordeaux et Brown, ISV (Ima 8) Biosensors: Clik reaction to track pharmacological agents in cultured cells (R. Rodriguez, ICSN)

(Ima9) Copper effects on EdU-revealed clik reaction. M. Bourge, S. Brown, Gif sur Yvette. Collaboration with Cecile Raynaud team, IBP; J.S. Jly team, INAF.

<u>Relation with industry</u> Vilmorin, AgriObtention (confidential study).

Additional grant obtained: CPER Total amount spent:

Publications with FBI members/Imagif platform in co-autors:

<u>Communication and training courses</u>: annual course of confocal microscopy (theory and practical one week) each year 25 persons; 50 users trained on the equipment; workshops Bio-Imaging advanced Technology; posters for IFR 87 and SPS annual congress.



8- Detailed scientific perspectives of the WPs per node

Detailed perspectives for WP4 and WP5 being in close continuity with actual achievements, they are described in the previous chapters.

WP 1a – Super Resolution & Single Molecule Tracking

MARSEILLE

CIML

<u>FBI support</u>: Equipment: 45 000 €. Functioning: 20 k€ / year Supervision & development D. Marguet (CNRS, DR)

Recruited personnel:

<u>Other personnel</u>: R. Fabre (CNRS, IR CDD starting on 04/14); S. Brustlein (INSERM, SANOFI CDD); S. Mailfert (CNRS, IR 50% on R&D and 50% on platform activity); M. Fallet (CNRS, IE, 50% on R&D and 50% on platform activity); Y. Hamon (Inserm, CR); R. Lasserre (Inserm, CR); HT HE (CNRS, DR); two-year research fellow to be recruited in 09/14, CNRS.

Objectives of the project:

- To complete the ongoing work on sample preparations

- To develop and produce fluorescently labeled probes for specific dSTORM application (work in collaboration with the MarseilleImmunopôle MI-mAbs platform and Innate Pharma)

- To stabilize a new version of our previous algorithm (Multiple Target Tracing) to analyse data from super-resolution imaging

- To improve the spatial resolution on the optical axis

<u>- To extend the whole method (sample preparation, setup and data analysis) for imaging molecular</u> complexes by multicolor staining.

Scientific projects (tile & collaborator name):

Ongoing projects

1C- - THe Immune function of Natural Killer cells) / coord. Eric VIVIER, coll. D. MARGUET

2C - nanoDIGICODE - Deciphering the molecular dynamics & organization of the Ras signaling node in the control of the analog/digital signal processing / coord. D. MARGUET, coll.H. RIGNEAULT 3C - ReceptOrient - Observing the T cell receptor (TCR) activation in real time: monitoring the intracellular domain orientation dynamics of the receptor complex in live cells with polarized time-resolved fluorescence imaging / coord. Hai-Tao HE, coll. D. MARGUET & H. RIGNEAULT New projects

6C - TC21 & TCR cotranslocation / Coord. D. Marguet, coll. B. Alarcón (CBMSO, Madrid)

Funding pending

7C – Combining optico-mechanical manipulations, high-resolution microscopy and physical modelling to decipher the role of caveolae in mechanosignaling / Coord. P. SENS, coll. Ch Lamaze Institut Curie, D. Marguet CIML.

<u>Additional grant (obtained/required):</u>

Funding agency: Inform LABEX (partner, obtained), AMIDEX interdisciplinary project (partner, obtained), ANRs (decision pending)



IBDM

<u>FBI support</u> : Equipment: 320 K€, Functioning: 50 k€ Supervision & development; PF Lenne & C Matthews (user support)

Recruited personnel: Binh-An Truong Quang (01-01-2014/31-08-2014)

<u>Other personnel</u>: IR Sept 2013-Aug 2014 (PALM-3D) - IE- june 2014-May 2015 50% (STED multicolor, Development and User Support)

<u>Objectives of the project</u>: 1- 3D PALM imaging using light sheet illumination. While we have tested the localization precision of this set-up in single sections of a whole tissue, 3D imaging at high resolution will be the main goal of this second phase. 2- STED Multicolor

Scientific projects (tile & collaborator name):

11 Polarisation and binary cell fate decisions in the neuronal system (V. Bertrand IBDM)

2I New physical approaches to dissect the principles of apical junction supramolecular organization in cancer epithelial cells (A. Le Bivic, IBDM)

31 Rôle de Crumbs dans le remodelage des jonctions adhérentes de l'épithélium de l'aile de drosophile (A. Le Bivic, IBDM)

4I Dissection moléculaire des mécanismes assurant l'architecture et la plasticité d'un épithélium (T. Lecuit, IBDM)

5I The Biomechanics of Epithelial cell and Tissue morphogenesis (T. Lecuit, IBDM)

6I In vivo mechanics during tissue morphogenesis (PF Lenne, IBDM)

7I Mesoscopic origins of cell behaviours during tissue morphogenesis: mechanics and biochemical signaling (PF Lenne, IBDM)

8I Expression et fonction des VEGFRs (Vascular Endothelial Growth Factor Receptors) dans la biologie des cellules neurales du SNC: aspects développementaux et physiologiques; potentielles utilisations pour la réparation tissulaire (F. Mann, IBDM)

91 Régulation de la réponse anti-bactérienne dans les épithélia respiratoires et intestinaux chez l'insecte modèle Drosophila melanogaster (J Royet, IBDM)



MONTPELLIER

CBS

<u>FBI support</u> : Equipment: 54476 € , Functioning: 2507 € Supervision & development: Marcelo Nollmann

Recruited personnel:

Other personnel: JB Fiche, IR. Laura Oudjedi, research fellow. Mariya Georgieva, PhD candidate.

Objectives of the project:

Development of super-resolution microscopy methods to study the architecture and dynamics of proteins within cells. In particular, we focus on extending PALM/STORM towards 3D and 2-colors and on combining PALM/STORM with other methods such as SIM and multi-focal plane microscopy. We specialize in studies involving nuclear organization.

Scientific projects (tile & collaborator name):

Development of muti focal point SIM (MSIM) coupled to 3D-PALM. This setup will use adaptive optics for 3D-PALM (already functioning) and for 3D-SIM. In collaboration with Hari Shroff (NIH).
 Development of multi focal plane microscopy for 3D-STORM, in collaboration with S. Abrahmson (Rockefeller University and Janilia Farms).

- Architecture of M. Xanthus adventurous gliding machine, Tam Mignot, LCB, CNRS Marseille

- In Vivo organization and Dynamics of the nucleoprotein complex assembled on the bacterial centromer at the single molecule level, JY Bouet, Laboratoire de microbiologie et genetique moleculaires, CNRS Toulouse

- Analysis of the super-resolution distribution of Polycomb proteins and topologically associated domains by PALM and STORM, Giacomo Cavalli, IGH, CNRS Montpellier and Ting Lab, MIT.

Additional grant (obtained/required): Funding agency ANR, HFSP, ERC. Total amount 100 000 €



BORDEAUX

LP2N - Institut d'Optique Graduate School

<u>FBI support</u> : salary for Pierre Vermeulen (Research Engineer) Supervision & development: Brahim Lounis and Laurent Cognet

Recruted personnel: Pierre Vermeulen - 2 years (FBI)

<u>Other personnel</u>: Philippe TAMARAT (R&D, PR UB), Jean-Baptiste TREBBIA (R&D CR CNRS), Zhenghong Gao (Research fellow, LP2N), Edakkattuparambil Shibu (Research fellow, LP2N), Antoine Godin (Research fellow, LP2N)

Objectives of the project:

Our objectives are to improve super-localization and super-resolution microscopy techniques on several aspects by developing (i) a photothermal microscope prototype to track single gold nanoparticles, (ii) correlative microscopy between photothermal microscopy and electron microscopy, (iii) low temperature super-resolution microscopy to reach true nanometer resolutions (iv) parallelized STED and RESOLFT microscopy (v) FRET based super-resolution by uPAINT to study interacting molecules and (vi) developing single molecule tracking in the near-infrared.

