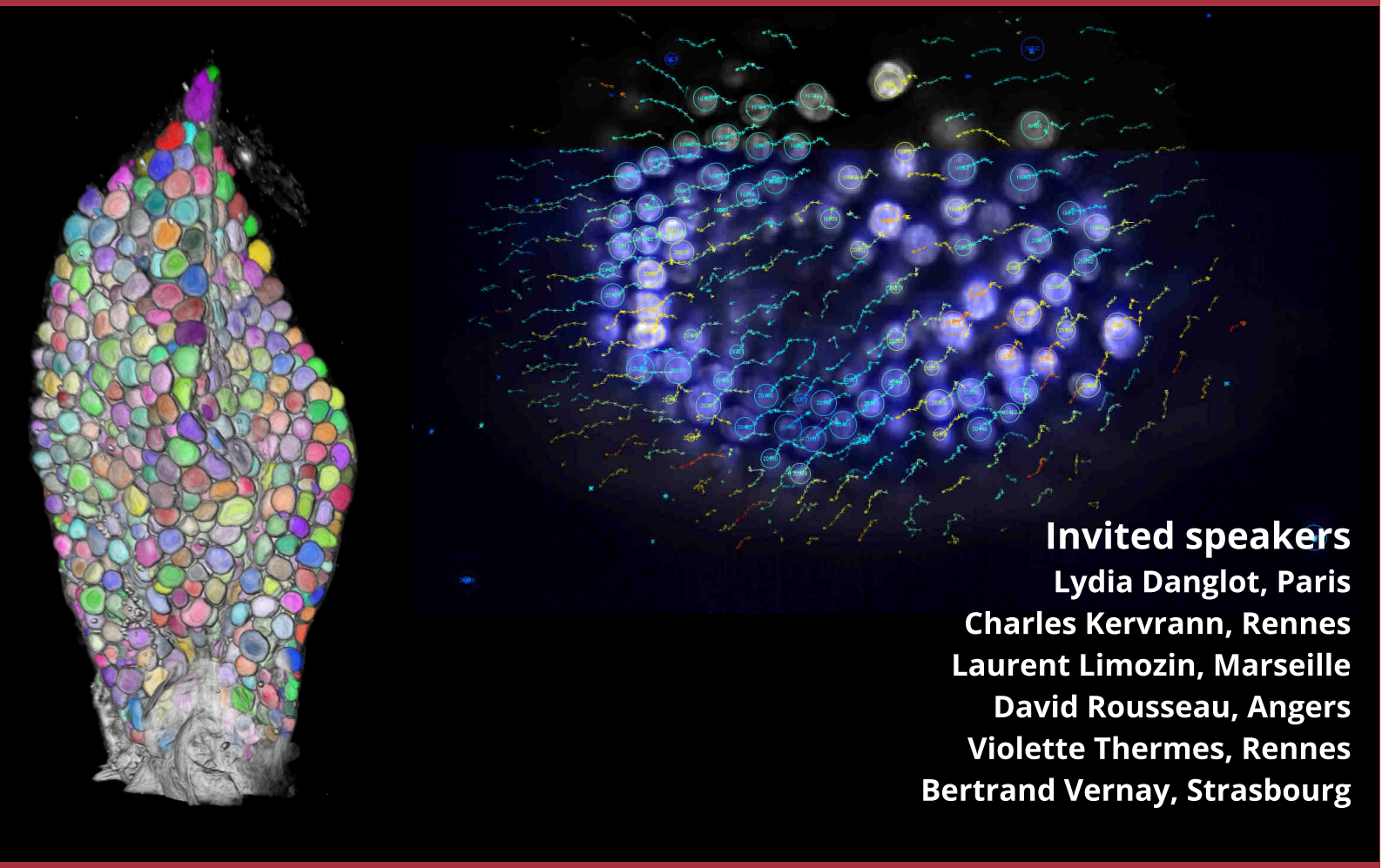


Invited speakers

Lydia Danglot, Paris
Charles Kervrann, Rennes
Laurent Limozin, Marseille
David Rousseau, Angers
Violette Thermes, Rennes
Bertrand Vernay, Strasbourg

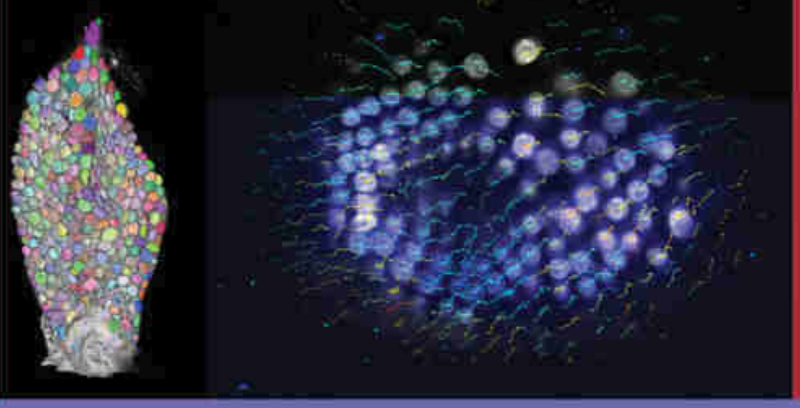
AI & imaging for Cell and developmental biology: insights, limits and perspectives

