BatchEval Pipeline: Batch Effect Evaluation Workflow for Multiple Datasets Joint Analysis

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ABSTRACT

As genomic sequencing technology continues to advance, it becomes increasingly important to perform multiple dataset joint analysis of transcriptomics to understand complex biological systems. However, batch effect presents challenges for dataset integration, such as sequencing measured by different platforms and datasets collected at different times. Here, we develop a BatchEval Pipeline, which is used to evaluate batch effect of dataset integration and output a comprehensive report. This report consists of a series of HTML pages for the assessment findings, including a main page, a raw dataset evaluation page and several built-in methods evaluation pages. The main page exhibits basic information of integrated datasets, comprehensive score of batch effect and the most recommended method for batch effect removal to current datasets. The residual pages exhibit the evaluation details of raw dataset and evaluation results of many built-in batch effect removal methods after removing batch effect. This comprehensive report enables researchers to accurately identify and remove batch effects, resulting in more reliable and meaningful biological insights from integrated datasets. In summary, BatchEval Pipeline represents a significant advancement in batch effect evaluation and is a valuable tool to improve the accuracy and reliability of the experimental results.

Availability & Implementation: The source code of BatchEval is available at https://github.com/STOmics/BatchEval.

INTRODUCTION

Advancements in gene sequencing technology have facilitated the integrated analysis of multiple batches of gene transcription data, resulting in more reliable information extracted from datasets. However, batch effects caused by various sequencing platforms, experimental designs, experimentalists, laboratory circumstances, and experimental reagent batches are often neglected during joint analysis. The batch effect introduces technical biases into sequencing to reduce the dependability of downstream analysis, which must be addressed. Several efficient approaches have been proposed to minimize or lessen technical biases and batch effect in integrated datasets, while retaining the most significant biological variance. These approaches include non-linear models, such as Seurat's classic correlation analysis [1], linear regression models, such as Combat [2], and implementations based on matching mutual nearest neighbors, such as MNNs [3]. In some differential gene expression analysis models, such as MSAT [4], DESeq [5] and Limma [6], batch effect is integrated into the model as a significant component to eliminate it from the dataset. More recently, works have been published to improve the integration of spatially resolved transcriptomics by exploiting spatial information, such as spatiAlign [7] and PRECAST [8].

Visualization tools are useful for initially assessing dataset integration and quickly comparing results after removing batch effect. Density distribution visualizations show differences in gene expression counts between different tissue sections, and overlays of probability density functions compare variations across sections. Quantitative statistics, such as the chi-square test [9] and the local inverse Simpson index (*LISI*) [10], precisely quantifies dataset integration. When integrating multiple different tissue sections, it may not be clear whether batch effect exists or which removal method should be used, thus requiring detailed analysis. Even though user-friendly software like BatchQC has been published [11], there is still a shortage of tools suitable for analyzing large-scale dataset integration.

To address these issues, we develop the BatchEval Pipeline[1], a comprehensive evaluation batch effect workflow for large-scale dataset integration and output a summary evaluation report. It evaluates dataset integration from different perspectives related to data mixing and biological

[1] https://github.com/STOmics/BatchEval.git

variance preservation after batch effect removal. The report consists of a series of HTML pages, including a main page, a raw integration dataset evaluation page and a series of pages of evaluation results for each built-in batch effect removal method. The main page exhibits the details of integration datasets, comprehensive score of evaluation batch effect for raw datasets, and summaries batch effect removal results using different built-in methods (including single-cell-based methods, such as Harmony [10] and BBKNN [12], and spatially resolved transcriptomics method, such as spatiAlign [7]), and the most recommended method for testing datasets to remove batch effect. Users can conveniently access the detailed report of raw integrated datasets and removal batch effect method through super link in the main page. The raw dataset page consists of statistical evaluation, biological variance preservation metric score and visualization. And the batch effect removal method pages also consist of biological variance preservation metric score and visualization. All of the detailed pages can conveniently go back to the main page through the top left button. BatchEval Pipeline workflow is shown as in Figure 1. The main contributions of this study are as follows:

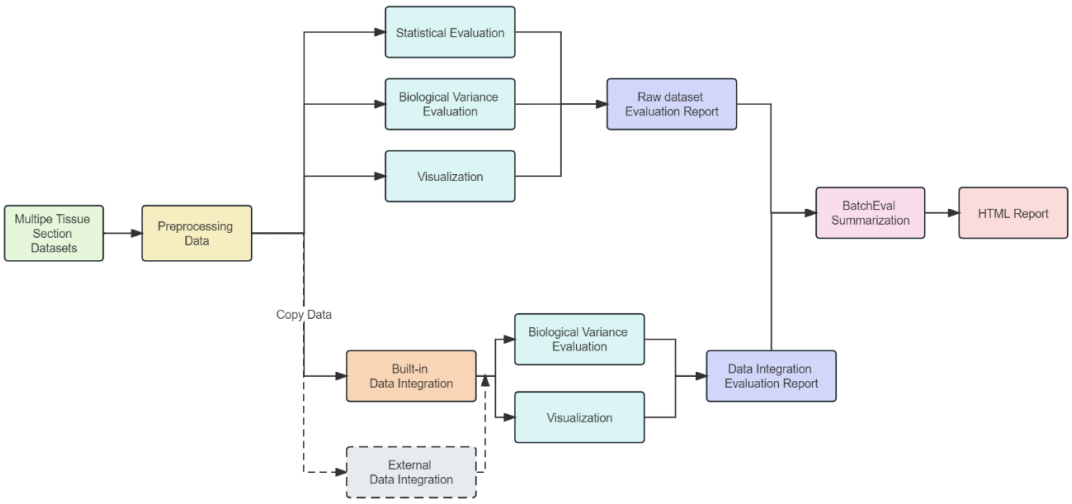

Figure 1. BatchEval Pipeline workflow

1. The BatchEval Pipeline carefully analyzes batch effect from integrated datasets, considering various factors related to data mixing and biological variance preservation between dataset integration before and after.

2. The BatchEval Pipeline can provide extensible remove batch effect benchmarking and output an evaluation report, which includes single-cell-based and spatially resolved transcriptomics methods. BatchEval Pipeline can also recommend suitable batch effect removal

methods according to different datasets.

3. The evaluation results of the BatchEval Pipeline are crucial in determining whether and how to correct batch effects in datasets.

RESULTS

Dataset

We collected two groups of spatially resolved transcriptomics datasets to evaluate BatchEval Pipeline. (1) Two mouse brain olfactory bulb (OB) datasets, which measured by Stereo-seq [13] and 10x Genomics Visium [14], respectively. Both OB datasets contained five identical cell type annotations. The Stereo-seq dataset included 1123 spots, each of which had an average of 6028 genes and an average gene expression of 16681. In contrast, the 10x Genomics Visium dataset included 1184 spots, each of which had an average of 4580 genes and an average gene expression of 16916. (2) Five time-series mouse embryonic brain datasets, which were measured by Stereo-seq. These brain sections were collected at different embryonic tissues from E9.5 to E15.5, which included a total of 81181 spots/cells and 22864 genes in the merged dataset.

Statistical analysis with BatchEval Pipeline

Batch effect and sequencing outcomes are typically complex and influenced by various factors, such as experimental conditions, operators, reagents, and timing, which are not directly related to the experiment's goal. However, the batch effect can impact the accuracy of downstream results. The dataset includes a variety of batch effects, as well as associations between linear and non-linear processes, which can affect the distribution of gene expression probability density for each spot across subsequent data batches. Additionally, different sequencing techniques can result in varying sequencing depths across data batches. To address these issues, BatchEval Pipeline performs Min-Max normalization and logarithmic mapping preprocessing on each spot/cell gene expression levels and integrates multiple batches of gene expression data into low-dimensional representations.

BatchEval Pipeline employs the Kruskal-Wallis H test [15] to evaluate the variation in the average level of gene expression across different tissue sections and performs variance analysis on gene expression total counts for each tissue section. However, when comparing data from different platforms, such as Stereo-seq and 10x Genomics Visium, it is important to note that they may have

considerable differences and not satisfy the homogeneity of variance assumption. Therefore, it may not be clear how gene expression consistency differs between the data from two platforms. To determine if gene expression data from several batches originated from the same distribution, a Kolmogorov-Smirnov Test [16] is performed. By observation, the data from Stereo-seq and 10x Genomics Visium expression do not fit together properly (Table 1).

