VeloViz: RNA-velocity informed embeddings for visualizing cellular trajectories

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15 **0 Abstract**

16 Single cell transcriptomic technologies enable genome-wide gene expression measurements in individual
 17 cells but can only provide a static snapshot of cell states. RNA velocity analysis can infer cell state changes
 18 from single cell transcriptomics data. To interpret these cell state changes as part of underlying cellular
 19 trajectories, current approaches rely on visualization with principal components, t-distributed stochastic
 20 neighbor embedding, and other 2D embeddings derived from the observed single cell transcriptional
 21 states. However, these 2D embeddings can yield different representations of the underlying cellular
 22 trajectories, hindering the interpretation of cell state changes. We developed VeloViz to create RNA-
 23 velocity-informed 2D and 3D embeddings from single cell transcriptomics data. Using both real and
 24 simulated data, we demonstrate that VeloViz embeddings are able to consistently capture underlying
 25 cellular trajectories across diverse trajectory topologies, even when intermediate cell states may be
 26 missing. By taking into consideration the predicted future transcriptional states from RNA velocity
 27 analysis, VeloViz can help visualize a more reliable representation of underlying cellular trajectories.
 28 VeloViz is available as an R package on GitHub (<https://github.com/JEFworks-Lab/veloviz>) with
 29 additional tutorials at <https://JEF.works/veloviz/>.

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32 **1 Introduction**

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34 Current technologies for high-throughput single cell transcriptomics profiling provide a static snapshot of
 35 the transcriptional states in individual cells. Still, the continuum of transcriptional states for cells along
 36 dynamic processes such as organ development or tumorigenesis can be used to infer how cell states may
 37 change over time (Tritschler *et al.*, 2019; Saelens *et al.*, 2019). Notably, RNA velocity analysis can be
 38 applied to infer dynamics of gene expression and predict the future transcriptional state of a cell from
 39 single cell RNA-sequencing and imaging data (La Manno *et al.*, 2018; Xia *et al.*, 2019).

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41 To interpret such cell state changes from RNA velocity analysis, current approaches project the observed
 42 current and predicted future transcriptional states onto 2-dimensional (2D) embeddings in order to
 43 visualize the putative directed cellular trajectory (La Manno *et al.*, 2018; Zywitzka *et al.*, 2018; Bastidas-
 44 Ponce *et al.*, 2019; Zhang *et al.*, 2019). Previously used 2D embeddings include those derived from
 45 principal components (PC), t-distributed Stochastic Neighbor Embeddings (t-SNE), Uniform Manifold
 46 Approximation and Projection (UMAP), and diffusion maps (Coifman *et al.*, 2005; Maaten and Hinton,
 47 2008; McInnes *et al.*, 2018) established using the observed single cell transcriptional states. However,
 48 these approaches can yield different representations of the underlying cellular trajectory. Furthermore, in
 49 dynamic processes where intermediate cell states are not well represented due to their transient nature or
 50 due to technical limitations in sample collection and processing, current 2D embeddings may be unable
 51 to capture global relationships between cell subpopulations thereby hindering downstream interpretation
 52 of cell state changes (Kester and Oudenaarden, 2018; Weinreb *et al.*, 2018). Although alternative non-
 53 visual methods such as identifying dynamic driver-genes have been developed to help interpret
 54 information from RNA velocity analysis (Bergen *et al.*, 2020), visual representation of cellular trajectories
 55 remains an important approach to understanding the overall relationships between cell states.

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57 Here, we developed VeloViz to visualize cellular trajectories by incorporating information about each
 58 cell's predicted future transcriptional state inferred from RNA velocity analysis. Using both real and
 59 simulated data representing cellular trajectories, we demonstrate that VeloViz embeddings are better able
 60 to consistently capture underlying cellular trajectories across diverse trajectory topologies compared to
 61 other evaluated methods. Likewise, given simulated cellular trajectories with missing intermediate cell
 62 states, we find that VeloViz embeddings are able to more robustly retain the overall cell state relationships
 63 in the underlying trajectories compared to other evaluated methods.