<u>Scientific projects</u> (tile & collaborator name):

(a) User Friendly Photothermal Microscopy Prototype for User Plateform, Lounis/Cognet (LP2N)

(b) Parallel STED & RESOLFT, Lounis/Trebbia (LP2N)

(c) SUPERCLEM: Correlative microscopy - Photothermal vs Electron microscopy, Lounis (LP2N)

(d) SmFRET-uPAINT Single molecule FRET+UPAINT, Cognet/Lounis (LP2N)

(e) Low temperature super-resolution imaging, Lounis/Tamarat (LP2N),

(f) Near infrared microscopes for single molecule tracking, Cognet/Lounis (LP2N), Groc (IINS)



PARIS CENTRE

IJM/IMAGOSEINE: SUPER-RESOLUTION PALM-STORM-SIM

<u>FBI support</u> : Equipment: 52 000€; Functioning:

Supervision & development Orestis Faklaris; France Lam (user support)

<u>Recruited personnel</u>: Olivier Blanc (IR: 09/2013-10/2015); To be recruited (40% shared with WP 1b & d BioImage analysis)

Other personnel: Vincent Contremoulins (IE CNRS)

Objectives of the project:

Short term: validation of PALM/ STORM/ SIM techniques (acquisition + data analysis).

Mid-term: Development of 3D PALM microscopy to answer biological questions, using adaptive optics.

Long-term: 1. Development of in depth PALM microscopy using 2photon excitation (DeepSee laser) and Light Sheet Illumination. ; 2. Development of single molecule FRET-PALM detection in cells. <u>Scientific projects</u> (tile & collaborator name):

-Membrane dynamics and vesicular traffic (T. Galli)

-Migration of transient populations of neurons (A. Pierani)

-Mechanics of cell adhesion and mechanosensitivity at cell-cell junctions (B. Ladoux – R. Mege) -Study of the organization of GPCR proteins (T. Durroux)

-Netrin-1 receptors recruitment and dynamics in living cells. (M. Coppey-Moisan)

-Test for the importance of the conformational changes of the Iga/Igb tail in the initiation of BCR signalling (J. Yang)

Additional grant (obtained/required): 40k€ for adaptive optics component. Project submitted

Laboratoire Physico-Chimie Curie – PCC

<u>FBI support</u> : Equipment:75 000 €, Functioning: 34 898 € Supervision & development; Maxime Dahan (DR CNRS)

Recruited personnel: To be recruited (64 860 €)

<u>Other personnel</u>: Bassam Hajj (Research fellow), Davide Normanno (Research fellow), Mohamed El Beheiry (PhD candidate)

Objectives of the project:

We aim to further develop optical and computational tools for 3D multifocal microscopy. First, we use novel multiphase diffractive grating (instead of binary phase gratings) in order to improve the sensitivity of the multifocal microscope. Second, we will test novel deconvolution schemes and compressed-sensing based methods to improve the density of detectable molecules and their localization accuracy in single molecule PALM/STORM images. Finally, we will further expand the functionalities of the visualization software VISP that was developed in the lab.

Scientific projects (tile & collaborator name):

- Mapping the nanoenvironment in living cells using high-density single molecule imaging, with J.B. Masson (Pasteur)

- Analysis of the 3D organization of the neuronal membrane, with A. Triller (IBENS)
- 3D Organization and dynamics of telomeres in the yeast nucleus, with A. Taddei (Institut Curie)
- 3D dynamics of TALE proteins, with J. Lopes (MNHM)

Additional grant: grant application under evaluation at ANR for the implementation



IBENS:

FBI support :

Supervision & development; (user support): DARZACQ (DR CNRS) / SPECHT (CR INSERM);

Recruited personnel:

Other personnel: Ignacio Izzedin (Research fellow 01/2012-03/2014); Xavier Marques (IE CDD 01/2012-12/2016)

Objectives of the project:

We will evaluate the performance and capabilities of the 3D PALM microscope for different biology projects in vitro and in vivo.

Scientific projects (tile & collaborator name):

- The "Functional Imaging of Transcription" group (Darzacq lab) will analyze transcription and transcription regulation in mammalian cells thanks to 3D PALM, studying the role of the nuclear organization and dynamics of the molecules and macromolecular complexes involved in this process.

- The "Cell biology of Synapses" group (Triller's lab) will exploit the 3D PALM to analyze the dynamics of receptors at synapses.

- We will designed Localization-based super-resolution (PALM/STORM) set-up combined with local activation/imaging configuration (local activation, pattern activation/imaging, combined FRAP/FLIP with PALM/STORM).



WP 1b - Functional Imaging

MARSEILLE

CIML

FBI support: Equipment: 30 000 €. Functioning: 20 k€ / year Supervision & development: D. Marguet (CNRS, DR)

Recruited personnel: Anastasia LOMAN (IR: 03/2013-02/2017)

<u>Other personnel</u>: S. Brustlein (INSERM, SANOFI CDD); S. Mailfert (CNRS, IR 50% on R&D and 50% on platform activity); M. Fallet (CNRS, IE, 50% on R&D and 50% on platform activity); M. Bajénoff (CNRS, CR); Y. Hamon (Inserm, CR); R. Lasserre (Inserm, CR); HT HE (CNRS, DR); a one-year post-doc (AMU, to be recruited on 05/14).

Objectives of the project:

We will pursue the methodological and instrumentation developments as listed for the CIML in the Achievement report on WP1b with a special focus on the multimodal FCS at theoretical and experimental level (short-term development). The designed ALBA module (ISS) will be combined to our current setup. We aim at investigating the potential of spot variation Fluorescence Cross-Correlation Spectroscopy (svFCCS) and related FCS measurements on the biological models developed by different teams at the CIML or within the frame of collaboration, both at national and intentional levels.

<u>Scientific projects</u> (tile & collaborator name):

Ongoing projects

1C- - THe Immune function of Natural Killer cells) / coord. Eric VIVIER, coll. D. MARGUET

2C - nanoDIGICODE - Deciphering the molecular dynamics & organization of the Ras signaling node in the control of the analog/digital signal processing / coord. D. MARGUET, coll.H. RIGNEAULT 3C - ReceptOrient - Observing the T cell receptor (TCR) activation in real time: monitoring the intracellular domain orientation dynamics of the receptor complex in live cells with polarized time-resolved fluorescence imaging / coord. Hai-Tao HE, coll. D. MARGUET & H. RIGNEAULT

New projects

4C - Immunobiologie des cellules stromales lymphoïdes / Coord. M. Bajénoff CIML

5C - Mechanisms of Tumor-Associated Macrophage (TAM) Homeostasis in Pancreatic Cancer / Coord. T. Lawrence, coll M. Bajénoff

6C - TC21 & TCR cotranslocation / Coord. D. Marguet, coll. B. Alarcón (CBMSO, Madrid)

Funding pending

7C – Combining optico-mechanical manipulations, high-resolution microscopy and physical modelling to decipher the role of caveolae in mechanosignaling / Coord. P. SENS, coll. Ch Lamaze Institut Curie, D. Marguet CIML.

