DANISH SINGLE-CELL NETWORK NEWSLETTER

May - 2023

THIS MONTH'S HIGHLIGHTS:

A relay velocity model infers cell-dependent RNA velocity

Li, S., Zhang, P., Chen, W. et al.

Spatiotemporally resolved transcriptomics reveals the subcellular RNA kinetic landscape

Ren, J., Zhou, H., Zeng, H. et al.

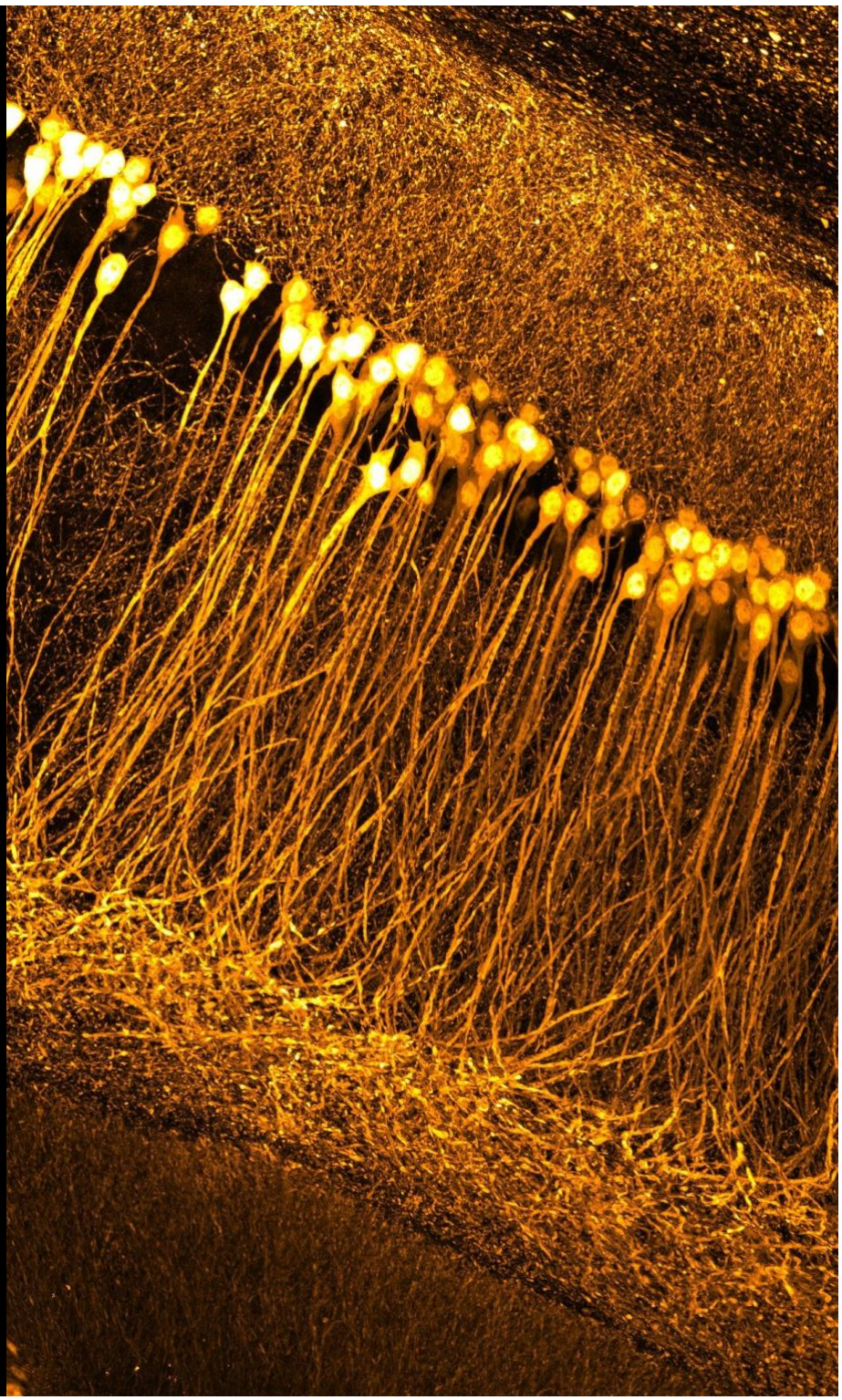
Multimodal charting of molecular and functional cell states via *in situ* electro-sequencing

Li, Q., Lin, Z., Liu, R. et al.

Brain-wide correspondence between neuronal epigenomics and long-distance projections

Zhou, J., Zhang, Z., Wu, M. et al.

