Cell imaging undergoes a revolution

>>> Philippe COCHARD, CNRS senior scientist, Centre de Biologie du Développement, (CBD, joint UPS/CNRS lab) and Alain JAUNEAU, CNRS research engineer (Microscopy and Imaging Platform IFR40).

Over the past fifteen years, microscopy has developed considerably thanks to collaborative teamwork by biologists, mathematicians, computer scientists, physicists and chemists. New equipment and new techniques are continuously appearing, allowing researchers to image living specimens in three dimensions, real-time, in depth and with very high resolution.

The techniques have developed in two complementary directions: imaging molecules and their interactions, and analyzing them within complex samples, cells, embryos and even whole organisms. We can now observe how live cells behave under the objective of the microscope and even follow how proteins move and interact thanks to fluorescent markers derived from marine animals. Indeed, the Nobel prize in chemistry was attributed in 2008 to O. Shimomura, M. Chalfie et R. Tsien for their work in these fields. We can also interact with cells at the nanoscale by means of lasers. Future applications are many, and will only be limited by the imagination of researchers.

However, such spectacular developments mean that biology laboratories need to acquire the latest equipment if they want to remain competitive at the international level. But, modern optical and electron microscopes are expensive and require highly qualified staff. For this reason, the different microscope facilities in and around Toulouse were grouped together very early on in a regional platform of cell imaging called the Toulouse Imaging Network (TRI) - recognized at the national level by the GIS IBiSA.

This platform was initially set up at the Developmental Biology Center and then at the Institute of Functional Exploration of Genomes (IEFG, IFR 109). It now coordinates resources in optical imaging, electron microscopy and cytometry in the entire Midi-Pyrénées region at the facilities of the Research Federation in Biology of Toulouse (FRBT), of the Institute of Agrobiosciences, Interactions and Biodiversity (IFR 40, Auzeville Agrocampus), of the Medical Biology Institute of Toulouse (IFR 150), located on the campuses of Purpan and Rangueil regional hospitals, and of the Pierre Potier Center (ITAV). Six core facilities, with more than 30 teams and 20 engineers and technicians, welcome over 800 researchers each year.
Despite being spread across the region, the platform is managed through a quality approach (certification ISO 9001, obtained in January 2010), which allows it to function optimally. The platform's website (http://tri.genotoul.fr/) describes the activities, equipment and services available in all of the facilities, irrespective of their location. The platform is a member of Toulouse Genopole (http://genopole-toulouse.prd.fr/), a network that coordinates most large platforms in life sciences in the Midi-Pyrénées region. It is open to all researchers in the field, be they public or private. Its main missions are to provide scientists with expertise and high technology in cell imaging; to establish a framework for discussion and exchange in this rapidly evolving field; train researchers and students in all techniques related to microscopy; and to contribute to the development of new equipment and methodologies.

On the whole, the TRI platform provides skills, equipment and technical support for any project requiring cell imaging in its diverse forms. It has state-of-the-art equipment, some of which is unique in France, and even Europe. Examples include confocal and multiphoton microscopy, wide-field microscopy with deconvolution, fluorescence life-time imaging (FLIM), single-plane imaging microscopy (SPIM), high-resolution microscopy (TIRF), single-particle imaging, intra-vital and whole body imaging of small animals, tomography and cryo-methods in electron microscopy.

This report illustrates some of the research projects being carried out thanks to the resources available at the TRI's imaging platform.

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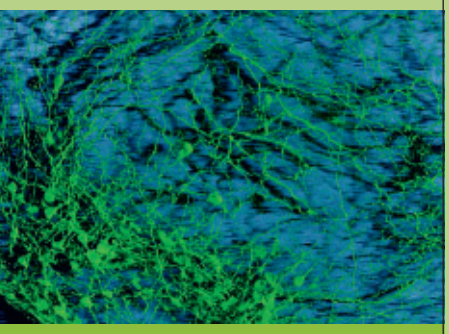

>>> Three-dimensional reconstruction of neurons and their processes labelled with the fluorescent protein GFP in a slice of rodent brain. Image acquired on a two-photon microscope. © B. Ronsin, A. Le Ru & A. Lorsignol.