8C - BiRefringence for Anisotropy imaging in Histological Melanoma Signatures analysis / Coord. S. Monneret Institut Fresnel, coll. B. Wattelier PHASICS, D. Marguet CIML

<u>Additional grant</u>: Funding agency: Inform LABEX (partner, obtained), AMIDEX interdisciplinary project (partner, obtained), ANRs (decision pending)



IBDML

<u>FBI support</u> : Equipment: 70 k€, Functioning: 40 k€ Supervision & development; PF Lenne & Claire Chardès IE (Dvt), Aix-Marseille University

Recruited personnel:

Other personnel: Research fellow (Dev), CNRS, 01/05/14 - 30/04/16

Objectives of the project:

Implementation of two-colors FCS imaging for measurements in small organisms

Scientific projects (tile & collaborator name):

- 1I Polarisation and binary cell fate decisions in the neuronal system (V. Bertrand IBDM)
- 2I New physical approaches to dissect the principles of apical junction supramolecular organization in cancer epithelial cells (A. Le Bivic, IBDM)
- 5I The Biomechanics of Epithelial cell and Tissue morphogenesis (T. Lecuit, IBDM)

INSTITUT FRESNEL

<u>FBI support:</u> Equipment: 30 000 € Supervision & development S. Brasselet (CNRS, DR)

Recruited personnel: None

<u>Other personnel</u>: Cesar Valades Cruz (PhD, Erasmus Mundus), Haitham Ahmed (PhD, Erasmus Mundus), 2 Research fellows to be recruited

<u>Objectives of the project</u>:Providing a new molecular orientational information in imaging for functional cell and tissue imaging.

Scientific projects (tile & collaborator name):

Ongoing projects

- nanoDIGICODE - Deciphering the molecular dynamics & organization of the Ras signaling node in the control of the analog/digital signal processing / coord. D. Marguet, coll. S. Brasselet / H. Rigneault

- ReceptOrient - Observing the T cell receptor (TCR) activation in real time: monitoring the intracellular domain orientation dynamics of the receptor complex in live cells with polarized time-resolved fluorescence imaging / coord. HT HE, coll. D. Marguet & S. Brasselet / H. Rigneault

New projects

- Correlation between lipid molecular order in membrane and cytoskeleton architecture (S. Brasselet, S. Scheuring INSERM)

- Relation between tissue mechanic and molecular order (S. Brasselet, P. F. I Lenne IBDML, M. Mavrakis IBDML)

Additional grant obtained: Amidex Light in Bio (200 000€)



MONTPELLIER

CBS

FBI support : Equipment: 0€, Functioning: 30000€

Supervision & development name: Emmanuel Margeat ; (Caroline Clerte : user support)

Recruited personnel:

Other personnel: C. Clerte (IR INSERM, 50% R&D, 50% Platform); JB Fiche (IR INSERM, 50% R&D, 50% Platform); V. Georget (IR, 100% Platform; S. De Rossi (IE, 100% Platform)

Objectives of the project:

- Continue the development of fluctuations techniques. Establish benchmarks for quantitative analysis

- Development of a FCS in vivo microscope (collab. P. Mollard, see also WG1d and WP3)

- Design and implementation of a laser scanning system for our Pulsed Interleaved Excitation microscope for cross-talk free crosscorrelation, cross N&B and cross RICS experiments.

<u>Scientific projects</u> (tile & collaborator name):

(2) Analysis of nuclear pre-ribosomal stalk complexes in yeast by Fluorescence micro-microscopy techniques; CSIC, Madrid, Instituto de quimica fisica Rocasolano Dep Quimica Fisica Biologica

(3) Dynamic remodeling of scafforld interactions in dendritic spines controls synaptic transmission; IGF, CNRS Montpellier

(6) Analysis of G protein-coupled receptor oligomerization in native tissues-Importance of monomer/oligomerization equilibrium; IGF, CNRS Montpellier

(8) Probabilistic differentiation in the Bacterial Heat-shock response, Dajkovic, Faculté de Médecine, Paris

(9) Detection of GFP-DspA in the yeast S. cerevisae, Barny, INRA paris

Additional grant: None for the moment

BORDEAUX

LP2N - Institut d'Optique Graduate School

<u>FBI support</u> : salary for Pierre Vermeulen (Research Engineer) Supervision & development: Brahim Lounis and Laurent Cognet

<u>Recruited personnel</u>: Pierre Vermeulen – 2 years (FBI) <u>Other personnel</u>: Antoine Godin (Postdoc LP2N)

Objectives of the project:

Our objectives are to achieve functional imaging in super-resolution microscopy techniques and single molecule related techniques. In particular, using uPAINT, we aim at demonstrating and developing the first FRET based super-resolution microscope in order to study interacting molecules. We also wish to provide to the community a correlation based photothermal microscope to study diffusing nanoparticles.

Scientific projects (tile & collaborator name):

(a) User Friendly Photothermal Microscopy Prototype for User Plateform, Lounis/Cognet (LP2N)(b) SmFRET-uPAINT Single molecule FRET+UPAINT, Cognet/Lounis (LP2N)



PARIS CENTRE

IJM/IMAGOSEINE

<u>FBI support</u>: Equipment: 10000€ (beam scanning) + 80 000 € (novel Spinning disk with FRAP head)

Supervision & development: Nicolas Audugé (IR CNRS), France Lam (IE CDD CNRS) (user support)

<u>Recruited personnel</u>: To be recruited (30 %, shared with WP1-a & d activities, Bioimage analysis) <u>Other personnel</u> (name, status and lab): Nicolas Borghi (CR CNRS), Philippe Girard (MCU P7), Karen Uriot (PhD candidate), Charlène Gayrard (PhD candidate), Cynthia Seiler (IE CDD ANR), 2year research fellow (ANR, to be recruited 2014).

Objectives of the project:

We want to implement scanning capabilities on our system that performs FCS/FCCS and FLIM-FRET to alleviate or remove several distinct problems often encountered in standard FCS: poor statistical accuracy in measurements with slowly moving molecules, photobleaching, optical distortions affecting the calibration of the measurement volume, membrane instabilities, etc. Simultaneouly, we want to keep the capacity to access the fluorescence lifetime of the tagged protein for FLIM-FRET measurements.

In addition, we want to expand the range of excitation wavelengths on a home-made TIRF system to perform smFRET in adhesion proteins.

<u>Scientific projects</u> (tile & collaborator name): Ongoing:

Focal Adhesion mobilities and interactions. N. Audugé, N. Borghi, M. Coppey-Moisan (IJM).

Photonic crystals for enhanced FCS/FCCS excitation. N. Audugé, coll. A. Levenson and A. Giacometti (LPN, CNRS UPR20)

Live-cell imaging of cytoskeleton regulators activity during Shigella invasion. N. Audugé, coll. G. Tran Van Nhieu (Collège de France, Paris)

Live cell imaging of B cell antigen receptor signaling cascade. N. Audugé, coll. J. Yang (Max-Planck-Institute of Immunbiology, Freiburg).

Future:

Interactions and tensions in adhesion proteins. N. Audugé, N. Borghi, coll. R. Seddiki, B. Ladoux, RM Mège (IJM)

Mechanotransduction and signalling at Adherens Junctions. N. Borghi, N. Audugé, C. Gayrard, C. Seiler (IJM)

Mechanotrnsduction and signalling at the nuclear envelope. N. Borghi, N. Audugé, C. Seiler, coll. E. Gomes (UPMC, IMM Lisbon), M. Balland (U. J. Fourier).

Mechanotransduction and signalling by Netrin receptors. P. Girard, N. Audugé, K. Uriot, coll. E. Bloch-Gallego (Institut Cochin).

