PhD PROJECT PROPOSAL

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Quantification and modeling of embryonic lymph node organogenesis at the single cell scale

Supervisor 1 (with name, email, affiliated laboratory and doctoral school affiliation)
Léo Guignard, IBDM

Supervisor 2 (with name, email and affiliated laboratory and doctoral school affiliation)
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Abstract (10 lines)*
Lymph node (LN) formation requires hematopoietic lymphoid tissue inducer cells (LTi) to interact with mesenchymal cells at precise locations within the embryo, where they subsequently form aggregates. We have postulated that the peripheral nervous system outgrowth initiates the earliest events in LN formation. Indeed, preliminary data show that LTi aggregate morphology and cell density is affected in whole-mount stained mouse embryos lacking neuronal subsets. To understand the relationship between neuronal outgrowth and lymph node formation, we will develop new computational methods to reconstruct and quantify LTi aggregates and peripheral nervous system morphology at the single cell scale in the whole embryo. The reconstructions will then be used to develop a machine learning framework to systematically quantify phenotypes in perturbed mouse embryos. These quantifications will in turn allow to model the effects of neuronal outgrowth on LN formation.

Keywords*
Quantitative embryogenesis, whole-mount analysis, peripheral nervous system, immune system, image analysis, machine learning, big data analysis

Scientific question and Objectives (10 lines)*

How are lymph nodes always positioned at exactly the same location? Are these related to the initial events leading to the clustering of the involved cell types at precise locations? Based on previous studies (van de Pavert, Nature Immunology 2009), we postulated that the outgrowing peripheral nervous system could be involved in providing key molecules to allow differentiation of the cells involved. Indeed, in an associated study financed by A*Midex Blanc, preliminary data obtained from a mouse model where specific subsets of neurons are deleted, suggest that the cell aggregates which will form the lymph node are smaller and positioned differently. However, quantification of the size, shape and location of these aggregates in relation to the neurons is impossible to establish using available tools. Therefore, our objectives are:

- To build a library of whole-mount mouse embryo images
- To develop computational methods for the analysis of the generated library, including:
- Reconstruction and mapping of the neuronal network
- Quantification of LTi aggregate morphologies and positions
- To develop computational methods to automatically stage mouse embryos

*: Mandatory

- To model LN formation function of the neuronal network morphology

Proposed approach (experimental / theoretical / computational) and research plan (20 lines)*

Different mouse models in which specific neuronal subsets are deleted are already available. Embryos will be isolated at the time during which LN are initiated and whole-mount stained, cleared and acquired on the new Blaze Lightsheet microscope in the CIML. In the Guignard lab, the datasets generated will be computationally segmented using algorithms to constitute a quantitative single cell atlas of mouse LTi morphology. Machine learning algorithms will then be developed to quantify LTi morphology phenotypes for the set of mouse models. The quantification will drive potential new experiments and the development of a model of LTi aggregate formation. Both partners have published on the proposed methods before and thus have the necessary background to carry out this project. The labs will meet frequently to discuss progress and ensure the alignment between the biological and the computational questions.

Interdisciplinarity and Implication of the two labs (15 lines)*
(In this section the collaboration of the two laboratories will be explained in details to explain why the project cannot be conducted by one team alone)

The proposed project aims at developing computational algorithms and methods to answer a precise biological question for which new experiments will be necessary. While the van de Pavert lab at the CIML harbors the mouse lines, isolate, stain and acquire the mouse embryos, the Guignard lab at the IBDM will be responsible for developing the algorithms which will quantify and compare the aggregates and neurons morphologies. The analysis of the quantitative outputs while help designing and orienting future experiments that will be carried out in the van de Pavert lab. The collaboration between both labs is essential to propose a model for LN formation.

Specify with whom the person recruited will collaborate and on what aspects *

The main focus is to develop a pipeline to analyze the different embryos in 3D. However, regular contact with both teams is essential to safeguard the model making and the biological questions and progress. Therefore, we will setup;

- Contact with both supervisors (Guignard and van de Pavert)
- Common project meeting at least once a month
- Embedded within both laboratories; Invite to both laboratory labmeetings
- An office in both laboratories

PhD student's expected profile*

Enthusiastic, creative and ambitious, good communication skills and eager to learn.

Master degree with major or minor in computer science. Affection for developmental biology. Note: exception can be made for students who have not studied computer science if the student can prove coding skills.

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Is this project the continuation of an existing project or an entirely new one?
In the case of an existing project, please explain the links between the two projects (5 lines)*
This project concerns the re-application of a previous, awarded, project (2022). We were unfortunate that the appointed PhD student choose to pursue a direction out of the scope from the computational image analysis into AI modelling outside the university.