Table 1. Statistical evaluation results of mouse olfactory bulb dataset before data integration

Variation Analysis	*n_batch*	*n_sample*	*F*	*p* value	*F* ref (2, 3116)
	3	3119	252.6876	0.	2.9986
K-S Test	*n_sample*		*k-s* stat		*p* value
batch0-1	1935		0.6924		0.
batch0-2	1996		0.6115		0.
batch1-2	2307		0.1579		0.
Cramer's Test	Pearson Correlation Coefficient		Cramer's V Coeffient		
	0.9270		0.8190		

The BatchEval Pipeline utilizes a contingency table to analyze the correlation between experimental conditions and dataset batches. For example, Cramer's V correlation coefficient [17] in Table 1 is calculated based on it. By assessing the correlation between different experimental conditions for each batch, the pipeline determines that the experimental conditions in the Stereo-seq and 10x Genomics Visium dataset batches are closely related, with a Cramer's V correlation coefficient of approximately 0.8190 (the statistical evaluation results of mouse embryonic brain datasets are shown Supplementary Table S1).

Biological variance preservation evaluation with BatchEval Pipeline

BatchEval Pipeline adopts a non-linear neural network classifier to estimate data mixing across multiple tissue sections. The model takes cells/spots gene expression matrices as inputs and predicts which tissue sections for each cell/spot come from. If the prediction accuracy is low, it means that the integrated dataset is mixed well, otherwise, the integrated dataset is mixed poor. As shown in Table 2, the batch/domain estimate score accept rate of mouse embryonic brain datasets is around 2%, indicating that the classifier can successfully differentiate each spot/cell come from which tissue sections. Therefore, it is concluded that there is a definite batch effect between each time-series mouse embryonic brain dataset.

Exploratory transcriptome downstream analysis, especially low-dimensional embedding techniques such as principal component analysis (PCA) and clustering, are commonly used in

batch effect evaluation methods. These methods are used to assess the efficiency of dataset integration, which can be divided into two parts: retaining biological variances and testing the validity of dataset integration/data mixing. BatchEval Pipeline employs k-BET test [9] to evaluate the mixing level between different tissue sections in the neighborhood surrounding each spot/cell. This method first projects integrated datasets into a low-dimensional embedding by PCA or Uniform Manifold Approximation and Projection (UMAP) [18], then applies Pearson's Chi-square test. If the neighborhood of each spot/cell is from various tissue sections, the relevant data mixing level is more acceptable, indicating that the data mixing well. In the low-dimensional embedding, the dataset can be clearly distinguished, and the batch effect is evident. As shown in Table 2, the distributions of time-series mouse embryonic brain datasets differ significantly, and the k-BET score accept rate is approximately 0.0085 ($p = 0.0027 < 0.05$).

Table 2. Biological variance evaluation results of mouse embryonic brain datasets before data integration

Batch/Domain Estimate score	*n_batch*	*n_sample*	train size	accept rate
	5	81181	56826	0.0194
k-BET score	chi mean	95% *p* value	accept rate	reject rate
	58.0453	0.0027	0.0085	0.9915
Local inverse Simpson's index		*iLISI*	*cLISI*	F1 score
		0.0154	0.0386	0.0303
Silhouette score		*iSS*	*cSS*	F1 score
		0.6675	0.6155	0.4318

The BatchEval Pipeline utilizes the *LISI* [10] and silhouette coefficient (*SS*) to estimate the data mixing and biological variance preservation while remove batch effect, presuming that cell type labels are accessible for the data. After integrating and projecting the data into a low-dimensional embedding, the Pipeline computes the *LISI* and *SS* using two different groupings, respectively: (1) grouping using different tissue sections as the batch *LISI* (*iLISI*)/batch *SS* (*iSS*) score, and (2) grouping known cell types or clustering types as the cell/domain-type *LISI* score (*cLISI*) or cell/domain-type SS score (*cSS*). And then using F1 score to summarize the *LISI* and SS score, respectively. A larger F1 score of *LISI*/*SS* suggests better dataset integration that preservers the biological variations between domain cluster types while removing batch effect across multiple tissues. As shown in Table 2, the F1 scores of *LISI* and *SS* are not well, indicating that the raw datasets of time-series mouse embryonic brain datasets are mixed worse, and it requires further batch effect removal processing (the statistical evaluation results of mouse olfactory bulb

dataset are shown Supplementary Table S2).