Additional grant obtained/required:

Funding agency: CNRS "DEFI instrum. Limites" (pending)/ CNRS IJM Total amount: 30 000€ + 200 000 € (Spinning)



INSTITUT CURIE: Physico-Chimie Curie – UMR 168

<u>FBI support</u> : Equipments: 0 € ; Fonctioning: 0 € Supervision & development : Maxime Dahan (DR, CNRS), Davide Normanno (Post-Doc, Institut Curie)

<u>Recruited personnel</u>: None <u>Other personnel</u>: None

Objectives of the project:

Study of the dynamics of recruitment of individual proteins at specific nuclear loci and of the effect of local chromatin organization and re-organization with human chromatin remodeling factors. Quantification of the interplay between specific and off-target interactions of artificial proteins (TALEs) used for genome editing.

Scientific projects (tile & collaborator name):

Study of chromatin remodeling factors – B. Burman and T. Misteli (Center for Cancer Research, National Cancer Institute, NIH, Bethesda, MD - USA)

Study of nonspecific interactions of TALE proteins – J. Lopes (Muséum National d'Histoire Naturelle, CNRS UMR 7196 / INSERM U565, Paris)

Study of chromatin accessibility at the X inactivation center – L. Giorgetti, T. Pollex, and E. Heard (Institut Curie, CNRS UMR 3215 / INSERM U934, Paris)

Additional grant: None

INSTITUT PASTEUR/IMAGOPOLE

FBI support : Equipment:, Functioning:

Supervision & development; Spencer Shorte; Joe Dragavon (user support)

<u>Recruited personnel</u>: Joe Dragavon (1/1/2013 – 28/2/2014) Other personnel:

Objectives of the project:

The primary drawback of the current FLIM-SD device is the lack of system automation. Currently the exchange between the FLIM-SD and traditional SD imaging is performed manually, making time-lapse imaging difficult and cumbersome In the upcoming months efforts will be given to resolving this issue that will allow for the long-term visualization of living biological samples while providing multivalent information on host-pathogen interactions.

Scientific projects (tile & collaborator name):

3D mapping of the internal oxygen concentration of single cells during bacterial invasion, Ellen Arena (Philippe Sansonetti).

The determination of Avanti polar lipid accumulation upon actin modification in neuron and fibroblast networks, Soraya Victoria and Stéphanie Lebreton (Chiarra Surzolo).

<u>Additional grant</u> obtained/required:

Funding agency: Institut Pasteur, Pasteur Foundation, Nichols Foundation, Institut Carnot, ANR, Region Île de France, Conny-Maeva Charitable Foundation. Total amount: 300 000 €



WP 1c - CLEM & SuperCLEM

BORDEAUX

BIC (UMS CNRS 3420, US INSERM 004) / IINS (UMR CNRS 5297) / LBM (UMR CNRS 5200)

FBI support: Equipment: 40 269 €, Functioning: 23 544 € (Lucie GEAY salary)

Supervision & development: Landry Marc, Prof. Univ. Bordeaux, IINS/BIC ; Moreau Patrick, DR-CNRS, UMR5200/BIC ; Gontier Etienne, IR Univ. Bordeaux, BIC; Brocard Lysiane, IR Univ. Bordeaux, BIC.

<u>Recruited personnel</u>: GEAY Lucie (User support, ITA , 15/10/2013-14/8/2014) <u>Other personnel</u>: personnel from BIC and some people from IINS and LBM involved.

<u>Objectives of the project</u>: Correlative Light Electron Microscopy (CLEM) in various experimental models in the domains of *neurosciences and plant sciences*:

-Development of "In-Resin Fluorescence" approaches (IRF).

-Development of Super-CLEM to correlate high resolution photonic microscopy with EM. -Development of 3D-CLEM to correlate photonic microscopy with electron tomography.

Scientific projects:

Regarding the R&D activities, based on proposed development projects, the work that has been carried out was as follows:

At first, our work consisted in developing the method of Neuronal Culture on Photo-etched coverslips suitable for the Correlation with TEM. We have performed the 3D CLEM after cryo-fixation project as follows:

In anticipation of the 200kV TEM acquisition that will allow to perform tomography studies, the electron imaging facility of the BIC was able to develop one protocol for high pressure freezing on neuronal culture and 2 Freeze-substitution protocols, one dedicated to morphological study and another for immune-detection studies. <u>These methods are now available in routine as a service to</u> <u>users</u>. The acquisition of tomography softwares has just been achieved in order to implement the important Tomography analysis which is under progress in the BIC plant imaging and electron imaging facilities.

Intra-node collaborations:

1/ Collaboration with Landry's Team in IINS: the objective is to study the changes of GABAB receptor distribution with correlative microscopy to explore possible disinhibition mechanisms in chronic pain conditions. The technical objective is to establish the correlation in neuronal cultures endogenously expressing recombinant GABAB-GFP by using squared glass support.

2/ Collaboration between the BIC plant imaging facility and electron imaging facility for the development of "In-Resin Fluorescence" approaches (IRF). The aim of the project is to keep fluorescence on biological samples. For that, the BIC plant facility tests plant samples containing YFP or m-cherry fluorochromes, with chemical fixation or HPF. The electronic facility tests animal tissue samples containing GFP or rhodamine, also with chemical fixation or HPF. Regarding chemical fixation, we are focusing our developments on protocols using lowicryl resin embedding. 3/ Collaboration with Moreau's Team in LBM and the BIC photonic imaging facility: STED on plant tissue samples has been succeeded.

Communications and Training courses:

Etienne Gontier has participated in 2012 to the France-BioImaging Kick-Off meeting held at the Institut Curie, Paris. Lysiane Brocard has participated in 2013 to the national workshop on CLEM, Institut Curie and Institut Pasteur (October 21st- 22nd) supported by GDR 2588 and FBI.
 Marc Landry is also member of the Training and dissemination WP5.

<u>Additional grant obtained/required</u>: Complementary financial support will be purchased from the next CPER program 2015-2020, National Research Organisms and Region Aquitaine



PARIS CENTRE

IBENS

<u>FBI support</u>: Equipment: 72 486 euros. Functioning: Supervision & development:

Recruited personnel:

Other personnel: Rostaing Philippe, research engineer INSERM

<u>Objectives of the project</u>:Molecular organization of synapses in normal and pathological conditions using innovative technologies.

Scientific projects:

Electron microscopic studies on central nervous system are often used to characterize the subcellular organization of molecular complexes of synapses. Recently, we have developed a method using high pressure freezing , allowing instant freezing of unfixed and non cryo-protected tissues with few crystal formation, before electron tomographic reconstruction.

However, dehydration of tissues alters the cellular structure with extraction and precipitation of cytoplasmic proteins. In order to avoid these artifacts, cryoultramicrotomy allows to realize ultra thin sections of frozen hydrated tissue.

For our current projects, we need to access to the number of molecules of gephyrin at inhibitory synapses in spinal cord. We want to analyze data collected on a same specimen both at photon and electron levels.

Quantitative and 3 D analysis based on single-molecules detections showed that there is a close correspondence between the spatial organization of gephyrin scaffolds and inhibitory receptors at spinal cord synapses.

The correlative light and electron microscopy allows to directly measure absolute number of molecules in correlation with the structure of the synapse, the function and synaptic plasticity. We are able to combine live-cell imaging and ultra-structure or immunolabeling on ultra thin cryosections.