CPTP: Centre de Physiopathologie de Toulouse Purpan / Research Center for Pathophysiology at Toulouse Purpan.
CBD: Centre de Biologie du développement / Center of Developmental Biology.
LIPM: Laboratoire des Interactions Plantes Micro-organismes / Plant-Micro-organisms Interactions Laboratory.
IPBS: Institut de Pharmacologie et Biologie Structurale / Institute of Pharmacology and Structural Biology.
LBME: Laboratoire de Biologie Moléculaire Eukaryote / Laboratory of Eukaryotic Molecular Biology.
ITAV: Institut des Technologies Avancées en sciences du Vivant / Advanced Technology Institute in Life Sciences.

How plants protect themselves from pathogens

>>> Laurent DESLANDES, CNRS scientist, and Susana RIVAS, CNRS scientist, at the Laboratory of Plant-Microbes Interactions (joint CNRS/INRA lab)

Similar to the immune system in animals, plants have developed complex defense mechanisms to protect themselves against constant attack by a variety of microbial pathogens, including viruses, bacteria and fungi. Modern imaging techniques have allowed researchers to elucidate the intricacy of the proteins involved in such attacks. Unravelling the molecular mechanisms behind plant immunity is a major scientific challenge that aims at eventually reducing the environmental risks associated with the use of chemicals to improve crop yield.

We are concerned with understanding the mechanisms behind plant resistance. We are investigating how a plant perceives the presence of its attacker on the cellular level and the mechanisms by which the plant is able to mount a specifically adapted defence response.

Our model system involves the plant Arabidopsis thaliana or "thale cress" and two bacteria, Xanthomonas campestris and Ralstonia solanacearum, which cause plant diseases responsible for agricultural losses amounting to millions of euros each year.

Like other bacterial pathogens, these two bacterial species are able to inject proteins into their host plant cells. These proteins are called effectors and truly resemble homing missiles that target plant proteins involved in defense, thereby neutralizing and short-circuiting plant immunity. In response to bacterial effectors, plants have developed surveillance mechanisms involving "guard" proteins that can detect the presence of either the effectors themselves or the effect of their action(s) on targeted plant proteins. Once the pathogen is unmasked, the immune response is triggered. Otherwise, the plant is not able to defend itself and the attacker gets the upper hand.

The plant cell nucleus: the site of the battlefield

We are currently working with protein pairs respectively coming from the invading bacterium and its host plant (in other words, the missile and its target). In close collaboration with the IFR40 Microscopy and Cellular Imaging Facility, physical interactions between different protein partners have been demonstrated thanks to the FRET-FLIM technique. This technique is based on measuring the transfer of energy between fluorescent molecules fused to the proteins of interest. One big advantage of FRET-FLIM is that protein-protein interactions can be revealed in living cells with intact cellular structures and we are therefore able to monitor the dynamic movement of protein complexes at the subcellular level.

We are only beginning our surprising journey in this field. Beyond characterizing true protein complexes, our work will bring to light molecular mechanisms of great complexity. These scientific advances are crucial for understanding the molecular mechanisms involved in plant-pathogen perception that determine the activation of the plant immune response.

To learn more: Froidure et al (2010) Proc. Natl. Acad. Sci. 107, 15281-15286; Tasset et al (2010) Plos Pathog. (in press).

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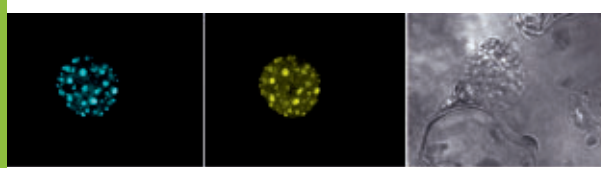


>>> A bacterial effector protein and its plant target respectively fused to CFP (Cyan Fluorescent Protein) and YFP (Yellow Fluorescent Protein) are transiently expressed in Nicotiana benthamiana cells. The FRET-FLIM technique allows to detect the energy transfer between the two fluorophores when they are fused to two proteins that are able to physically interact.

