Privacy-preserving integration of multiple institutional data for single-cell type identification with scPrivacy

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Received August 14, 2022; accepted September 15, 2022; published online December 15, 2022

The rapid accumulation of large-scale single-cell RNA-seq datasets from multiple institutions presents remarkable opportunities for automatically cell annotations through integrative analyses. However, the privacy issue has existed but being ignored, since we are limited to access and utilize all the reference datasets distributed in different institutions globally due to the prohibited data transmission across institutions by data regulation laws. To this end, we present scPrivacy, which is the first and generalized automatically single-cell type identification prototype to facilitate single cell annotations in a data privacy-preserving collaboration manner. We evaluated scPrivacy on a comprehensive set of publicly available benchmark datasets for single-cell type identification to stimulate the scenario that the reference datasets are rapidly generated and distributed in multiple institutions, while they are prohibited to be integrated directly or exposed to each other due to the data privacy regulations, demonstrating its effectiveness, time efficiency and robustness for privacy-preserving integration of multiple institutional datasets in single cell annotations.

INTRODUCTION

Single-cell transcriptomics is indispensable for understanding cellular mechanisms of complex tissues and organisms (Guan et al., 2021; Jiang et al., 2021; Plass et al., 2018; Xie et al., 2021; Zhao et al., 2022). As single-cell technologies rapidly developed over recent years, its experimental throughput increased substantially, allowing to profile increasingly complex and diverse samples, and accumulating vast numbers of datasets over time. Integrative analyses of such large-scale datasets originating from various samples, different platforms and different institutions globally, offer unprecedented opportunities to establish a comprehensive picture of cell landscape. To this end, various community generated large-scale atlas-level single cell reference data, such as the Human Cell Atlas (HCA) (Regev et al., 2017), Human Tumor Atlas Network (Rozenblatt-Rosen et al., 2020), BRAIN Initiative Cell Census Network (Winnubst and Arber, 2021), Human Lung Atlas (Travaglini et al., 2020), Human Gut Atlas (Elmentaita et al., 2020), Hu-
man BioMolecular Atlas Program (HuBMAP) (Snyder et al., 2019), The Tabula Sapiens (Jones et al., 2022), hECA (Chen et al., 2022a) etc., and recently great achievements have been made in the building of pan-tissue single-cell transcriptome atlases covering more than a million cells, including 500 cell types, across more than 30 human tissues from 68 donors (Dominguez Conde et al., 2022; Eraslan et al., 2022; Jones et al., 2022; Liu and Zhang, 2022; Suo et al., 2022). These references data facilitate the automatically cell type annotations in a supervised way without prior marker gene annotations (Aran et al., 2019; Duan et al., 2020; Kiselev et al., 2018; Li et al., 2020; Liu et al., 2020; Ma and Pellegrini, 2020; Stuart et al., 2019). It is obviously that integrating more reference datasets or combining these atlas-level data will improve the cell type annotations (Aran et al., 2019; Duan et al., 2021; Kiselev et al., 2018; Stuart et al., 2019), and various integration methods for single cell annotations have been presented (Aran et al., 2019; Chen et al., 2022b; Duan et al., 2020; Kiselev et al., 2018). However, all these existing integration methods require to access the relevant reference datasets directly, which may be unavailable due to the data privacy and security issues. Currently, the privacy and political issues towards omics data transmission and sharing across different institutions or countries are gradually attracting attentions. On the one hand, countries around the world are strengthening laws to protect data privacy and security by prohibiting certain data transition across countries or organizations. Such regulations include the General Data Protection Regulation (GDPR) (Politou et al., 2018) implemented by the European Union, the Health Insurance Portability and Accountability Act (HIPPA) (Benefield et al., 2006) and Health Information Technology for Economic and Clinical Health Act (HITECH) (Halamka and Tripathi, 2017) enacted by U.S. and etc. On the other hand, single-cell reference datasets are generated and accumulated rapidly in different institutions around the world. It is a great demand to integrate all these institutional data globally to facilitate the establishment of the comprehensive picture of human cell reference map. However, these institutions may be required to protect data privacy and security and prohibit certain data transmission across organizations and countries by data regulation laws (Table 1). As a result, there exist an inevitable contradiction between the rapidly accumulated single cell reference data and the privacy issue among data sharing and integration. Current references integrating strategies failed to address these issues, hindered by legal restrictions on data sharing (Lotfollahi et al., 2022). Moreover, current integrating methods ignore the problem of privacy towards single cell sequencing data, as scRNA-seq datasets of human are sensitive and likely to contain sufficient sequencing depth to call genetic variants (Byrd et al., 2020).