WP 1d - New Contrast and In-Depth Imaging

MARSEILLE

IBDM

FBI support : Equipment: 105 k€. Functioning: 40 k€

Supervision & development; (user support) Pierre-François Lenne & Claire Chardès <u>Recruited personnel</u>: IR to be recruted Sept 2014 - May 2017

Other personnel: Research fellow, Labex June 2014-May 2016

Objectives of the project:

- Providing a versatile fast imaging system for developmental biology

- We will develop and implement Structured illumination in light sheets.

<u>Scientific projects</u> (tile & collaborator name): New physical approaches to dissect the principles of apical junction supramolecular organization in cancer epithelial cells (A. Le Bivic, IBDM) The Biomechanics of Epithelial cell and Tissue morphogenesis (T. Lecuit, IBDM) In vivo mechanics during tissue morphogenesis (PF Lenne, IBDM) Polarisation and binary cell fate decisions in the neuronal system (V. Bertrand IBDM)

INSTITUT FRESNEL

<u>FBI support</u> : Equipment: 389383€:, Functioning: 75545€ Supervision & development: Hervé Rigneault (DR1-IF)

<u>Recruited personnel</u>: J. Savatier (IR, 01/12/13-30/11/14), H. de Aguiar (IR, 01/04/14–31/03/15) <u>Other personnel</u>: P. Guenuche (Research fellow), J. Wenger (CR1), S. Brasselet (DR2), J. Duboisset (MCF AMU), E. Andesen (Research fellow), P. Ferrand (MCF AMU), S. Monneret (CR1)

Objectives of the project:

Following the work done during the first working period we wish to develop

- Wavefront control for in-depth imaging in scattering tissue
- Nonlinear imaging resolved in polarization
- Phase imaging resolved in polarization
- Enhanced FCS by nano-antennas
- Lensless endoscope for in-depth imaging

Scientific projects (tile & collaborator name):

- Molecular oder: receptor trans-membrane proteins, lipid probes (S. Brasselet IF, D Marguet CIML)

- Molecular order of actin filament in furrow canals of drosophila embrios (S. Brasselet IF, T. Lecuit IBDML)

- Molecular lipd probe engineering for near infrared detection imaging (Olivier Maury ENS Lyon)
- Molecular order in spinal cord myelin (S. Brasselet IF, F Debardieux INT)
- Multimodal NLO imaging of melanoma (H. Rigneault IF, D Marguet CIML)
- Imaging drug penetration in skin (H. Rigneault IF, L'Oreal & Galderma)
- Deep brain imaging (H. Rigneault IF, R Cossart INMED)

Additional grant obtained: EU FP7 ITN – FINON – Nonlinear optical imaging – 500 000€ Fondation pour la recherche médicale – Deep brain imaging – 250 000€ Amidex Aix-Marseille Univ – Light in Bio – 112 500€



BORDEAUX

LP2N / PHOTONIC, DIGITAL & NANOSCIENCE LABORATORY

<u>FBI support</u> : Equipments: 400k€ ; Functioning: 100k€ Supervision & development: Brahim Lounis, PRCE LP2N

<u>Recruited personnel</u>: Pierre Vermeulen (IR, 01/11/2013 – 31/11/2015) <u>Other personnel</u>: Laurent Cognet (DR2, LP2N)

Objectives of the project:

Following the work done during the first working period we wish to:

- Achieve the assemblage of the different parts of the photothermal machine prototype
- Test the performances of the system
- Install a system on a commercial confocal microscope
- transfer a prototype to an bioimaging platform

IdF SUD NODE

ECOLE POLYTECHNIQUE-LOB

FBI support: <u>Equipment</u>: 378 600 €. <u>Functioning</u>: 100 000 € Supervision & development Emmanuel Beaurepaire

<u>Recruited personnel</u>: C.Stringari (method developments, research fellow, 01/01/14 - 01/12/14) <u>Other personnel</u>: LOB CNRS UMR7645 : MC.Schanne-Klein (dvt, DR), W.Supatto (dvt, CR), M. Joffre (dvt, DR), N.Vuillemin (dvt, PhD candidate), S.Psilodimitrakopoulos (dvt, research fellow), X.Solinas (dvt, IE), JM.Sintes (dvt, IE)

Objectives of the project:

Following the work done during the first working period we will work on the following

- Wavefront control & adaptive optics for in-depth tissue imaging
- Nonlinear polarimetric imaging (SHG, THG)
- Multicolor 2PEF imaging and its combination with coherent contrasts

Scientific projects (with external teams):

* Multiphoton imaging of engineered tissue - I.Georgakoudi, Tufts University, Boston, USA.

* SHG imaging of corneas - F. Behar-Cohen & JL. Bourges, Centre de Recherches des Cordeliers, Hôtel-Dieu Hospital, Paris.

* Multicolor 2PEF imaging of nervous tissue development - J. Livet, Institut de la Vision, Paris & X.Morin, IBENS, Paris.

* Multiphoton imaging of brain samples - G. Latour & F. Pain, IMNC, (Orsay)

* Multiphoton imaging of bone - D. Débarre & A. Gourrier, LIPhy (Grenoble)

Additional grant obtained: Equipex Morphoscope2 project - start 12/2013.



PARIS CENTRE

NEUROPHOTONIC LABORATORY/ UNIV PARIS-DESCARTES

FBI support : Equipment:, Functioning:

Supervision & development; Valentina Emiliani (*Wave front engineering microscopy group*; DR)

<u>Recruited personnel</u>: R. Conti (1P holographic light patterning & electrophysiology, IR 01/14-12/14); V. Zampini (2P holographic light patterning in vivo & in vitro, IR 01/14-01/16) <u>Other personnel</u>: E. PAPAGIAKOUMOU (in vivo & in vitro 2P holography, CR INSERM), C. TOURIN (electrical circuits, AJT), V. DESARS (software developement 1P & 2P holography, IR); M. GUILLON (1P and 2P holography, MCU); E. Ronzitti (video rate Hilo Microscopy; 2P in vivo imaging & photoactivation, research fellow); E. Chaigneau (2P in vivo imaging & electrophysiology, research fellow). A. Foust (2P imaging & photoactivaiotn in vitro, research fellow).

<u>Objectives of the project</u>: Development of 1P and 2P optical microscopes for patterned photoactivation *in vitro* and *in vivo*.

Projects:

Research: The activity of the group will continue to be focused on the design of optical systems for 1P and 2P patterned photoactivation. The main goal of the next years will be to demonstrate the validity of these approaches for *in vivo* application on head restrained and freely moving mice. The use of the microscope for imaging and activation on zebrafish larvae will be also tested. Finally the use of patterned light for imaging of voltage sensitive dyes will be tested.

Technology transfer & Relation with industry: The commercial prototype for 1P holography will be

opened the more and more to external users.

Starting from 2015 the system will be also for a one week training course on the principle and use of holographic light patterning.

The upgrade of the system for 2P imaging and photoactivation is in progress and should be finished by the end of 2105.

ENS/IBENS

FBI support : Equipment: 110090 euros, Functioning: 77 780 euros

Supervision & development : Laurent Bourdieu (DR CNRS, dvt), Stéphane Dieudonné (DR INSERM, dvt); Benjamin Mathieu (IE INSERM, dvt & user support)

<u>Recruited personnel</u>: Walther AKEMANN (IR, 02/05/2013 - 01/05/2016) <u>Other personnel</u>: Jean-François Léger (CR, CNRS, IBENS), Cathie Ventalon (CR, CNRS, IBENS)

Objectives of the project:

Once the 3D-ultrafast two-photon microscope will be setup, we will first continue to improve its performance by implementing dual paths for either two-color imaging or simultaneous imaging and opto-genetic stimulation. We will in parallel use this new microscope for in vivo studies of plasticity and coding in the cerebellum and the cortex. Finally, we will implement adaptive optics strategies to improve depth penetration on scanning microscopes.