COVER IMAGE
Credit: Anna Maria Reuss (USZ) & Fabian Voigt (UZH)
Title: The Schmidt objektive produces detailed images of neurons in a mouse brain (2023)



UNIVERSITY OF COPENHAGEN

A relay velocity model infers cell-dependent RNA velocity

Li, S., Zhang, P., Chen, W. et al. *Nature Biotechnology* (2023), https://doi.org/10.1038/s41587-023-01728-5

The existing RNA velocity models assume uniform kinetics of all cells in an scRNA-seq experiment, which can lead to poor predictive performance when cell subpopulations have dissimilar RNA velocity kinetics. To overcome this limitation, researchers have developed **cellDancer**, a **model-based deep neural network (DNN) framework**, to locally infer velocity for each cell from its neighbors and then to relay a series of local velocities to provide **single-cell resolution inference of velocity kinetics**. Specifically, cellDancer trains separate DNNs for each gene to calculate cell-specific transcription, splicing, and degradation rates. It uses an RNA velocity model to predict future mRNA based on these rates. The crucial step is defining a loss function that measures similarity between predicted and observed mRNA in neighboring cells. By optimizing global similarity, cellDancer infers rates at a single-cell resolution, unlike existing methods that use bulk rates. The cellDancer introduces four innovations. First, it overcomes the challenge for inferring RNA velocity with multiple kinetics. Secondly, it offers flexibility by being adaptable to other velocity ordinary differential equations using the same framework. Additionally, cellDancer is highly modularized and extensible to multi-omics velocity models. Lastly, the DNN is scalable, which allows us to customize an optimal network structure based on the complexity of the velocity model and experimental data.

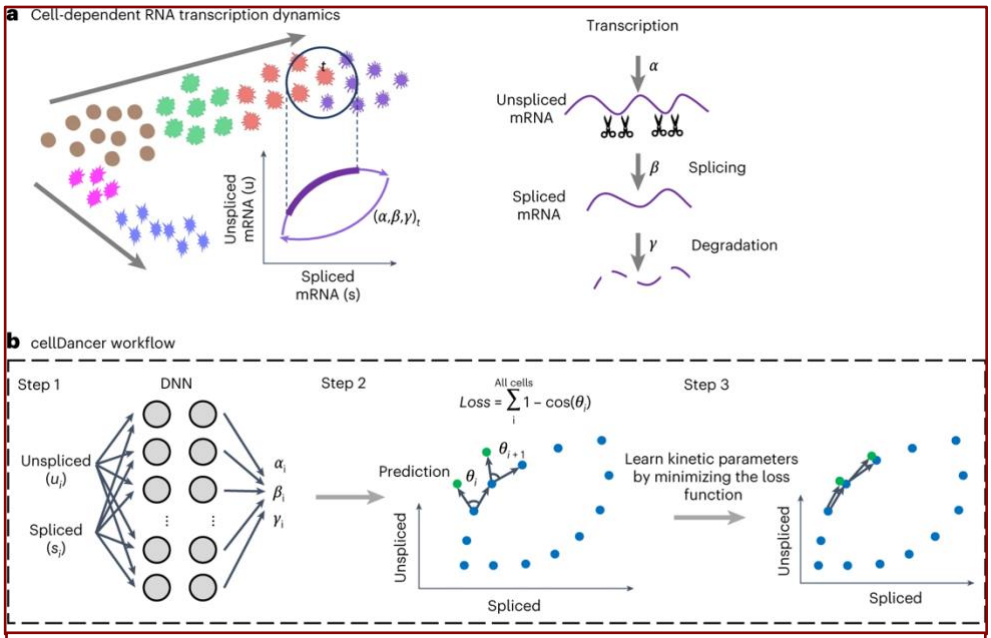


Figure 1. Overview of cellDancer. The cellDancer uses a DNN to predict cell-specific transcription (α), splicing (β) and degradation (γ) rates for each gene. The DNN consists of an input layer with the spliced and unspliced mRNA abundances (u_i, s_i) i = 1,2, …, n_{cells}, two fully connected hidden layers each with 100 nodes and an output layer yielding cell-specific α, β and γ. The loss function is defined as the sum of every cell's cosine similarity of predicted and observed velocity vectors. The DNN is iteratively optimized by minimizing the loss function.

