A-399

RNA Velocity Comparison Method Based on Contrastive Learning for Cross-Condition Analysis

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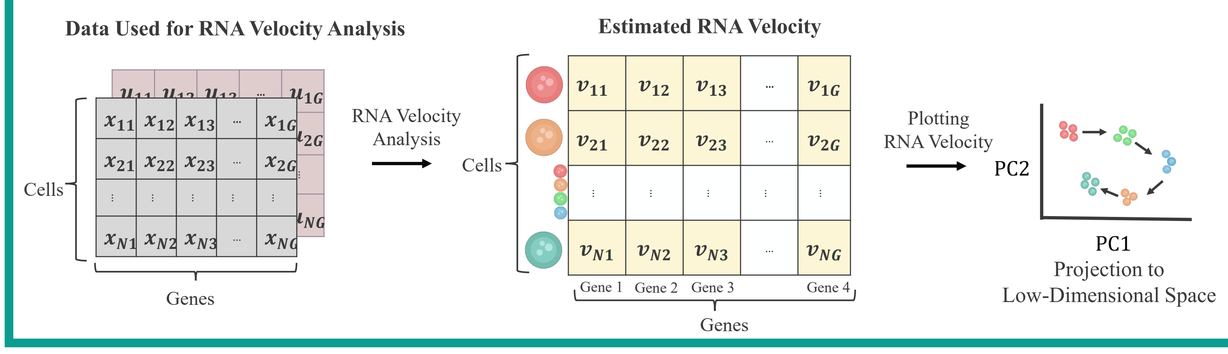
Code : https://github.com/Keybo2066/Velo_compare

1. Introduction

What is RNA Velocity ?

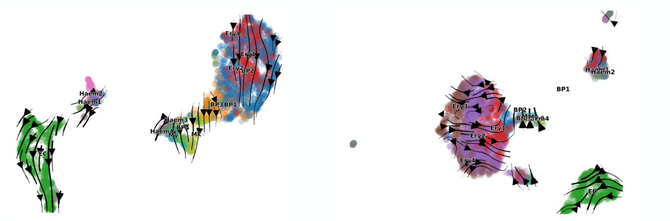
An index for evaluating the **direction of cell differentiation trajectories** - assessing which direction cells will take in terms of gene expression states in the future. RNA velocity estimation enables prediction of future **cell differentiation trajectories.**

Standard RNA Velocity Analysis Workflow



Issues with Current Research

When comparing RNA velocities estimated from two conditions of samples (e.g., **Wild Type** and **Knock Out** type), there is no shared low-dimensional embedding space, making it impossible to compare differentiation processes between the two conditions or identify specific cell types.



RNA velocity vector field estimated for **WT group**

RNA velocity vector field estimated for **KO group**

Limitation of Cross-Condition Analysis :
Differentiation processes of erythroid lineage cells from WT and KO conditions collected from chimeric mice.
WT condition : Wild Type cells
KO condition : Gata1 knockout cells
Motivation for Comparison :
To quantitatively compare the effects of Gata1 gene presence/absence on cell differentiation trajectories.

Goal :

Development of a framework enabling RNA velocity comparison between two conditions
Proposal 1 : Proposal of embedding method for shared latent space
Proposal 2 : Proposal of comparison method for RNA velocity vectors between two groups

2. Methods

Proposal 1 : Embeddings

Objective Function : Optimized using Adam optimizer.

$$\min_{\{\theta,\phi,\mathbf{z}\}} \sum \left[\mathcal{L}_{ELBO}(\mathbf{x},\mathbf{z};\theta,\phi) + \lambda_{cont}\mathcal{L}_{contrast}\left(Z^{\mathrm{WT}},\mathrm{Z}^{\mathrm{KO}},Y^{\mathrm{WT}},Y^{\mathrm{KO}}\right) + \lambda_{align}\mathcal{L}_{align}(Z,Y)\right]$$

Variational autoencoder term — Contrastive learning term — Regularization term promoting cluster alignment

Definition

- $\mathcal{C}=\{c_1,c_2,\dots,c_C\}$: Set of cell type labels
- $y_i^{KO}\in\mathcal{C}$: Cell type label of cell i in KO group
- $y_j^{WT}\in\mathcal{C}$: Cell type label of cell j in WT group
- $Y^{\mathrm{WT}}=\{y_i^{\mathrm{WT}}\}_{i=1}^{N_{\mathrm{WT}}}$: Cell labels for WT group
- $Y^{\mathrm{KO}}=\{y_j^{\mathrm{KO}}\}_{i=1}^{N_{\mathrm{KO}}}$: Cell labels for KO group
- $\mathcal{S}^{d-1}=\{\mathbf{z}\in\mathbb{R}^d \mid \|z\|=1\}$: Unit hypersphere
- d : Dimension of latent space
- $\mathcal{P}=\{(i,j)\mid y_i^{WT}=y_j^{KO}\}$: Set of pairs belonging to the same cell type between WT and KO
- θ,ϕ : Neural network parameters