Visualization of BatchEval Pipeline

In Figure 2, we exhibit the statistical analysis results of mouse embryonic brain datasets before data integration. The kernel distribution curve of gene expression total counts (Figure. 2a) and cumulative density curve (CDF) (Figure. 2b) are significant differences. And if the maximum difference value between CDF curve is lagrer, the more significant of batch effect. The total counts of gene expression for each spot/cell also have differences (Figure. 2c). Moreover, we evaluate the mean and variance of each gene (Figure. 2d), and we observed that “batch 4” genes are more discrete than in other tissues.

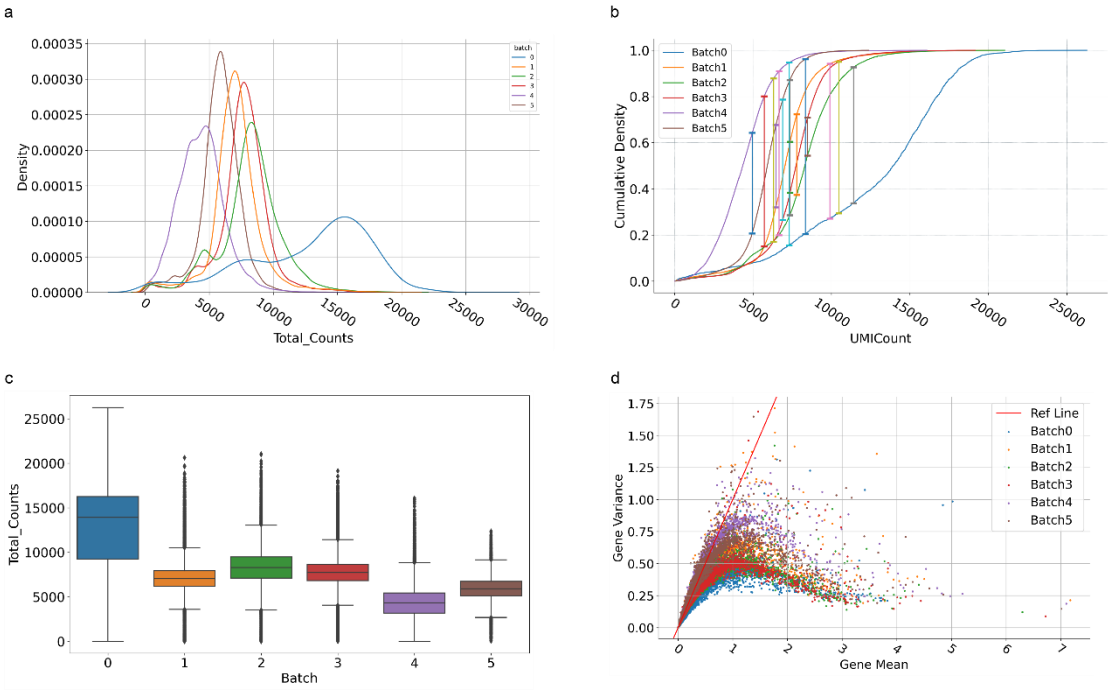

Figure 2. Visualization of statistical analysis for time-series mouse embryonic brain datasets. (a) Kernel distribution curve of total gene expression counts for each tissue section, different color represents different tissues. (b) Cumulative density curve of total gene expression counts for each tissue section. (c) Boxplot of total gene expression counts. (d) The scatter plot of mean gene and gene variance.

Furthermore, BatchEval Pipeline employs PCA to reduce the dimension of merged dataset. The scatter plots and edge probability density (PDF) curves are used to illustrate the variations in distribution across multiple data batches in PCA dimensions. As shown in Figure 3a, the first two PCA dimensions are used as horizontal and vertical coordinates, and a PDF curve is fitted to capture the variations in the distribution of gene expression in different batches. The BatchEval

Pipeline can quickly display the level of data mixing across multiple batches using UMAP and various color displays based on batch metadata. We utilized the integrated dataset in UMAP space and colored by “batch”. As shown in Figures 3b, c and d, we observed that spatiAlign can efficiently mix time-series mouse embryonic brain datasets, however, other methods cannot mix datasets as well as spatiAlign.

