scDEED: a statistical method for detecting dubious 2D single-cell embeddings

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Abstract

Two-dimensional (2D) embedding methods are crucial for single-cell data visualization. Popular methods such as t-SNE and UMAP are commonly used for visualizing cell clusters; however, it is well known that t-SNE and UMAP's 2D embedding might not reliably inform the similarities among cell clusters. Motivated by this challenge, we developed a statistical method, scDEED, for detecting dubious cell embeddings output by any 2D embedding method. By calculating a reliability score for every cell embedding, scDEED identifies the cell embeddings with low reliability scores as dubious and those with high reliability scores as trustworthy. Moreover, by minimizing the number of dubious cell embeddings, scDEED provides intuitive guidance for optimizing the hyperparameters of an embedding method. Applied to multiple scRNA-seq datasets, scDEED demonstrates its effectiveness for detecting dubious cell embeddings and optimizing the hyperparameters of t-SNE and UMAP.

Keywords: t-SNE; UMAP; cell cluster locations; global topology; hyperparameter optimization

Introduction

In the burgeoning field of single-cell biology, two-dimensional (2D) data visualization is an indispensable exploratory step that allows researchers to inspect the similarities and differences among single cells, so as to discern the putative existence of discrete cell types and continuous cell trajectories. 2D data visualization is achieved by embedding methods, also known as dimension reduction methods. Among the many embedding methods developed for single-cell data [1–10], t-SNE [11,12] and UMAP [13,14] are the most popular because they can enhance the similarities of cells in the same type and increase the contrasts of disparate cell types.

However, the enhancement of similar cells’ proximity in the 2D space comes at the cost of distorting the overall distances among all cells. In the trade-off between highlighting local cell clusters and preserving the global cell topology, t-SNE and UMAP focus on the local end, while the classic PCA sits at the global end by optimally preserving the global variance of cells. Despite the popularity of t-SNE

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and UMAP, cautionary messages have been raised against using t-SNE and UMAP embeddings to interpret the distances of cell clusters [15–19], which are related to the global cell topology.

Aligned with those cautionary messages, strategies have been proposed to optimize the hyperparameters of t-SNE and UMAP from the perspective of preserving the global cell topology [17,20–26], and new embedding methods have been developed to improve upon t-SNE and UMAP [8,15]. Nevertheless, none of these optimization strategies or new methods can guarantee to what extent the resultant cell embeddings will accurately preserve the distances between cell clusters. Hence, researchers are still perplexed about whether a particular cell cluster’s neighboring clusters are trustworthy in the 2D embedding space. A related question is whether distant clusters in the 2D embedding space are truly distinct. Moreover, it remains questionable whether a visual cluster in the 2D embedding space is trustworthy or should be divided into more than one cluster.

To address these questions, we present scDEED (single-cell dubious embedding detector), a statistical method that decides whether each cell’s 2D embedding is dubious or trustworthy. The core idea of scDEED is to assess if a cell has similarly ordered neighbors up to a mid-range before and after the 2D embedding (Figure 1I). Based on this idea, scDEED assigns every cell a “reliability score,” whose large value indicates that the cell’s immediate to mid-range neighbors are well preserved after the embedding. Then scDEED compares each cell’s reliability score to a null distribution of reliability scores (when all cells have random neighbors) and identifies the cell’s embedding as dubious (or trustworthy) if the cell’s reliability score is worse (or better) than 95% of the null reliability scores. The results from scDEED will help researchers avoid using dubious cell embeddings to interpret cell similarities and feel confident about using trustworthy cell embeddings.

This dubious embedding detection functionality also gives scDEED another use: optimizing the hyperparameters of a 2D embedding method by minimizing the number of dubious embeddings (Figure 1II). Unlike existing optimization strategies for t-SNE and UMAP [17,20,21,23–27], scDEED offers users the flexibility to optimize hyperparameters in an intuitive and graphical way (users can see which cell embeddings are dubious under each hyperparameter setting), without modifying the embedding method’s algorithm. Furthermore, scDEED’s definition of dubious cell embeddings distinguishes scDEED from DynamicViz [26], a method that optimizes hyperparameters by minimizing the variance of cell embeddings’ Euclidean distances across multiple bootstraps. Instead of checking the stability of 2D embeddings, as DynamicViz does, scDEED evaluates the preservation of cells’ immediate to mid-range neighbors in the 2D embedding space.

Notably, scDEED is compatible with all 2D embedding methods, and here we show its use for t-SNE and UMAP for demonstration purposes. This general applicability distinguishes scDEED from EMBEDR [25], a method that assigns every cell a quality score based on the t-SNE loss function and is therefore unsuitable for comparing embeddings from different methods. In contrast, scDEED can compare embeddings from different methods at various resolutions, including individual cells, cell types, and all cells.
In this study, we applied scDEED to multiple scRNA-seq datasets. Our results show that scDEED successfully identified dubious cell embeddings in the original studies. Moreover, the hyperparameters optimized by scDEED resulted in cell embeddings that better preserved the biological relationships of cell types compared to the original studies. We also demonstrated the advantages of scDEED over EMBEDR in optimizing the perplexity hyperparameter of t-SNE, even though EMBEDR was designed based on the t-SNE loss function.

Results

scDEED detected dubious cell embeddings and optimized the perplexity hyperparameter for t-SNE visualization of the Hydra dataset.

The Hydra scRNA-seq dataset was the first cell atlas of the adult Hydra polyp. The transcriptomes of 24,985 Hydra cells were sequenced by Drop-seq [28]. In the original study, the Hydra cells were visualized by t-SNE with the perplexity hyperparameter set to 40 (Figure 2a, a reproduction of the original study’s Figure 1F in [28]).

We first applied scDEED to this original t-SNE visualization to detect dubious cell embeddings. The results had 4.77% of cells with dubious embeddings (Figure 2b) and 92.84% with trustworthy embeddings (Figure 2c). The remaining cell embeddings, whose reliability scores were between the 5th and 95th percentiles of null reliability scores, were neither dubious nor trustworthy. Interestingly, most dubious cell embeddings appeared as small clusters, suggesting that these clusters might not represent disparate cell types. Moreover, there are three notable examples. First, the annotated nematocytes contained many cells with dubious embeddings (Supplementary Figure S1a). We verified this finding by showing that these cells had gene expression profiles largely distinct from the other nematocytes with trustworthy embeddings (Supplementary Figure S1b). Second, the annotated neuron ec1 (neuron ectodermal 1) cells were divided into two non-neighboring clusters (Figure 3a), but one cluster consisted of dubious cell embeddings (Figure 2b). We examined the gene expression profiles of these two clusters and found the two clusters hardly distinguishable (Figure 3c). Third, the annotated male germline cells had many dubious embeddings (Supplementary Figure S2a). Checking the gene expression profiles of male germline cells with dubious embeddings, we found that these cells should belong to two clusters (Supplementary Figure S2b) instead of the continuum shown in the original visualization. These results confirmed that scDEED effectively identified dubious cell embeddings worth further investigation.

Next, we used scDEED to optimize the t-SNE perplexity hyperparameter by minimizing the number of dubious cell embeddings (Figure 2d); the optimized perplexity was 210 (Supplementary Figure S3a, left). With this optimized perplexity, dubious cell embeddings decreased from 4.77% to 0.53% of all cell embeddings (Figure 2e), and trustworthy cell embeddings increased from 92.84% to 99.1% (Figure 2f). Notably, this optimized perplexity was close to Kobak and Berens’ suggested t-SNE perplexity 251, defined as the number of cells divided by 100 [20]. Compared to this suggested perplexity, scDEED’s optimized perplexity was data-driven and thus accounted for other data characteristics besides the cell number. In addition to finding the perplexity that minimized the number of dubious cell embeddings, we
also considered the “kneedle” method [29] for finding the perplexity at the elbow point in the scatterplot of the number of dubious cell embeddings (y-axis) vs. perplexity (x-axis). The resulting “kneedle” perplexity was 170 (Supplementary Figure S3a, right), which gave similar cell embeddings to those output by scDEED’s optimized perplexity 210 (Supplementary Figure S3b).

With scDEED’s optimization, the t-SNE visualization (Figure 2d) exhibits several key differences from the original t-SNE visualization (Figure 2a). First, the two clusters of neuron ec1 cells at the original perplexity of 40 (Figure 3a) became one continuum at the perplexity of 210 optimized by scDEED (Figure 3b). Using ecEP_sc (ectodermal epithelial_single cell) as a reference cell type, we observed a clearer picture: ecEP_sc was a close neighbor (with similar Euclidean distances in the 2D embedding space) of the two separated neuron ec1 clusters at the original perplexity (Figure 3a); however, ecEP_sc became far away from the unified neuron ec1 continuum at the perplexity optimized by scDEED (Figure 3b). Gene expression profiles supported the visualization optimized by scDEED: the unified neuron ec1 cells indeed had similar gene expression and a large distinction from ecEP_sc (Figure 3c).

Second, at the original perplexity, the annotated neuron ec1 and neuron ec3 cells were distinct clusters that appeared similarly close to ecEP_sc cells (Figure 3d); however, at the perplexity optimized by scDEED, neuron ec1 and neuron ec3 cells were unified as one large cloud far away from ecEP_sc (Figure 3e). Again, the visualization optimized by scDEED was supported by those cells’ gene expression profiles (Figure 3f), which confirmed that neuron ec1 and neuron ec3 cells are similar to each other and distinct from ecEP_sc cells.