Input

- $\mathbf{X}^{\mathrm{WT}}\in\mathbb{R}^{N_{\mathrm{WT}}\times G}$: Gene expression matrix for WT group
- $\mathbf{X}^{\mathrm{KO}}\in\mathbb{R}^{N_{\mathrm{KO}}\times G}$: Gene expression matrix for KO group
- $\mathbf{x}_i^{\mathbf{WT}}\in\mathbb{R}^G$: Expression vector of cell i in WT group $(i=1,\dots,N_{\mathrm{WT}})$
- $\mathbf{x}_j^{\mathbf{KO}}\in\mathbb{R}^G$: Expression vector of cell j in KO group $(j=1,\dots,N_{\mathrm{KO}})$
- $N_{\mathrm{WT}},N_{\mathrm{KO}}$: Number of cells in both groups
- G : Number of genes common to both groups
- τ : Temperature parameter
- $\lambda_{cont},\lambda_{align}$: Tuning parameters

Output

- $\mathbf{z}_i^{\mathrm{WT}}\in\mathcal{S}^{d-1}$: Latent representation vector of cell i in WT group
- $\mathbf{z}_j^{\mathrm{KO}}\in\mathcal{S}^{d-1}$: Latent representation vector of cell j in KO group
- $Z^{\mathrm{WT}}=\{z_i^{\mathrm{WT}}\}_{i=1}^{N_{\mathrm{WT}}}$: Latent representations for WT group
- $Z^{\mathrm{KO}}=\{z_i^{\mathrm{KO}}\}_{i=1}^{N_{\mathrm{KO}}}$: Latent representations for KO group

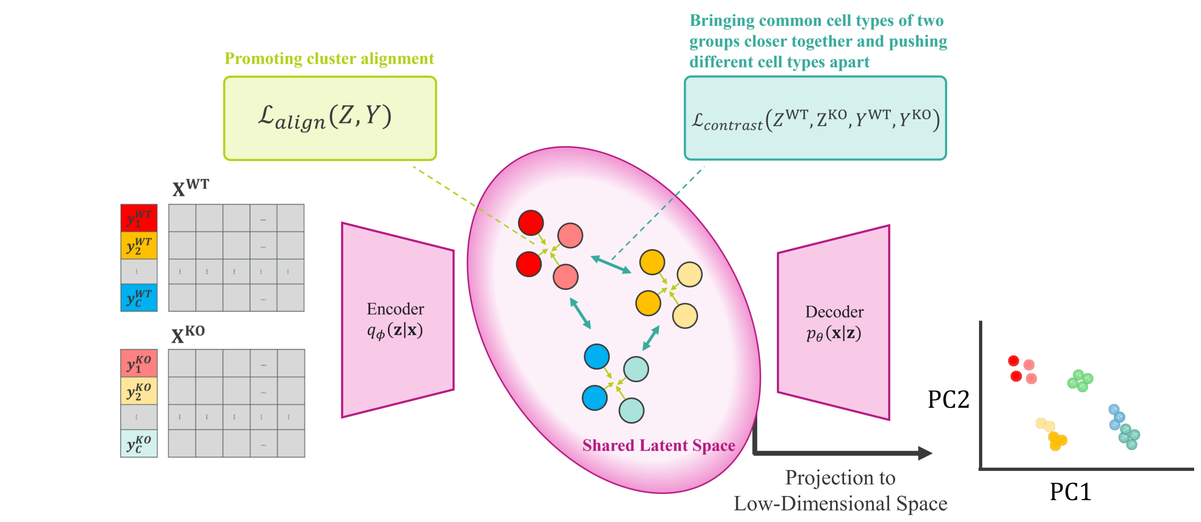
Proposal 1 : Model Architecture

Correspondence between same cell types across two conditions

Learn latent representations to bring representations of the same cell types between WT and KO conditions closer together

Preservation of cell type structure

Encourage cells of the same type to cluster together within the latent space



Variational autoencoder term :

In this method, we define latent variables $z_n\in\mathbb{S}^{d-1}$ on the unit hypersphere, using a uniform distribution $p(z_n)=\mathcal{U}(\mathbb{S}^{d-1})$ as the prior distribution and a Power Spherical distribution $q_\phi(z_n|x_n)$ as the approximate posterior distribution. The variational autoencoder-derived term consists of the following [2].

$$\mathcal{L}_{ELBO}(\mathbf{x_i},\mathbf{z_i};\theta,\phi) = \underbrace{-\mathbb{E}_{q_\phi}[\log p_\theta(\mathbf{x_i}|\mathbf{z_i})]}_{\text{Expected negative log-likelihood}} + \underbrace{D_{KL}(q_\phi(\mathbf{z_i}|\mathbf{x_i})\|p(z))}_{\text{Latent variable regularization term}}$$