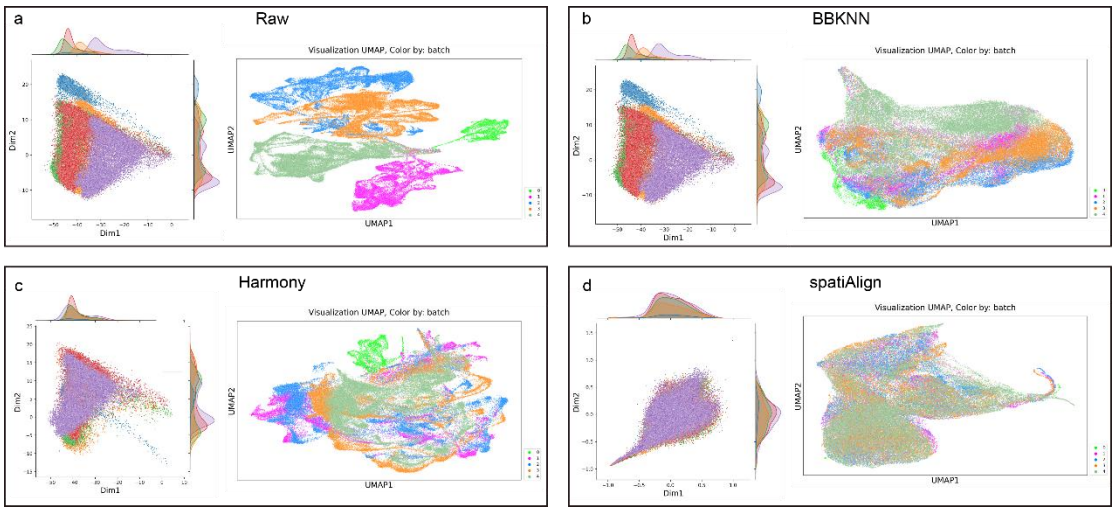

Figure 3. Visualization of integration for raw datasets and built-in batch effect removal methods. (a) Visualization of raw dataset. (b) Visualization of dataset integrated by BBKNN. (c) Visualization of dataset integrated by Harmony. (d) Visualization of dataset integrated by spatiAlign. All sub-figures left, joint plot of PCA, which using first two PC component to plot, and right, UMAP plot, colored by different data tissue sections.

Since the distribution of gene expression levels in different batches is usually unknown, we also employed quantile-quantile plots (Q-Q plot) to evaluate data distribution, which can be used to assess whether data from different batches follow the same distribution [19]. Data from multiple batches were coupled with each other to generate Q-Q maps for gene expression. When the gene expression of the matched data was less different, the cumulative density distribution of the corresponding gene expression was more tightly spaced.

BatchEval score evaluation

We employ the biological variance preservation score between batch effect removal before and after and data integration mixing score to summaries the batch effect of dataset integration. For example, as shown in Table 3, we utilize mouse embryonic brain datasets for test, and the conclusion is that this dataset should further perform batch effect removal processing and the most recommended method for users is “spatiAlign”. This information is essential for researchers to

understand the reliability of downstream analyses and to make informed decisions about the suitability of the dataset for their research goals (the summarizes evaluation results of mouse olfactory bulb are shown Supplementary Table S3).

Table 3. The summary evaluation of time-series mouse embryonic brain datasets

	Raw	spatiAlign	Harmony	BBKNN
k-BET score	0.0085	0.7419	0.1055	0.1598
95% *p* value	0.0027	0.3183	0.0266	0.0478
BatchEval score	0.1605	0.6315	0.5064	0.3086
Conclusion	This dataset has batch effect and requires further processing and recommend “spatiAlign”. More details of “spatiAlign” can be found in 'https://github.com/STOmics/Spatialign.git'.			