September 26, 2024

Paris

Amphitheater Buffon, 15 rue Hélène Brion, 75013 Paris

Registration



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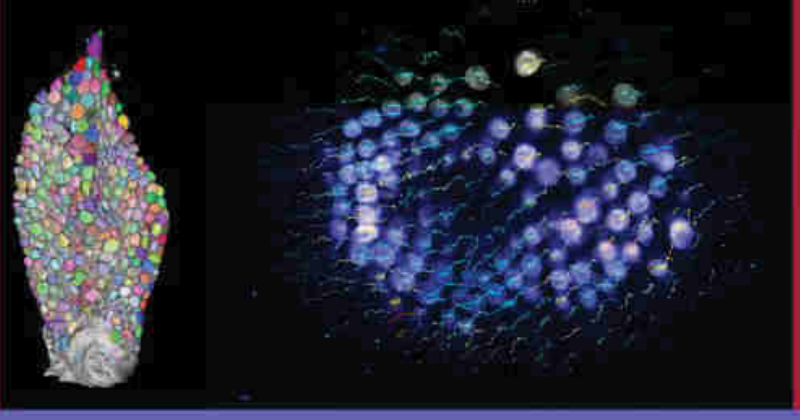
Organizing committee

Frédérique Clément, Inria
Thierry Galli, Inserm
Laurent Héliot, GDR Imabio - University of Lille
Imane Lahmami, Inserm
Christine Lemaitre, Inserm
David Rousseau, University of Angers



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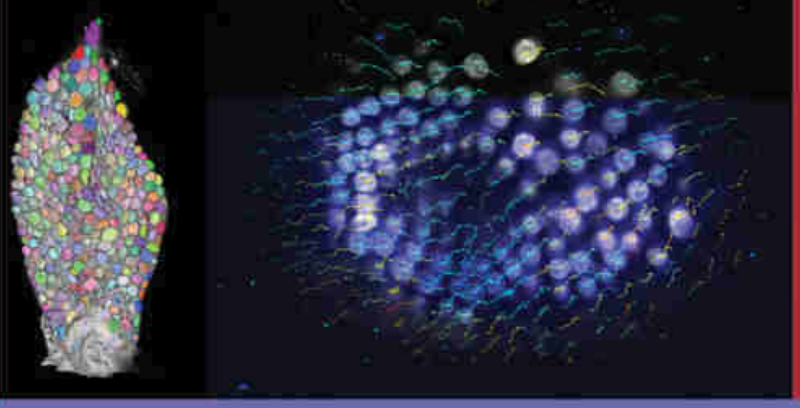
- **9.50 am** — **Welcome by the organizers**
  Thierry Galli, Director of the thematic Institute "Cell Biology, Development and Evolution"
- **10.00 am** — **Session 1: Methods and tools**
  Chairperson: David Rousseau, Angers, France
- **10.00 am** — "Statistical and Artificial Intelligence Methods for Live-Cell Fluorescence Imaging and Cryo-Electron Tomography", **Charles Kervrann**, Inria Center at University of Rennes, Rennes, France
- **10.45 am** — "Artificial Intelligence and the light microscopy core facilities", **Bertrand Vernay,** IGBMC - CNRS UMR 7104 - Inserm U1258, Illkirch, France
- 11.30 am — Coffee break

  Chairperson: Charles Kervrann, Rennes, France
- **11.45 am** — "Machine learning sucks", **David Rousseau**, Angers University, Angers, France
- 12.30 pm — Lunch
- **12.30 pm** — **Poster and Demonstration session**
- **2.30 pm** — **Session 2: The benefits of imaging**
  Chairperson: Frédérique Clément, Paris, France
- **2.30 pm** — "Studying dynamic cell interactions with Celldetective", **Laurent Limozin**, Biophysics of immune recognition, Inserm, Marseille, France
- **3.15 pm** — "Using Artificial Intelligence to study ovarian development in model fish", **Violette Thermes**, Fish Physiology and Genomics Institute, UR 1037 INRAE, Rennes, France
- **4.00 pm** — "Segmenting individual neurons within a dense tissue network using a new pipeline of clearing and multiscale 3D STED imaging on thick brain tissue", **Lydia Danglot**, Institut de Psychiatrie et Neurosciences de Paris, Inserm 1266, Paris, France
- **4.45 pm** — **Conclusions**
- **5.00 pm** — **Meeting end**

REGISTRATION : AI & imaging for Cell and developmental biology_REGISTRATION (inserm.fr)



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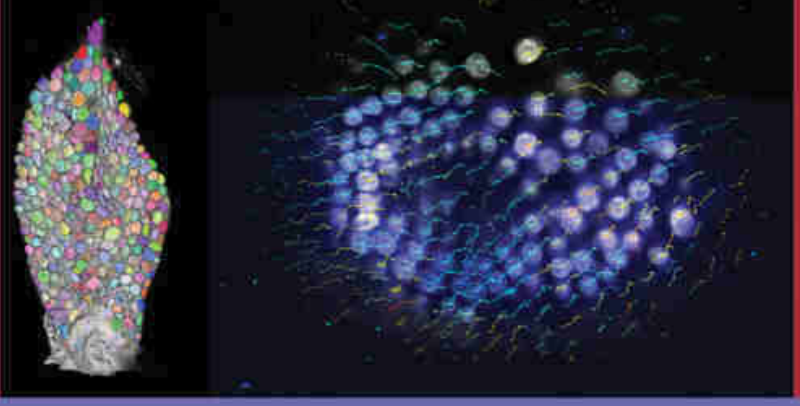
INVITED SPEAKERS



