Spatiotemporal Multiplexing of Single-Cell RNA Velocity Trajectories via Graph-Regularized Optimal Transport for Lineage Deconvolution in Murine Hematopoiesis

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Abstract. Reconstructing developmental lineage hierarchies from single-cell transcriptomic data remains a fundamental challenge in systems biology, particularly when asynchronous cell-state transitions obscure pseudotemporal ordering. Here, we present SCRVOT, a graph-regularized optimal transport framework that integrates RNA velocity vectors with spatially resolved transcriptomic anchors to resolve hematopoietic progenitor bifurcations at single-cell resolution. Applied to murine bone marrow datasets comprising 84,000 cells across five developmental timepoints, SCRVOT demonstrated superior branch-point disambiguation compared to existing trajectory inference tools, achieving a 31% improvement in lineage assignment accuracy as measured by clonal barcoding ground truth. The framework further incorporates a Wasserstein-penalized regularization term to mitigate dropout-induced trajectory distortion. These findings establish SCRVOT as a robust computational strategy for dissecting multipotent progenitor fate decisions with direct translational relevance to leukemogenesis modeling.

Keywords: single-cell RNA sequencing, RNA velocity, optimal transport, hematopoietic lineage tracing, trajectory inference, graph regularization, pseudotime analysis, bone marrow progenitor cells, Wasserstein distance

Introduction

The precise delineation of cell fate decisions during hematopoiesis constitutes one of the most intensively studied problems in developmental biology and translational hematology. Hematopoietic stem cells (HSCs) residing within the bone marrow niche give rise to a hierarchically organized continuum of progenitor populations, each committed to distinct myeloid, lymphoid, or erythroid fates through a series of epigenetically reinforced transcriptional bifurcations. Despite decades of investigation using surface immunophenotyping and bulk transcriptomics, the precise molecular determinants governing multipotent progenitor (MPP) fate allocation have remained incompletely resolved, largely due to the population-averaging inherent to ensemble-level assays. The advent of single-cell RNA sequencing (scRNA-seq) technologies has fundamentally transformed our capacity to interrogate transcriptional heterogeneity at cellular resolution. Landmark studies employing droplet-based microfluidic platforms have catalogued unprecedented diversity among phenotypically homogeneous progenitor compartments, revealing that canonical surface marker-defined populations harbor functionally distinct transcriptional substates. However, static transcriptomic snapshots are intrinsically limited in their ability to infer directionality of state transitions, motivating the development of RNA velocity—a computational framework that leverages the kinetics of unspliced-to-spliced mRNA ratios to infer instantaneous transcriptional momentum within individual cells. Despite the transformative potential of RNA velocity-based trajectory inference, current implementations suffer from several well-characterized limitations: sensitivity to sparse count matrices introduced by stochastic transcript capture, systematic bias in dynamical model fitting under conditions of low transcriptional activity, and the absence of spatial context that can constrain biologically plausible cell movement within the marrow microenvironment. These limitations become particularly acute when resolving the Megakaryocyte-Erythroid Progenitor (MEP) versus Granulocyte-Monocyte Progenitor (GMP) bifurcation, which proceeds through transient intermediary states detectable only within narrow temporal windows. Concurrently, optimal transport theory has emerged as a mathematically principled approach for mapping probability distributions across metric spaces, offering a natural formulation for modeling cellular transitions between transcriptional states across developmental time. Recent applications of Waddington-Optimal Transport (WOT) have demonstrated compelling utility in embryonic developmental atlases; however, their integration with directional RNA velocity information and spatially resolved transcriptomic data remains an open methodological challenge. In the present study, we address these gaps by introducing SCRVOT (Single-Cell RNA Velocity Optimal Transport), a unified computational framework that couples graph-regularized optimal transport with RNA velocity vector fields and Slide-seq-derived spatial anchors. We validate the framework against high-resolution clonal barcoding datasets and benchmark its performance across multiple published murine hematopoietic atlases. The manuscript is organized as follows: Section 2 details the mathematical formulation of the SCRVOT algorithm and its regularization strategy; Section 3 describes the experimental datasets and preprocessing pipelines; Section 4 presents benchmarking

results and lineage-resolved trajectory maps; Section 5 discusses biological implications for HSC fate commitment and potential applications to malignant hematopoiesis modeling; and Section 6 provides concluding remarks alongside future methodological directions.

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