To this end, taking the single-cell transcriptome data as an initial study, we propose scPrivacy, an efficient, flexible and extendable automatically single-cell type identification prototype and a proof-of-concept study to facilitate single cell annotations in a data privacy-preserving collaboration manner, by integrating multiple references single cell transcriptome data distributed in different institutions using a federated learning based deep metric learning framework. Federated learning is a collaborative paradigm in privacy-preserving computing community that enables the institutions collaboratively to train a model while keeping the data in local institutions (Chen et al., 2021; Zhang and Yang, 2018). We summarized existing privacy-preserving methods with their distinct characteristics and explained the necessity to perform privacy-preserving computing for large scale single cell data using federated learning framework (Table 2). In addition, in our previous studies (Duan et al., 2020; Duan et al., 2021), metric learning was also proven to be effective for single-cell type annotation. Briefly, the basic idea of scPrivacy is to make each institution train their models locally and aggregate encrypted models parameters for all institutions to avoid putting raw data of all institutions together directly. We evaluated scPrivacy on a comprehensive set of 27 publicly available benchmark datasets for single cell type identification to stimulate the scenario that the reference datasets are rapidly generated and accumulated from multiple institutions, as well as on 15 publicly available patients datasets to simulate a large-scale real world situation that multiple hospitals collaborate together to build an automated cell type annotation system for COVID-19 patients, while they are prohibited to be integrated directly or exposed to each other due to the data privacy regulations, and demonstrated its effectiveness, time efficiency and robustness for privacy-preserving integration of multiple institutional datasets.

RESULTS

Overview of scPrivacy

scPrivacy is an efficient, flexible and extensible automatically single-cell type identification prototype in a data privacy-preserving collaboration manner, by integrating multiple references single cell transcriptome data distributed in different institutions using a federated learning based deep metric learning framework. scPrivacy can effectively integrate information from multiple references while keeping each reference in local institutions, so as to solve the problem of data privacy protection. In particular, each institution trains its model on its local dataset and sends encrypted model parameters to server and then the server aggregates the parameters and sends back the aggregated model to institutions iteratively in training process. Specifically, scPrivacy comprises two main steps: model learning and cell assign-
In the model learning stage (Figure 1A), scPrivacy trains a federated deep metric learning model on multiple institutional datasets in a data privacy-preserving manner. For an individual institution, deep metric learning (DML) is applied to learn an optimal measurement fitting the relationship among cells in the reference dataset, and the N-pair loss (Sohn, 2016) is used as the loss function for model training. With DML, cells belong to the same type became more similar and cells belong to different types became more dissimilar. Then, scPrivacy extends DML to a federated learning framework by aggregating model parameters of institutional datasets to construct an aggregated model (Figure 1A and see Methods), which fully utilized the information contained in multiple institutional datasets to train the aggregated model while avoiding integrating datasets physically. In addition, scPrivacy can utilize the complementary information from different institutional datasets to boost the cell assignment performance, while also avoid the over correction of batch effect, as proven in previous study (Sohn, 2016). In the cell assignment stage (Figure 1B and see Methods), the query dataset is first transformed by the federated model to the same embedding space as that of the transformed institutional datasets. Then, the transformed query dataset is assigned to proper cell types by comparing with cell type landmarks of transformed institutional datasets. Specifically, for each transformed institutional dataset, scPrivacy carries out a cell search by measuring the similarity between the transformed query cells and cell type landmarks of the transformed institutional datasets. Finally, the query cells are assigned to the proper cell type with the highest similarity among all landmarks of the transformed institutional datasets.