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AI & imaging for Cell and developmental biology: insights, limits and perspectives

Lydia Danglot

NeurImag facility, Institut de Psychiatrie et Neurosciences de Paris, Inserm 1266, Université Paris Cité, Paris, France

Dr. Lydia Danglot is a cell biologist (Inserm CRHC) with a BSc in Biochemistry and a PhD in Neuroscience (Pierre & Marie Curie University) realized in the lab of Antoine Triller at Ecole Normale Supérieure Paris where she studied the formation of inhibitory synapses in hippocampal neurons. She focused on EGF receptors dynamics in cancers during her post-doc at the Institut Jacques Monod in the lab of Thierry Galli. She was recruited in 2010 as permanent Inserm researcher within the lab of Thierry Galli, where she led several projects focused on the trafficking of vesicular synaptic proteins during morphogenesis. She became Scientific Director of the NeurImag imaging facility at the Institut de Psychiatrie et Neurosciences de Paris (IPNP) in 2017 and received the National Research Premium Award PEDR in 2020. She developed super-resolution microscopy methods (SIM, STED and 3D-STORM) and segmentation software's to analyze the dynamics of membranes nanodomains and the geometry of synaptic complexes (Nature Comm 2018, 2022 and 2024). Lydia Danglot is part of the CNRS GDR Imabio, ICON Europe nanoscopy, and from French Club ExoEndocytose steering comities.

Website

Lydia Danglot, PhD, NeurImag facility, Institut de Psychiatrie et Neurosciences de Paris, Inserm 1266, Université Paris Cité

Segmenting individual neurons within a dense tissue network using a new pipeline of clearing and multiscale 3D STED imaging on thick brain tissue

The development of robust tools for segmenting cellular and sub-cellular neuronal structures lags behind the massive production of high-resolution 3D images of neurons in brain tissue. The challenges are principally related to high neuronal density and low signal-to-noise characteristics in thick samples, as well as the heterogeneity of data acquired with different imaging methods. To address this issue, we design a framework which includes sample preparation for high resolution imaging and image analysis. Specifically, we set up a method for labeling thick samples and develop SENPAI, a scalable algorithm for segmenting neurons at cellular and sub-cellular scales in conventional and super resolution STimulated Emission Depletion (STED) microscopy images of brain tissues. Further, we propose a validation paradigm for testing segmentation performance when a manual ground-truth may not exhaustively describe neuronal arborization. We show that SENPAI provides accurate multi-scale segmentation, from entire neurons down to spines, outperforming state-of-the-art tools. The framework will empower image processing of complex neuronal circuitries.

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AI & imaging for Cell and developmental biology: insights, limits and perspectives

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Charles Kervrann

Space-time imaging, artificial intelligence and computing for cellular and chemical biology (SAIRPICO) Team leader, Inria Center at University of Rennes and Cellular & Chemical Biology Unit, U1143 INSERM, UMR3666 CNRS, Institut Curie, PSL Research University, France

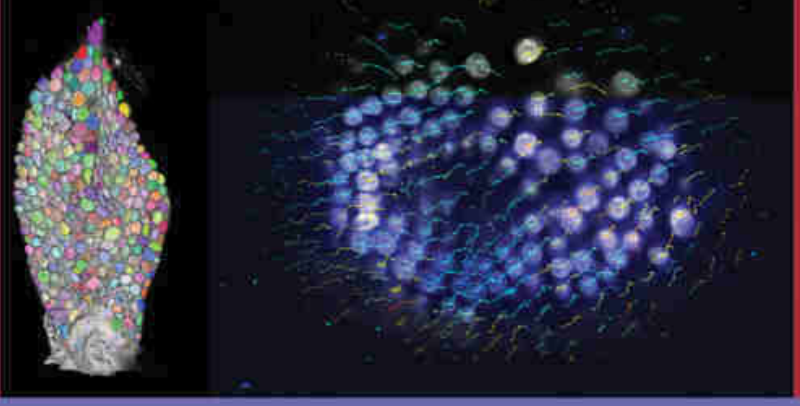
Charles Kervrann was appointed as Inria Research Director in 2010 (DR1 Inria in 2017), and is currently heading the Sairpico Project-Team (Inria Rennes, U1143 INSERM/UMR 3666 CNRS, Institut Curie, PSL University) jointly located in Rennes and Paris since April 1st, 2023. Previously, he was head of Serpico Project-Team (Inria, UMR 144 CNRS, Institut Curie, PSL University) (2018-2023). His research interests include mathematical and statistical methods for biological image processing. He focuses on image sequence analysis, motion estimation, object detection, noise modeling for microscopy and traffic, and dynamics modeling in cell biology.

Website

Charles Kervrann, Inria Center at University of Rennes, SAIRPICO Team, Cellular and Chemical Biology Unit, U1143 INSERM, UMR3666 CNRS, Institut Curie, PSL Research University, SAIRPICo Team-Projet, Centre Inria de l'université de Rennes INSERM-U1142/CNCRS-UMR366.