Contrastive learning term :

Brings latent representations closer between the same cell types in WT and KO conditions. Increases similarity for same cell type pairs across both conditions while expanding distances from other pairs.

$$\mathcal{L}_{contrast}\left(Z^{\mathrm{WT}},\mathrm{Z}^{\mathrm{KO}},Y^{\mathrm{WT}},Y^{\mathrm{KO}}\right) = \frac{1}{2}\left(\underbrace{\sum_{(i,j)\in\mathcal{P}} -\log\frac{\exp\left(\frac{\cos(z_i^{\mathrm{WT}},z_j^{\mathrm{KO}})}{\tau}\right)}{\sum_{l=1}^{N_{\mathrm{KO}}}\exp\left(\frac{\cos(z_i^{\mathrm{WT}},z_l^{\mathrm{KO}})}{\tau}\right)}}_{\text{WT group}\rightarrow\text{KO group}} + \underbrace{\sum_{(j,i)\in\mathcal{P}} -\log\frac{\exp\left(\frac{\cos(z_j^{\mathrm{KO}},z_i^{\mathrm{WT}})}{\tau}\right)}{\sum_{m=1}^{N_{\mathrm{WT}}}\exp\left(\frac{\cos(z_j^{\mathrm{KO}},z_m^{\mathrm{WT}})}{\tau}\right)}}_{\text{KO group}\rightarrow\text{WT group}}\right)$$

Regularization term promoting cluster alignment :

Attracts latent representations of cells belonging to the same cell type toward their cluster centers.

$$\mathcal{L}_{align}(Z,Y) = \frac{1}{C}\sum_{c=1}^{C}\frac{1}{|S_c|}\sum_{i\in S_c}\|z_i-\hat{\mu}_c\|^2$$

- $Z=Z^{\mathrm{WT}}\cup Z^{\mathrm{KO}}$: Set of all cell latent representations
- $Y=Y^{\mathrm{WT}}\cup Y^{\mathrm{KO}}$: Set of all cell labels
- $\mu_c=\frac{1}{|S_c|}\Sigma_{i\in S_c}Z_i$: Centroid of latent representations for cell type c
- $\hat{\mu}_c=\frac{\mu_c}{\|\mu_c\|}$: Centroid normalized on the unit hypersphere
- $S_c=\{i\mid y_i=c\}$: Set of cell indices for cell type c

Proposal 2 : Comparison Method for RNA Velocity Vectors

Plot vector fields of both conditions in the shared embedding space of the estimated shared space. Divide the low-dimensional space into grid units and calculate average velocity vectors for each grid to capture local structures of the two groups. The same calculation is performed for the KO conditions as well.

Step 1

Calculation of transition probability matrix between cells

$$\pi_{ii'}^{\mathrm{WT}} = \exp\left(\frac{\mathrm{corr}(\rho(\mathbf{x}_{i'}-\mathbf{x}_i),\rho(\mathbf{v}_i))}{\sigma}\right)$$

- $\mathbf{v_i}\in\mathbb{R}^G$: RNA velocity of cell i
- $\rho(x)=\mathrm{sgn}(x)\cdot\sqrt{|x|}$: Variance stabilizing transformation
- corr : Pearson correlation
- σ : Scaling factor

Step2

Projection to embedding space

$$\Delta\mathbf{u}_{ii'}^{\mathrm{WT}} = \frac{\mathbf{u}_{i'}^{\mathrm{WT}}-\mathbf{u}_i^{\mathrm{WT}}}{\left\|\mathbf{u}_{i'}^{\mathrm{WT}}-\mathbf{u}_i^{\mathrm{WT}}\right\|}$$

$$\Delta\mathbf{u}_i^{\mathrm{WT}} = \sum_{i'\in\mathcal{N}(i)}\left(\pi_{ii'}^{\mathrm{WT}}-\frac{1}{n_i}\right)\cdot\Delta\mathbf{u}_{ii'}^{\mathrm{WT}}$$

- $\mathbf{u}_i^{\mathrm{WT}}\in\mathbb{R}^2$:Coordinate vector of cell i
- $\mathbf{u}_{i'}^{\mathrm{WT}}\in\mathbb{R}^2$: Coordinate vector of cell i' neighboring cell i
- $\Delta\mathbf{u}_{i,i'}^{\mathrm{WT}}=$: Directional vector from cell i to cell i'
- $\mathcal{N}(i)$: Set of neighboring cells cell i
- $\Delta\mathbf{u}_i^{\mathrm{WT}}$: RNA velocity vector of cell i

Step3

Calculation of average velocity vectors in grid units

$$\bar{v}_{mn}^{WT} = \frac{1}{N_{mn}^{WT}}\sum_{i\in\mathcal{C}_{mn}^{WT}}\Delta\mathbf{u}_i^{\mathrm{WT}}$$

Divide the low-dimensional space into grid units and calculate average velocity vectors for each grid (m,n) for each condition. $\mathcal{C}_{mn}^{WT}$ represents the set of cell indices contained in each grid.