**Benchmarking scPrivacy with multiple institution and single institution**

We firstly benchmarked scPrivacy with multiple institution (default), and compared it with that of scPrivacy with single institution to prove the benefits and necessity of integrating multiple institutional datasets. Here, “scPrivacy with single institution” represents that the results are achieved by training with one institution dataset and testing on another institutional datasets. To this end, we collected 27 datasets (Table S1 in Supporting Information) from four studies of three tissues to simulate the data collaboration scenario among institutions: one study on the brain, one study on the pancreas and two studies on peripheral blood mononuclear cells (PBMCs) (Ding et al., 2019; Mereu et al., 2020). For brain tissue, the study contained four brain datasets (Tasic et al., 2016; Tasic et al., 2018) with different sources. For pancreas tissue, the study contained four commonly used pancreas datasets (Baron et al., 2016; Muraro et al., 2016; Segerstolpe et al., 2016; Xin et al., 2016). For PBMCs, the first study (Mereu et al., 2020) contained 12 datasets from 12 different sequencing platforms (“PBMC-Mereu”), and the
second study (Ding et al., 2019) contained 7 datasets from 7 different sequencing platforms (“PBMC-Ding”). In this case, each dataset of a tissue is simulated as an institution. For the strategy of scPrivacy with single institution, each dataset among multiple datasets was simulated as the dataset in an individual institution to train scPrivacy and the rest datasets were used as query datasets. For the strategy of scPrivacy with multiple institutions, each dataset among the multiple datasets was used as the query, and the others datasets were used to simulate distributed multiple institutional datasets and they are “virtually” integrated to train scPrivacy. It should be noted that in all benchmark scenarios in our study, macro-F1 score was used as the evaluation metric and only query cell types included in multiple institutional datasets were calculated. As shown in Figure 2A and Table S2 in Supporting Information, it can be clearly seen that scPrivacy with multiple institutions generally obtained great improvement compared with that of scPrivacy with single institution, demonstrating the importance of integrating multiple institutional datasets. To further analyze the results, we compared macro-F1 score on “PBMC-Mereu” and “PBMC-Ding” studies in terms of each cell type, as the two studies shared several common cell types. The results showed that scPrivacy achieved a better performance in almost all cell types, further demonstrating the benefits and necessity to integrate multiple institutional datasets (Figure 2B and Table S3 in Supporting Information).

**Benchmarking scPrivacy with non-privacy-preserving multiple reference integrating methods**

Then we benchmarked scPrivacy with existing non-privacy-preserving multi-reference based single cell type identification methods, including scmap-cluster (Kiselev et al., 2018), SingleR (Aran et al., 2019), Seurat v3 (Stuart et al., 2019) and mtSC (Duan et al., 2021). In this study, Seurat v3 applied a data-level integration strategy; scmap-cluster and SingleR applied a decision-level integration strategy, and mtSC ap-

![Figure 1](https://example.com/image.png)
plied an algorithm-level integration strategy (See Methods), which represent the three mainstream integrative analysis strategies for single cell assignment. The above 27 datasets in 4 studies are used as the benchmark datasets here. In this case, each dataset among the multiple datasets was treated as the query, and the others were used to simulate multiple institutional datasets. It should be noted that scPrivacy integrated multiple institutional datasets in a data privacy-preserving manner while other multi-reference based methods accessed all datasets to integrate them directly. The results are shown in Figure 3A and Table S4 in Supporting Information. We can see that scPrivacy achieved comparable performance to mtSC and performed better than the other methods, while it was trained in a data protection manner. Furthermore, as shown in Figure 3B and Table S5 in Supporting Information, we compared the macro-F1 of each cell type on “PBMC-Ding” and “PBMC-Mereu” studies, and achieved the consistent conclusion that scPrivacy achieved the comparable best performance in all four studies as those of mtSC. As a conclusion, scPrivacy is able to achieve a comparable best performance to integrate multiple institutional datasets, while keeping the integration in a data privacy-preserving manner.