METHOD

The gene expression profiles are stored in a matrix with rows representing cells/spots and columns representing genes in the dataset. To ensure consistent and reliable results in subsequent analyses, the datasets are preprocessed using Min-Max normalization and log mapping, which standardizes the values to the same range and transforms them to a logarithmic scale. The preprocessing can be calculated using the follow parameterization:

$$\hat{x} = \log 1p\left(\frac{x - x_{\min}}{x_{\max} - x_{\min}}\right) \tag{1}$$

where x is the original expression of each spot/cell, x_{min} and x_{max} are the minimum and maximum expressions captured in dataset, respectively.

Statistical test

The Kruskal-Wallis H test [15] mixes the gene expression of each spot in different batches, sorts them from the smallest to largest, and records the ordinal number (rank). If the sorted spot gene expression values are the same, the corresponding rank is the same. Then, rank sum of each value is calculated with the Student’s T test. The value of H indicates the distribution of rank in k batches. The larger the value of H, the greater the difference in rank is. The calculation formula is shown as follows:

$$H = \frac{12}{N\ \ N+1}\sum_{i=1}^{k}\frac{R_i^2}{n_i} - 3\ \ N+1 \tag{2}$$

where N denotes the number of all spots in different batches, k denotes the number of data

batches, n_i denotes the number of spots in batch i, and R_i^2 denotes the square of the rank sum of each spot in batch i.

Kolmogorov-Smirnov Test [16] can be used to test whether the cumulative density distributions of two datasets are different:

$$stat = \sup_x \left| F_1(x) - F_2(x) \right| \tag{3}$$

where x denotes the gene expression of spot and $F(\cdot)$ denotes cumulative density function.

Cramer's V correlation coefficient [17] can be used to determine correlation between several experimental conditions in each dataset. Generating a cross-tabulation between each experimental condition and batch is the first step. By default, the BatchEval Pipeline generates the cross-tabulation using several experimental conditions for every batch. The calculation formula is as follows:

$$\phi_c = \sqrt{\frac{\chi^2}{N(k-1)}} \tag{4}$$

where χ^2 denotes Pearson chi-square statistic, N is the total number of samples tested in the cross-tabulation table, and k number of categories of smaller variables in cross-tabulation table. Cramer's V correlation coefficient reflects the magnitude of correlation between data from different batches under different experimental conditions.

Neural network models can fit complex relationships between datasets. BatchEval Pipeline constructs domain consistency assessment neural network models for estimating the magnitude of variation in different batches of datasets:

$$\hat{x} = relu\left(\sum_k w_k x_k + b\right) \tag{5}$$

where $relu = \max(0, x)$, x denotes the gene vector of each spot, w denotes the model weight, and b denotes the model offset. The number of spots included in each batch will be inconsistent, and BatchEval Pipeline employs Focal Loss [20] to measure the difference between model prediction results and labels, and the stochastic gradient descent method to update model parameters. The Focal Loss is calculated as follows:

$$Focal_Loss = -\alpha (1 - P_t)^{\gamma} \log(P_t) \tag{6}$$

where, α, γ are hyperparameters and P_t is predicted probability value of model output. The more accurate results predicted by the model, the higher level of differentiability of the data domain in different batches and more obvious the batch effect is.

Metric score

The k-BET test [9] is based on the Pearson chi-square test to test the level of mixing of different batches of data within a local area and is calculated as follows:

$$\kappa_j^k = \sum_{i=1}^{l} \frac{n_{ji}^k - f_i \times k}{f_i \times k}^2 \sim \chi_{l-1}^2 \tag{7}$$

where χ_{l-1}^2 denotes the χ^2 distribution with degree of freedom $l-1$ and n_{ji}^k denotes the number of cells in subset j of size k in batch i. f denotes the probability distribution of batch i samples in the overall population.