Statistical and Artificial Intelligence Methods for Live-Cell Fluorescence Imaging and Cryo-Eelectron Tomography

During the past two decades, biological imaging has undergone a revolution in the development of new microscopy techniques that allow visualization of tissues, cells, proteins and macromolecular structures at all levels of resolution. Thanks to recent advances in optics, digital sensors and labeling probes, one can now visualize sub-cellular components and organelles at the scale of a few dozens nanometers to several hundreds of nanometers. As a result, fluorescent microscopy and multimodal imaging has become the workhorse of modern biology. All these technological advances in microscopy, created new challenges for researchers in statistical image analysis and machine learning. In this talk, we present statistical and machine learning methods and algorithms to build an integrated imaging approach that bridges the resolution gaps between the molecule and the whole cell, in space and time. We will present statistical AI-based methods for microscopy image restoration, macromolecule localization, molecule dynamics and interaction analysis inside the cell. The proposed image processing methods and algorithms can be applied to a large range of problems in cell imaging and can be integrated in generic image-based workflows, including for high content screening applications.



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Laurent Limozin

Biophysics of immune recognition, Inserm, Marseille, France

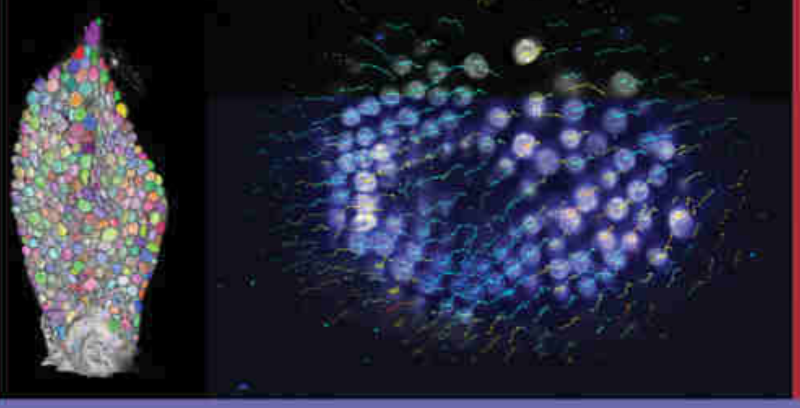
Laurent Limozin is director of research at CNRS. His current research interests include molecular and cellular biophysics of immune recognition and immunotherapy, surface engineering and optical microscopy, micromanipulations, and advanced image analysis.

Website

Laurent Limozin, Biophysics of immune recognition, Inserm, Marseille, France

Studying dynamic cell interactions with Celldetective

A current bioimaging challenge in immunology and immunotherapy research is the analysis of multimodal and multidimensional data recording of dynamic interactions between diverse cell populations. We developed Celldetective, a software that integrates AI-based cell segmentation and tracking algorithms as well as automated signal analysis into a user-friendly graphical interface. It offers complete interactive visualization, annotation, and training capabilities. We present original experimental data of spreading immune effector cells on activating surfaces imaged by interferential microscopy. Harnessing Celldetective, we extract a detailed chronological description of immune cell interactions and decisions within large populations. We also showcase antibody-dependent cell cytotoxicity events using multimodal fluorescence microscopy, with the aim of characterizing new potential anticancerous agents.



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David Rousseau

Angers University, - Imaging for Horticulture and Phenotyping - ImHorPhen – Team leader, Angers, France

David Rousseau is a full professor at the Université d'Angers, where he heads the ImHorPhen bioimaging group. He develops image processing and analysis methods based on machine learning or not.
https://scholar.google.fr/citations?user=33IO_m4AAAAJ&hl=fr

Website

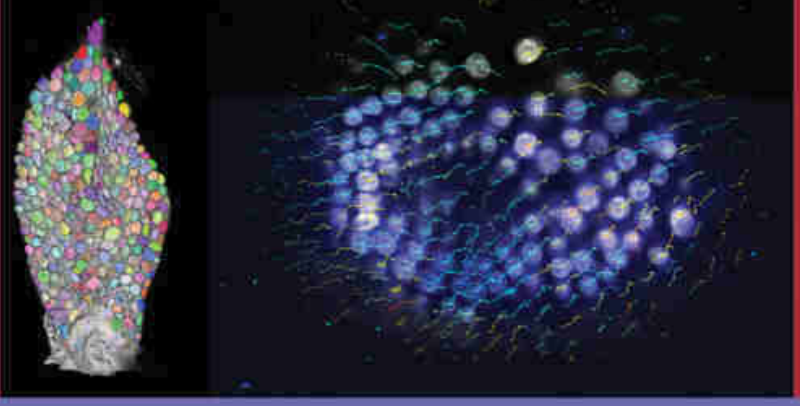
David Rousseau, Angers University, ImHorPhen Team, Angers, France

Machine learning sucks

In this communication, I will introduce limitation of classical machine learning and show how to overcome them based on some of our recent works.

Gilet, V., Mabilleau, G., Loumaigne, M., Coic, L., Vitale, R., Oberlin, T., ... & Rousseau, D. Superpixels meet essential spectra for fast Raman hyperspectral microimaging. Optics Express, 2024.