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66 **2 Method**

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68 In order to create an RNA-velocity-informed embedding, VeloViz uses each cell's current observed and
69 predicted future transcriptional states inferred from RNA velocity analysis to represent cells in the
70 population as a graph (Figure 1, Supplementary Information 1). Briefly, starting with spliced and
71 unspliced RNA counts from single-cell RNA-sequencing (scRNA-seq) data or cytoplasmic and nuclear
72 RNA counts from single cell molecular imaging data, the predicted future transcriptional state of cells are
73 inferred using RNA velocity pipelines such as velocity (La Manno *et al.*, 2018) or scVelo (Bergen *et al.*,
74 2020). We then optionally restrict to overdispersed genes (Fan *et al.*, 2016) and unit scale each gene's
75 variance, as well as mean center each gene's expression for the observed current and predicted future
76 transcriptional states, followed by dimensionality reduction by projecting these observed current and
77 predicted future transcriptional states into a common PC space. Using this reduced dimensional
78 representation of the observed current and predicted future transcriptional states, VeloViz then computes
79 a composite distance ($D_{A \rightarrow B} = -\cos(\theta_{AB}) * \frac{1}{\omega * d_{AB} + 1}$) between all cell pairs in the population. The
80 composite distance between two cells, Cell A and Cell B, takes into account: (1) their transcriptional
81 dissimilarity, defined as the Euclidean distance in the common PC space between Cell A's predicted future
82 state and Cell B's observed current state (d_{AB}) and (2) their velocity similarity, defined as the cosine
83 correlation between Cell A's velocity vector and the change vector representing the transition from Cell
84 A to Cell B (θ_{AB}). An additional tuning parameter (ω) weighs the relative importance of the transcriptional
85 similarity and the velocity similarity components. In this manner, the composite distance will be
86 minimized when Cell A's predicted future transcriptional state is similar to Cell B's observed current
87 transcriptional state and when the direction of Cell A's RNA velocity is similar to the direction of the
88 transition from Cell A to Cell B. Based on these composite distances, VeloViz creates a k-nearest neighbor

graph by assigning k directed, weighted edges from each cell to the k neighboring cells with smallest composite distances. Edges are further pruned based on parameters that specify the minimum transcriptional and velocity similarity in order to remove spurious cell state relationships. Finally, the pruned graph can be visualized in 2D or 3D using graph layout or graph-embedding approaches such as force-directed layout algorithms (Fruchterman and Reingold, 1991) or UMAP (McInnes *et al.*, 2018).

3 Results

3.1 Comparing VeloViz to other embeddings

To evaluate the performance of VeloViz, we first assessed VeloViz's ability to capture cellular trajectories in simulated data representing cycling or branching trajectories (Supplementary Information 2). We compared the VeloViz embeddings to more conventional PC, t-SNE, UMAP, and diffusion map embeddings. To evaluate how accurately each embedding captured the ground truth trajectory, we calculated a trajectory consistency (TC) score (Supplementary Information 3, (Boggust *et al.*, 2019)) where high TC scores indicate more accurate representations of the ground truth trajectory. For the simulated cycling trajectory, all evaluated embeddings were able to capture the cycling structure of the trajectory except for the PC embedding (Supplementary Figure 1A). The TC score for the VeloViz embedding was further higher than that of the PC, t-SNE, and UMAP embeddings. For the simulated branching trajectory, the TC score for the VeloViz embedding was higher than TC scores for the t-SNE, UMAP, and diffusion map embeddings (Supplementary Figure 1B-C). Likewise, we evaluated VeloViz's ability to capture simultaneously cellular trajectories in conjunction with terminally differentiated cell-types using simulated data representing both cycling or branching trajectories with stable a cell population. For the simulated cycling trajectory with a stable cell population, all evaluated

114 embeddings were able to correctly distinguish the cycling and stable populations except for the PC
 115 embedding (Supplementary Figure 1 D). Likewise, the VeloViz, t-SNE, UMAP, and diffusion map
 116 embeddings preserved the cycling trajectory, while the PC embedding did not. The TC score for the
 117 VeloViz embedding was higher than that of the other embeddings. For the simulated branching
 118 trajectories with a stable cell population, all embeddings were able to separate the dynamic and stable
 119 populations, but only the VeloViz and PC embeddings were able to capture the underlying branching
 120 trajectory of the dynamic population (Supplementary Figure 1E-F). This is again reflected in the TC
 121 scores, which are consistently higher for the VeloViz and PC embeddings compared to the TC scores for
 122 the t-SNE, UMAP, and diffusion map embeddings. These simulation results demonstrate that VeloViz is
 123 able to capture trajectories of various topologies compared to other embeddings, which may be better
 124 suited for specific topologies.