Third, at the original perplexity, ectodermal neurons were shown in six clusters labeled as five subtypes: neuron ec1, neuron ec2, neuron ec3, neuron ec4, and neuron ec5 (Figure 3g). In contrast, at the perplexity optimized by scDEED, the five subtypes were unified (Figure 3h). Using battery cell 2 (mp) as a reference cell type, we found that mp’s relative location to the five subtypes changed drastically after scDEED optimized the perplexity: mp was closer to neuron ec5 than the other four subtypes at the original perplexity (Figure 3g), but mp became far away from all five subtypes at the optimized perplexity (Figure 3h). Again, gene expression profiles supported the visualization optimized by scDEED (Figure 3i). To further justify that the five subtypes should be unified, we calculated the ROGUE value, a cell cluster purity metric that ranges from 0 to 1 and whose higher value indicates a purer cluster [30], for every subtype and the unified cluster. The ROGUE values of the five subtypes had an average of 0.768 and a standard deviation of 0.055, while the ROGUE value of the unified cluster was 0.744. Hence, unifying the five subtypes did not significantly reduce the subtypes’ purity and was thus reasonable. These results confirmed that scDEED effectively optimized the t-SNE perplexity hyperparameter on this Hydra dataset.

**scDEED detected dubious cell embeddings and optimized the perplexity hyperparameter for t-SNE visualization of the CAR-T dataset.**

The CAR-T scRNA-seq dataset contained 62,167 CD8+ chimeric antigen receptor modified-T (CAR-T) cells from 10 patients undergoing CD19 CAR-T immunotherapy [31]. In the original study, CAR-T cells
were visualized by t-SNE with the perplexity 30 (Figure 4a, a reproduction of the original study’s Figure 5A with cell clusters highlighted as in [31]).

We first applied scDEED to this original visualization to detect dubious cell embeddings. The results had 1.81% of cells with dubious embeddings (Figure 4b) and 83.99% with trustworthy embeddings (Figure 4c). Most of the dubious cell embeddings were in cluster 7; at the original perplexity 30, these dubious cell embeddings were not visually distinguishable from the trustworthy cell embeddings in cluster 7 (Figure 5a), suggesting the need for further investigation. To understand the differences between the dubious and trustworthy cell embeddings in cluster 7, we performed the differential gene expression analysis and found that the groups of cells indeed had distinct gene expression profiles (Figure 5c). In particular, many ribosomal genes were more highly expressed in the trustworthyly embedded cells, a phenomenon associated with higher transcriptional activities [32,33]. This observation was consistent with the fact the trustworthyly embedded cells were more enriched with CAR-T cells from the early simulation stage than the dubiously embedded cells (48.05% of trustworthyly embedded cells and 28.51% of dubiously embedded cells were from the early simulation stage; p-value = 3.304e-12). Since early-stage CAR-T cells right after simulation were expected to have higher transcriptional activities than other CAR-T cells, it is reasonable that the trustworthyly embedded cells had higher ribosomal gene expression than the dubiously embedded cells.

Next, we used scDEED to optimize the t-SNE perplexity hyperparameter by minimizing the number of dubious cell embeddings; the resulting perplexity was 750 (Supplementary Figure S4a, left). With this optimized perplexity, dubious cell embeddings decreased from 1.81% to 0.09% of all cell embeddings (Figure 4e), and trustworthy cell embeddings increased from 83.99% to 95.87% (Figure 4f). The “kneedle” method resulted in a perplexity of 170 (Supplementary Figure S4a, right), which gave similar cell embeddings to those of the optimized perplexity 750 (Supplementary Figure S4b).

With the perplexity of 750 optimized by scDEED, the t-SNE visualization (Figure 4d) exhibited several key differences from the original t-SNE visualization (Figure 4a). First, the dubiously embedded and trustworthyly embedded cells in cluster 7 were no longer clustered (Figure 5a) but separated far apart at the perplexity optimized by scDEED (Figure 5b). Taking cluster 5 and cluster 14 as references, we observed that the dubiously embedded cells in cluster 7 became close to cluster 14, while the trustworthyly embedded cells in cluster 7 became close to cluster 5 (Supplementary Figure S5a). We validated this visualization using the cell trajectory reconstruction method STREAM [34] (Supplementary Figure S5b): the reconstructed cell trajectory had the dubiously embedded cluster 7 cells in one branch with the cluster 14 cells (branch S6), while the trustworthyly embedded cluster 7 cells were in another branch with the cluster 5 cells (branch S4).

Second, at the original perplexity, cluster 7 was between cluster 14 and cluster 6; in particular, cluster 7 was next to cluster 14 and at some distance from cluster 6 (Figure 5d). However, this pattern changed after we optimized the perplexity. In particular, at the perplexity optimized by scDEED, most of the dubiously embedded cluster 7 cells were in one small cluster next to cluster 14, but the rest of cluster 7 cells (including the trustworthyly embedded cluster 7 cells) were no longer between cluster 14 and
cluster 6 (Figure 5e). This optimized visualization was consistent with the gene expression profiles (Figure 5f).

Third, the four clusters under the stage IP (no stimulation), i.e., clusters 3, 4, 9, and 11 (Figure 5a in the original study [28]), have little overlap at the original perplexity (Figure 5g). Nevertheless, at the perplexity optimized by scDEED, cluster 9 stayed distinct, while the other three clusters (clusters 3, 4, and 11) had large overlaps (Figure 5h). We found supportive evidence in the gene expression profiles, showing that cluster 9 is distinct from the other three clusters (Figure 5i). To confirm this result quantitatively, we computed the ROGUE values for cluster 3, the combination of clusters 3 and 4, and the combination of clusters 3, 4, and 11, obtaining 0.887, 0.885, and 0.886, respectively. These ROGUE values suggested no clear separation among the three clusters. Moreover, the ROGUE value dropped to 0.832 after we combined clusters 3, 4, 9, and 11, confirming cluster 9 had distinct gene expression profiles from those of clusters 3, 4, and 11. Together, the ROGUE values were consistent with the t-SNE visualization at the perplexity optimized by scDEED.

Fourth, at the original perplexity, cluster 8 and cluster 9 appeared to have similar distances from cluster 14 (Supplementary Figure S6a left). However, cluster 8 and cluster 9’s relative locations changed after we optimized the perplexity, with cluster 8 standing between cluster 14 and cluster 9 (Supplementary Figure S6a right). Again, their gene expression profiles and the corresponding hierarchical clustering of cells supported the t-SNE visualization under the optimized perplexity (Supplementary Figure S6b).

**scDEED detected dubious cell embeddings and optimized the min.dist and n.neighbors hyperparameters for UMAP visualization of the Alveolar dataset.**

The Alveolar dataset was collected to learn the cellular dynamics during the regeneration process after bleomycin-induced lung injury [35]. Measured by Dropseq, the Alveolar dataset contained 29,297 cells from 28 mice, with about 1000 cells per mouse. In the original study, cells were visualized by UMAP with hyperparameters min.dist = 0.3 and n.neighbors = 10 (Figure 6a, a reproduction of the original study’s Figure 1a [35] with the annotated cell clusters highlighted).

We applied scDEED to this original visualization to detect dubious cell embeddings. The results show that 3.14% of cells had dubious embeddings (Figure 6b), and 50.71% had trustworthy embeddings (Figure 6c). Unlike t-SNE, UMAP has two hyperparameters: min.dist and n.neighbors, which jointly determine the 2D cell embeddings. The scDEED R package allows users to optimize min.dist and n.neighbors marginally or jointly, and the goal is to minimize the number of dubious embeddings. In marginal optimization, we applied scDEED to optimize min.dist or n.neighbors by fixing the other hyperparameter at the original value. Hence, marginal optimization considers fewer hyperparameter combinations and is thus more computationally efficient than joint optimization.

From marginal optimization, we obtained n.neighbors = 5 (with min.dist = 0.3) and min.dist = 0.6 (with n.neighbors = 10) (Supplementary Figure S7). In particular, the first hyperparameter set (min.dist = 0.3 and n.neighbors = 5) reduced the dubious embeddings to 2.28% of cells and increased the trustworthy embeddings to 56.73% (Supplementary Figure S7a, left); the second hyperparameter set (min.dist =
0.6 and n.neighbors = 10) reduced the dubious embeddings to 2.45% and increased the trustworthy embeddings to 54.75% (Supplementary Figure S7a, right). When we jointly optimized the two hyperparameters, we obtained min.dist = 0.7 and n.neighbors = 6 (Figure 6d), which further reduced the dubious embeddings to 2.04% (Figure 6e) and increased the trustworthy embeddings to 58.84% (Figure 6f).

Among the cell clusters that contained many dubious cell embeddings, we focused on the relative locations of the following clusters: lec, b_cells, vcam1_vec, vec, and t_cells. We noted two key differences between the original UMAP visualization (Figure 7a) and the three optimized visualizations, which largely agree with each other (joint hyperparameter optimization in Figure 7b; marginal hyperparameter optimizations in Supplementary Figure S8a).

First, under the original hyperparameter setting (min.dist = 0.3 and n.neighbors = 10), the lec cluster was close to the b_cells cluster and far away from the vcam1_vec and vec clusters (Figure 7a). In contrast, under the three optimized hyperparameter settings, lec was no longer adjacent to b_cells but became close to vcam1_vec and vec, with b_cells lying far away (Figure 7b and Supplementary Figure S8a). We found supporting evidence in the gene expression profiles, which showed that the b_cells cluster was distinct from the lec, vcam1_vec, and vec clusters (Figure 7c).