Real time morphogenesis

A major challenge in life sciences is to decipher how macromolecules that compose living species assemble together in organized three dimensional structures. Recent advances in cell imaging now allow real-time analysis of the changes in cell shape that occur during development and can unravel the underlying mechanisms.

>>> Members of the Morphogenesis and cell signalling team, Centre de Biologie du Développement (CBD, joint UPS/CNRS lab). From left to right, front row: Serge PLAZA, Hélène CHANUT, Delphine MENORET, François PAYRE, back row: Pierre FERRER, Yvan LATAPIE, Philippe VALENTI, Ahmad ALSAWADI, Emilie BENRABAH.

headline

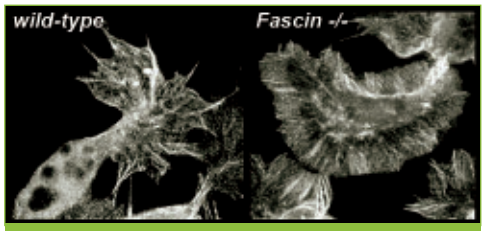
A fundamental feature of embryonic development is morphogenesis, the finely tuned control of the shape of cells, tissues, and organs, which is critical to their specific functions within an organism. Cells display a wide range of three dimensional organization, which when altered can lead to a variety of human pathologies, including cancers. The molecular mechanisms of morphogenesis are still poorly understood and require access to the dynamics of cell shape changes. Coupled with novel technologies, scientific advances now open the way to functional analyses in living cells using fluorescent imaging at high spatial and temporal resolution (5D).

Input from physics, information technology and even marine biology!

The last part of the XXth century saw multiple (r)evolutions in the conception of light microscopes, light (lasers), digital cameras and their computer-assisted control. Nevertheless, a major step in live imaging came thanks to the discovery of a fluorescent protein (Green Fluorescent Protein ou GFP) in jellyfish. Thanks to molecular genetics, it has now become possible to fuse any protein of interest with GFP derivatives, allowing researchers to explore its dynamic distribution and function in live cells.

Cell Division

Division in animal cells requires a stereotyped series of changes in their form, ultimately leading to the separation of two daughter cells. While it is well established that actin filaments are important in modifying cell shape, the mechanisms regulating reorganization of the actin network have not been fully elucidated. Our team at the Centre de Biologie du Développement, making use of dynamic analyses of the actin cytoskeleton in normal and genetically engineered cells, has recently discovered the role of Ezrin, Radixin and Moesin (ERM) proteins in controlling cell shape and chromosome segregation for cell division. The function of ERM proteins relies on a localized control of their activity and we are developing large-scale functional studies to identify the regulators involved and their specific functions.



>>> Living macrophages undergoing active migration in the drosophila embryo. Actin filaments are visualized through a GFP fusion protein, revealing alteration in the polarized organization of cells lacking Fascin (at right). © J. Zanet

Invasive migration during development

Shaping the developing embryo also requires the migration of specific cell populations that are guided by attractive gradients emanating from neighbouring tissues. Unravelling these mechanisms can thus only be done by directly analyzing cell migration within the whole embryo. Again, combining the use of fluorescent protein derivatives, live confocal imaging and genetic approaches, we recently identified the Fascin protein, as a key regulator for the shape of migrating cells. Fascin acts to bundle actin filaments in large intracellular cables that then support polarized organisation of migrating cells. In the absence of Fascin, cells become depolarized and are unable to properly migrate.

Both ERM proteins and Fascin are deregulated in various cancers, so causing aggressive metastases to form. Work on model organisms would thus help us better understand how their function is disrupted in tumours. Constant advances in live imaging at Paul Sabatier University will continue to bring novel insights into fundamental mechanisms of morphogenesis, directly relevant to human health.

Related publications: Carreno et al, 2008, Journal of Cell Biology, 2009 Aug; 136(15):2557-65; Zanet et al, 2009, Development, 2008 Feb 25;180(4):739-46.