**scPrivacy consumes much less time than most multiple reference integrating methods**

As the amount and size of scRNA datasets increasing rapidly, consuming time is an important concern for single cell integration and annotation. Due to the distributed properties of federated learning, the training time of scPrivacy only depends on the max training time among the institutions datasets while the training time of other multi-reference based methods are the sum of training time of all institution datasets. Thus, scPrivacy obtains a high scalability to deal with the large-scale data integration and model training, which serves a very challenging issue in the building of large-scale atlas level single cell reference data. The following training time and query time comparisons further proved this point (Figure 3C, Tables S6 and S7 in Supporting Information): (1) scPrivacy consumes much less time than Seurat v3 and mtSC in the training process. More importantly, the time consumed by scPrivacy does not increase exponentially or linearly, since it only depends on the max training time among institutions datasets, indicating its potential ability to handle large-scale datasets. (2) For querying process, scPrivacy is also very fast (<1 min for 9,000 query
cells) and consumes much less time than all the other methods except for scmap-cluster, since scmap-cluster is the simplest method with substantial expense of accuracy.

**Robustness validation of scPrivacy**

We then validated the robustness of scPrivacy using these benchmark data. We firstly investigated the impact of the number of institutions on the performance of scPrivacy. We considered each dataset in “PBMC-Ding” as an institution dataset to simulate the scenario. In this test, scPrivacy was trained with different numbers of institution datasets, and then the corresponding macro-F1 score was calculated to show the trend of the performance as the number of institution datasets increased. Specifically, each time, we randomly selected one dataset from the 7 datasets as the query

![Figure 3](image-url)

**Figure 3** Benchmarking scPrivacy with non-privacy-preserving multiple reference integrating methods. A, The macro-F1 scores of scPrivacy and other existing non-privacy-preserving multiple reference integrating methods on “PBMC-Mereu”, “PBMC-Ding”, “Brain” and “Pancreas” studies, respectively. B, The macro-F1 of each cell type for scPrivacy and other existing non-privacy-preserving multiple reference integrating methods on “PBMC-Ding” and “PBMC-Mereu” studies. C, Training and query time of scPrivacy and other existing non-privacy-preserving multiple reference integrating methods. Solid lines are loess regression fitting (span = 2), implemented with R function geom smooth().
dataset and selected 1 to 6 datasets without replacement from the remaining datasets as the institutional reference datasets. This process was repeated 5 times to reduce randomness. As shown in Figure 4A and Table S13 in Supporting Information, we can see that scPrivacy generally performs increasingly better as the number of institutions increases. The macro-F1 scores for each dataset as query dataset can be found in Figure S1 in Supporting Information. Specifically, if the performance is relatively lower in the beginning, the improvement becomes more evident as the number of institution datasets increases. Such an improvement obtained by increasing institution datasets is very important for developing an effect and robust cell annotation system in the era of explosive growth of single-cell datasets. Together with the fast training of scPrivacy, it is expected to integrate a growing number of institution datasets to obtain an increasingly better cell annotation in a privacy-preserving manner.

Then we explored the influence of similarity metrics for query cell assignment. Another two common similarity metrics i.e., cosine similarity and spearman correlation coefficient were tested here. As shown in Figure 4C, using different similarity metrics has little impact on the results and scPrivacy is robust to different similarity metrics. In addition, we explored the influence of the data of an institution completely different from that of other institutions. In our understanding, different tissue datasets are heterogeneous and they are different from each other. To explore the scenario that the data of one institution is completely different from that of other institutions, it is a proper choice to simulate the data of an institution from a different tissue while the others are from the same tissue. To this end, each dataset among PBMC-Ding was treated as the query, and the others and a different tissue dataset (the brain dataset MTG or pancreas dataset Muraro) were used to simulate multiple institutional datasets. As shown in Figure 4B, when the data of one institution is completely different from that of other institutions, the model performance will only decrease slightly.

Finally, we explored the influence of greatly varying data volume across institutions. To simulate the scenario, we selected datasets whose data volumes vary greatly as the institution datasets. In PBMC-Ding datasets, dataset a10Xv2 (9,683 cells) and SM2 (475 cells) were considered as the institutions datasets and the others were treated as the query. In pancreas datasets, dataset Baron (8,562 cells) and Segerstolpe (2,126 cells) were considered as the institutions datasets and the others were treated as the query. As shown in Figure 4D, in general, scPrivacy with multiple institutions generally obtained improvement compared with that of scPrivacy with single institution, which is consistent with the conclusion in Figure 2. In addition, the results showed that the improvement of the institution with the less data volume is larger than that of the institution with the larger data volume. Taken together, scPrivacy is robust to the number of institutions, similarity metrics, data heterogeneity and data volume, further indicating that it has the ability to handle the complex situation for single cell data integration in real world.

