3D Cell Segmentation for Image-Based Spatial Transcriptomics in MERFISH Data

Master Project (Lyon, France)

The Institut de Génomique Fonctionnelle de Lyon (IGFL) announce the opening of a six-month internship position, ideally starting in March 2026, in the Spatial-Cell-ID facility, co-led by Dr. Jonathan Enriquez.

Keywords Cell segmentation, Deep learning, Spatial transcriptomics, 3D MERFISH, Visualization tools, Napari.

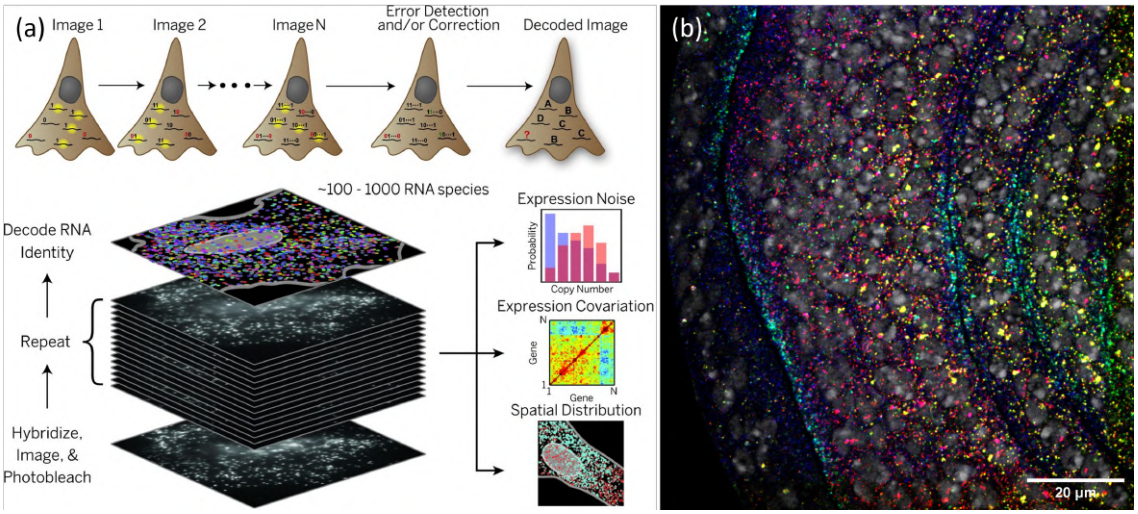

Figure 1: (a):MERFISH for transcriptome imaging [1], (b):3D MERFISH microscopy image of a Drosophila leg disk at L3 stage. 5 bit channels (colored) and DAPI nuclear staining (gray).

Scientific context Fundamental biological processes such as development, maintenance of homeostasis, cell differentiation, and adaptation to environmental or pathological conditions rely on the precise spatiotemporal regulation of gene expression at the single-cell level. Historically, technical limitations restricted biologists to measuring average transcriptomes across groups of cells within a tissue, thereby masking the diversity of cell states that coexist in complex organisms. Tissues, however, are composed of individual cells with distinct shapes, functions, and developmental stages. The emergence of single-cell sequencing technologies has revealed a previously unsuspected heterogeneity among neighboring cells and has opened new questions regarding transcriptome homeostasis, gene expression robustness, noise filtering, and the temporal averaging of cell identities.

It is now essential to characterize the spatial organization of this heterogeneity and its dynamics directly within biological tissues. Spatial transcriptomics, which aims to measure each cell's transcriptome in situ, was recognized as a major scientific breakthrough (named Science magazine's "Breakthrough of the Year" in 2020 [2]) because it provides a means to link gene expression with tissue architecture and cellular interactions [3].

Within this context, we are building methods to identify and characterize the transcriptome of every cell, along with its temporal dynamics, in animal and plant organisms across developmental, physiological, and pathological conditions. To this end, we are establishing a national spatial transcriptomics facility, called Spatial-Cell-ID (funded by the ANR and co-led by Jonathan Enriquez together with Yad Ghavi-Helm and Teva Vernoux) that combines single-cell RNA profiling, advanced microscopy (confocal and STED), targeted genetic manipulations, and a novel 3D image-based spatial transcriptomic technique known as 3D MERFISH. MERFISH (Multiplexed Error-Robust Fluorescence In Situ Hybridization)

relies on combinatorial barcoding and iterative imaging [1] to reconstruct three-dimensional gene expression maps with subcellular resolution, enabling the detection of hundreds to thousands of RNA species in intact tissues and producing rich volumetric datasets [4, 5].

Preliminary results Our full-scale 3D MERFISH experimental pipeline, including probe design, sample preparation, and multi-round imaging, is nearing completion. We have already obtained proof-of-concept results using small gene panels and simplified experimental conditions. In parallel, we are developing a Python-based image processing pipeline that transforms raw microscopy data into spatially resolved transcriptomic information suitable for downstream analyses [6].