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Fluorescence imaging of small animals

>>> Aurélie PAGANIN-GIOANNI, post-doctoral researcher and Elisabeth BELLARD, CNRS engineer, Muriel GOLZIO, CNRS scientist and Justin Teissié, CNRS senior scientist, all at the Institute of Pharmacology and Structural Biology (IPBS, joint UPS/CNRS lab).

Fluorescence imaging of small animals allows us to observe the cellular behaviour inside a live animal. The technique is very instructive, and above all ethical.

The technology complies with the 3R rule (Replacement, Refinement and Reduction), developed to limit the use of laboratory animals. Indeed, the same animal can be used over a pre-defined time period (with lapses of over several months) without sacrificing the animal, as is the case with classical end point methods. Reducing the number of animals necessary for reliable statistical data is not only economically important but also kinder to animals.

Optical imaging by fluorescence consists of visualizing the biological target thanks to molecules injected into an anaesthetized animal. These molecules are coupled with a fluorescent marker and bind to the targets and emit light.

The main technical difficulty in observing the interior of animals without surgically cutting them open involves light propagation in tissues -- non-homogeneous environments in which the inherent turbidity deviates incident and transmitted light beams and deteriorates the detection of emitted light signals. To overcome this challenge, we need to introduce mathematical corrections to take into account absorption, diffusion, reflection and refraction of photons. Therefore, the optimal optical window is located in the near-red region, between 600 and 1000 nm.

Whole body

The facility thus integrates highly advanced technologies (powerful illuminators, lasers, LEDs and ultra-sensitive cameras) to greatly increase the sensitivity of detection and spatial and temporal resolutions. We chose spectral imagery to eliminate autofluorescence and our studies were performed on a "Leica macroscope" or devices specifically designed for whole body imaging. In this way, we can locate the zones of expression of fluorescent tumours in the animal (for example, at the time of metastatic progression). In the future, we should also be able to visually target tumour tissues during surgery.

The skin itself also limits spatial resolution. One way to overcome this is to work in "intra-vital" mode where minimal surgery allows researchers to have direct view of the target body. An alternative is to mount a glass window in the place of skin.

Detection is performed on an anaesthetized animal placed under the stage of a biphotonic macroscope, a wide field microscope or a dedicated microscope with video recording. The major advantage is that the skin is not present any more in the optical path, so there is no absorption, reflection and autofluorescence. Resolution on the cellular scale is possible thanks to multiphotonic imaging, which makes it possible to resolve more in-depth emitted light signals because it uses wavelengths of excitation in near infrared that deeply penetrate tissues. This technique allows to reach a depth of 200 to 600 µm and three-dimensional images of very high-resolution are acquired. Out-of-focal plan fluorescence is eliminated too and there is less photodegradation and photo-toxicity as well.

ONIPA facility

ONIPA stands for non-invasive optical imagery of the small animal and many joint projects are currently running, such as imaging of metastatic progression of prostate cancer with Olivier Cuvillier and Bernard Malavaud. Biphoton microscopy allows us to observe the organization of both cancer cells expressing the GFP (a green fluorescent protein) and tumour blood vessels. It is possible to detect vessels by intravenously injecting rhodamine dextran (red fluorescence) into the animal. The images can then be analyzed by digital processing to determine permeability (via Labview) and vascular density. How these structures evolve in time provides information on the dynamics of various internal processes. One can, for example, observe how blood flow changes during tumour treatment (for example, electro-chemotherapy). Digitized imaging allows for a quantitative follow-up of how vessel membrane permeability evolves and enables us to detect trapped active injected drugs, all of which increase the effectiveness of treatment.

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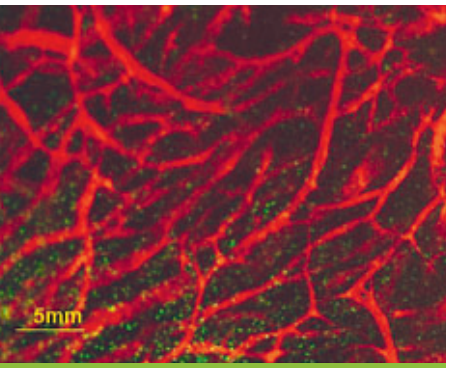

>>> Organization of tumour vessels of B16 melanomas: visualization by "intra-vital macroscopy". The injection of a red fluorescent molecule (rhodamine dextran) allows us to directly visualize the complex organization of vessels in the tumour.