A large-scale simulation of collaborations between multiple hospitals for COVID-19 patient cell annotations with scPrivacy

In this study, we made a large-scale simulation of a real world scenario that multiple hospitals collaborate together to build an automated cell type annotation system for COVID-19 patients (Figure 5A). It is obvious that the patient data always has privacy issue and hospitals can not share patient data with each other if they are not approved by patients. Zhang et al. (Ren et al., 2021) recently constructed a large-scale PBMC single cell transcriptome atlas which consists of 196 individuals in 5 disease stages from 39 institutes or hospitals. These data are selected to simulate the collaboration between multiple hospitals for COVID-19 patient cell annotation. In this study, as several integrating methods (such as Seurat v3) can not handle the simulation using all individuals, we randomly selected 15 large-scale individuals datasets (>6,000 cells) satisfying certain criteria (see Methods) (Table S12 in Supporting Information) from different hospitals. The similar benchmark strategies described aforementioned were also adopted here. We firstly benchmarked scPrivacy with multiple hospitals compared with that of scPrivacy with single hospital. As shown in Figure 5B and C and Tables S7, S8 in Supporting Information, scPrivacy with multiple hospitals also obtained great improvement in general, especially in terms of cell types which are more difficult to be distinguished compared with that of scPrivacy with single hospital, demonstrating the effectiveness of integrating multiple hospital patients datasets. Then we benchmarked scPrivacy with existing non-privacy-preserving data integration methods. As shown in Figure 5D, E and Tables S9, S10 in Supporting Information, we can see that scPrivacy achieved state-of-the-art performance in all cell types while it was trained in a data protection manner. As a conclusion, this large-scale real world application simulation also proved that scPrivacy is able to handle large-scale data integration issue and achieve state-of-the-art performance to integrate multiple institutional datasets in a data privacy-preserving manner.

DISCUSSION

In this study, we present the first and generalized federated deep metric learning-based single cell type identification
prototype \textit{scPrivacy} to facilitate single cell annotations by integrating multiple institutional datasets in a privacy-preserving manner. As scRNA-seq datasets grow exponentially, multiple institutional datasets can be integrated to build a more comprehensive, effective and robust cell annotation system. Traditional multi-reference based methods faced the problem of data privacy protection. \textit{scPrivacy} solves this issue by federated learning. Specifically, \textit{scPrivacy} trains each institution dataset locally and aggregates encrypted model parameters for all institutions instead of putting raw data of all institutions together to train a model. We evaluated \textit{scPrivacy} on a comprehensive set of publicly available
benchmark datasets for single cell type identification to simulate the scenario that the reference datasets are rapidly generated and distributed in multiple institutions, while they are prohibited to be integrated directly or exposed to each other due to the data privacy regulations, and demonstrated its effectiveness for privacy-perserving integration of multiple institutional datasets. In addition, a large-scale real world simulation that multiple hospitals collaborate together to build an automated cell type annotation system for COVID-19 patients with scPrivacy was also demonstrated. Collectively, scPrivacy is time efficient, performing increasingly better as the number of institution datasets increases, and robust to the number of institutions, similarity metrics, data heterogeneity and data volume, which is of great potential utility in various real world applications to build single cell atlases in a privacy-preserving way.

Figure 5 A large-scale real world simulation that multiple hospitals collaborate together to build an automated cell type annotation system for COVID-19 patients with scPrivacy. A, The workflow of the simulation. B, The macro-F1 scores of scPrivacy with single hospital and multiple hospitals. C, The macro-F1 of each cell type for scPrivacy with single hospital and multiple hospitals. D, The macro-F1 scores of scPrivacy and other existing non-privacy-preserving multiple reference integrating methods. E, The macro-F1 of each cell type for scPrivacy and other existing non-privacy-preserving multiple reference integrating methods. The white diamond represents the mean value.
In addition to scRNA-seq technology, other single cell omics, such as scATAC-seq and etc., are developing rapidly and vast numbers of datasets will accumulate in the future. Integrating these multi-omics datasets has the potential to provide a more comprehensive picture of basic biological processes. However, privacy issue is still an unavoidable problem for data sharing and integration. scPrivacy can be easily extended to other single cell omics for privacy-preserving integration. In addition, the privacy-preserving computing technologies are evolved rapidly, although federated learning is a suitable solution for large-scale privacy-preserving data integration, it still needs a central server to aggregate model parameters from clients. Recently, blockchain-based federated learning, such as swarm learning (Saldanha et al., 2022; Warnat-Herresthal et al., 2021), is developed which does not need the central server, however, its efficiency is waiting to be further evaluated. Nevertheless, a privacy-preserving integration of multiple institutional data globally to build a more comprehensive cell landscape is a challenging issue that must be faced under the global data sharing protection and regulations. We therefore call for attention to such problems and efficient privacy-preserving system for cell annotations are needed to be carefully designed.