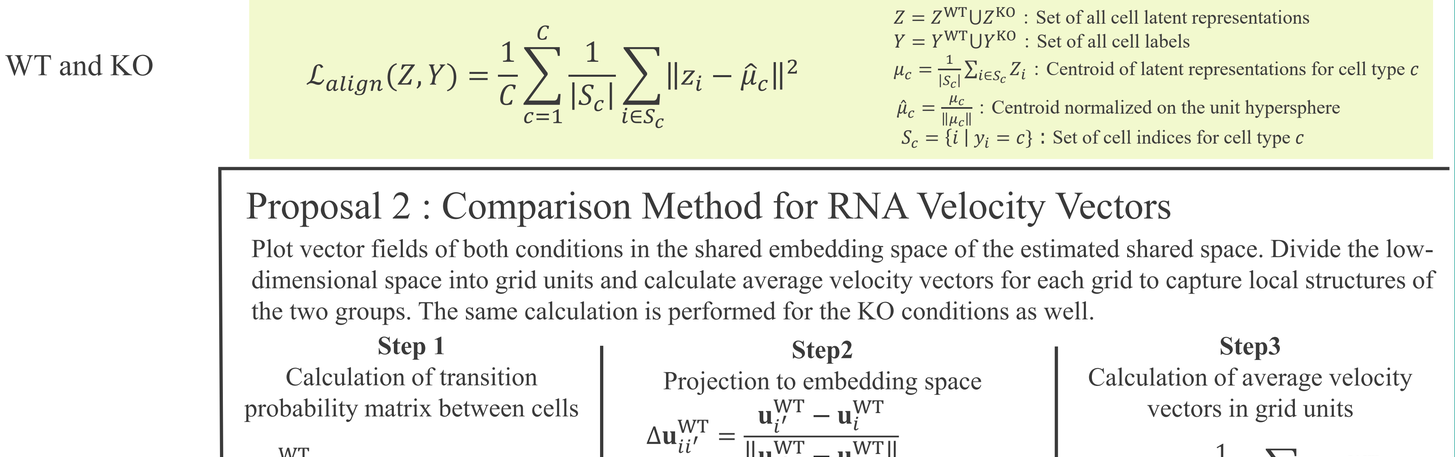
3. Results

Objective :

Apply the proposed method to RNA velocity data from Gata1 gene knockout (KO) and non-knockout (WT) conditions to compare **cell differentiation trajectories** between both conditions and validate the effectiveness of the proposed method.

Dataset :

We used a scRNA-seq dataset (GSE167576) from Gata1 chimeric embryonic mice. Hematopoietic cell clusters were extracted, and VeloVI [3] was applied to KO/WT conditions to estimate RNA velocity.



WT group vector field — KO group vector field — Cross-condition comparison (cosine similarity)

Results:

In the WT condition, smooth velocity vectors along the direction of erythroid lineage differentiation were observed, confirming normal hematopoietic processes. In contrast, the KO condition showed a prominent differentiation process where progression beyond Ery2 was inhibited, visually capturing the differentiation defects due to Gata1 deficiency reported in [1]. Furthermore, from the cosine similarity map of velocity vectors, we were able to quantitatively identify cell populations (particularly the BP population) with significantly different directionality between WT and KO conditions.

Future Works

Addressing cases with few common cell types: Since our method utilizes contrastive learning based on common cell types between two conditions, learning becomes unstable when common cell types are limited. As a solution, we consider extending to models that enable matching based on continuity of cell states rather than cell type levels.

Determination of tuning parameters: The shared latent space embedding depends on parameters. Development of specific parameter determination methods is needed.

References :

[1] Barile, M., Imaz-Rosshandler, I., Inzani, I., Ghazanfar, S., Nichols, J., Marioni, J. C., ... & Göttgens, B. (2021). Coordinated changes in gene expression kinetics underlie both mouse and human erythroid maturation. *Genome biology*, **22**, 1-22.

[2] De Cao, N., & Aziz, W. (2020). The power spherical distribution. *arXiv preprint arXiv:2006.04437*.

[3] Gayoso, A., Weiler, P., Lotfollahi, M., Klein, D., Hong, J., Streets, A., ... & Yosef, N. (2024). Deep generative modeling of transcriptional dynamics for RNA velocity analysis in single cells. *Nature methods*, **21**(1), 50-59.