Selective Plane Illumination Microscopy for 3D imaging

One of the challenges of biological optical imaging is to non-destructively visualize structures and organisms in three dimensions. A new instrument, based on selective plane illumination microscopy (SPIM), allows scientists to acquire multiples views of a live sample from different angles with minimum disruption.

>>> Valérie LOBJOIS, research scientist and Corinne LORENZO, engineer, working at the UMS3039- ITAV (joint UPS/CNRS/INSA lab).

SPIM works by shining a very thin slice of light through a sample and then systematically moving the specimen through the light sheet to capture images from each layer. The light sheet is obtained using a cylindrical lens. The technique, invented at the EMBL (in Heidelberg) by Ernst Stelzer and colleagues, allows scientists to image relatively large live samples (up to 3 mm in size) without the need to cut them up and fix them to a slide, as in conventional microscopy. What's more, SPIM gives a sharp image of the sample without the usual background blur because no out-of-focus light is created. And since only thin slices of light are employed, the sample suffers less light-induced damage

SPIM is also very fast - images can be obtained in just minutes. To further improve resolution and to correct for distortions that depend on the viewing angle, the sample can be rotated by 360° and scanned again. Combining these different views yields hitherto unparalleled 3D images of a sample.

Thick samples

At the moment, there are no commercial SPIMs. To address the need to image thick samples, we have developed a prototype SPIM with the help of cellular biologists specialized in imaging (ITAV/LBCMCP, Bernard Ducommun), mathematicians at the IMT (Jérôme Fahrenbach, Pierre Weiss) and computer scientists at the IRIT (Denis Kouamé).

At the international level, a scientific community specialized in SPIM is emerging, with the aim of sharing advances in this new type of microscopy.

Emerging microscopy

This new microscope technique is attracting the attention of scientists worldwide because it allows for a much deeper look into living organisms than ever before. It is particularly adapted to deep imaging of samples between several microns and several millimetres in size. Areas diverse as cellular biology (figure 1a), development biology (figures 1e-f), marine biology, plant biology and tissue engineering could benefit as the images below contest. These images were taken using the prototype SPIM at the ITAV imaging platform.

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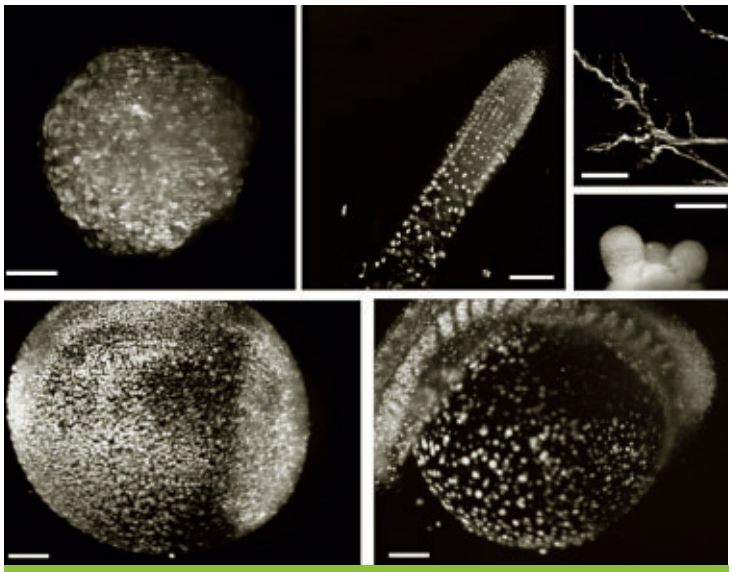


>>> Images of different model organisms obtained by the SPIM. Illumination objective is 10X NA 0.25, detection objective 10X physiological NA 0.3, ex 532 nm, em 560 nm. (a) Capan2-DsRed spheres, (b) Arabidopsis thaliana root, (c) Mouse embryo neurofilaments, (d) Arabidopsis thaliana meristem, (e-f) Zebrafish embryo. Scale 100 µm.