Second, under the original hyperparameter setting, the b_cells cluster lay between the t_cells and lec clusters at approximately equal distances (Figure 7d). In contrast, under the three optimized hyperparameter settings, the b_cells and t_cells clusters became close to each other but far away from the lec cluster (joint hyperparameter optimization in Figure 7e; marginal hyperparameter optimizations in Supplementary Figure S8b). Again, to evaluate the relative locations of the b_cells, t_cells, and lec clusters, we examined the gene expression profiles. Figure 7f shows that lec was the most distinctive among the three clusters, thus supporting the optimized visualizations found by scDEED.

**scDEED detected dubious cell embeddings and optimized the min.dist and n.neighbors hyperparameters for UMAP visualization of the Samusik_01 dataset.**

The Samusik_01 dataset was from a mass cytometry study of bone marrow hematopoiesis. It contained more than 86,000 cells, 38 features, and 24 annotated cell types [36]. In the original study [36], cells were visualized by UMAP with hyperparameters min.dist = 0.2 and n.neighbors = 15 (Figure 8a, a reproduction of the original study’s Figure 2a [36]).

We first applied scDEED to this original visualization, finding 1.13% dubious cell embeddings (Figure 8b) and 98.74% trustworthy cell embeddings (Figure 8c). Next, we applied scDEED to optimize min.dist and n.neighbors marginally by minimizing the number of dubious cell embeddings. From this marginal optimization, we obtained n.neighbors = 160 (with min.dist = 0.2) and min.dist = 0.05 (with n.neighbors = 15) (Supplementary Figure S9). In particular, the first hyperparameter set (min.dist = 0.2 and n.neighbors = 160) reduced the dubious embeddings to 0.68% and increased the trustworthy embeddings to 99.19%; the second hyperparameter set (min.dist = 0.05 and n.neighbors = 15) reduced the dubious embeddings to 0.837% and increased the trustworthy embeddings to 99.028%
When we jointly optimized the two hyperparameters, we obtained min.dist = 0.7 and n.neighbors = 160 (Figure 8d). This hyperparameter set further reduced the dubious embeddings to 0.641% (Figure 8e) and increased the trustworthy embeddings to 99.269% (Figure 8f). As expected, the joint optimization achieved the lowest percentage of dubious cell embeddings and the highest percentage of trustworthy cell embeddings. Meanwhile, the joint optimization and the marginal optimization of n.neighbors shared the same n.neighbors = 160 and similar visualizations (joint hyperparameter optimization in Figure 8d; marginal hyperparameter optimizations in Supplementary Figure S9b).

Among the cell types that contained dubious cell embeddings, we focused on the relative locations of the following cell types: Non-Classical Monocytes (ncm), Intermediate Monocytes, Basophils, Plasmacytoid Dendritic Cells (pDCs), Myeloid Dendritic Cells (mDCs), and Macrophages. Notably, the joint optimization and the marginal optimization of n.neighbors (Figure 8d and Supplementary Figure S9b left) exhibited two key differences from the original UMAP visualization (Figure 8a).

First, under the original hyperparameter setting (min.dist = 0.2 and n.neighbors = 15), Macrophages lay between pDCs and ncm with approximately equal distances and were far away from mDCs (Figure 9a). In contrast, under the marginally optimized n.neighbors setting (min.dist = 0.2 and n.neighbors = 160) and the jointly optimized hyperparameter setting (min.dist = 0.7 and n.neighbors = 160), which had the two lowest percentages of dubious embeddings, Macrophages became adjacent to mDCs but farther away from ncm and pDCs (Figure 9b). The gene expression profiles confirmed that Macrophages were more similar to mDCs than ncm and pDCs (Figure 9c).

Second, under the original hyperparameter setting, looking at pairwise distances among the three cell types—Plasma Cells, ncm, and NK cells, we found similar distances between Plasma Cells and ncm, and between ncm and NK cells, and the distance between Plasma Cells and NK cells was the largest (Figure 9d). In contrast, under the jointly optimized hyperparameter setting (min.dist = 0.7 and n.neighbors = 160), the distance between ncm and NK cells became the smallest among the three pairwise distances, with the other two distances becoming similar to each other (Figure 9e). To evaluate the relative locations of these three cell types, we calculated the cell-to-cell Euclidean distances in the 38-dimensional feature space (before UMAP embedding) between every two cell types, confirming that NK cells and ncm were closer to each other than to Plasma Cells (Figure 9f).

Not that the above two differences were not as apparent in the embeddings obtained from marginally optimizing the hyperparameter min.dist (min.dist = 0.05 and n.neighbors = 15). A possible reason was that this hyperparameter set had the highest percentage of dubious embeddings and the lowest percentage of trustworthy embeddings among the three optimized hyperparameter sets.

scDEED improved the consistency of relative distances among human PBMC cell types across three scRNA-seq technologies and between t-SNE and UMAP.
The Human PBMC dataset was collected to compare multiple scRNA-seq technologies [37]. It contained 31,021 cells with cell type labels, and the gene expression levels were log-transformed UMI count per 10,000. We accessed the dataset “pbmcsca.SeuratData” in the R package “SeuratData.”

We considered three scRNA-seq technologies that measured more than 500 cells in the dataset: Dropseq, inDrops, and SeqWell. For each technology, we visualized its measured cells using t-SNE and UMAP at the default hyperparameters or the hyperparameters optimized by scDEED (Figure 10). We observed that the optimized hyperparameters led to more consistent relative distances among the cell types, both across scRNA-seq technologies and between t-SNE and UMAP. Specifically, we had the following three observations.

First, when used to visualize the Dropseq data at the default hyperparameters, t-SNE and UMAP showed different relative distances among four cell types: t-SNE ordered the cell types Cytotoxic T cell, CD4+ T cell, CD14+ monocyte, and B cell clockwise (Figure 10a, left), but UMAP switched the order of CD14+ monocyte and B cell (Figure 10a, right). In contrast, at the hyperparameters optimized by scDEED, t-SNE and UMAP had the same counterclockwise order of the four cell types: Cytotoxic T cell, CD4+ T cell, B cell, and CD14+ monocyte (Figure 10b). That is, the hyperparameters optimized by scDEED improved the consistency between t-SNE and UMAP.

Second, when used to visualize the inDrops data at the default hyperparameters, t-SNE ordered Cytotoxic T cell, CD4+ T cell, B cell, and CD14+ monocyte counterclockwise, while UMAP ordered the same four cell types clockwise (Figure 10c). In contrast, at the hyperparameters optimized by scDEED, both t-SNE and UMAP ordered the four cell types clockwise (Figure 10d). Notably, this clockwise order was consistent with the counterclockwise order of the four cell types in the t-SNE and UMAP visualizations of the Dropseq data. Hence, the relative distances of the four cell types became consistent between Dropseq and inDrops after scDEED’s optimization.

Third, when used to visualize the Seqwell data at the default perplexity hyperparameter, t-SNE separated the CD14+ monocyte cell type into two clusters, with the two cell types Cytotoxic T cell and CD4+ T cell in between (Figure 10e, left); however, the two CD14+ monocyte clusters became attached at the perplexity optimized by scDEED (Figure 10f, left). The optimized t-SNE and UMAP visualizations had a consistent counterclockwise order of the four cell types Cytotoxic T cell, CD4+ T cell, B cell, and CD14+ monocyte (Figure 10f). Hence, the relative distances of the four cell types became consistent across the three scRNA-seq technologies after scDEED’s optimization.

**scDEED improved t-SNE visualization of RNA velocities.**

We performed RNA velocity analysis [38] on a dentate gyrus dataset [39]. RNA velocity vectors were calculated in the high-dimensional space, and a 2D vector field was used for visualization. The visualized 2D vectors were calculated based on a small neighborhood of cells defined in the 2D embedding space. Hence, 2D embedding affected velocity visualization.
Supplementary Figure S10a shows a subset of the cells in the t-SNE visualization of the original study [39], and Supplementary Figure S10c shows the same cells in the t-SNE visualization under the perplexity optimized by scDEED. Of particular note was the trajectory from nIPC to neuronal subtypes 1 and 2 (Neuro1 and Neuro2), then to immature granules, and ending at mature granules [39]. This velocity trajectory became more evident in the t-SNE visualization at the perplexity optimized by scDEED (Supplementary Figure S10d) than at the original perplexity (Supplementary Figure S10b). Aside from the cells in the trajectory, the mature granules continued to have velocities close to zero, consistent with the fact that mature granules are at the end of differentiation. Hence, scDEED’s optimized perplexity improved the t-SNE visualization of RNA velocities.

**scDEED outperformed EMBEDR in optimizing t-SNE and UMAP visualizations**

We benchmarked scDEED against EMBEDR, a method designed based on the t-SNE loss function, on three datasets: (1) the Hydra dataset (used in the Results section “scDEED detected dubious cell embeddings and optimized the t-SNE perplexity hyperparameter for the Hydra dataset”), (2) the Tabula Muris Consortium marrow dataset [40] (used in the EMBEDR paper [25]), and (3) the synthetic data generated by scDesign3 [41]. We compared scDEED with EMBEDR in three aspects: detection of dubious embeddings at the default t-SNE perplexity, optimization of the t-SNE perplexity, and computational time.

