Evaluating the use of Dynamo Trajectory Inference for Non-Kinetic scRNA-seq Data

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Abstract

This project evaluates the **Dynamo** framework for reconstructing developmental trajectories in single-cell RNA sequencing (scRNA-seq) data that lack spliced and unspliced RNA counts. These counts are typically used to estimate RNA velocity to order cells along developmental trajectories. In their absence, alternative methods can impose a **pseudotime trajectory** based on relative gene expression. This work explores whether Dynamo's vector field projections remain informative under such constraints. It was found that the resulting vector fields reflect only the imposed pseudotime gradient, rather than independently inferred cell state transitions. This highlights a key limitation of velocity-based models when applied to static transcriptomic data and emphasises the distinction between **descriptive trajectory mapping** and **mechanistic modelling** of cell fate.

Introduction

Single-cell RNA sequencing (scRNA-seq) measures gene expression in individual cells, untangling cellular diversity and dynamics. With computational tools, it is possible to predict cell fates and infer how cells transition from one state to another—a process known as trajectory inference.

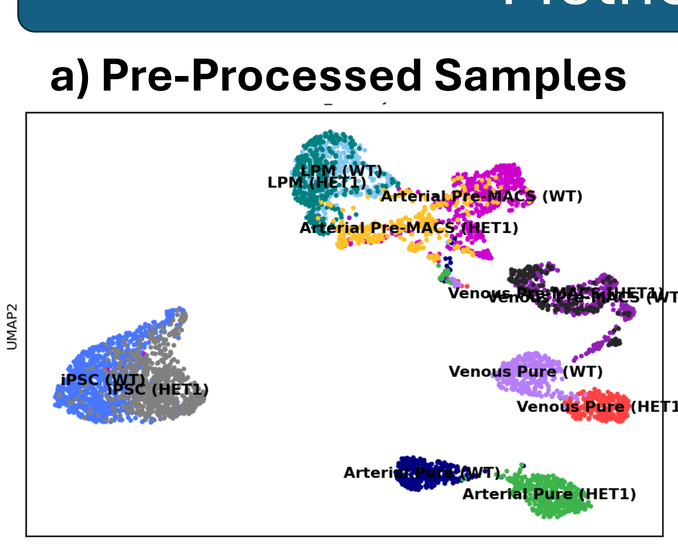
Dynamo[1] is one such method. It uses **RNA velocity**—the rate of gene expression change estimated from the ratios of spliced to unspliced RNA—to **reconstruct transcriptional vector fields**. These vector fields model how cells move through gene expression space over time, helping to **infer developmental paths** and **cell-state transitions**. However, when RNA velocity data is unavailable, a virtual trajectory can be constructed based on relative gene expression. Although this **pseudotime** does not capture the true temporal directionality of differentiation, it can approximate developmental progression from static transcriptomic data.

Aims

This project aims to assess Dynamo's accuracy in reconstructing trajectories using Pseudotime on real world data.

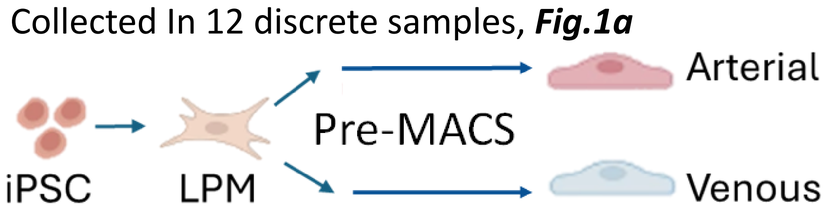
Impact: If successful, Dynamo could open new avenues for modelling developmental processes in systems where spliced RNA data is unavailable

Methods and Materials



Endothelial developmental Drop-seq scRNA-seq dataset from University of Dundee.

- Tracks differentiation: stem cell (iPSC) → Lateral Plate Mesoderm (LPM) → bifurcation → precursor pre-macrophage (Pre-MACS) → arterial and venous cells. Collected In 12 discrete samples, ***Fig.1a***



- Includes both wild-type (WT) and HET1 mutant cell types.
- Analysed using best-practice scRNA-seq in Python (ScanPy, anndata, Dynamo, Palantir) and R (Seurat, Monocle).

Analysis followed **Current Best Practices in scRNA-seq**[2]:

1. **Pre-Processing:** Normalisation, Quality control, Dimensionality reduction, Batch correction
2. **Clustering:** Grouped cells by similarity in gene expression within UMAP embedding.
3. **Annotation: Identified** and **labelled** cell identities by comparing sample/clustering plots ***Fig.1b***
4. **Pseudotemporal Ordering:** placed cells along developmental timeline, "root" LPM to terminal pure states. Using Palantir, diffusion Pseudotime(DPT), Monocle ***Fig.1c***
5. **Trajectory Inference:** Dynamo pipeline utilised to generate and plot vector field of RNA expression. ***Fig.2***
6. **Analysis:** Vector field and key gene expression analysed and compared to the literature

Figure 1: A collection of UMAP plots showing the endothelial cell developmental trajectory for Wild Type and Heterozygous(HET1) backgrounds: ***a:*** Pre-Processed iPSC to Pure states, each labelled with corresponding cellular identities. ***b:*** Transition from LPM to terminal arterial and venous fates. Displays clear bifurcation into intermediate pre-MACS with isolated pure states. ***c***: DPT pseudotime values across UMAP reflecting progressive transcriptional maturation, across both cell types.