Shedding light on calcium

Calcium ions control several cellular activation processes in the body, particularly during embryonic development. This role has been studied by Marc Moreau's team at the Centre de Biologie du développement, who has found that is important during the formation of the nervous system (neurogenesis) and kidney development (nephrogenesis).

>>> Marc MOREAU, CNRS senior scientist, director of the GDR 2688, and Catherine LECLERC CNRS research scientist, both researchers in the Centre de Biologie du développement (CBD, joint UPS/CNRS lab).

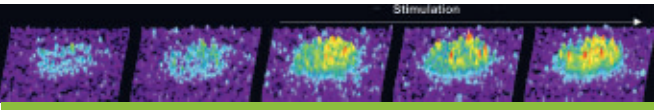
The South African toad, or Xenopus, is a star in the laboratory. With this animal, Marc Moreau's team study the role of calcium during development. The main focus of the research is on dynamic imaging, which has allowed the researchers to visualize, in real time, the movement of calcium movement in Xenopus embryonic cells. This innovative imaging technique works by counting emitted photons from a luminescent probe sensitive to calcium and measures in vivo calcium signals in a single cell in both time and space.

Channel opening

The imaging has shown, that in specified regions of the embryo, some cells trigger a spontaneous and transitory increase in intracellular calcium. These increases are modulated in space and time and correspond to specific calcium channels opening at the plasma membrane level. In the long-term, these very early elementary events will control specific gene transcription, something that irreversibly orientates the fate of cells in precise differentiation pathways. With this technique we have deciphered calcium dependent pathways involved at the origin of nervous system or kidney formation in the embryo.

Allergies

The elements involved in calcium signalling could also be a source of potential therapeutic targets in several pathologies. Indeed the characteristics of a calcium signal (the so-called calcium signature) in response to a given stimulus are different when we compare a normal cell to a diseased one. The modification of the calcium signature by pharmacological agents can induce a shift from a pathological to a normal situation in cells or organisms. This aspect of our research is illustrated by a fruitful collaboration with Lucette Pelletier's team at the Centre de physiopathologie de Toulouse Purpan (CPTP, UPS/INSERM). In this work we have shown that calcium signalling plays a crucial role during activation and differentiation of T-lymphocytes. Among these lymphocytes, the TH2 population is responsible for allergic diseases, the frequency of which are increasing in the modern world. During this collaboration we have identified calcium channels specifically expressed in TH2 lymphocytes that play a determining role in their function.



>>> Calcium movement through single calcium channels in a TH2 lymphocyte measured by evanescent waves (TIRFM). In these pictures the calcium flux through a single channel is coded in false colours. The blue corresponds to low calcium concentration and the red to higher concentrations. Cells have been stimulated by an antigen that binds to a receptor located at the membrane surface of the lymphocyte.

Evanescent waves

It is difficult to study the function of these channels using conventional electrophysiological techniques. A new imaging method that allows to monitor the activity of these channels has been developed: TIRFM (Total Internal Reflection Fluorescence Microscopy). With this technique, we can image an individual calcium ion channel (that is, one protein) by using evanescent waves on cells that have been preloaded with a calcium-sensitive fluorescent probe. This is a non-invasive technique providing simultaneous readout from hundreds of channels in real time. In addition, channel locations can be mapped at the membrane surface and the technique can provide high-throughput screening for channel-related drugs.

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Gene expression under the microscope

One of the major challenges in biology today is to place molecular biology processes in their cellular context to better understand how living structures are organized. Matching up these two scales, molecular and cellular, in living organisms is a challenge that could now be addressed.

>>> Isabelle LÉGER-SILVESTRE, assistant professor at UPS, Olivier GADAL, CNRS research scientist, Pierre-Emmanuel GLEIZES, UPS professor, Célia PLISSON-CHASTANG, CNRS scientist and Franck DELAVOIE, UPS assistant professor, all at the Laboratoire de Biologie Moléculaire Eucaryote, (LBME, joint UPS/CNRS lab).