On the Hydra dataset, at the original t-SNE perplexity of 40, unlike scDEED (Figure 2b; Supplementary Figure S11a), EMBEDR found most of the cell embeddings dubious (Supplementary Figure S11b). We argue that the EMBEDR result was unlikely true because if most of the cell embeddings were dubious, then the t-SNE visualization used in the Hydra study [28] would be meaningless; however, this was not the case.

Besides the number of dubious embeddings, scDEED and EMBEDR had two notable differences in their detection results on the Hydra dataset. First, among the nematocytes, the cells with dubious embeddings are more distinct from those with trustworthy embeddings in the scDEED result (Supplementary Figure S11c and e; Supplementary Figure S1) than in the EMBEDR result (Supplementary Figure S11d and f). To quantify the difference between the scDEED and EMBEDR results, we calculated the pairwise distances between dubious-embedding cells and trustworthy-embedding cells in the scDEED and EMBEDR results separately; then we compared the two sets of pairwise distances using the one-sided Wilcoxon rank-sum test and obtained a p-value less than $10^{-16}$, suggesting that the dubious embeddings and trustworthy embeddings found by scDEED were more distinct and thus more reasonable.

Second, for the neuron ec1 cells divided into two non-neighboring clusters (Figure 3a), scDEED identified one cluster as dubious and the other cluster as trustworthy (Supplementary Figure S11a). In contrast, EMBEDR identified dubious cell embeddings in both clusters (Supplementary Figure S11g), but the dubious embeddings and trustworthy embeddings identified by EMBEDR did not exhibit obvious differences in gene expression files (Supplementary Figure S11i). Since both scDEED and EMBEDR unified the two clusters in their respective optimized embeddings (Figure 3b for scDEED;
Supplementary Figure S11h for EMBEDR), scDEED’s dubious detection result under the original perplexity 40 was more reasonable than EMBEDR’s because the neuron ec1 cells in each cluster should be either jointly dubious (far away from similar cells) or jointly trustworthy (close to similar cells).

Regarding optimizing the perplexity hyperparameter on the Hydra dataset, since EMBEDR does not have a default list of candidate perplexity values, we provided EMBEDR with the default candidate perplexity values of scDEED, and EMBEDR selected the highest candidate perplexity of 410 (Supplementary Figure S12). This result is expected because EMBEDR’s loss function is the t-SNE loss function (i.e., the KL divergence), and it was reported that the t-SNE loss function tends to decrease as perplexity increases [24]. That is, EMBEDR is expected to choose the largest candidate perplexity—a conceptually undesirable property.

Regarding the computational time on the Hydra dataset, scDEED is advantageous over EMBEDR. Running without parallelization, scDEED completed the analysis (dubious embedding detection and perplexity optimization) in 4 hours, while EMBEDR finished in 18.5 hours using all available processors (the default setting in EMBEDR).

On the Tabula Muris Consortium marrow dataset used in the EMBEDR paper [25], EMBEDR reported an optimal t-SNE perplexity of 1000, and we were able to reproduce EMBEDR’s optimized visualization using the processed dataset of 4,821 cells (Supplementary Figure S13b). In contrast to EMBEDR, scDEED found an optimal t-SNE perplexity of 100 (Supplementary Figure S 13a). This example again highlights EMBEDR’s preference for high perplexity values. Note that the scDEED optimal perplexity of 100 falls within the suggested range of perplexity as 1–10% of the number of cells [20,22], while the EMBEDR optimal perplexity of 1000 is far beyond the range.

Also on the Tabula Muris Consortium marrow dataset, comparison of EMBEDR’s and scDEED’s optimized visualizations shows a striking difference in the locations of the cluster of early mouse hematopoietic stem cells expressing genes Kit, Lin, and Sca-1 (referred to as KLS cells) and the cluster of granulocytes (Supplementary Figure S13a–b). While these two clusters had a large separation in EMBEDR’s optimized visualization (Supplementary Figure S13b), they shared some neighboring cells in scDEED’s optimized visualization (Supplementary Figure S13a). We randomly picked a KLS cell close to granulocytes and examined the cell’s 50 nearest neighboring cells in the 50-dimensional principal component (PC) space (50 PCs were used as the input into t-SNE). We found the KLS cell close to its 50 nearest neighbors in scDEED’s optimized visualization (Supplementary Figure S13c), but not in EMBEDR’s optimized visualization (Supplementary Figure S13d), suggesting that scDEED’s optimized visualization better preserves neighboring information.

Finally, we used the simulator scDesign3 [41] to generate 20 simulated scRNA-seq datasets from a model fitted on a real scRNA-seq dataset of mouse small intestinal epithelial cells (Methods). For each simulated dataset, we used scDEED or EMBEDR to optimize the t-SNE perplexity or marginally optimize the UMAP n.neighbors hyperparameter. We fixed the UMAP min.dist hyperparameter at 0.1 to match the EMBEDR algorithm, which only allows optimization of the t-SNE perplexity or the UMAP n.neighbors. Comparing scDEED’s optimized hyperparameters with EMBEDR’s (Supplementary
Figure S14a–d), we observed that EMBEDR tends to choose the highest perplexity value (consistent with the previous real data results) and the highest n.neighbors value, while scDEED did not exhibit this conceptually undesirable phenomenon. Moreover, scDEED reduced the number of dubious embeddings to zero for both t-SNE and UMAP, while EMBEDR consistently preferred t-SNE to UMAP due to using the t-SNE loss function (Supplementary Figure S14e). Hence, while scDEED can compare 2D embedding methods, EMBEDR biasedly favors t-SNE by design.

Also using the simulated data, we evaluated two metrics regarding the preservation of neighboring information as in [20]. The first metric is the K-nearest neighbors (KNN) reflecting the preservation of local information, i.e., the average proportion of the 10 nearest neighbors in the input space that remain in the set of 10 nearest neighbors in the 2D embedding space (a proportion is calculated for every cell, and the average is taken over all cells' proportions). The second metric is the K-nearest clusters (KNC) reflecting the preservation of global information, i.e., the average proportion of the 4 nearest clusters in the input space that remain in the set of 4 nearest clusters in the 2D embedding space (a proportion is calculated for every cell, and the average is taken over all cells' proportions). Comparison of the KNC and KNN metrics (Supplementary Figure S14f) shows that scDEED outperformed EMBEDR in preserving the local information (i.e., scDEED led to significantly higher KNN values than EMBEDR; one-sided paired t-test p-value = 7.88×10^{-15}), and scDEED performed similarly to EMBEDR in preserving the global information (i.e., scDEED and EMBEDR had similar KNC values; two-sided paired t-test p-value = 0.208). Hence, scDEED is the better method for preserving neighboring information.

Discussion

scDEED is a flexible statistical method for detecting cells with dubious embeddings produced by 2D embedding methods such as t-SNE and UMAP. scDEED detects dubious embeddings based on statistically significant low similarities between ordered neighbors before and after 2D embedding. Based on the detected dubious embeddings, scDEED enables the optimization of an embedding method’s hyperparameter setting (e.g., t-SNE’s perplexity and UMAP’s min.dist and n.neighbors) by minimizing the number of dubious cell embeddings.

Using multiple scRNA-seq datasets and their embeddings from published studies, we demonstrate that the dubious cell embeddings detected by scDEED indeed have dubious locations relative to other cells. We also show that the hyperparameter settings optimized by scDEED lead to cell embeddings that better align with biological knowledge and pre-embedding cell distances, compared with the original hyperparameter settings used in published studies.

By default, scDEED sets a conservative threshold for detecting dubious cell embeddings: cells are flagged as dubious if their reliability scores are no greater than the 5th percentile of the null reliability scores. However, users of the scDEED R package can increase or decrease the percentile threshold based on their knowledge and preference so that more or fewer cell embeddings will be flagged as dubious.
Minimizing the number of dubious cell embeddings can also help optimize other hyperparameters, such as random seed and learning rate, which are known to have impacts on t-SNE and UMAP [17,21]. Further, detecting dubious cell embeddings may help discern the topology of a dataset. For example, if the number of dubious cell embeddings exhibits a complex trend as a hyperparameter value increases, then the cells might have a complex topology, and users should be cautious when interpreting the 2D visualization.

scDEED is advantageous over competing methods, EMBEDR and DynamicViz, in terms of dubious embedding detection, hyperparameter optimization, and computational time. EMBEDR uses the t-SNE loss function and thus biasedly favors t-SNE over other embedding methods. Even for t-SNE, EMBEDR’s optimization is undesirable because it prefers high perplexity values. DynamicViz only evaluates cell embeddings from a stability perspective and cannot decide if cell embeddings are dubious based on cell distances before embedding. Moreover, both EMBEDR and DynamicViz are computationally expensive because they require many runs of bootstraps followed by embedding. In contrast, scDEED does not have these limitations: scDEED is fair for all embedding methods, effective at detecting dubious embeddings, and computationally efficient.

An interesting observation from our evaluation of scDEED is that t-SNE and UMAP could have more similar visualizations after scDEED's optimization. This finding questions the common belief that t-SNE preserves global information worse than UMAP and suggests that the lack of hyperparameter optimization might be a contributing factor. It is important to note that while we demonstrate the utility of scDEED on scRNA-seq data using t-SNE and UMAP as proof of concept, scDEED is applicable to other data types (e.g., multimodal assays [42]) and other embedding methods as well.

Therefore, we expect scDEED to be a valuable computational tool for single-cell researchers to generate and interpret visualization plots, which play an essential role in observation-based scientific discoveries.