Ahmad, A., Salac, F., Paièd, P., Candeoc, A., D'Annunzioe, S., Zippoe, A., ... & Rousseau, D. On the robustness of machine learning algorithms toward microfluidic distortions for cell classification via on-chip fluorescent microscopy. Lab On Chip, 2023.



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AI & imaging for Cell and developmental biology: insights, limits and perspectives

Violette Thermes

Fish Physiology and Genomics Institute, UR 1037 INRAE, Rennes, France

Violette Thermes is Laboratory of Fish Physiology and Genomics (LPGP)' deputy director, and Group leader of the Research Group "Sex, Oogenesis and Behavior". Violette Thermes received her undergraduate degree in cellular and molecular biology from the University of Paris (UPMC, France). She then joined the Erasmus Master Programme and moved to the university La Sapienza in Rome (Italie), where she received a master training in molecular biology. She then moved to the CNRS of Gif-sur-Yvette (France) and gained her PhD degree from the University of Paris-Sud, working on the embryonic fish brain.She then underwent a postdoctoral training at the Academia Sinica in Taipei (Taiwan), working on osmoregulatory cells developmental dynamics in fish, before joining the INRAE (National Research Institute for Agriculture, Food and the Environment) Fish Physiology and Genomics Institute (LPGP) in Rennes. She is now working on the ovary development in fish and the role of miRNAs.

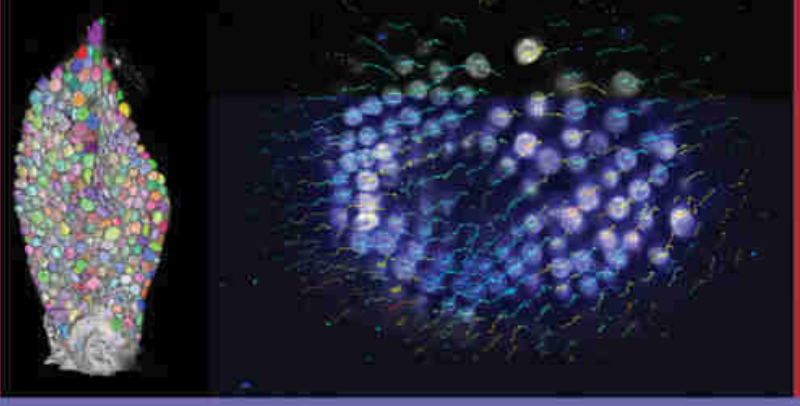
Website

Violette Thermes, Laboratoire de Physiologie et Génomique des Poissons, UR 1037 INRAE RENNES

Using Artificial Intelligence to study ovarian development in model fish

The study of organ development most often requires a step of quantification of the biological structures of interest, including measurements of size and number. The ovaries are made up of anatomical structures (follicles) within which female gametes (oocytes) are formed. In fish, these structures, whose diameter varies from 20 µm to more than 1000 µm, have the particularity of being constantly renewed during adulthood.

For a long time, biologists have been working to decipher the growth dynamics of these structures but, despite recent progress in three-dimensional (3D) imaging, access to the total cellular contents of the ovaries remained limited due to the absence of efficient methods for analyzing microscopy images, particularly 3D images. The emergence, a few years ago, of Artificial Intelligence for image analysis made it possible to resolve this technological obstacle. We used open-source Deep Learning algorithms accessible to biologists, which simplifies the segmentation step and overcomes the methodological biases of classic 2D stereological approaches. These approaches provided us with quantitative data of unprecedented precision on the different oocyte populations in the Medaka, at different stages of development in normal and disturbed conditions. All of these data now allow us to better understand the dynamics of asynchronous growth of oocytes and to decipher the role of molecular players in determining fecundity. Nowadays, the explosion in the number of AI tools made available for the analysis of 2D and 3D images gives hope for new perspectives for the study of ovarian development in fish.



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AI & imaging for Cell and developmental biology: insights, limits and perspectives

Bertrand Vernay

Coordinator RTmfm Working Group MAIIA Light Microscopy Facility, IGBMC - CNRS UMR 7104 - Inserm U 1258, Illkirch, France

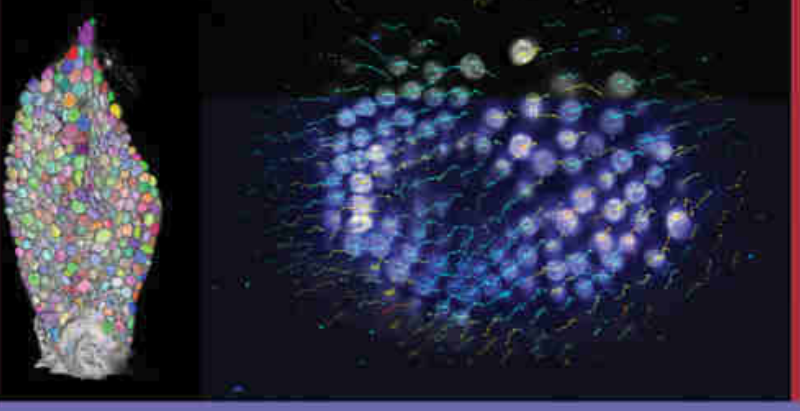
Bertrand Vernay leads the Photonic microscopy Platform at IGBMC, that offers access to state-of-the-art imaging techniques in optical microscopy and specialises in imaging dynamic life processes at the molecular, cellular and whole organism levels.