Spatiotemporally resolved transcriptomics reveals the subcellular RNA kinetic landscape

Ren, J., Zhou, H., Zeng, H. *et al.* *Nature Methods* (2023), http://doi.org/10.1038/s41592-023-01829-8

Understanding the intricate gene regulatory mechanisms in cells and tissues requires systematic profiling of transcriptomes at a single-cell level, considering both temporal and spatial dimensions within intact cellular networks. However, existing transcriptomics methods have limitations in capturing simultaneous spatial and temporal RNA profiles. To address this, the authors introduced **TEMPOmap** (temporally resolved *in situ* sequencing and mapping), a method that **tracks the spatiotemporal evolution of nascent transcriptomes at subcellular resolution**.

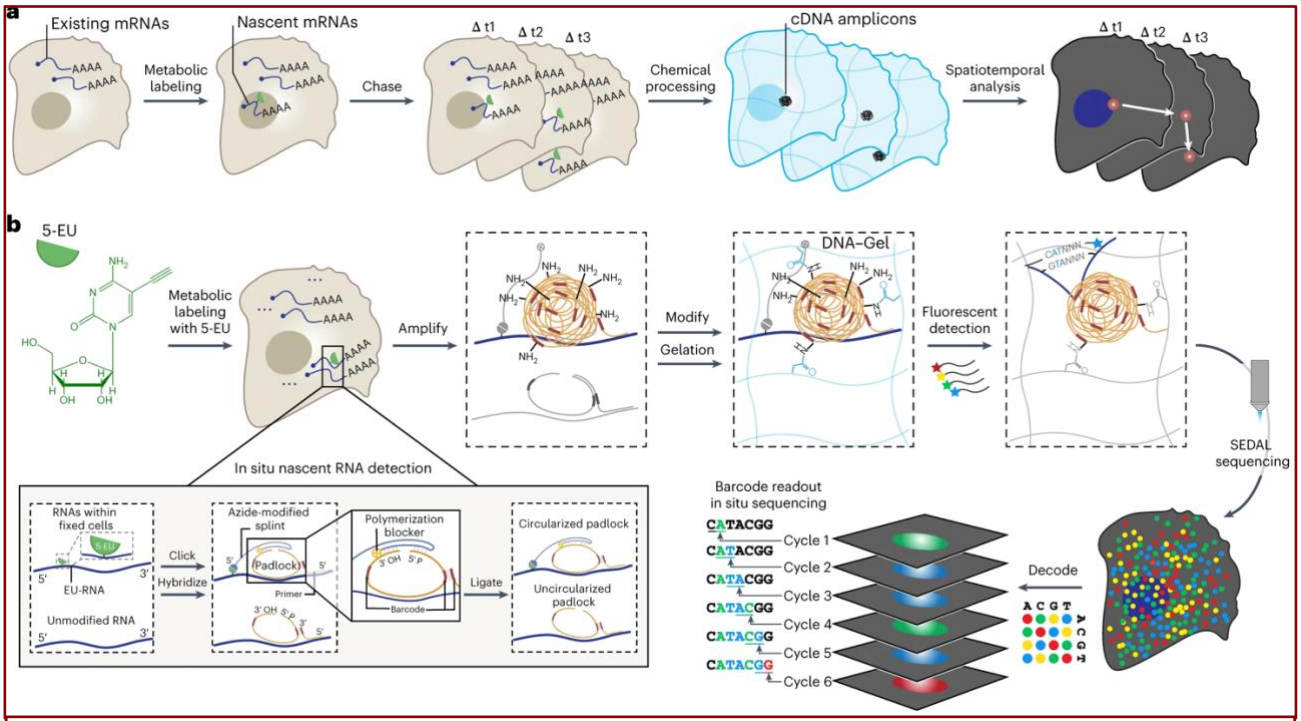


Figure 2. Overview of TEMPOmap workflow. After 5-EU-labeled cells are prepared, a set of tri-probes (splint, primer and padlock) are conjugated or hybridized to cellular mRNAs, resulting in the enzymatic replication of each padlock sequence into cDNA amplicons. The amplicons are anchored *in situ* via a functionalized acrylic group (blue) to a hydrogel mesh to create a DNA-gel hybrid (blue wavy lines). The five-base barcode on each amplicon is read out by six rounds of SEDAL. Thus, multiplexed RNA quantification reveals gene expression in nascent subcellular locations.