Recent advances in microscopy and in situ molecular detection techniques have allowed scientists to match up cellular organization with cell function. At the same time, the structure of many macromolecules has been elucidated at the atomic scale thanks to X-ray crystallography or NMR. Developments in transmission electron microscopy (TEM) have also allowed scientists to detail a cell in 3D at the nanometre scale and to directly detect macromolecular structures inside cells. The images obtained using these techniques are closer to reality than ever before because the sample is prepared by ultrafast freezing and observed under transparent ice using electronic cryomicroscopy (cryo-TEM). This method is ideal for studying cellular structures or complex macromolecules.

Ribosome production

These advanced techniques are now available at the IFR109 electron microscopy platform and are used at the LBME to better understand the way in which ribosomes are produced. These molecular machines, made from RNA and proteins, catalyze the synthesis of proteins in all living organisms. The way ribosomes are produced is extremely complex and begins with the production of the RNA component from ribosomal genes. Isolated from the nucleus by chromatine spreading, a ribosomal gene can be visualized using TEM (figure A). The structure looks like a Christmas tree in which each branch corresponds to a ribosome being synthesized.

In a cell, these genes are repeated and assembled into a nuclear domain, the nucleolus, whose morphology reflects the state of production of the ribosomes (figure B). Analysis of these structures using electron tomography, currently under way, will allow us to add to our knowledge of how nucleolar chromatine is organized on a scale that lies between the molecular and the ultrastructure of the nucleole.

During its synthesis, RNA folds and assembles with proteins. Determining the three dimensional structure of the ribosomal particles (also known as pre-ribosomes) to better understand assembly mechanisms and transport to the cytoplasm requires the analysis of thousands of images of these particles isolated and observed under the transparent ice of the cryo-TEM. By coupling the analysis of these structures with electron tomography of cells, it should now be possible to localize the pre-ribosomes inside the cell volume directly and so understand the mechanisms behind their synthesis (figure C). These developments have been made possible by the automation of the TEM image acquiring processes and the possibility of applying image analysis techniques that require a lot of computing power.

Following the dynamics of complex macromolecules

As well as these spectacular advances in TEM for elucidating the structure of live biological cells, other advances just as important in fluorescence microscopy have allowed scientists to follow the dynamics of complex macromolecules in situ. Developing correlative microscopy (visualizing the same structures using TEM and FM) represents another study scale. Combining such complementary information obtained using different imaging techniques at different scales will allow us to better understand the complexity and function of macromolecules like ribosomes.

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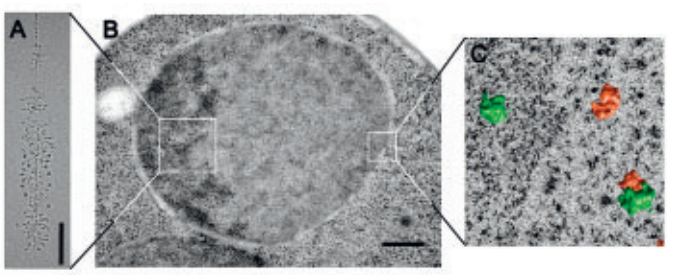


>>> Cellular organization in isolated molecules: how to detect a ribosomal gene during transcription (observed on chromatine spreading in A) or how to identify the molecular structure of ribosomal particles (C) in a yeast nucleus (B).

The immunological synapse in human tumour pathologies

New imaging techniques at the Federative Institute of Biomedical Research at Toulouse-Purpan (IFR 150) have revealed the dangerous liaisons formed between cells of the immune system and their targets, tumour cells.

>>> Salvatore VALITUTTI, INSERM senior scientist, Centre de physiopathologie de Toulouse Purpan (CPTP, joint UPS/CNRS lab).

Our research team has recently embarked on a program in which we employ different imaging techniques to observe the interactions between immune system cells and cancer cells.