Two to five references related to the project*

Simic M. et al. Cell Reports, 32(6):108004
Wang et al., Developmental Cell, 56(22), 3128-3145.e15
van de Pavert S.A. et al., Nature Immunology 10(11):1193-9
van de Pavert S.A. et al. Nature 508(7494):123-7
McDole K., Guignard L. et al. Cell, 175(3):859-876

Two main publications from each PI over the last 5 years*
Simic M. *et al.,* Cell Reports 2020, 32(6):108004 (van de Pavert team)
Siret C. *et al.*, Nat Commun. 2022;13(1):7366(van de Pavert team)
Gros A., Vanaret J., Dunsing-Eichenauer V. et al. bioRxiv 2024.08.13.607832 (Guignard team)
Sampath Kumar, A., Tian, L., Bolondi, A. et al. Nat Genet 2023 55, 1176–1185 (Guignard team)

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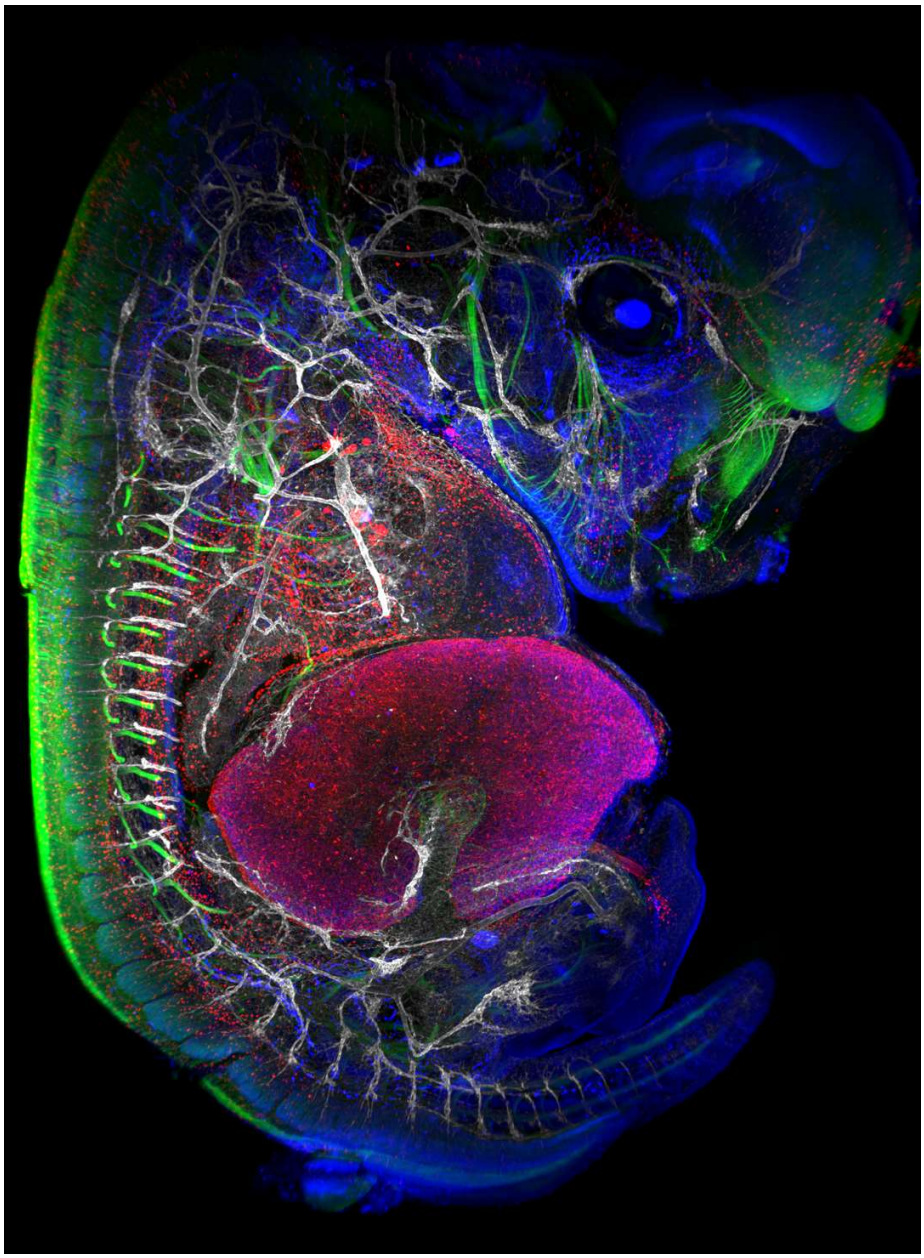
Project's illustrating image



A maximum fluorescence projection of a whole mount stained E13.5 mouse embryo, acquired with the Lightsheet microscope in the team of Serge van de Pavert. Macrophages and lymphatic endothelial cells are stained by anti-Lyve1 (red), or only lymphatic endothelial cells by anti-Prox1 (blue), neurons are stained by anti-ßIII Tubulin (green), blood vasculature by anti-CD31 (Pecam, white). This technique will also be used in this project and the datasets from the microscope processed within the team of Léo Guignard.

*: Mandatory