TEMPOmap integrates metabolic labeling and selective amplification of pulse-labeled nascent transcriptomes with state-of-the-art 3D *in situ* RNA sequencing at 200 nm resolution within a hydrogel-cell scaffold. By implementing pulse-chase labeling, TEMPOmap enables the simultaneous tracking of key kinetic parameters for hundreds to thousands of genes throughout their RNA life cycle, including assessing rates of transcription, decay, nuclear export, and cytoplasmic translocation. By using these spatiotemporal parameters, the study's findings demonstrate that different genes undergo distinct regulatory processes at various stages of the RNA life cycle and across different cell-cycle phases, thereby contributing to their respective gene functions. It is noteworthy that TEMPOmap's detection efficiency may have sequence biases, such as U-rich sequence, since it requires the metabolic labeling of U analogs and relies on DNA probe design. In summary, spatiotemporally resolved transcriptomics profiled by TEMPOmap provides a gateway to uncovering new spatiotemporal gene regulation principles.

Multimodal charting of molecular and functional cell states via *in situ* electro-sequencing

Li, Q., Lin, Z., Liu, R. *et al. Cell* (2023), https://doi.org/10.1016/j.cell.2023.03.023

Integrating single-cell gene expression and electrophysiology in intact cells and tissues across time and space is crucial for understanding gene-to-function relationships in diverse fields, from developmental biology to cardiology and neuroscience. In this paper, the authors developed a method called ***in situ* electro-sequencing (electro-seq)** that **combines flexible bioelectronics with *in situ* RNA sequencing**. This integrated technique enables **simultaneous and stable mapping of millisecond-timescale electrical activity and single-cell gene expression within the same cells across intact biological networks**, including cardiac and neural patches. By applying *in situ* electro-seq to human-induced pluripotent stem-cell-derived cardiomyocyte patches, the authors conducted multimodal analysis to gain comprehensive insights into cardiomyocyte electrophysiology, cellular level gene expression, jointly defining cell states, and developmental trajectories. The authors employed machine-learning-based cross-modal analysis, which successfully unveiled gene-to-electrophysiology relationships throughout cardiomyocyte development. Moreover, leveraging long-term stable electrical measurements, they accurately reconstructed gene expression profiles over time. The findings highlight the potential of *in situ* electro-seq to create spatiotemporal multimodal maps in electrogenic tissues, thus advancing our understanding of the specific cell types and underlying gene programs contributing to normal and pathological electrophysiological function. This integrated approach provides a powerful tool for deciphering the intricate interplay between gene expression and electrophysiology in complex biological systems. It is important to note that, although cross-modal correlation and inference have been conducted, the resulting gene-to-electrophysiology relationship should be interpreted as correlation, not causality. Further genetic perturbation or molecular inhibition experiments are needed to fully establish the causal relationship and underlying mechanisms between genes and their functions.