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126 Next, we assessed VeloViz's ability to capture cellular trajectories in scRNA-seq data. We applied
 127 VeloViz to scRNA-seq data of mouse spermatogenic cells (Supplementary Information 4), where we
 128 expect a developmental progression from spermatogonial stem cells to more differentiated spermatids
 129 (Hermann *et al.*, 2018). For this simple, linear cellular trajectory, VeloViz was able to capture the overall
 130 expected trajectory from secondary spermatocytes to early, mid, then late round spermatids
 131 (Supplementary Figure 2). Generally, PCA, t-SNE, UMAP, and diffusion map were also able to capture
 132 this expected trajectory. To assess VeloViz's ability to capture more complex trajectory structures, we
 133 applied VeloViz to scRNA-seq data of the developing mouse pancreas (Supplementary Information 5),
 134 where we expect to see both cycling and branching topologies at different stages of the trajectory. Briefly,
 135 we expect cycling ductal cells to give rise to endocrine progenitor-precursor (EP) cells, which become
 136 pre-endocrine cells that then differentiate into four hormone producing endocrine cell-types (Alpha, Beta,
 137 Delta, and Epsilon cells) (Bastidas-Ponce *et al.*, 2019). We observed that while all evaluated embeddings
 138 captured the progression of EP cells towards pre-endocrine cells, VeloViz, UMAP, and t-SNE embeddings

also captured the terminal branching differentiation into the different endocrine cell-types, which is not clear in the PC or diffusion map embeddings (Figure 2). In addition, VeloViz was better able to capture the cycling structure of ductal cells. Overall, these results indicate that VeloViz embeddings are able to recapitulate expected trends from real scRNA-seq data

To explore the potential of using VeloViz with velocity estimated from other data types, we further applied VeloViz to multiplexed error-robust fluorescent in situ hybridization (MERFISH) data (Xia *et al.*, 2019) of cycling cultured U-2 OS cells (Supplementary Information 6). Again, we compared the VeloViz embedding to embeddings constructed using PCA, t-SNE, and UMAP and found that all evaluated embeddings, including VeloViz, were able to capture the expected cycling trajectory (Supplementary Figure 3). In this manner, we find that VeloViz is able to capture cellular trajectories of diverse topologies using both simulated and real data from multiple single cell transcriptomics technologies

3.2 Performance with missing intermediate cell states

While uniform sampling of the continuum of transcriptional states for cells along dynamic processes can be used to infer how cell states may change over time, when sampling trajectories with rare or short-lived intermediate cell states or when different cell states are differentially impacted by cell isolation protocols, intermediate cell states may be lost leading to gaps in the observed cellular trajectory (Krishnaswami *et al.*, 2016; Villani *et al.*, 2017; MacLean *et al.*, 2018; Moffitt *et al.*, 2018; Slyper *et al.*, 2020; Fan *et al.*, 2020). We hypothesized that incorporating information about each cell's predicted future transcriptional state could enable VeloViz to more robustly construct representative cellular trajectories even when the sampled cell states contain missing intermediate cell states or gaps in the underlying trajectory.

163 To evaluate the robustness of VeloViz in visualizing trajectories with such missing intermediate cell states,
 164 we used simulated and real single cell transcriptomics data where some intermediate cells were removed,
 165 creating a trajectory gap. Because t-SNE and UMAP preferentially preserve local cell-cell relationships,
 166 we hypothesized that these embeddings would result in two distinct clusters of cells before and after the
 167 simulated gap (Kobak and Berens, 2019; Heiser and Lau, 2020). Therefore, in addition to TC scores, we
 168 calculated a gap distance (Supplementary Information 3), which measures the distance in the 2D
 169 embedding space between cells before and after the simulated gap in the trajectory. Embeddings that
 170 preserve the underlying trajectory despite this simulated gap will have a smaller gap distance. A small gap
 171 distance between cells that are part of the same trajectory will facilitate a clearer depiction of the
 172 underlying cell transitions compared to a large gap distance which may erroneously suggest that the cells
 173 are unrelated.

174

175 Indeed, for the simulated cycling trajectory where cells corresponding to a segment of the cycle were
 176 removed (Supplementary Information 2), VeloViz was the only embedding able to clearly represent the
 177 cycling structure of the trajectory (Supplementary Figure 4A). The gap distance in the VeloViz embedding
 178 was also smaller than in t-SNE, UMAP, and diffusion map embeddings. Likewise, for the simulated
 179 branching trajectories where cells corresponding to a segment of an intermediate branch were removed
 180 (Supplementary Information 2), only VeloViz and PCA were able to preserve the underlying topology
 181 (Supplementary Figure 4B-C). The gap distance in the VeloViz embedding was smaller than that in the t-
 182 SNE, UMAP, and diffusion map embeddings. In contrast, t-SNE and UMAP split cells before and after
 183 the simulated gap into distinct clusters as expected. TC scores were also consistently higher for VeloViz
 184 than with t-SNE, UMAP, and diffusion map embeddings. Similar trends were observed with simulated
 185 data that included both dynamic cycling and branching populations with missing intermediate cell states
 186 along with a stable cell population (Supplementary Figure 4D-F).