**Methods**

**The scDEED algorithm.**

Given a gene-by-cell matrix (after appropriate normalization and logarithmic transformation, i.e., the input into the principal component analysis (PCA) with users’ discretion) with n columns (corresponding to n cells) and a 2D embedding method, e.g. t-SNE and UMAP, with a given hyperparameter setting, scDEED finds dubious cell embeddings in the following six steps.

Step 1. scDEED constructs a permuted data matrix by independently permuting the n cells for each gene (i.e., independently shuffling the n values in each row) in the original data matrix.

Step 2 (optional). In most single-cell data analysis pipelines, e.g., the Python package Scanpy [43] and the R package Seurat [44], users perform the PCA with a chosen K (the number of principal components (PCs)) on the original data matrix before applying the nonlinear embedding method.
Similarly, scDEED asks users to input $K$, and it performs PCA with $K$ on both the original data matrix and the permuted data matrix. Users may choose $K$ based on their preferred method. For the results in this study, we chose $K$ following the original studies or based on the elbow plot if the original studies did not provide $K$.

Step 3. scDEED applies the 2D embedding method (with a given hyperparameter setting and rand.seed = 100) to the two PCA-reduced-dimension matrices (original and permuted) or directly to the original and permuted matrices (if Step 2 is not performed). Hence, each cell receives two 2D embeddings, one original and one permuted.

Step 4. Based on the original data before and after the 2D embedding, scDEED defines a reliability score for each cell $i = 1, \ldots, n$, based on cell $i$'s $x\%$ (default $x = 50$, the only hyperparameter of scDEED) closest neighbors in the 2D embedding space and those in the pre-embedding space (the PC space if Step 2 is performed or the original space otherwise), with the neighbors in each space defined based on the Euclidean distance. Given the two sets of neighbors, scDEED calculates cell $i$'s Euclidean distances to the ordered neighbors (from the closest to the farthest) in each set in the 2D embedding space, obtaining two distance vectors of length $x\% \times n$ (rounded to the closest integer). Finally, scDEED defines the reliability score as the Pearson correlation of the two distance vectors. That is, each cell’s reliability score ranges from -1 to 1; a higher reliability score indicates a better agreement between the cell’s ordered neighbors before and after the 2D embedding. We use the Pearson correlation because the actual values of the Euclidean distances in the embedding space matter (for our visualization and interpretation), not just the ranks of the Euclidean distances used in the Spearman correlation.

Step 5. Based on the permuted data before and after the 2D embedding, scDEED applies the same procedure in Step 4 to obtain the null reliability scores of the $n$ cells. Because of the permutation, the similarities among cells are disrupted, and no biological neighboring relationships are preserved by the 2D embedding. Hence, each cell’s neighbors are purely determined by random chance, and its reliability score reflects the random agreement between its ordered neighbors before and after the 2D embedding. Leveraging the $n$ null reliability scores, scDEED finds the thresholds for calling a cell’s reliability score low or high.

Step 6. scDEED defines dubious cell embeddings as the embeddings of the cells whose reliability scores are less than or equal to the 5-th percentile of the $n$ null reliability scores. On the other end, scDEED defines trustworthy cell embeddings as the embeddings of the cells whose reliability scores are greater than or equal to the 95-th percentile of the $n$ null reliability scores. After the above steps, scDEED reports the number of dubious cells given a parameter setting. From a grid search of candidate hyperparameter settings, scDEED finds the setting that minimizes the number of dubious cell embeddings.

In the scDEED R package, we use the following candidate hyperparameter values by default, but users can specify their own candidate hyperparameter values. For t-SNE, the default candidate perplexity values are 20, 50, \ldots, 380, 410, 450, 500, \ldots, 750, and 800. For UMAP, the default n.neighbors values
are 5, 6, …, 29, 30, 35, 40, 45, and 50; the default min.dist values are 0.0125, 0.05, 0.1, 0.2, …, 0.7, and 0.8.

**Alternative hyperparameter optimization via the “kneedle” method.**

Instead of looking for the hyperparameter value (e.g., the t-SNE perplexity) to minimize the number of dubious embeddings over a default grid, we implemented another algorithm that searches for the hyperparameter value as the elbow point in the plot of the number of dubious embeddings (i.e., the y-axis) versus the hyperparameter value (i.e., the x-axis). We investigated this alternative optimization approach on two datasets and found the resulting t-SNE visualizations highly similar to those from the grid search approach (Figures S3–S4).

**Sensitivity analysis of scDEED’s only hyperparameter (x: the percentage of closest neighbors).**

In Steps 4 and 5 of the scDEED algorithm, x, the percentage of closest neighbors, is required as an input, which is also the only hyperparameter of scDEED. While the default value is $x = 50$, we performed a sensitivity analysis over a list of values: $x = 40, 50,$ and $60$, for t-SNE on the *Hydra* dataset. Corresponding to these three values in an increasing order, scDEED found the optimized t-SNE perplexity values as 250, 210, and 270, which minimized the number of dubious embeddings for each $x$. The t-SNE visualizations under these three perplexity values are highly similar (top panels in Figure S15). Alternatively, we used the “kneedle” method to find the perplexity values as 150, 170, and 170, respectively, and found the t-SNE visualizations to remain stable (bottom panels in Figure S15). Hence, we conclude that scDEED’s optimization of the t-SNE perplexity hyperparameter is robust to the choice of $x$ on the *Hydra* dataset.

**Implementation of t-SNE and UMAP.**

We performed t-SNE and UMAP using the functions `RunTSNE()` and `RunUMAP()` respectively in the R package Seurat (version 3.2.3). The hyperparameters scDEED optimizes are perplexity in the `RunTSNE()` function and n.neighbors and min.dist in the `RunUMAP()` function. We used “seed.use = 100” when running `RunTSNE()` and `RunUMAP()` and kept the rest of the arguments as default.

**Assessing the purity of cell clusters in the *Hydra* dataset.**

We used the function `CalculateRogue()` in the R package Rogue (version 2.0.0) to calculate the ROGUE statistic, which measures the purity of a cell cluster. The larger the ROGUE value, the purer (or more homogeneous) the cell cluster. In the *Hydra* dataset, the ROGUE values of the five clusters of neuron ectodermal cells are 0.710 (*neuron ec1*), 0.784 (*neuron ec2*), 0.714 (*neuron ec3*), 0.793 (*neuron ec4*), and 0.839 (*neuron ec5*).

**Selection of genes in heatmaps.**
In every heatmap, unless otherwise specified, we plotted the top 30 genes that have the largest expression variances (based on the gene expression values before the PCA step in the Seurat package) across the cells shown in the heatmap.

**Evaluation metrics for the preservation of local and global information.**

We evaluated the preservation of information as in [20], using the following two metrics.

K-nearest neighbors (KNN) reflects the preservation of local information, i.e., the average proportion of the 10 nearest neighbors in the input PC space that remain in the set of 10 nearest neighbors in the 2D embedding space.

K-nearest clusters (KNC) reflects the preservation of global information, i.e., the average proportion of the 4 nearest clusters in the input PC space that remain in the set of 4 nearest clusters in the 2D embedding space. Note that for KNC, we deviated from [20] by defining each cluster center through the median rather than the mean because the median is more robust to outliers.

**Real datasets.**

Whenever preprocessed datasets were available, they were directly used in this study. Otherwise, datasets were preprocessed in the same way as in the original studies generating the data. Below is the preprocessing detail for every dataset. The preprocessed data and code are available on Zenodo (DOI 10.172022/scDEED).

**Hydra.** The dataset *Hydra/Hydra_Seurat_Whole_Transcriptome.rds* (from the original study) contains the transcriptomes of $n = 25,052$ single *Hydra* polyp cells sequenced by Drop-seq, with the cells labeled as cell clusters, and 33,391 genes’ scaled expression levels processed by Seurat [28]. Following the original study, we used $K = 31$ principal components in Step 2 of scDEED, and the default `RunTSNE(dims = 1:5)` in the Seurat R package as the 2D embedding method. The preprocessing code is in *Hydra/data_processing_hydra.Rmd*, and the preprocessed data is in *Hydra/Hydra.rds*.

We ran scDEED with the candidate perplexity values 10, 30, ..., 390, and 410, as well as the value 40 used in the original study. The running time was 2.80 hours (see “Computer environment”).

**CAR-T.** In patients with B cell malignancies, lymphodepletion chemotherapy followed by infusion of CD19-specific chimeric antigen receptor modified-T (CAR-T) cells is known to generate anti-tumor responses. The dataset was produced to understand the clonal composition of CAR-T cells in the infusion products (IP) after the adoptive transfer. In particular, the dataset contains a sample of 10 patients who received CD19-specific CAR-T cells, and it is representative of the population in terms of age, sex, adverse events, clinical outcome, lymphodepletion therapy and cell dose. Using the 10x Genomics platform, single-cell RNA-seq data were generated from $n = 62,167$ CD8+ CAR-T cells sorted based on truncated human epidermal growth factor receptor (EGFRt) expression from the IP and blood at the early (day 7–14), late (day 26–30), and very late (day 83–112) time points after
infusion. This dataset is in the file \textit{CART/raw.expMatrix.csv}, downloaded from the Gene Expression Omnibus (GEO) with the accession GSE125881. Following the original study, we used $K = 15$ principal components in Step 2 of scDEED and the default \texttt{RunTSNE(dims = 1:5)} in the Seurat R package as the 2D embedding method. The preprocessing code is in \texttt{CART/data\_processing\_CART.Rmd}, and the preprocessed data is in \texttt{CART/seuratObj\_v3.RData}.