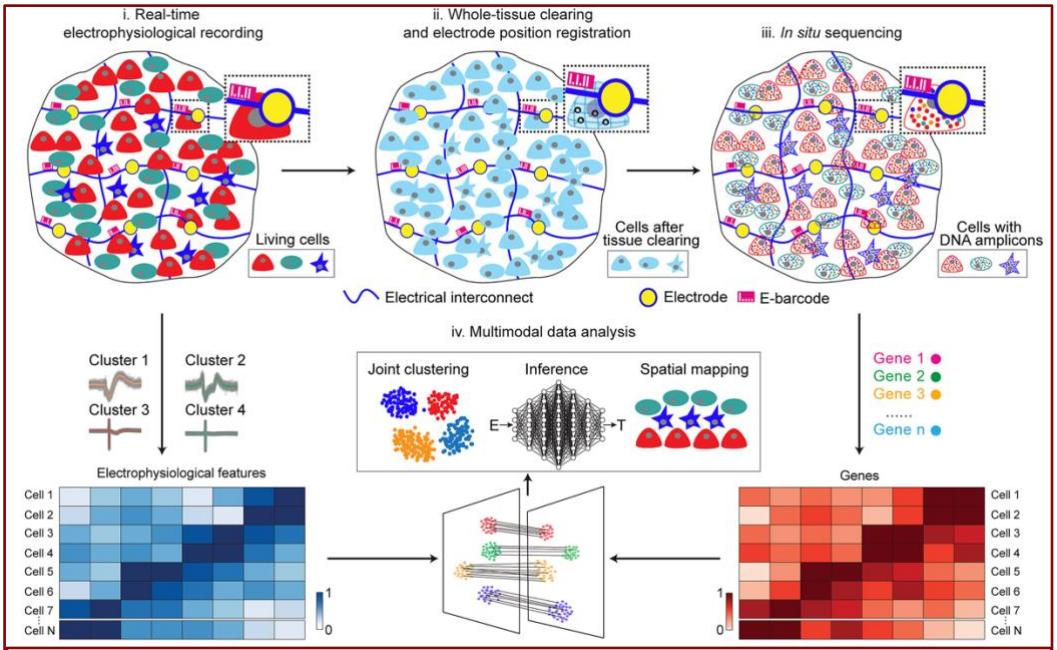


Figure 3. Overview of *in situ* electro-seq method. The in situ electro-seq consists of four key steps: (1) the mesh electronics with E-barcoded electrodes are embedded in the biological samples, such as cardiac and neural patches for continuous electrical recording; (2) the entire cell-electronics hybrid is fixed, embedded in hydrogels, and cleared for *in situ* sequencing; (3) gene identities and E-barcodes are simultaneously read out by multiple cycles of fluorescence imaging to integrate electrical recording with gene expression profiling at single-cell resolution; and (4) the integrated data are analyzed using multimodal and cross-modal visualization and correlation to reconstruct spatiotemporal gene-to-function relationships.

Brain-wide correspondence between neuronal epigenomics and long-distance projections

Zhou, J., Zhang, Z., Wu, M. *et al. bioRxiv* (2023), https://doi.org/10.1101/2023.05.01.538832

Single-cell genetic and epigenetic analyses categorize the brain's neurons into distinct cell-type clusters residing in different brain structures. These cell types play specific roles through targeted long-distance axonal projections, facilitating interactions between them. In this preprint paper, the authors utilized **Epi-Retro-Seq**, a method **combining retrograde labeling with single nucleus methylation sequencing (snmC-Seq)**, to investigate the **association between single-cell epigenomes, cell types, and long-distance projections** in the mouse brain. They examined 33,034 neurons from 32 source regions projecting to 24 targets across the entire brain. The dataset provided insights into the general principles linking projection cell types to their transcriptomic and epigenomic properties and allowed the exploration of specific hypotheses about cell types and connections in relation to genetics. Statistical comparisons were performed to assess the discriminability of neurons projecting to each target from different source regions. Integration of this dataset with the BICCN cell type atlas enabled the linkage of projection cell types to consensus clusters. Additionally, spatial transcriptomic data facilitated the assignment of projection-enriched clusters to smaller source regions. The authors conducted detailed analyses of neurons with projections from the hypothalamus, thalamus, hindbrain, amygdala, and midbrain, uncovering new insights into their properties, including differentially expressed genes, associated cis-regulatory elements and transcription factor binding motifs, and neurotransmitter usage.

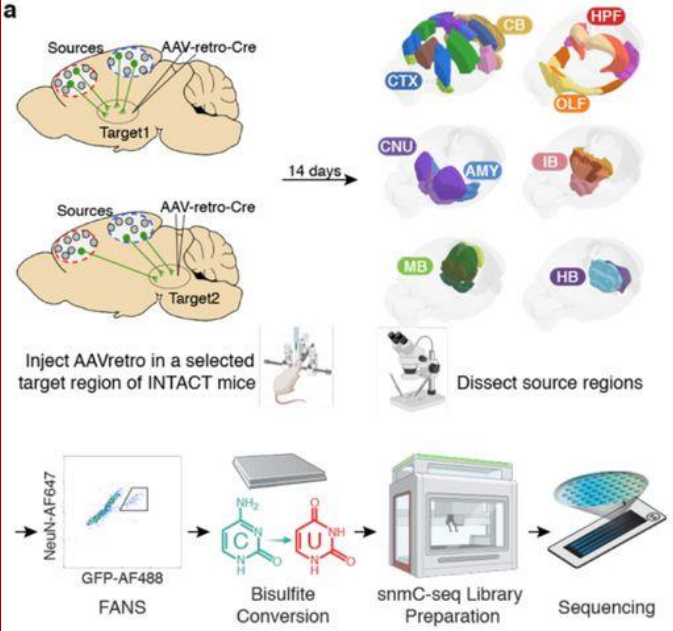


Figure 4. Overview of Epi-Retro-Seq workflow for retrogradely labeling and epigenetically profiling single projection neurons. The retrograde tracer rAAV2-retro-cre was injected into a specific target area in INTACT knock-in mice to label the nuclei of neurons that project to the target area with cre-dependent nuclear GFP. Source regions of interest with projections to the target areas were dissected 14 days after injection. Single GFP+/NeuN+ nuclei were isolated using fluorescence activated nuclei sorting (FANS), and then subjected to snmC-seq library preparation and sequencing for epigenome profiling.

Next Single Cell Seminar

Time and place: TBA

Speakers: TBA

If you would like to announce anything single cell related, e.g. job announcement, event, your published paper, technology development etc., please contact us.

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