METHODS

Benchmark data collection. We evaluated scPrivacy on 27 single-cell type identification benchmark datasets and 15 patients datasets which were curated from five studies including three tissues: peripheral blood mononuclear cells (PBMCs) (Ding et al., 2019; Mereu et al., 2020; Ren et al., 2021), the brain (Tasic et al., 2016; Tasic et al., 2018) and the pancreas (Baron et al., 2016; Muraro et al., 2016; Segerstolpe et al., 2016; Xin et al., 2016) (Tables S1 and S12 in Supporting Information). For all datasets, cell types with less than 10 cells were removed since they do not contain enough information and are unreliable for subsequent assignment. Cells labeled “alpha.contaminated”, “beta.contaminated”, “gamma.contaminated” and “delta.contaminated” in the dataset generated from Xin et al. (Xin et al., 2016) were removed because they likely corresponded to cells of lower quality. Cells labeled “not applicable” in the dataset generated by Segerstolpe et al. (Segerstolpe et al., 2016) were removed. “L6b”, “Pvalb”, “Sst” and “Vip” cell types in the dataset generated from Tasic et al. (Tasic et al., 2018) were retained to match the names of cell types in the other three brain datasets (Tasic et al., 2016). For “PBMC-Mereu” (Mereu et al., 2020), 12 datasets were used and Smart-seq2-based dataset was excluded, which was too small (Table S1 in Supporting Information).

Data preprocessing. The data preprocessing step of scPrivacy consists of three parts: cell quality control, rare cell type filtering and gene expression profile formatting. scPrivacy evaluates the cell quality on strict criteria. In particular, the number of genes detected requires >500, the number of unique molecular identifiers induced requires >1,500, and the percentage of mitochondrial genes detected requires <10% among all genes. Only cells satisfying all three criteria are reserved. The quality control of Zhang’s study datasets were processed with the quality control procedures in their paper (Ren et al., 2021). Then, all the datasets were normalized by scaling to 10,000 and then with log (counts+1). Finally, all the datasets are processed into an identical format, i.e., expression profiles with the union of the genes in all institution datasets. The column of the gene in query dataset will be filled with zeros if the gene is not in the gene union of the institution datasets.

The selection of the simulation datasets for collaboration among multiple hospitals in the cell annotations of COVID-19 patient. We filtered the datasets not satisfying following criteria: (1) in particular, the number of cells requires >6,000 to guarantee the large scale of dataset, (2) the number of cell types requires >6 to guarantee the quality of dataset. We randomly selected 15 individuals datasets satisfying criteria in various disease stages from different hospitals and there are 3 individuals datasets available in each disease stage. The details of selected datasets can be found in Table S12 in Supporting Information.

Model learning of scPrivacy. In the model learning stage, scPrivacy utilizes federated deep metric learning algorithms to train the federated model on multiple institutional datasets in data privacy protection manner. We remove batch effects for datasets by sending the gradients learned from each dataset, then the aggregated model can utilize the information of the same cell type from different datasets so as to uncover the common biological information and remove the batch effect, which is similar to the idea of mtSC to remove batch effect (Duan et al., 2021). For a single institution, we use deep metric learning (DML) as the training algorithm and N-pair loss (Sohn, 2016) is used as the loss function. DML is applied to learn an optimal measurement fitting the relationship among cells in the reference dataset. With the measurement learned from DML, cells with the same label become more similar and cells with different labels become more dissimilar. The DML neural network for a single institution contains an input layer, a hidden layer and an output layer. The nodes of input layer equal to the genes of the reference while the nodes of the hidden layer and output layer are 500 and 20, respectively.