Scientific projects (tile & collaborator name):

1) Implementation on the new 3D two-photon microscope of simultaneous wavefront control and 3D addressing for optimal imaging and opto-genetic stimulation.

2) Use of the 3D two-photon microscope for neurophysiology studies: it will include (this list being obviously not exhaustive),

- the recordings of neuronal activity in S1 and M1 cortices during active sensation in awake head-restrained behaving mice (collaboration L. Bourdieu and C. Léna's groups);



RANCE-BIOIMAGING

- the study of plasticity rules at the parallel fiber - Purkinje cell synapse (collaboration S. Dieudonné, M. Casado)

3) Implementation on different scanning microscopes of the IBENS platform (confocal, 2-photons) of adaptive optics strategies to improve the depth penetration and the control of the point spread function in the volume of a tissue.

Additional grant : None at this time. Application to new grants is planned in 2014

IJM/IMAGOSEINE

FBI support: 150 000 €

Supervision & development: Philippe Girard (MCU-P5), Nicolas Tissot (IE), Olivier Blanc (IR)

<u>Recruited personnel</u> (name and duration): Nicolas Tissot (IE 04/2012-04/2015) Olivier Blanc (IR-CNRS), to be recruited (30% shared with activities of WP1-a & b, BioImage analysis) <u>Other personnel</u> (name, status and lab): Orestis Faklaris (IR-CNRS), Aude Jobart-Malfait (IE-INSERM), Xavier Baudin (IE-CNRS)

Objectives of the project:

Short-term: Validation of 2PEF, SHG, THG, photomanipulation techniques (activation, ablation, conversion).

Mid-term: (1) We will develop the multimodal multiphoton microscope in order to do intravital imaging, and CARS (collaboration with Institut Fresnel, Marseille).

(2) We are developing a home-made Light Sheet Microscope called MacroSPIM dedicated to study thick samples in clearing medium (mouse brain, old stage mouse embryo, etc...) with low photobleaching.

Long-term: The second project aims to set up a Light Sheet Microscope with 2Photons illumination (Insight Laser) to perform PALM in thick samples (collaboration with Paris Descartes, Paris-Centre).

Scientific projects (tile & collaborator name):

-Membrane dynamics and vesicular traffic (T. Galli)

-Migration of transient populations of neurons (A. Pierani)

-Relationship between nucleus and microtubules arrays in drosophila oocyte (A.Guichet)

-Development of mouse embryo: how identical cells come to adopt different identities (J. Collignon)

-Mechanics of cell adhesion and mechanosensitivity at cell-cell junctions (B. Ladoux)

-Tissue mechanics: create blood vessel and force propagation in 3D active tissue (C. Wilhelm)



INSTITUT CURIE (PICT-IBISA/UMR144)

FBI support: Functioning: 20 000 €

Supervision & development: J. Salamero (DR1 CNRS); P. Chavrier (DR1 CNRS); F. Waharte (IR2 CNRS)

<u>Other personnel</u>: Marie Irondelle (IE-Pic BIM UMR144/PICT, 31/12/2012 - 31/12/2014); Sylvie YU (IE-PICT/Nikon) (01/10/2013-01/10/2014)

Objectives of the project:

- Building the NLO Intravital imaging platform with all needed environmental devices for high-resolution imaging over periods of up to 8 hours. These constraints require that:

1) Animals are positioned stably on imagers with devices necessary for anesthesia, perfusion, and monitoring of vital signs (ECG, RMIC, SpO2, ...)

2) Acquisition of images is synchronized with the respiratory movements of the animal and images recalibration (online image registration) based on these movements.

3) At any time of the imaging cycle, checking the constants of the animal, withdrawals or injections.

- Characterization of performances in axial and lateral resolution and other tests and measurements to assess the added value of the AZC2MP prototype and its coupling with AO for identifying rare events in a complex environment (a cell in the multicellular context) or to monitor the growth of tissue or tumor development.

- Tests of multimodal imaging (TPEF/SHG/THG) on A1RMP and AZC2MP.

Scientific projects (tile & collaborator name):

-Continuation of started projects (see ongoing projects in WP1d achievements)

-Use of Harmonic Nanoparticles in tumor progression and cell migration (L. Bonacina, GAPbiophotonic, University of Geneva; EPFL)

-Centrosome duplication and tumorigenesis in Mutant-mouse model (R. Basto, UMR 144)

Additional grants obtained/required:

Canceropole IdF DIM-Cancer "CaNOLI project"; FRM-Grands Equipements; Inca: "MP microscopy in cancer research"; Inca: "Image data base CID: sharing and processing very large data sets". Total budget: 2.8 M€, until end of 2015. Waiting for SIRIC support (march 2014). ANR Swiss/France



WP 2 - High Throughput & High Content Screening

IdF SUD

BIOMERGENCES/IMAGIF

FBI support : Equipment:, Functioning: Supervision & development; (user support) IDFsud has no specific funding for this project.

Recruited personnel: none

<u>Other personnel</u>: Sylvia Bruneau (IE, INRA Amagen), Louise Duloquin (CDD Sympactis, prestataire pour BioEmergences), Nadine Peyriéras (DR CNRS N&D), Raphaël Rodriguez (CR CNRS, ICSN), Beatrice Satiat-Jeunemaitre (DR CNRS IMAGIF)

Objectives of the project:

Multistep phenotypic screening with non-mammalian and non-vertebrate model organisms

The aim of the present project is to develop and implement a workflow of 3D+time image data acquisition and computation analysis for high content phenotypic screening of developing embryos. The proof of concept of the BioEmergences PharmaTox screening workflow is established in collaboration with the Institute of Chemistry Natural Substances http://www.icsn.cnrs-gif.fr/. The ICSN, known for the development of the Taxol as an anticancer drug, is in great need of the complete renewal of its drug screening strategies based so far on the assessment of cell survival in cell cultures. Phenotypic drug screening on developing embryos in the context of the 3Rs principles is expected to readily lead to the identification of potential hits in signaling pathways conserved throughout evolution including in human. We will standardize, systematize and robotize a screening chain from the observation of macroscopic phenotypes to the observation of cell behaviors by 2-photon microscopy and automated processing of 3D+time image data and the quantification of cell displacements, shape and fate changes. The project will deliver its protocols and a database gathering raw and processed data for further evaluation, comparison and modeling, leading to a new paradigm in fundamental and applied biological investigations.

Scientific projects (title & collaborator name):

Project ID: BioEM1

Multistep phenotypic screening with non-mammalian and non-vertebrate model organisms Collaboration: Nadine Peyriéras (BioEmergence FBI), Raphaël Rodriguez (ICSN), Béatrice Satiat Jeunemaitre (Imagif FBI).

<u>Additional grant</u> obtained/required: no additional funding so far, the project is funded on the BioEmergences and IMAGIF platform resources and uses FBI tranche 2 for the maintenance of a Leica LSI matrix screening microscope.



MONTPELLIER

MRI

FBI support : Equipment:Tecan robot for plate handling and upgrade of automated microscope (Cellomics) Functioning: funds for siRNA libraries, consumables

Supervision & development; (user support)

Recruited personnel Julien BELLIS (feb. 2013 for 3 years)

<u>Other personnel</u> : Cédric HASSEN-KHODJA (CDD IE nov.2013 for 1 year), Frédérique LIONNETON (IR2 CNRS, MGC facility), Virginie GEORGET (IR2 CNRS, MRI facility), Edouard BERTRAND (DR1 CNRS, IGMM and MRI)

Objectives of the project:

Our general aim is to two-fold: (i) to provide a service for cellular phenotypic screening using drugs and RNAi, using HCS; (ii) to develop innovative screening approches, in particular using smFISH.