We ran scDEED with the candidate perplexity values 20, 50, \ldots, 380, 410, 450, 500, \ldots, 750, and 800, as well as the value 30 used in the original study. The running time was 14.37 hours (see “Computer environment”).

\textbf{Alveolar.} This dataset was constructed to learn the cell-cell communication during the regeneration process after bleomycin induced lung injury. It contains whole-organ single cell suspensions from mice, from six time points after injury and uninjured control lungs with four replicate mice per time point on average. Single cell transcriptomes from about 1000 cells per individual mouse were carried out using the Dropseq workflow, leading to a sample of $n = 29,297$ cells in the final dataset. Following the original study, we used $K = 50$ independent components in Step 2 of scDEED and as the input for UMAP in the Seurat R package, \texttt{RunUMAP(dims = 1:50)}. Unlike \texttt{RunTSNE}, Seurat requires the user to specify the input dimension for UMAP. The preprocessing code is in \texttt{Alveolar/data\_processing\_Alveolar.Rmd}, and the preprocessed data is in \texttt{Alveolar/Seurat\_v3.RData}.

We ran scDEED with the candidate n.neighbors values 5, 6, \ldots, 9, 10, 15, 20, \ldots, 45, 50, 80, 160, \ldots, 240, and 320; and candidate min.dist values 0.0125, 0.05, 0.1, 0.2, \ldots, 0.7, and 0.8. The running time was 1.37 hours for marginal optimization of n.neighbors, 38.59 minutes for marginal optimization of min.dist, and 12.42 hours for joint optimization (see “Computer environment”).

\textbf{Samusik.} Cells were gathered from bone marrow samples, and cell surface markers were used for CyTOF analysis. Data was normalized and annotated with clusters and the hand gated populations. Doublets and neutrophils were removed. The final dataset has $n = 841,644$ cells. Following the original study, we used $p = 38$ genetic markers as the input for Step 2 of scDEED and as the input for UMAP, \texttt{RunUMAP(features = feature\_list)}, where \texttt{feature\_list} refers to the 38 genetic markers. The preprocessing code is in \texttt{Samusik/data\_processing\_samusik01.Rmd}, and the preprocessed data is in \texttt{Samusik/samusik01\_seurat.Rdata}.

We ran scDEED with the same candidate n.neighbors and min.dist values as in \textbf{Alveolar}. The running time was 12.43 hours for marginal optimization of n.neighbors, 4.89 hours for marginal optimization of min.dist, and 3.77 days for joint optimization (see “Computer environment”).

\textbf{HumanPBMC.} This dataset was gathered by the Broad Institute to compare seven single cell/single nucleus sequencing methods [37]. The original study manually annotated cells based on canonical cell markers. Here, we focused on three sequencing methods (inDrops, CEL-Seq, and SeqWell) and four common cell types \textit{Cytotoxic T cell}, \textit{CD4+ T cell}, \textit{CD14+ Monocyte}, and \textit{B cell}. This resulted in $n = 5,858$ cells for inDrops, $n = 445$ cells for CEL-Seq, and $n = 3,626$ cells for SeqWell. The entire dataset is available as \texttt{pbmcsca.SeuratData} in the R package SeuratData. The subset of data we analyzed...
is in Across_Techniques/Seurat.Rdata. We used $K = 50$ principal components in Step 2 of scDEED. The low dimensional space was obtained using `RunUMAP(dims = 1:50)` and `RunTSNE(dims = 1:5)`. We ran scDEED with the candidate perplexity values 5, 10, ..., 135, and 140; n.neighboirs values 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 80, 160, and 240; and min.dist values 0.0125, 0.05, 0.1, 0.3, 0.5, 0.7, and 0.9.

**Marrow.** This dataset was used in the EMBEDR paper [25] and is a subset of a single-cell transcriptome of *Mus. musculus*. Cells were harvested from mice and sorted with fluorescent activated cell sorting (FACS). Sequencing was done using the Smart-seq2 protocol with Illumina sequencing. The original data is available as Marrow/Marrow_counts at [https://figshare.com/projects/Tabula_Muris_Transcriptomic_characterization_of_20_organs_and_tissues_from_Mus_musculus_at_single_cell_resolution/27733](https://figshare.com/projects/Tabula_Muris_Transcriptomic_characterization_of_20_organs_and_tissues_from_Mus_musculus_at_single_cell_resolution/27733). The original dataset contained $n = 5,037$ cells. Following the preprocessing notebook available at EMBEDR’s GitHub (Marrow/Marrow_preprocessing.ipynb), we obtained $n = 4,821$ cells (Marrow/Marrow_processed.csv). This differs from EMBEDR’s reported $n = 4,771$ cells after the preprocessing (in the EMBEDR publication). However, the EMBEDR’s authors’ code indicated that all $n = 5,037$ cells were used for analysis. Despite this discrepancy, our preprocessed $n = 4,821$ cells with 17,303 genes replicated the EMBEDR results fairly well. For fair comparison, the processed data was used for all analysis. Following the EMBEDR tutorial at [https://github.com/ejohnson643/EMBEDR/blob/master/projects/Figures/Figure_04v1_GlobalParameterSweep.ipynb](https://github.com/ejohnson643/EMBEDR/blob/master/projects/Figures/Figure_04v1_GlobalParameterSweep.ipynb), we used $K = 50$ principal components as input for EMBEDR optimization. For scDEED optimization, we used $K = 16$ principal components (chosen from an elbow plot) in Step 2 of scDEED. The low dimensional space and visualizations were obtained using the default Seurat R command `RunTSNE(dims = 1:5)`.

We ran scDEED with the candidate values 10, 15, 20, 30, 40, 50, 60, 80, 100, 150, 200, 300, 350, 500, 600, 800, 1000, 1300, 1700, 2200, 2900, and 3700 to mimic the $K_{eff}$ values in the original Figure 4 in the EMBEDR paper [25]. The running time was 10 minutes (see "Computer environment").

**DG.** This dentate gyrus dataset was measured to elucidate the gyrus cell lineage. The 10x Genomics processed data used in the tutorial of the R package Velocyto (version 0.6) was analyzed ([Velocyto/10X43.1_loom](https://htmlpreview.github.io/?https://github.com/satijalab/seurat.wrappers/blob/master/docs/velocity.html)). The tutorial is available at [https://htmlpreview.github.io/?https://github.com/satijalab/seurat.wrappers/blob/master/docs/velocity.html](https://htmlpreview.github.io/?https://github.com/satijalab/seurat.wrappers/blob/master/docs/velocity.html).

Cells were annotated using the Louvain clustering at the default resolution (0.8) and marker genes from the original paper ([Velocyto/data_annotated.Rds](http://pklab.med.harvard.edu/velocyto/DG1/10X43_1.loom)). The dataset consists of $n = 3,396$ cells and 92,135 features across the spliced and unspliced assays and is accessible at [http://pklab.med.harvard.edu/velocyto/DG1/10X43_1.loom](http://pklab.med.harvard.edu/velocyto/DG1/10X43_1.loom). For scDEED optimization, we used $K = 12$ principal components in Step 2 of scDEED and obtained the low dimensional space using the default Seurat R command `RunTSNE(dims = 1:5)`. Final visualization used the command `RunTSNE(dims = 1:12)`. 18
We ran scDEED with the candidate perplexity values 20, 50, ..., 380, 410, 450, 500, 600, 700, and 800.

**Simulated Data.** The 20 simulated datasets (Simulated_Data/Simulated_data_1.Rds, ..., Simulated_Data/Simulated_data_20.Rds) were generated by scDesign3 [41], which was trained on a built-in dataset of mouse small intestinal epithelial cells of the R package scDesign2 [45] (GEO number GSE92332 [46]). To increase the distances between three cell types (Enterocyte, Progenitor, TA.Early, and Stem), we independently permuted each cell type’s gene expression mean values (every gene has a mean parameter value in each cell type) across all genes. To ensure gene expression did not largely deviate from the specified cluster means, the 100 largest dispersion parameters were divided by 150.

In total, we had 10,000 genes and n = 7,217 cells. For scDEED optimization, we used K = 12 principal components in Step 2 of scDEED. For the low dimensional space, we used RunUMAP(dims = 1:12) and the default RunTSNE(dims = 1:5). For EMBEDR, we used p = 12 principal components for the optimization process.

To compare t-SNE and UMAP fairly, the final 2D embeddings used for KNN and KNC comparison all used p = 12 principal components as input.

We ran scDEED with the candidate perplexity values 20, 50, ..., 380, 410, 450, 500, ..., 750, and 800 (default settings in scDEED).

**RNA velocity**

RNA velocity was performed using Velocyto (version 0.6) with default settings using the tutorial available at: https://htmlpreview.github.io/?https://github.com/satijalab/seurat.wrappers/blob/master/docs/velocity.html.

**Comparison with EMBEDR and DynamicViz**

EMBEDR is available as a Python package. Hyperparameter sweeps were performed following the available tutorials with default settings, including the option to use all available processors. Applying EMBEDR to the Hydra dataset, we used the suggested 25 data embeddings with 15 null embeddings. Applying EMBEDR to the 20 simulated datasets, we used 5 data embeddings with 10 null embeddings to save computational time. Since EMBEDR requires the user to provide a list of candidate hyperparameter parameters, we used the default lists of perplexity and n.neighbors values in scDEED. EMBEDR does not sweep over min.dist, so for a fair comparison, we fixed min.dist at 0.1 (default EMBEDR setting) when using scDEED.