187

188 Likewise, for the U-2 OS MERFISH data, to simulate missing intermediate cell states, we removed cells
189 in the G2/M cell cycle phase. Briefly, we identified cells in the G2/M cell cycle phase by computing for
190 each cell a G2/M score based on the aggregated expression of canonical G2/M phase genes
191 (Supplementary Information 6). As before, we compared the VeloViz embedding to those constructed
192 with PCA, t-SNE, and UMAP. We found that VeloViz was better able to retain the cycling trajectory
193 despite the missing G2/M cells compared to the other evaluated embeddings (Supplementary Figure 5).

194
195 Similarly, for the developing mouse pancreas scRNA-seq data, to simulate missing intermediate cell
196 states, we removed pre-endocrine cells and used cell latent time (Bergen et al., 2020) to identify cells
197 before and after pre-endocrine cells in the developmental trajectory and to calculate gap distances in the
198 recalculated embeddings (Supplementary Information 5). Notably, while all embeddings depicted the
199 transition from ductal cells to endocrine progenitors, the subsequent transition from endocrine progenitors
200 into terminal endocrine cell-types was best captured by VeloViz. As expected, t-SNE and UMAP split
201 ductal and endocrine progenitor cells from terminal endocrine cell-types, which is reflected in the gap
202 distances (Figure 3). In particular, the position of endocrine progenitors and terminal endocrine cells and
203 the resulting velocity streams may lead to the interpretation that these two cell populations are
204 differentiating in two separate trajectories.

205
206 Still, because low dimensional representations can vary depending on parameter choices, we explored
207 the effect of changing these parameters on t-SNE and UMAP visualizations to see if certain parameter
208 choices would yield visualizations more representative of the underlying cellular trajectory. For t-SNE
209 embeddings, the perplexity parameter affects the extent to which the embedding reflects global vs. local
210 structure, with higher values resulting in embeddings that better preserve global structure (Kobak and
211 Berens, 2019). However, with a gap in the trajectory, the t-SNE embeddings result in two distinct
212 clusters of cells before and after the trajectory gap, even at large perplexity values (Supplementary

Figure 3). Likewise, for UMAP, we varied the values of two parameters: minimum distance, which controls how densely packed points are in the embedding with small values resulting in more dense clusters, and the number of neighbors, which functions similarly to perplexity in t-SNE (McInnes *et al.*, 2018). As with t-SNE, when embedding data with a simulated gap, UMAP is unable to capture the expected trajectory even at large values of number of neighbors (Supplementary Figure 7). This indicates that when intermediate cell states are missing, t-SNE and UMAP embeddings may be unable to recapitulate the expected underlying trajectory structure regardless of parameter choices. Overall, we find that VeloViz is able to visualize a more reliable representation of underlying trajectories even when intermediate cell states may be missing.

3.3 Scalability

Given the increasing availability of large single cell transcriptomics datasets (Lähnemann *et al.*, 2020), we sought to evaluate the scalability of VeloViz with increasing cell numbers. Briefly, we down-sampled a dataset of approximately 10,000 cells (10X Genomics, 2020) to create datasets ranging from 100 cells to 9295 cells. For each dataset, we calculated velocity using *velocyto.R* and constructed an embedding using VeloViz while evaluating runtime and memory usage (Supplementary Information 7). We find that both runtime and memory usage of VeloViz scales linearly with the number of cells and is comparable to that of RNA velocity estimations (Supplementary Figure 8).

4 Discussion

In order to facilitate better visual representation of relationships between cell states in single cell transcriptomic data, we developed VeloViz to create low dimensional embeddings that incorporate dynamic information inferred from RNA velocity. We find that VeloViz is able to visualize cellular trajectories of diverse topologies and capture global cell state relationships, even when intermediate cell

238 states may be missing. Particularly when intermediate cell states are missing, we find that visualization
239 with t-SNE and UMAP may result in distinct clusters containing cells before and after trajectory gaps,
240 leading to erroneous interpretation that cells are part of biologically distinct subpopulations rather than
241 the same biological trajectory.