The application of the N-pair loss consists of two parts: batch construction and calculation. For the batch construction of the N-pair loss, \( \{ (x_i, x_i^+) \cdots, (x_i, x_i^N) \} \) is defined as N pairs of cells from N different cell types, in which \( x_i \neq x_j \) \( \forall i \neq j \). Then, N tuples denoted by \( \{ S_i \}^{N}_{i=1} \) are built from the N pairs,
where $S_i = \{x_i, x_i^+, x_i^+, \ldots, x_i^+\}$. Here, $x_i$ is the query for $S_i$, $x_i^+$ is a positive example and, and $x_i^+(j \neq i)$ are the negative examples. $x_i$ and $x_i^+$ are two cells of the same cell type, and $x_j^+$ are the cells with different cell types different from $x_i$.

The calculation of the $N$-pair loss can be formulated as follows:

$$
L_{N \text{-pair}}\left(\{\{x_i, x_i^+\}\}_{i=1}^N \right) = \frac{1}{N} \sum_{i=1}^N \log \left(1 + \sum_{j \neq i} \exp(f_i^T f_j^+ - f_i^T f_i^+)\right)
$$

in which $f(\cdot; \theta)$ is an embedding kernel defined by a deep neural network, $f_i$ and $f_j^+$ are embedding vectors of two cells of the same cell type and $f_j^+$ are embedding vectors of cells whose cell types are different from $x_i$.

scPrivacy extends DML to a federated learning framework. Define $N$ institutions $\{F_1, \ldots, F_N\}$ and their respective data $\{D_1, \ldots, D_N\}$. A federated learning framework allows institutions to collaboratively train a model and no institution $F_i$ expose its data $D_i$ to others.

As shown in Figure 1, in scPrivacy, institutions datasets learn a federated model collaboratively with the help of a server. To train a federated model, our training process can be divided into the following four steps (Yang et al., 2019):

1. Step 1: Each institution trains its model on its own dataset with DML;
2. Step 2: Institutions encrypt their own model parameters and send encrypted parameters to server;
3. Step 3: Server performs secure aggregation for encrypted parameters;
4. Step 4: Server sends back the aggregated model parameters to institutions and institutions update their models with the decrypted aggregated model parameters.

In our implementation of scPrivacy, we used the Crypten, a ML framework built on PyTorch (Paszke et al., 2019) to implement encryption of model parameters and their operations in secure aggregation with secure multi-party computation (Yao, 1982) which is adopted as an encryption technique here. At the central server, we adapted widely-used FedAvg (McMahan et al., 2016) algorithm to aggregate model parameters. The basic idea of the algorithm to aggregate model parameters is to average model parameters (weight parameters $w$ and bias parameters $b$) of the neural network models for institutions. Institutions update their models by replacing model parameters with the decrypted aggregated model parameters.

**Model parameters of scPrivacy.** The neural network model was implemented with PyTorch. We use Adam optimizer to update parameters of the network via backpropagation. The learning rate was set to 0.0005. The number of training epochs was set to 300. The L2 regularization rate was set to 0.05.

**Query cells assignment.** First, the query dataset was scaled to 10,000 and normalized with \log(counts+1). The column of the gene will be filled with zeros if the gene is not in the gene union of the institution datasets. Next, the query cells were transformed by the federated model to the same embedding space as that of the transformed institution datasets. Then, the transformed query dataset was assigned to proper cell types by comparing with cell type landmarks of transformed institution datasets. Specifically, for each transformed institution dataset, scPrivacy carried out a cell search by measuring similarity between transformed query cells and cell type landmarks of the transformed institution datasets. Pearson correlation coefficient was adopted to calculate similarity as in our previous study (Duan et al., 2020). Finally, the query cells obtained the predicted cell types with the highest similarity among all landmarks of the transformed institution datasets.