Website

Bertrand Vernay, Coordinator RTmfm Working Group MAIIA Light Microscopy Facility IGBMC - CNRS UMR 7104 - Inserm U 1258 Illkirch, France

Artificial Intelligence and the light microscopy core facilities

With the advent of the open source tools based on machine learning/deep learning such as Weka based pixel segmentation and instance segmentation generalist models (StarDist and Cellpose), bioimage analysis has dramatically changed for core facilities, their staff and their users. Microscope manufacturers are also integrating artificial intelligence modules within their software promising easy denoising, segmentation, object classification, etc. We will present how core facilities are embracing these new tools, the positives changes they brought, and the effort of the community to train staff and users to adopt best practices and to avoid pitfalls inherent to these tools in Artificial Intelligence tools.



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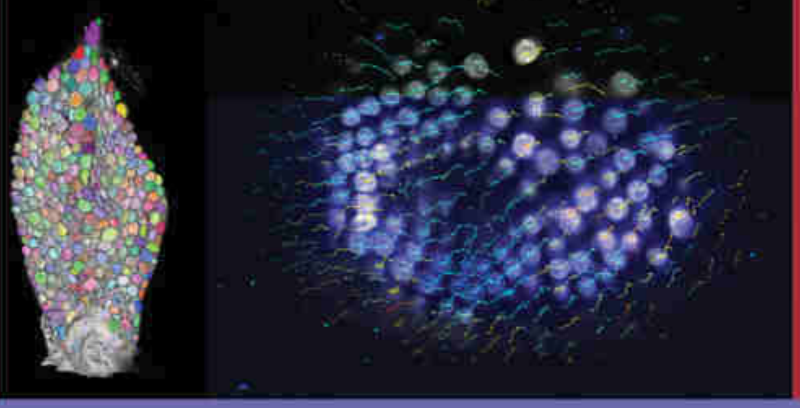
POSTERS ABSTRACTS



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3D imaging-based analysis of the germline in teleost

Marlène Davilma *1*, Stéphanie Gay *1*, Manon Thomas *1*, Laurence Dubreil *2*, Frédérique Clément *3*, Violette Thermes *1*
***1* Team SOCS, LPGP INRAE, Campus de Beaulieu, 35042 Rennes**
***2* Oniris, INRAE, APEX, PAnTher, 44300 Nantes, France**
***3* Equipe-projet commune CNRS-INRAE-INRIA MUSCA, Centre INRIA de Saclay**

In teleost fish, female fecundity depends essentially on the oocyte reserve, which determines the number of eggs laid in each reproductive cycle. Unlike mammals, which have a limited and predefined stock of oocytes at birth, this reserve can be renewed throughout female's life. In adult teleost, this reserve is, on the one hand, used to generate mature oocytes ready to be laid and, on the other hand, replenished from germline stem cells present in specialized structures called germline cradles. A main issue is to understand the contribution of these germline stem cells in the renewal of the oocyte reserve in both juveniles and adults, as well as the involved regulatory mechanisms.

To this end, we have implemented a 3D whole ovary imaging strategy in Medaka to provide quantitative data and study the cellular dynamics of the germinal cradle. We have refined ovary clearing protocols combined with immunolabelings (e.g., anti-vasa, anti-pH3, anti-GFP), and imaged the ovaries using light sheet microscopy. In addition, we have set up 3D image analysis pipelines that integrates pre-trained open-source neural networks suitable for precise segmentation. These deep-learning based pipelines have greatly improved our ability to manage complex 3D analysis of the germinal cradle and allow us to access quantitative data at the level of the entire ovary. We are now analyzing the number, the distribution and the composition of germinal cradles in wild-type females, as well as in two KO lines (miR-202 -/- and miR-187 -/-) showing a drastic decrease in female fecundity, to uncover the miRNA-mediated regulatory mechanisms. In the future, this approach should also provide us with the means to explore in depth the interactions between the somatic and germ cells within germinal cradles, including their spatial organisation.

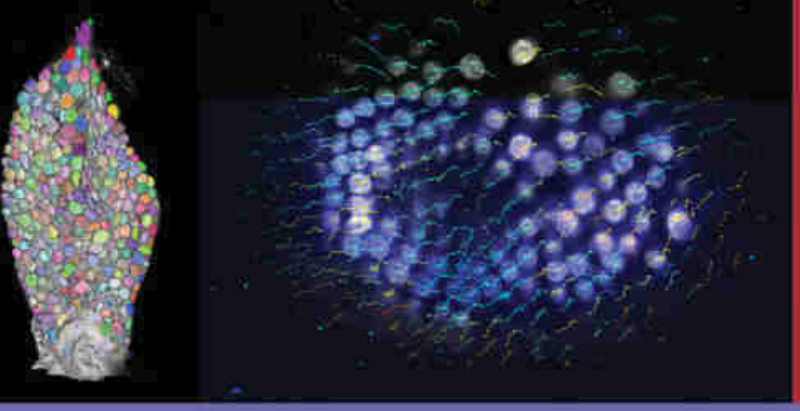
Keywords : Light sheet microscopy – 3D image analysis – fish oogenesis – deep-learning