Challenges A first challenge concerns visualization. During image processing, MERFISH datasets are stored in a custom Zarr-based datastore and consist of multiple volumetric layers, including raw and processed images, decoded RNA localizations, segmentation masks, and various intermediate outputs. Inspecting these different types of information, and especially their spatial relationships, is essential for validating algorithmic developments and ensuring the quality of both data and processing.

The second major challenge is achieving accurate 3D segmentation of cells. Three-dimensional segmentation is difficult because cell boundaries can be faint or incomplete, cell densities are high, and tissue morphology is often irregular, making cell contours hard to define. Errors at this stage propagate through downstream analyses and can severally distort biological interpretation. Although recent deep-learning approaches perform well in 2D, adapting these methods to 3D volumes, and more specifically to the imaging characteristics of our MERFISH datasets, remains an unresolved problem.

Work plan The internship will focus on two complementary objectives.

- The first objective is to design and implement a visualization tool based on the napari ecosystem [7] to facilitate efficient exploration of our MERFISH datastore and support rapid quality control during algorithm development. The intern will develop a functional napari plugin that follows good software engineering practices and integrates seamlessly with our processing pipeline.
- The second objective is to improve the 3D segmentation module of our image processing pipeline. We aim to extend recent 2D segmentation approaches, such as RNA2Seg [8] based on the gold standard Cellpose [9, 10, 11, 12], to full 3D volumes. This includes analyzing the model architecture, adapting it to 3D, training and validating it on real experimental data, and optimizing the method for performance, robustness, and scalability.

Throughout the internship, the student will contribute directly to our growing open-source Python packages for MERFISH image processing. Collaborative code development, documentation, participation in lab meetings, and regular interactions with researchers and engineers will be integral to the project.

Skills We are seeking an enthusiastic and autonomous candidate enrolled in a Master's or Engineering program (M2), ideally with a background in applied mathematics, image processing, computer vision, or deep learning. Although previous experience in these areas is beneficial, the internship will provide training in Python programming, collaborative development using Git and GitHub, deep learning, and the scientific foundations of spatial transcriptomic imaging. Within the Spatial-Cell-ID project, the intern will join a multidisciplinary team of researchers, engineers, and other interns working on related topics, offering a dynamic and stimulating environment at the interface of biology, physics, and computational science.

How to apply? Send CV, motivation letter, and academic records to hugo.blanc@ens-lyon.fr, nicolas.ducros@creatis.insa-lyon.fr and jonathan.enriquez@ens-lyon.fr.

References

[1] K. H. Chen *et al.*, "Spatially resolved, highly multiplexed RNA profiling in single cells," *Science*, vol. 348, no. 6233, p. aaa6090, Apr. 2015, publisher: American Association for the Advancement of Science.

[2] "Method of the Year 2020: spatially resolved transcriptomics," *Nature Methods*, vol. 18, no. 1, pp. 1–1, Jan. 2021, publisher: Nature Publishing Group.

[3] D. Bressan *et al.*, "The dawn of spatial omics," *Science*, vol. 381, no. 6657, p. eabq4964, Aug. 2023, publisher: American Association for the Advancement of Science.

[4] R. Fang *et al.*, "Three-dimensional single-cell transcriptome imaging of thick tissues," *eLife*, vol. 12, p. RP90029, Dec. 2024, publisher: eLife Sciences Publications, Ltd.

[5] Y. Wan *et al.*, "Whole-embryo Spatial Transcriptomics at Subcellular Resolution from Gastrulation to Organogenesis," Aug. 2024, pages: 2024.08.27.609868 Section: New Results.

[6] R. Kruithoff *et al.*, "GPU-accelerated, self-optimizing processing for 3D multiplexed iterative RNA-FISH experiments," Nov. 2025, iSSN: 2692-8205 Pages: 2025.10.10.681751 Section: New Results.

[7] N. Sofroniew *et al.*, "napari: a multi-dimensional image viewer for Python," Oct. 2025.

[8] T. Defard *et al.*, "RNA2seg: a generalist model for cell segmentation in image-based spatial transcriptomics," Mar. 2025, pages: 2025.03.03.641259 Section: New Results.

[9] C. Stringer *et al.*, "Cellpose: a generalist algorithm for cellular segmentation," *Nature Methods*, vol. 18, no. 1, pp. 100–106, Jan. 2021, publisher: Nature Publishing Group.

[10] M. Pachitariu *et al.*, "Cellpose 2.0: how to train your own model," *Nature Methods*, vol. 19, no. 12, pp. 1634–1641, Dec. 2022, publisher: Nature Publishing Group.

[11] C. Stringer *et al.*, "Cellpose3: one-click image restoration for improved cellular segmentation," Feb. 2024, pages: 2024.02.10.579780 Section: New Results.

[12] M. Pachitariu *et al.*, "Cellpose-SAM: superhuman generalization for cellular segmentation," May 2025, pages: 2025.04.28.651001 Section: New Results.