The MRI screening facility is expanding its present expertise in automatic image acquisition/analysis with a service of plate preparation/handling for RNAi, expression and drug screening experiments. Of not, we have access to a collection of 17 000 human ORFs cloned in high-throughput cloning Gateway system. The major goal is to deliver an integrated screening service for the Montpellier scientific community spanning all steps : siRNA and ORFs library management, plate design and making, cell plating, fixation/immunocytochemistry, image acquisition/analysis and finally statistical analysis. While our primary target is the analysis of cellular phenotypes through high-content image analysis, the screening facility can also deliver simpler read-outs such as a fluorescent or luminescence measurement for *in vitro* or *in vivo* assays. Our focus will be on targeted small libraries of genes opposed to a genome wide approach. Having a strong expertise in imaging put us in a very good position to tackle technical problems associated with automatic imaging and image analysis. We aim to develop even further our specificity in imaging by integrating "intelligent acquisition" in the near future, in particular using Leica microscopes equipped with MatrixScreener.

Besides this "general purpose" screening activities, our aim is to develop an innovative screening approach using single molecule FISH (smFISH). This technique allows to detect every molecule of a given mRNA in native, fixed cells, and it thus a great tool to study gene expression, and its perturbations. Compared with traditional approaches, SmFISH has the great advantages to provide information about mRNA localization, absolute mRNA quantification (in copy number /cell), and its cell-to cell variability. The use of smFISH in high-throughput mode will certainly open new doors to study gene expression.

<u>Scientific projects</u> (title & collaborator name):

1) screening of chemicals on CDK-cyclin activity reported by fluorescent sensor (in vitro assay)-May Morris Montpellier

2) HCS of siRNA family of genes on cell cycle assay-Bénédicte Delaval CRBM Montpellier

3) High Content Screening of siRNA family genes on smFISH techniques - Edouard Bertrand IGMM Montpellier

4) large scale identification of host factors involved in *Coxiella burnetii* infections- Matteo Bonazzi - CPBS Montpellier

Additional grant obtained/required:

Funding agency: 90 k€ from FRM (co-funding for the spinning disk), 30 k€ from MRI (for small equipments) and 37 k€ from MRI (for 1 CDD salary). 300k from FRM (for HT-smFISH) Total amount: 457 k€


BORDEAUX

IINS, Interdisciplinary Institute for Neuroscience

FBI support : Supervision & development: Daniel Choquet, Jean-Baptiste Sibarita

<u>Recruited personnel</u>: <u>Other personnel</u>: F. Cordelières (IR-CNRS)

Objectives of the project:

The IINS was created in 2011 to gather, in a unique spot, forces from both the neurobiology and cutting edge imaging techniques. One of our goals is to study, at the single molecule level, processes conducting to synaptic plasticity. Unravelling receptor dynamics, as part of multi-parametric assays, requires expertise in cell biology (D. Choquet, D. Genuer, E. Hosy), microscopy setup development (M. Bouazzi, JB. Sibarita) and automated image processing (A. Beghin, F. Cordelières, JB. Sibarita). Based of our expertise in high-density single molecule tracking (sptPALM and uPAINT) and advanced analysis methods (PALM_Tracer software), we are currently upscaling our acquisition and analysis capabilities towards HCS.



INTER NODES PROJECT

Supervision & development; Thomas Walter and Edouard Bertrand

General objectives of the project :

(1) Display facility information at the FBI web site in a structured and uniform way, such that FBI HCS activities appear as a whole: each Screening Facility should describe the service and resources (siRNA, Gateway ORFs, drug library) they offer in a structured way. This is supposed to be a very concise representation and hence complementary to the list of equipment we have already provided.

(2) High-Content-Screening Club: meeting 3 times a year. Corresponds to a Screening Technology Watch, and also a nest to initiate collaborative projects. First Meeting: January 28, 2014, where participants have presented their recent developments. In the future, we see these rather informal meetings as a useful way of discussing recent developments by ours and other groups and sharing our expertise in the field of HCS.

(3) High Content Screening Course: offered once a year by one of the platforms, ideally funded by FBI. With a maximum of 30 people, this course should allow course participants to perform a small screen and cover all aspects of HCS, including data analysis. We would like to underline the fact that adequate training is essential for HCS projects in general, but also beneficial for the visibility of the facilities inside our work group, and that much can be gained if we have a community effort to provide this training.

(4) International workshop on HCS. This event will be organized by Biophenics and will take place next 27 and 28th November 2014 and will be open to 150 participants.

(5) Exchange and sharing of ressources, such as siRNA, ORFeome, HT-cloning, & drug libraries, protocols, assays, etc...

(6) Initiate and develop specific, collaborative projects (see below).

<u>Suggestions for improvements</u>: The objective of this workgroup is to appear as a provider of a screening service to the community, where the France BioImaging web site serves as an entry point for the screening facilities. A few improvements could make this even more efficient:

(1) Organization of the FBI website. Currently, the organization is on a per-node basis. It might be more helpful for users to also have a technology-centered presentation, so that all entities offering a certain technique are listed. From a user perspective, it might be advantageous that users can directly access some technology menu, where they can choose a suitable technology and – in a second step – see the offers available in the different nodes.

(2) The procedure of project proposal submission and selection could be more transparent.

Specific projects:

Data storage and management. A main internode project will be with the IPDM (Image Processing & Data management) about two aspects: storage of dataset produced during the screening projects and management of analysed data associated with the datasets. Solutions exist for the data management aspect but they are not tailored for the needs of screening projects. Active discussion will be engaged between members of the WP2 and the IPDM to develop a resource that could fulfil both needs for regular and screening users.

Gene expression analysis by high-throughput smFISH. This project was initiated by the Montpellier node (see above), but the creation of this FBI WG has given the opportunity to developing it further, through an inter-node collaboration between Montpellier and Paris-Centre (Thomas Walter, Christophe Zimmer). Analysis of smFISH images requires dedicated tools, some of which being already available. However, systematic quantification of RNA localization and gene expression in single cells will require a dedicated pipeline, and the expertise of the Paris-Centre in this matter will be key to correctly modify and adapt current algorithms initially developed for analysis of proteins. The unique expertise brought by FBI will be key to the development of this highly innovative tool to study gene expression, which will presumably be interesting for many researchers on the long run and – once its power has been demonstrated – will be made available for the scientific community.



WP 3 - Probe Development, Optogenetics & Optomanipulation

PARIS-CENTRE

ENS Chemistry: Optical actuators and reading out systems for living systems

<u>FBI support</u>: Equipment: 0€. Functioning: 48 750 € (from 2012 up to 2017) Supervision & development: Isabelle Aujard, Thomas Le Saux, Emmanuelle Marie-Begue (user support)

Recruited personnel: Not applicable

<u>Other personnel</u>: Isabelle Aujard (IR ENS), Arnaud Gautier (MC ENS), Zoher Gueroui (CR CNRS), Ludovic Jullien (PR UPMC), Thomas Le Saux (MC UPMC), Emmanuelle Marie-Begue (CR CNRS), Sylvie Maurin (AJT ENS), Christophe Tribet (DR CNRS).