EMBEDR categorizes cells as well-embedded or noisy. For consistency in terminology between EMBEDR and scDEED, we considered well-embedded cells to have trustworthy embeddings and noisy cells to have dubious embeddings. Specifically, we defined dubious cell embeddings to be the cells
with EMBEDR p-values above 0.1 based on their suggested visualization tools, which consider all cells with p-values > 0.1 to have similar levels of noise.

DynamicViz is also available as a Python package. Parameter sweeps are not built in, but can be iterated. We were able to successfully use this package for t-SNE, yet for UMAP there were some errors. Due to this and the conceptual difference in the definition of dubious cell embeddings, DynamicViz was omitted from analysis.

**Versions of R packages.**

**Seurat** version 3.2.3: all the t-SNE and UMAP analyses except the EMBEDR analysis.

**SeuratData** version 0.2.2: for the dataset “pmbcsca.SeuratData”.

**doParallel** version 1.0.15; **foreach** version 1.5.0: for parallel computing and looping.

**ggsci** version 2.9: for plotting.

**Rogue** version 2.0.0: for assessing the purity of a cell cluster.

**distances** version 0.1.8: fast computation for pairwise distances between vectors.

**velocyto.R** version 0.6: RNA velocity analysis.

Other packages:

**Rfast** version 1.9.9; **VGAM** version 1.1.3; **pracma** version 2.2.9; **ggplot2** version 3.3.2; **SeuratWrappers** version 0.3.0

**Computer environment.**

All algorithms and code were executed on an iMac with 3.6 GHz Intel Core i9 processor, 64GB memory, and Mojave 10.14 system. For the data analysis performed in this paper, 6 cores were used.

**Data and Software Availability**

The scDEED R package is available at GitHub repository [https://github.com/JSB-UCLA/scDEED](https://github.com/JSB-UCLA/scDEED)

The computer code and processed data are available at Zenodo [https://zenodo.org/record/7216361#.ZDNgd-zMLJ8](https://zenodo.org/record/7216361#.ZDNgd-zMLJ8)
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Declaration of Interests

The authors declare no competing interests.
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Figure 1. Illustration of the two functionalities of scDEED. Functionality I decides whether each cell has a trustworthy or dubious embedding by calculating a reliability score, which is defined as the Pearson correlation between the cell’s distances to its closest 50% neighboring cells in the 2D embedding space and the same cell’s distances to its closest 50% neighboring cells in the original space (with the distances in each space ordered from the 1st neighbor to the \([n/2]\)th neighbor, where \(n\) is the total number of cells). Compared with a null distribution of reliability scores, cell 1’s reliability score falls into the highest 5%, so it has a trustworthy embedding; in contrast, cell 2’s reliability score falls into the lowest 5%, so it has a dubious embedding. Enabled by functionality I, functionality II optimizes the hyperparameter setting of an embedding method (e.g. t-SNE and UMAP) by minimizing the number of dubious embeddings.
Figure 2. Original t-SNE embeddings and t-SNE embeddings optimized by scDEED on the Hydra dataset. a, t-SNE plot of the Hydra dataset at the perplexity 40 used in the original study. b–c, Dubious cell embeddings (b) and trustworthy cell embeddings (c) defined by scDEED at the perplexity 40. d, t-SNE plot of the Hydra dataset at the perplexity 210 optimized by scDEED. e–f, Dubious cell embeddings (e) and trustworthy cell embeddings (f) defined by scDEED at the perplexity 210.
Figure 3. Evaluation of t-SNE embeddings optimized by scDEED on the Hydra dataset. a–b, Comparative t-SNE plots with the ecEP_sc, trustworthy cell embeddings in neuron ec1, and dubious cell embeddings in neuron ec1 highlighted, at the original perplexity 40 (a) and the perplexity 210 optimized by scDEED (b). C, Gene expression heatmap of the highlighted cells in a and b, where the cells are ordered by the default hierarchical clustering found by the R function heatmap.2().

d–e, Comparative t-SNE plots with the neuron ec1, neuron ec3, and ecEP_sc cells highlighted. At the original perplexity 40 (d), the neuron ec1 cells are in two separate clusters and have similarly short distances as the neuron ec3 cells have to the ecEP_sc cells; at the optimized perplexity 210 (e), the neuron ec1 and neuron ec3 cells are unified as one cluster far away from the ecEP_sc cells.

f, Gene expression heatmaps of the highlighted cells in e and f, where the cells are ordered by the default hierarchical clustering found by the R function heatmap.2().

g–h, Comparative t-SNE plots with the neuron ec1, neuron ec2, neuron ec3, neuron ec4, neuron ec5, and battery cell 2 (mp) cells highlighted. At the original perplexity 40 (g), the neuron ec1, neuron ec2, neuron ec3, neuron ec4, and neuron ec5 cells are in distinct clusters surrounding the battery cell 2 (mp) cells; at the optimized perplexity 210 (h), the five neuron ec clusters are unified as one cluster far away from the battery cell 2 (mp) cells.

i, Gene expression heatmaps of the highlighted cells in g and h, where the cells are ordered by the default hierarchical clustering found by the R function heatmap.2().
Figure 4: Original t-SNE embeddings and t-SNE embeddings optimized by scDEED on the CAR-T dataset. 

- **a**, t-SNE plot of the CAR-T dataset at the study’s original perplexity 30.
- **b–c**, Dubious embeddings (b) and trustworthy embeddings (c) defined by scDEED at the original perplexity 30.
- **d**, t-SNE plot of the CAR-T dataset at the perplexity 750 optimized by scDEED.
- **e–f**, Dubious embeddings (e) and trustworthy embeddings (f) defined by scDEED at the optimized perplexity 750.
Figure 5: Evaluation of cluster locations at the original perplexity and the perplexity optimized by scDEED on the CAR-T dataset. a–b, Comparative t-SNE plots of cluster 7’s dubious and trustworthy cell embeddings at the original perplexity 30 (a) and the perplexity 750 optimized by scDEED (b). C, Gene expression heatmap of highlighted cells in a and b. d–e, Comparative t-SNE plots of clusters 6, 7, and 14 at the perplexity 30 in the original study (d) and the perplexity 750 optimized by scDEED (e). We have recolored cluster 7 for better visualization in d–f. f, Gene expression heatmap of the highlighted cells in d and e, where cells are ordered by the default hierarchical clustering found by the R function heatmap(). g–h, Comparative t-SNE plots of clusters 3, 4, 9 and 11 at the perplexity 30 in the original study (g) and the perplexity 750 optimized by scDEED (h). We have recolored the clusters for better visualization in g–i. i, Gene expression heatmap of the highlighted cells in g and h.
Figure 6: Original UMAP embeddings and UMAP embeddings optimized by scDEED on the Alveolar dataset. 

**a**, UMAP plot of the Alveolar dataset at the study’s original hyperparameters, min.dist = 0.3 and n.neighbors = 10. 
**b–c**, Dubious embeddings (b) and trustworthy embeddings (c) defined by scDEED at the original hyperparameters. 
**d**, UMAP plot of the Alveolar dataset at the hyperparameters jointly optimized by scDEED, min.dist = 0.7 and n.neighbors = 6. 
**e–f**, Dubious embeddings (e) and trustworthy embeddings (f) defined by scDEED at the optimized hyperparameters.
Figure 7: Evaluation of cluster locations at the original hyperparameters and the hyperparameters jointly optimized by scDEED on the Alveolar dataset. a–b, Comparative UMAP plots of the Alveolar dataset with the lec, b_cells, vcam1_vec, and vec cells highlighted at the original hyperparameters of min.dist = 0.3 and n.neighbors = 10 (a) and the hyperparameters of min.dist = 0.7 and n.neighbors = 6 jointly optimized by scDEED (b). c, Gene expression heatmap of the highlighted cells in a and b. d–e, Comparative UMAP plots of the Alveolar dataset with the lec, b_cells, and t_cells cells highlighted at the original hyperparameters (d) and the hyperparameters jointly optimized by scDEED (e). f, Gene expression heatmap of the highlighted cells in d and e. Note we randomly downsampled b_cells and t_cells (from 911 and 2709 cells, respectively) so that each cluster has 256 cells (same as the number of cells in lec) to make a visually informative heatmap.
Figure 8: Original UMAP embeddings and UMAP embeddings optimized by scDEED on the Samusik dataset.  