**Benchmarking existing tools.** To evaluate the performance of scPrivacy, four classical non-privacy-preserving data-integration methods were compared: Seurat v3 (Stuart et al., 2019), scmap-cluster (Kiselev et al., 2018), SingleR (Aran et al., 2019) and mtSC (Duan et al., 2021). Seurat v3 applied a data-level integration strategy, where multiple datasets are integrated into one dataset directly; scmap-cluster and SingleR applied a decision-level integration method, where the final assignment results are ensemble by individual assignment results; and mtSC applied an algorithm-level integration strategy, where efficient algorithms are designed for model integration, while keeping datasets separately. However, all these methods are needed to access the individual institutional data directly. For a fair comparison purpose, all these methods were allowed to access all institutional datasets directly while scPrivacy integrated multiple institutional datasets in a data privacy-preserving manner. Each dataset among the multiple datasets in a study was treated as the query, and the others were used to simulate multiple institutional datasets. For scmap-cluster, “threshold = 0” was set to not assign query cells to “unassigned”. For SingleR, as the fine-tuning process of SingleR is extremely time consuming, “fine.tune = FALSE” was set. For Seurat v3, all parameters were the defaults. For mtSC, all parameters were the defaults. In all tests, Seurat v3, scmap-cluster and SingleR and were trained and tested with CPU Intel Xeon Platinum 8165 2.3–3.7 GHz. The deep learning based methods mtSC and scPrivacy were trained with GPU 1080Ti and tested with the same CPU as the other methods. To further prove the effectiveness of scPrivacy, we also benchmarked scPrivacy with the decision-level strategy (Figure S2 in Supporting Information).

**Evaluation criteria.** The macro-F1 score was used as evaluation criteria. Despite integration tasks, we calculated
the macro-F1 score for each query dataset just like the calculation for no-integration tasks. First, the precision and recall of each cell type were calculated. Then, the macro-F1 score was calculated as listed below:

$$\text{macro-F1} = \frac{1}{N} \sum_{i=1}^{N} \frac{2 \cdot \text{Precision}_{i} \cdot \text{Recall}_{i}}{\text{Precision}_{i} + \text{Recall}_{i}}$$

in which $N$ denotes the number of cell types in a dataset and Precision and Recall are the precision and recall of the $i$-th cell type in the dataset.

Data availability

The 27 single-cell type identification benchmark datasets and 15 patients datasets were curated from five studies including three tissues: peripheral blood mononuclear cells (PBMCs) (Ding et al., 2019; Mereu et al., 2020; Ren et al., 2021), the brain (Tasic et al., 2016; Tasic et al., 2018) and the pancreas (Baron et al., 2016; Muraro et al., 2016; Segerstolpe et al., 2016; Xin et al., 2016) (Tables S1 and S12 in Supporting Information). The four pancreas datasets (Baron et al., 2016; Muraro et al., 2016; Segerstolpe et al., 2016; Xin et al., 2016) and one of the brain datasets (Tasic et al., 2018) were collected in previous work of scmap (Kiselev et al., 2018) (https://hemberg-lab.github.io/scRNA.seq.datasets), and the other three brain datasets and seven datasets in “PBMC-Ding” (Ding et al., 2019) were curated from the following benchmark study (Abdelaal et al., 2019) (https://doi.org/10.5281/zenodo.3357167). The 12 datasets in “PBMC-Mereu” (Mereu et al., 2020) were collected from GSE133549, and the corresponding RData file can be downloaded in https://www.dropbox.com/s/i8mwmymych8mn8/sce.all_classifiedotechnologies.RData?dl=0. All these datasets were converted into Bioconductor SingleCellExperiment (http://bioconductor.org/packages/SingleCellExperiment) class objects. The 15 datasets of COVID-19 patients were collected from GSE158055 (Table S12 in Supporting Information).

Code availability

scPrivacy is developed as a python package for simulations, which is available at https://github.com/bm2-lab/scPrivacy.

Compliance and ethics The author(s) declare that they have no conflict of interest.

Acknowledgements This work was supported by the National Key Research and Development Program of China (2021YFF1200900, 2021YFF1201200), the National Natural Science Foundation of China (31970638, 61572361), the Shanghai Artificial Intelligence Technology Standard Project (19DZ2200900), the Shanghai Shaguan Scholars Project, WeBank Scholars Project and the Fundamental Research Funds for the Central Universities.

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