Detection and classification of ovarian follicles in histology images

Raphaël Corre *1*, Rosario Medina *2*, Céline Guigon *3*, Romain Yvinec *4*, Frédérique Clement *5*
***1*BFA , Université Paris Cité. (current address: CIML, Université Aix-Marseille).**
***2,4,5*Equipe-projet commune CNRS-INRAE-INRIA,Centre Inria de Saclay.**
***3*Inserm U1139, Université Paris Cité , Paris, France.**
***4*PRC, INRAE, CNRS, Université de Tours.**

The assessment of ovarian follicle counts at different developmental stages is critical in reproductive biology, with significant implications for fundamental research, pharmacological and toxicological studies, and the clinical management of fertility. Identifying all follicles requires invasive techniques that rely on histology. This process involves fixing the ovaries, slicing them into sections, staining them with specific dyes, and manually analyzing them using light microscopy. The traditional counting method is not only intricate, labourintensive, and operator-dependent but also lacks reliability and is highly time-consuming. Therefore, researchers in reproductive biology need an automatic solution to overcome the limitations of the traditional counting method, and our research aims to address this need. In this ongoing project, we have developed a pipeline for automatically detecting and classifying follicles in mouse ovary images. The pipeline consists of four modules utilizing open-source microscopy tools: (i) Whole slide histology image acquisition through light microscopy;
(ii) Creation of a section image dataset using QuPath;
(iii) Annotation of the dataset by the specialists using Omero; and,
(iv) Detection and classification of follicles based on a deep learning approach.
Our dataset comprises 2D ovarian section images in JPEG format corresponding to three mice's ovaries. During the annotation process, we only include follicles with visible oocytes. Therefore, the follicles in the images are categorised into six classes: Primordial, Primary, Healthy-Secondary, Atretic-Secondary, Healthy-Tertiary and Atretic-Tertiary. Additionally, the corpus luteum was also labelled and considered as another class. For the detection and classification process, we used a Fast R-CNN model pretrained on ImageNet. We also applied an IoU (Intersection over Union)-based non-maximum suppression to remove duplicates.
The initial findings are promising, indicating a good performance across most follicle stages in our test dataset, particularly the largest ones. Our future research will address the class imbalance and optimize the detection of primordial follicles.

Keywords : ovarian follicle, microscopy imaging, image recognition, deep learning



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AI & imaging for Cell and developmental biology:
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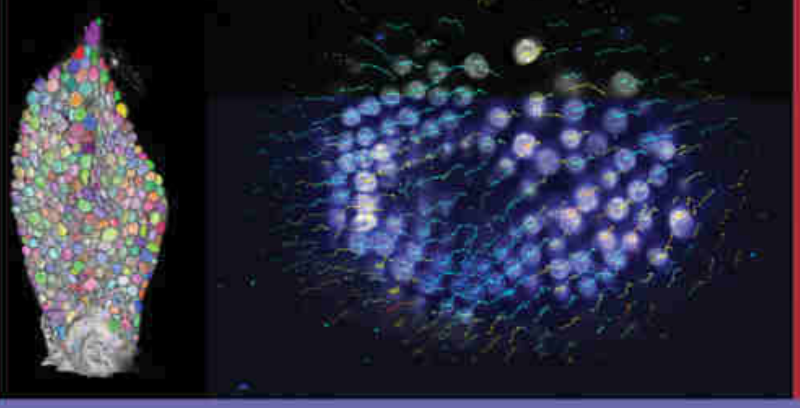
Developmental stereotypy assessment in ascidian embryos

Haydar JAMMOUL *1*, Kilian BIASUZ *2*, Benjamin GALLEAN *2,3*, Patrick LEMAIRE *2*, Grégoire MALANDAIN *4*
***1* Université Côte d'Azur, CNRS, I3S, France**
***2* CRBM, Université de Montpellier, CNRS, France**
***3* MRI, Biocampus, Université de Montpellier, CNRS, INSERM, France**
***4* Université Côte d'Azur, Inria, CNRS, I3S, France**

Many model organisms are used to study development. Among them, the C. elegans nematode is appealing since it exhibits a remarkable stereotyped development, every adult having exactly the same number of somatic cells, allowing to study the development at a cellular level. This stereotypy also exists, even across species, in the first developmental stages of ascidians, which are an appealing model since they are close relatives to vertebrates [Conklin, 1905].
However, little is known about variation in the stereotypy. In this project, we investigated quantitively geometric variation in cell divisions in ascidian, which is a key factor in embryo organization. More precisely, 1) Are there cells that exhibit a bimodal division pattern across individuals (e.g. left/right and anterior/posterior direction)? 2) How variable is the orientation of cell division along its major mode? 3) Is the observed variation correlated with other embryonic properties (e.g. cell fate, etc)?
The datasets used in our computational analyses came from a previous study [Guignard, 2020] where developing embryos of Phallusia mammillata have been imaged, resulting in a temporal series of 3D volumes. After segmentation, each cell can be tracked through embryo development up to its division. At each timepoint, we have access to its geometric properties (e.g. barycenter position) and lineage information.
As embryos adopt different positions under the microscope, their spatial alignment is required before any analyses to compare homologous cell divisions in the embryo population. As a first result, group analysis of division directions demonstrates that few 7th generation cells exhibit several modes of division orientations (e.g. A7.2). The variation in division plane orientation was further analyzed in other cells, paving the way to explore its correlation with specific cell characteristics, such as cell fate. Notably, we found that nervous system cells exhibit low variation in their division plane orientation.