- **a**: UMAP plot of the Samusik dataset at the study's original hyperparameters, min.dist = 0.2 and n.neighbors = 15. 
- **b**, Dubious embeddings and **c**, trustworthy embeddings defined by scDEED at the original hyperparameters. 
- **d**: UMAP plot of the Samusik dataset with the hyperparameters jointly optimized by scDEED, min.dist = 0.7 and n.neighbors = 160. 
- **e**, Dubious embeddings and **f**, trustworthy embeddings defined by scDEED at the optimized hyperparameters.
Figure 9: Evaluation of cluster locations at the original hyperparameters and the hyperparameters optimized by scDEED on the Samusik dataset. a–b, Comparative UMAP plots of the Samusik dataset with the non-classical monocytes (ncm), mDCs, pDCs, and macrophages highlighted at the original hyperparameters of min.dist = 0.2 and n.neighbors = 15 (a) and the hyperparameters of min.dist = 0.7 and n.neighbors =160 jointly optimized by scDEED (b). c, Gene expression heatmap of the highlighted cells in a and b. d–e, Comparative UMAP plots of the Samusik dataset with the NK cells (nk), ncm, and plasma cells highlighted at the original hyperparameters (d) and the hyperparameters optimized by scDEED (e). f, Comparison of distances between ncm, nk, and plasma cells. The two-sample t statistic p-values for between-boxplot comparisons are presented, with the null distribution computed based on 1000 random partitions of the cells in the three types by preserving the three cell type sizes (the theoretical t distribution should not be used because the distances are not independent). The two-sample t statistics are as follows: (nk vs. ncm) vs (ncm vs. plasma) = -728.580, (nk vs. ncm) vs (plasma vs. nk) = -370.042, (ncm vs. plasma) vs (plasma vs. nk) = -13.094. f confirms that e better preserves the three clusters’ relative distance than d does.
Figure 10. Original t-SNE and UMAP embeddings and embeddings optimized by scDEED on the PBMC dataset. a, t-SNE and UMAP plots for the DropSeq dataset at the original hyperparameters, perplexity = 30 (left) and min.dist = 0.3 and n.neighbors = 30 (right). b, t-SNE and UMAP plots for the Dropseq dataset at the hyperparameters optimized by scDEED, perplexity = 290 (left) and min.dist = 0.5 and n.neighbors = 5 (right). c, t-SNE and UMAP plots for the inDrops dataset at the original hyperparameters, perplexity = 30 (left) and min.dist = 0.3 and n.neighbors = 30 (right). d, t-SNE and UMAP plots for the inDrops dataset at the hyperparameters optimized by scDEED, perplexity = 320 (left) and min.dist = 0.5 and n.neighbors = 80 (right). e, t-SNE and UMAP plots for the SeqWell dataset at the original hyperparameters, perplexity = 30 (left) and min.dist = 0.3 and n.neighbors = 30 (right). f, t-SNE and UMAP plots for the SeqWell dataset at the hyperparameters optimized by scDEED, perplexity = 140 (left) and min.dist = 0.2 and n.neighbors = 7 (right).
Supplementary Figures

Figure S1. **a**, Comparative t-SNE plots at the original perplexity 40 with dubious (left) and trustworthy cell embeddings (right) in the nematocyte cluster. **b**, Gene expression heatmap of the highlighted cells in a.
Figure S2. a, t-SNE plot at the original perplexity 40, with the *male germline* cluster highlighted. b, Gene expression heatmap of the dubious cell embeddings in the *male germline* cluster.
Figure S3. **a,** Plots of the number of dubious cell embeddings (the y-axis) versus perplexity (the x-axis) with the original and optimized perplexities highlighted. The optimized perplexities correspond to the minimum number of dubious embeddings (left), or the elbow point selected by the “kneedle” method [29] (right). **b,** Comparative t-SNE plots corresponding to the optimized perplexities 210 (left) and 170 (right).
**Figure S4.**

**a,** Plots of the number of dubious cell embeddings (the y-axis) versus perplexity (the x-axis) with the original and optimized perplexities highlighted. The optimized perplexities correspond to the minimum number of dubious embeddings (left), or the elbow point selected by the “kneedle” method [29] (right).

**b,** Comparative t-SNE plots corresponding to the optimized perplexities 750 (left) and 170 (right).
Figure S5. a, t-SNE plot at the perplexity 750 optimized by scDEED, with cluster 14 and cluster 5 highlighted as the reference clusters for cluster 7's dubious and trustworthy embeddings defined by scDEED at the original perplexity 40. b, Cell trajectory reconstruction via STREAM, produced by Python functions st.plot_dimension_reduction() and st.plot_branches() in the Bioconda package “stream” [34].
Figure S6. a, Comparative t-SNE plots with clusters 8, 9, and 14 highlighted at the original perplexity 30 (left) and the perplexity 750 optimized by scDEED (right). b, Gene expression heatmap of the highlighted cells in a, where the cells are ordered by the default hierarchical clustering found by the R function `heatmap.2()`.
Figure S7. a, Plots of the number of dubious cell embeddings (the y-axis) versus n.neighbors (the x-axis) with the fixed min.dist = 0.3 (left) and the number of dubious cell embeddings (the y-axis) versus min.dist (the x-axis) with the fixed n.neighbors = 10 (right), having the original and the optimized hyperparameters highlighted. b, Comparative UMAP plots at the marginally optimized n.neighbors by scDEED (min.dist = 0.3 and n.neighbors = 5; left) and the marginally optimized min.dist by scDEED (min.dist = 0.6, n.neighbors = 10; right).
**Figure S8.**

**a.** Comparative UMAP plots of the *Alveolar* dataset with the *lec*, *b_cells*, *vcam1_vec*, and *vec* cells highlighted at the marginally optimized n.neighbors (min.dist = 0.3, n.neighbors = 5; left) and the marginally optimized min.dist (min.dist = 0.6, n.neighbors = 10; right).

**b.** Comparative UMAP plots of the *Alveolar* dataset with the *lec*, *b_cells*, and *t_cells* cells highlighted at the marginally optimized n.neighbors (left) and the marginally optimized min.dist (right).
Figure S9. a, Plots of the number of dubious cell embeddings (the y-axis) versus n.neighbors (the x-axis) with the fixed min.dist = 0.2 (left) and the number of dubious cell embeddings (the y-axis) versus min.dist (the x-axis) with the fixed n.neighbors = 15 (right), having the original and the optimized hyperparameters highlighted. b, Comparative UMAP plots corresponding to the marginally optimized n.neighbors by scDEED (min.dist = 0.2 and n.neighbors = 160; left) and the marginally optimized min.dist by scDEED (min.dist = 0.05, n.neighbors = 15; right).
Figure S10. Velocity analysis of the dentate gyrus dataset. **a**, t-SNE plot at the original perplexity of 30 (OL: oligodendrocytes; NOFL: newly formed oligodendrocytes; RGL: Radial glia-like; Neuro1: Neuroblast1; Neuro2: Neuroblast 2; nIPC: neuronal intermediate progenitor cells). **b**, Velocity visualization using the embeddings at the original perplexity of 30 with the default Velocyto [38] settings. **c** t-SNE plot at the perplexity of 450 optimized by scDEED. **d**, Velocity visualization using the embeddings at the perplexity of 450 optimized by scDEED with the default Velocyto [38] settings.
Figure S11. Evaluation of trustworthy and dubious embeddings defined by scDEED or EMBEDR on the Hydra dataset. 

a–b, Dubious cell embeddings defined at the perplexity 40 by scDEED (a) and EMBEDR (b). 

c–d, Dubious and trustworthy cell embeddings defined by scDEED (c) and EMBEDR (d) in the nematocyte cluster. 

e–f, Gene expression heatmaps of the cells with trustworthy and dubious embeddings in the nematocyte cluster defined by scDEED (e) and EMBEDR (f). The Euclidean distances calculated in the input PC space between the trustworthy- and dubious-embedding cells are greater for scDEED than EMBEDR ($\rho < 2.2 \times 10^{-16}$). 

g–h, Dubious and trustworthy cell embeddings defined by EMBEDR at perplexity 40 in the neuron ec1 cluster at the original perplexity of 40 (g) and the perplexity of 410 optimized by EMBEDR (h). 

i, Gene expression heatmaps of the trustworthy and dubious embeddings defined by EMBEDR in the neuron ec1 cluster.
Figure S12. t-SNE visualizations of the Hydra dataset using the optimized perplexity of 210 found by scDEED (left) and the optimized perplexity of 410 found by EMBEDR (right).
Figure S13. Comparison of scDEED and EMBEDR on the marrow dataset. a–b, t-SNE visualizations at the perplexity of 100 optimized by scDEED (a) and the perplexity of 1000 optimized by EMBEDR (b). c–d, t-SNE visualizations highlighting a random KLS cell’s 50 nearest neighbors in the input PCA space at the optimized perplexities found by scDEED (c) and EMBEDR (d).
Figure S14. a–b, Median proportion of dubious embeddings across 20 simulated datasets found by scDEED at varying perplexities for t-SNE (a) and n.neighbors for UMAP (b). c–d, Median proportion of dubious embeddings across 20 simulated datasets found by EMBEDR at varying perplexities for t-SNE (c) and n.neighbors for UMAP (d). We used exceedingly large n.neighbor values for EMBEDR to show the continual decrease in the number of dubious embeddings. Since scDEED had already achieved 0 dubious embeddings, these larger n.neighbor values were not used for scDEED (b). e, Proportions of dubious embeddings detected by EMBEDR in t-SNE and UMAP visualizations at each kEff value as calculated in the original paper [25]. The largest n.neighbors value used in UMAP was 850. f, Scatterplot of KNC and KNN metrics of the t-SNE and UMAP visualizations (optimized by scDEED and EMBEDR separately) on the 20 simulated datasets.
Figure S15. Sensitivity analysis of scDEED. a–b, Comparative t-SNE plots with perplexities optimized by scDEED with the hyperparameter ($x\%$, i.e., the percentage of closest neighbors, in Step 4 of the scDEED algorithm) set to 40%, 50%, and 60%, corresponding to the three columns. Given each $x\%$, the perplexity is optimized by minimizing the number of dubious cell embeddings (a) or the "kneedle" method (b). For easier visualization, the cell type labels are omitted, and the color key is the same as in Figure 2.
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