Results and Discussion

Dynamo Implementation

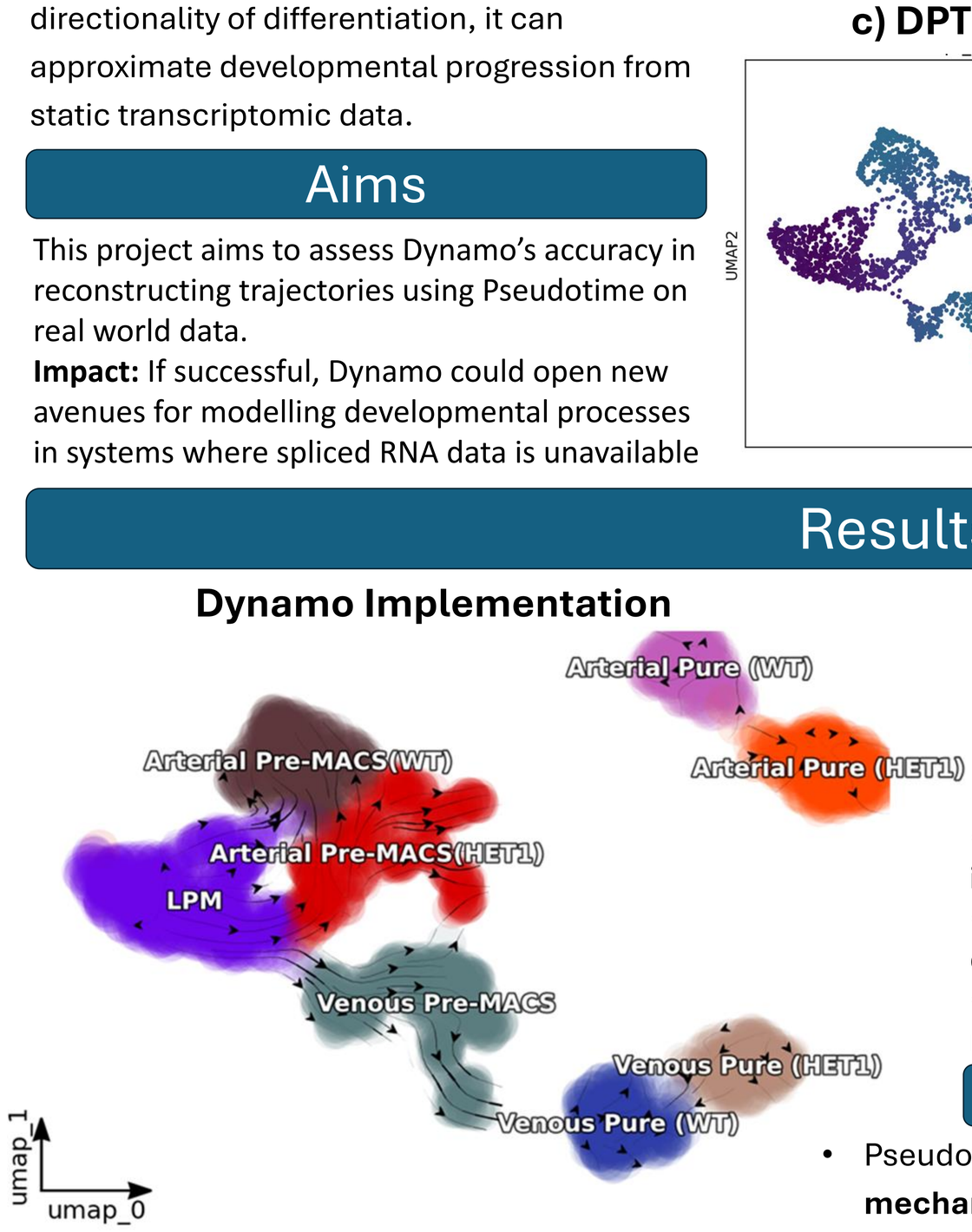


Figure 2: Shows an implementation of Dynamo on the clustered, temporally ordered data(DPT) embedded on the same UMAP as *Fig.1*. The vector field spines overlaying the clusters illustrate the projected change of RNA within the vector field

Pseudotime Accurately Reconstructs Expected Lineage Trajectories

- The pseudotime trajectory accurately **reflects known biological progression**, with LPM at early stages and terminal arterial/venous states at later stages, forming a **coherent linear path.**

Pseudotime Gradients Do Not Reflect Underlying Biological Dynamics

- **Dynamo's vector field** visually follows the pseudotime gradient, but this **reflects the imposed structure rather than independently inferred transitions**.
- The vector field spines **represent a projection of pseudotemporal ordering**—not true dynamic movement—due to the absence of RNA velocity data.
- However, this approach does not validate the inferred vector field or capture mechanistic insights into transcriptional dynamics.

Conclusion

- Pseudotime **reflects expected lineage relationships but not underlying genetic or kinetic mechanisms**. Dynamo can visualise these trajectories, but without RNA velocity, the **vector fields mirror-imposed structure rather than inferred developmental dynamics**. Therefore, to perform trajectory inference **Pseudotime is not a valid substitute for kinetic information**
- Drawing additional conclusions from this trajectory would not be rooted in scientific fact. Therefore, **different analysis pipelines** MOSLin or Mefisto are needed[3].

References

[1] Xiaojie Qiu et al. "Mapping transcriptomic vector fields of single cells". In: Cell 185.4 (2022), pp. 690–711. doi: 10.1016/j.cell.2021.12.045.

[2] Malte D Luecken and Fabian J Theis. "Current best practices in single-cell RNA-seq analysis: a tutorial". In: Molecular systems biology 15.6 (2019), e8746. doi: 10.15252/msb.20188746

[3] Lange, Marius, et al. "Mapping lineage-traced cells across time points with moslin." *Genome Biology* 25.1 (2024): 277. doi: 10.1186/s13059-024-03422-4