EG Conklin, Academy of Natural Sciences, 1905.
L Guignard et alii, Science, vol. 369, no. 6500, pp. 158, July 2020.

Keywords : Fluorescence microscopy, embryogenesis, division orientation, morphogenesis



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Spatial Exploration of Hepatoblastoma Tissue Bioarchitecture by Volumetric Imaging, AI and Applied Mathematics

Florian Robert *1,3,4*§*, Alexia Calovoulos *1,2§*, Emilie Indersie *5*, Olivier Déas *5*, Kathleen Flosseau *5*, Christophe Chardot *6*, Sophie Branchereau *7*, Etienne Gontier *2* , Baudouin Denis de Senneville *3,4* and Christophe F. Grosset *1*

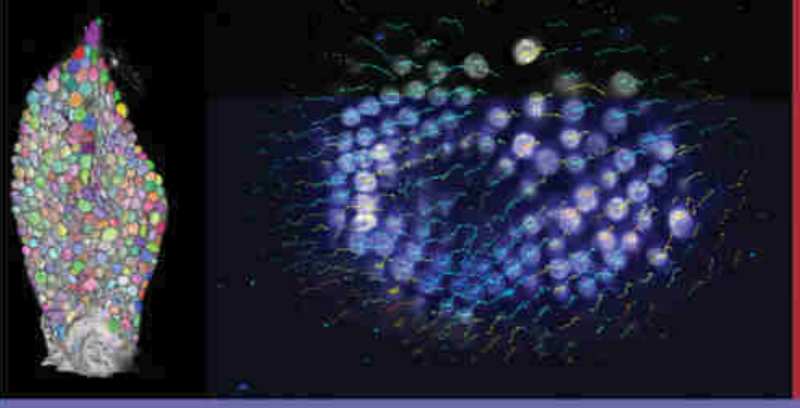
1 Univ. of Bordeaux, INSERM, Bordeaux Institute of Oncology, BRIC, U1312, MIRCADE team, 146 rue Léo Saignat, Bordeaux, 33000, France. *2* Univ. of Bordeaux, CNRS, INSERM, Bordeaux Imaging Center, BIC, UAR 3420, US 4, 146 rue Léo Saignas, Bordeaux, 33000, France. *3* Univ. of Bordeaux, CNRS Institut de Mathématiques de Bordeaux, IMB, UMR5251, 351 cours de la Libération, Talence, F-33400, France. *4* INRIA Bordeaux, MONC team, 200 avenue de la Vieille Tour, Talence, F-33400, France. *5* XenTech Company, Genopole Campus 3, 4 Rue Pierre Fontaine, F-91000 Évry-Courcouronnes, France. *6* Chirurgie Pédiatrique-Transplantation, Hôpital Necker-Enfants Malades, Assistance Publique Hôpitaux de Paris, 149 rue de Sèvres, F-75015 Paris, France. *7* Paediatric Surgery and Liver Transplant unit, Hôpital Bicêtre, Assistance Publique Hôpitaux de Paris – Université Paris Saclay, 78 rue du Général Leclerc, F-94270 Le Kremlin-Bicêtre, France.* Presenting author; § These authors contributed equally to this work; These authors contributed equally to the design and supervision of this work.

Despite a detailed characterisation of cellular components constituting the tumour tissue, the architecture of this pathologic tissue and the biological elements governing its structural organisation remain elusive. Recently, our consortium reported the first investigation on the complete ultrastructure of human tumour xenograft tissue by high-resolution 3D electron microscopy. We are currently analysing the internal organisation of 5 hepatoblastoma patient-derived xenograft tissues by serial block-face scanning electron microscopy (SBF-SEM) using an integrated workflow combining image processing, artificial intelligence (AI)-based segmentations and applied mathematics.

While current AI-based methods effectively segment and distinguish individual cells in scanning electron microscopy (SEM) images, persistent errors necessitate time-consuming manual corrections, especially in regions where cell contour quality is poor and gap filling is required. Our consortium has introduced a novel AI-driven approach for refining cell boundary delineation, which significantly enhances instance-based cell segmentation in SEM images and reduces the need for residual manual correction.

We investigated the influence of blood capillaries and haemorrhagic areas on the planar alignment, polarity and volume of tumour cell nuclei. We also discriminated tumour cells from immune cells and endothelial cells by developing and using dedicated bio-architectural parameters. Thanks to our integrated workflow, our final goal is to perform detailed intra- and inter-tumour sample comparisons, allowing a better understanding of the organisation of tumour tissuesand the bioarchitectural parameters influencing their response to drugs. These findings could potentially unveil differences and similarities among samples from different patients, providing valuable insights for clinicians and improving personalised treatment approaches.

Keywords: 3D scanning electron microscopy, Deep neural network, Tissue processing, Instance segmentation, Ultrastructure, Volume rendering



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AI & imaging for Cell and developmental biology:
insights, limits and perspectives

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