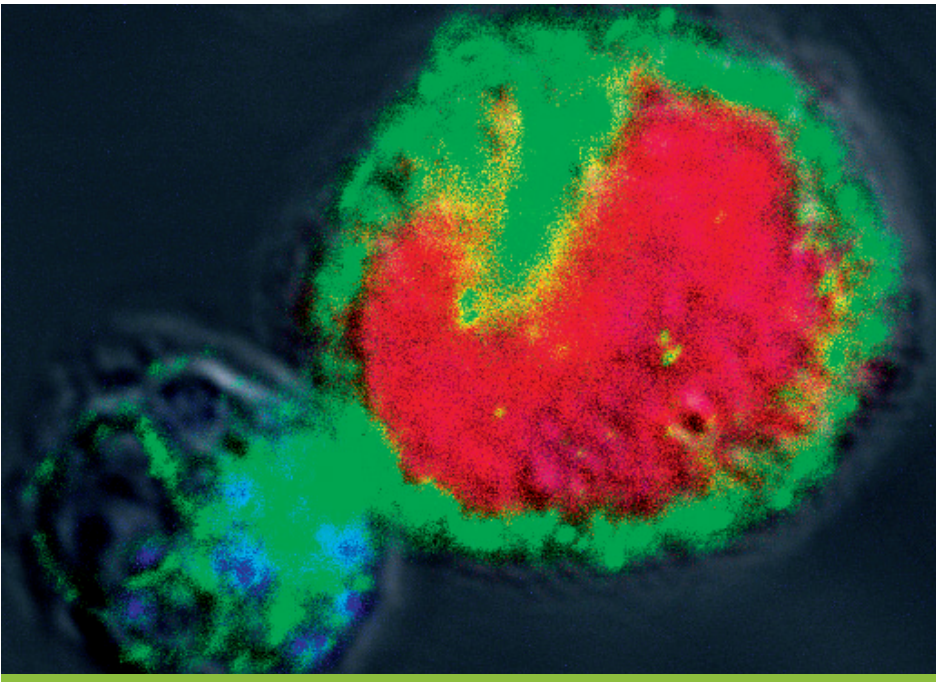
We have a long-standing interest in studying the molecular dynamics occurring at the immunological synapse formed between human T-lymphocytes and antigen presenting cells using morphological approaches in vitro. Although using human cells in vitro has certain advantages over studies on animal models, the approach has the obvious limitation of over simplifying the scenario of a physiological immune response based on multiple and heterogeneous cellular interactions.

To overcome this problem, our team recently set up a novel experiment to study human T-cell activation in a context that more closely resembles physiopathology. Using confocal and two-photon microscopy the team investigated the type, the three-dimensional location and the state of activation of immune cells infiltrating human tissues. Moreover, human antigen specific T-cell migration and interaction with APC is monitored, in living tissue explants, using time-lapse two-photon microscopy.

These approaches are employed to investigate the natural history and the progression of neoplastic diseases such as lymphomas and melanomas in collaboration with clinicians at the Department of Pathology of CHU Purpan (led by Prof. P. Brousset) and at the Department of Haematology of CHU Purpan (led by Prof. G. Laurent) and at the departments of Dermatology and Oncology in Toulouse.

Confocal and two-photon microscopy allowing three-dimensional multicolour visualization of tissues and quantification of morphological observations has rarely ever been applied before to human pathology, which, on the contrary, is routinely studied using immunohistochemistry and tissue micro-array techniques. Applying confocal and two-photon microscopy to human pathology has several advantages over classical techniques since it allows for an improved view of the contact site between cells of the immune system and tumour cells and better defines the molecular re-arrangements occurring at these cellular contacts. Close collaboration between the members of the microscopy facility and the research teams and clinicians in Purpan will further correlate detailed morphological results with the severity and prognosis of various diseases.

The study is still at its outset and is technically and conceptually challenging but we hope that the results obtained will have an important impact in understanding the mechanisms underlying immune surveillance in neoplastic diseases. In general, they should help us to develop new tools and know-how for treating human pathology with high-resolution morphological techniques.



>>> Immunological synapse formed at the contact site between a cytotoxic T-cell and a target cell infected by a virus (red). The cytotoxic T-lymphocyte polarizes its tubulin cytoskeleton (green) and its lytic granules (blue) towards the target cell for lethal hit delivery.

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