Objectives of the project:

Three actions have been planned for 2014: (i) The implementation of a forum on the FBI web site about identifying, characterizing, or using optical probes or photochemical actuators. The three WP3 supervisors will forward questions from users to the best national expert(s), who will post an answer (or access to instrumental platforms); (ii) The installation of a committee of expert chemists over the whole French territory that will act as advisers/trainers for users; (iii) The collection of information to build a list of instruments and know-how available in the laboratories of the expert chemists belonging to the preceding committee. This delocalized facility will allow us to be more reactive for local users while benefiting from a national platform.

<u>Scientific projects</u> (tile & collaborator name):

(1) Photocontrol of protein degradation by means of optogenetics. A. Gautier et al (ENS Chimie), Paris.

(2) Fluorescence turn-on strategy to label proteins. A. Gautier et L. Jullien (ENS Chimie), Paris; D. Bensimon (ENS Physics)+other national (to be defined) and international (S. Weiss, UCLA) collaborations.

(3) Fluorescence turn-on strategy to label RNA. A. Gautier, L. Jullien, and T. Le Saux (ENS Chimie), Paris; D. Bensimon (ENS Physics)+other national (to be defined) and international (S. Weiss, UCLA) collaborations.

(4) Characterization of photoactivatable self-immolative spacers for sensitive detection of enzymatic activity. F. Schmidt (Curie Institute, Paris) and L. Jullien (ENS Chimie), Paris.

(5) Photoporable microcapsules to release active proteins. E. Bloch-Gallego (Cochin), C. Tribet (ENS Chimie)

Additional grant (pending): ANR, PSL research grant/ANR. Funding agency >250 000 euros



LABORATOIRE PHYSICO-CHIMIE CURIE – PCC Group: Optogenetic control of intracellular

signaling

FBI support: not yet supported

Supervision & development: Mathieu Coppey (CR CNRS)

Recruited personnel:

<u>Other personnel</u>: Maxime DAHAN (DR CNRS), Marouen Bouazzi (DMD software development, research fellow, CNRS-PCC), Amanda Remorino (Application to cell polarity, research fellow, EMBO-PCC), Simon De Beco (Application to cell division, research fellow, CNRS-PCC), Léo Valon (Application to cell migration, PhD candidate, PCC).

Objectives of the project:

For 2014 we planned to: i) open our technical and practical knowledge to the community by setting up a wiki site to connect the scientific community involved in intracellular optogenetics, ii) tighten our collaboration with the chemists of the WP3 to offer a unified view of optomanipulation, iii) finalize our optoechanical device optimized for optogenetic applications in order to make it available to the community (hardware and software), iv) identify new signaling targets for optogenetic application.

Scientific projects (tile & collaborator name):

(1) Optogenetic control of RhoA and subcellular force measurement. Mathieu Coppey, Martial Balland (UJF, Grenoble), and Olivier Destain (UJF, Grenoble).

(2) Optogenetic control of RhoA and forces generated by tissue. Mathieu Coppey and Guillaume Charras (UCL, London)

(3) Optogenetic control of RhoA and forces at cell junctions. Mathieu Coppey and Virgile Viasnoff (ESPCI, Paris)

Additional grant required (pending): ANR, FBI. Funding agency >250 000 euros



BORDEAUX

Interdisciplinary Institute for Neuroscience

<u>FBI support</u>: not yet supported Supervision & development: Matthieu Sainlos (CR CNRS)

Recruited personnel: not applicable

Other personnel: Isabel Gauthereau (AI, IINS), Dolors Grillo-Bosh (research fellow, IINS).

Objectives of the project:

Along with the other WP3 supervisors, three actions have been planned for 2014: (i) implementation of a forum on the FBI web site to foster exchange within the developers and user communities; (ii) Installation of a committee of expert chemists over the whole French territory that will act as advisers/trainers for users; (iii) Creation of a list of instruments and know-how available in the laboratories of the expert chemists belonging to the preceding committee.

Scientific projects (tile & collaborator name):

Photocontrol and observation of PDZ domain-mediated interactions, Matthieu Sainlos
Photocontrol and observation of SH3 domain-mediated interactions, Matthieu Sainlos

Additional grant required (pending): ANR. Funding agency >250k€

LP2N - Institut d'Optique Graduate School

<u>FBI support</u>: salary for Pierre Vermeulen (Research Engineer) Supervision & development: Brahim Lounis and Laurent Cognet

<u>Recruited personnel</u>: Pierre Vermeulen – 2 years (FBI) <u>Other personnel</u>: Zhenghong Gao (Postdoc, LP2N), Edakkattuparambil Shibu (Postdoc, LP2N)

Objectives of the project:

Our projects concern the development new probes for single molecule tracking in complex environments for deep tissue. These probes will be based on small short gold nanorods (5x10nm) with optical resonance in the near infrared or ultrashort nanotubes (less than 20nm) or fully organic nanoparticles. Their functionalization will also be developed.

<u>Scientific projects</u> (tile & collaborator name):

(1) Ultrashort carbon nanotubes, Laurent Cognet (LP2N), Laurent Groc (IINS) Brahim Lounis (LP2N)

(2) NanoTubesInBrain, Laurent Cognet (LP2N), Laurent Groc (IINS) Mireille Blanchard-Desce (external collaborator (ISM)

Additional grant required (pending):



MONTPELLIER

MARS/IPAM-IGF & P. Mollard's team

<u>FBI support</u>: Montpellier « MARS » node: Development of in vivo 2-photon microscopy fitted with both long range objectives and optogenetic tools

Supervision & development: Patrice Mollard (DR CNRS, Scientific leader IPAM)

(see www.ipam.cnrs.fr and www.ipam.cnrs.fr an

Recruited personnel:

<u>Other personnel</u>: Chrystel Lafont (IE INSERM, cellular in vivo imaging & microsurgery), Marie Schaeffer (CR INSERM, 2-photon microscopy), François Molino (MCU-UM2, mathematician, software and model development), Pierre Fontanaud (AI CNRS, software development), Patrick Samper (CNRS fixed-termed contract, functional investigation)

Objectives of the project:

For 2014 we will: i) continue to propose to IPAM users the already developed combination of optogenetic tools (Cre-Lox strategy & viral injection) and 1P-in vivo imaging with long range objectives (w.d. 2cm, x20 magnification) (Lafont et al., ms in preparation), and ii) begin to combine the use of optogenetic tools with 2-P in vivo microscopy fitted with long wd objectives (Schaeffer et al., PNAS 2013).

Scientific projects (tile & collaborator name):

(1) Optogenetic control of endocrine rhythms (ANR grant "Opto-Rhythms" 2011-2015, P Mollard (coordinator), P. Le Tissier (NIMR&UCL, London) and U. Boehm (Homburg Univ., Germ.))

(2) Optogenetic control of perivascular space (ANR grant "Peri-pulse" 2013-2017, P. Mollard (coordinator), R. Lovell-Badge (NIMR-MRC, London), V. Prévot (INSERM, Lille) and V. Goffin (INSERM, Paris)

(3) Optogenetic control of pancreatic islets (ANR JCJC "Beta-Dyn" 2014-2018, M. Schaeffer)

(4) Combination of in vivo 2P-microscopy&other tools with MRI (CPER-LR "BNIF" project, nanoNMRI team, Institut de Physique, CNRS UMR 5587, Montpellier)(C. Goze-Bac, coordinator) (P. Mollard, Partner)

<u>Funding</u>: FBI (equipment, 470K€; consumables, 35K€; Data storage, 21K€) + ANR grants (animal costs, consumables...).

Additional grant required (pending): CPER "BNIF" (200 K€). Total amount: 726 k€





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