242

243 However, several limitations of VeloViz should be considered when using VeloViz embeddings to
244 interpret putative cellular trajectories. Embeddings constructed using VeloViz incorporate multiple user
245 inputted hyperparameters (Supplementary Information 1). We explored the effects of changing these
246 parameters on the visualization of simulated cycling trajectories with missing intermediates and the
247 resulting TC scores (Supplementary Figure 9). We found that the VeloViz embedding was most robust
248 to changes in cosine similarity threshold (t_i) and most sensitive to changes in k . However, without *a*
249 *priori* knowledge of expected relationships between cell subpopulations, it may be challenging to find
250 the optimal parameter set that yields the most representative embedding. Furthermore, different
251 components of the trajectory being visualized, such as gaps versus branching structures, may have
252 different optimal parameters. Thus, a range of hyperparameters may need to be explored to evaluate the
253 stability of visualized cellular trajectories. Further limitations of VeloViz extend from the limitations of
254 RNA velocity analysis in general. Notably, RNA velocity analysis can only infer cell state changes that
255 are determined by changes in gene expression. Other molecular features such as alternative splicing,
256 chromatin state, post-translational modifications, differential localization, and cell microenvironment
257 that contribute to cell state changes are not considered in RNA velocity analysis, and therefore these cell
258 state changes will not be represented in the VeloViz embedding (Weinreb *et al.*, 2018; Tritschler *et al.*,
259 2019). In addition, it remains unknown the degree to which cell state changes are stochastic i.e. the
260 probability that two cells with similar transcriptional states will develop differently. This stochasticity
261 may limit the accuracy of predicting future cell state based on current gene expression dynamics.
262 Ultimately, insights gained from RNA velocity analysis should be considered within the context of other

263 available data, such as differential gene expression, mutational analysis, and targeted experimental
264 validation.

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266 Overall, by taking into account the predicted future transcriptional states of cells from RNA velocity
267 analysis, VeloViz provides an additional approach for visualizing putative cellular trajectories to aid in
268 the interpretation of cellular dynamics from single cell transcriptomics data.

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272

273 **Conflict of Interest:** none declared.

274

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Figures

Figure 1. Overview of VeloViz. RNA-velocity informed embeddings are created by VeloViz in five steps: 1) The observed current (X_c) and predicted future (X_p) transcriptional cell states are inferred from RNA velocity and reduced into a common PC space; 2) composite distances (D) between all cell pairs are computed. The composite distance from Cell A to Cell X ($D_{A \rightarrow X}$) takes into account the similarity in transcriptional profiles (d_{AX}) between Cell X's observed current (X_c) and Cell A's predicted future transcriptional state (A_p), and the cosine correlation between Cell A's RNA-velocity (v_A) and the change vector (t_{AX}) representing a transition from Cell A's current state (A_c) to Cell X's current state (X_c). A distance weight (ω) is used to adjust the relative importance of transcriptional similarity and cosine correlation in the composite distance; 3) each cell is represented as a node in a graph, and for each cell, graph edges are assigned to the k cells with the minimum composite distances. Edge weights are computed based on composite distances as $weight_{AB} = \max(D) - D_{AB}$; 4) edges assigned in 3. are pruned (in grey) using transcriptional and velocity similarity thresholds. Edge shade corresponds to edge weight computed based on composite distance, with darker arrows representing edges with larger weights; 5) the resulting graph can be visualized as a 2D or 3D embedding using graph-based embedding approaches.

Figure 2. VeloViz reconstructs trajectories from pancreatic endocrinogenesis scRNA-seq. 2D

embeddings visualizing pancreatic endocrinogenesis generated using VeloViz (A), PCA (B), t-SNE (C), UMAP (D), and diffusion mapping (E). Cells are colored by cell state annotations provided in (Bergen *et al.*, 2020). Arrows show the projection of velocities derived from dynamical velocity modelling (Bergen *et al.*, 2020) onto the embeddings.

Figure 3. VeloViz reconstructs trajectories from pancreatic endocrinogenesis scRNA-seq data with missing intermediates. A) VeloViz 2D embedding visualizing pancreatic endocrinogenesis with pre-endocrine intermediates removed creating a gap in the developmental trajectory. Inset shows the VeloViz embedding of the full dataset. Cells are colored by cell state annotations provided in (Bergen *et al.*, 2020). Arrows show the projection of velocities derived from dynamical velocity modelling onto the VeloViz embeddings. Gap distances measure the median distance in the 2D embedding between the 300 cells before and after pre-endocrine cells in the developmental trajectory (Supplementary Information 3iii). Blue circle and green square indicate the median coordinates of cells before and after pre-endocrine cells in the developmental trajectory, respectively. B-E) 2D embeddings visualizing pancreatic endocrinogenesis with removed pre-endocrine intermediates using PCA, t-SNE, UMAP, and diffusion mapping, respectively with arrows showing the projection of velocities derived from dynamical velocity modelling.