To simultaneously evaluate the separation of each cell/batch cluster and mixing of multiple datasets, we calculate the average local inverse Simpson's index (*LISI*) [10] score of the datasets using two different groupings: (1) grouping using different datasets as the batch *LISI* score (*iLISI*), and (2) grouping known cell types or clustering types as the cell/domain-type *LISI* score (*cLISI*). In dataset integration, a larger value of *iLISI* indicates better mixing of datasets, and smaller *cLISI* indicates better preservation of the biological variance between cell/domain-types. These two metrics can be summarized using the F1 score as follows:

$$\text{F1 score}_{LISI} = \frac{2 \times (1 - cLISI) \times iLISI}{(1 - cLISI) + iLISI} \tag{8}$$

where $cLISI = MinMaxNorm(1/\sum_{b=1}^{B} p^2(b))$, $iLISI = MinMaxNorm(1/\sum_{c=1}^{C} p^2(c))$, B and C are the cell/domain-type or batch marker, $p(b)$ and $p(c)$ are the probability valus that the cell/domain-type or batch in the local area, respectively.

Furthermore, we also implement silhouette coefficient (*SS*) to evaluate the separation of each domain cluster and mixing multiple datasets. Similar to F1 score of *LISI*, we calculate the average *SS* of the datasets using two grouping label, domain cluster type (*cSS*), different dataset type (*iSS*), respectively. These two metrics also can be summarized using F1 score as follows,

$$\text{F1 score}_{SS} = \frac{2 \times (1 - iSS') \times cSS'}{(1 - iSS') + cSS'} \tag{9}$$

where, $iSS' = 1 + iSS\ /2$ and $cSS' = 1 + cSS\ /2$. A larger F1 score of SS suggests better dataset integration that preservers the biological variations between domain cluster types while removing batch effect across multiple tissues.

BatchEval score

Batch effect is confounded in the data, and BatchEval Pipeline provides a comprehensive assessment of the batch effect of aggregated data from different dimensions. BatchEval Pipeline is designed to calculate the final batch effect score by weighting the mean value with the formula as follows.

$$BatchEval\ score = Mean(\text{F1 score}_{LISI}, \text{F1 score}_{SS}, (1 - DomainAcc)) \tag{10}$$

where $DomainAcc$ is the accuracy of neural network domain classifier.

DISCUSSION

Although many approaches have been developed to remove batch effect, there is still a lack of effective methods to evaluate batch effect for large-scale dataset integration, especially spatially resolved transcriptomics. The sources and effects of batch effect can vary greatly from experiment to another, and it is essential to analyze the most common potential sources of batch effect to improve the effectiveness of their removal and to facilitate the integration of data from different batches. Additionally, there is a need for a more efficient way to accurately determine the extent to which data is affected by batch effect, as well as to remove batch effect more accurately.

The BatchEval Pipeline simplifies batch pre-testing by offering a comprehensive evaluation report for data integration, making it particularly beneficial for multi-omics studies with multiple datasets or samples collected at different times. It provides statistical testing, batch effect metrics evaluation, and visualization, allowing researchers to efficiently explore and correct for batch effect in their data.

Although substantial progress has been made in identifying and evaluating batch effect, there is still much for improvement to enhance the accuracy and effectiveness of batch effect removal. BatchEval Pipeline is a powerful tool for evaluation of integrated large-scale gene expression datasets. It provides a quantitative measure of biological variance preservation and data

integration mixing, and the conclusion indicates whether the batch effect is significant or not. Using the BatchEval Pipeline, users can objectively evaluate the presence and severity of batch effects in their integrated datasets. This feature makes the tool particularly valuable for researchers, who need to analyze large datasets, as it provides an easy and reliable way to assess data quality and ensures that downstream analyses are robust and reliable.

AVAILABILITY OF SOURCE CODE AND REQUIREMENTS

Project name: BatchEval

Project home page: https://github.com/STOmics/BatchEval

Operating system(s): Platform independent

Programming language: Python

Tutorials: https://batcheval.readthedocs.io/en/latest/index.html

License: MIT License

DATA AVAILABILITY

The mouse olfactory bulb and embryonic brain datasets measured by Stereo-seq can be download from: https://db.cngb.org/stomics/mosta, and the 10x Genomics Visium datasets can be download from: https://www.10xgenomics.com/resources/datasets/adult-mouse-olfactory-bulb-1-standard.

DECLARATIONS

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare they have no competing interests.

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Authors' contributions

Conceptualization: CZ; Project administration and supervision: XX; Software: CZ; Data collection, processing, and application: ZC and HX; Project coordination: SF; Manuscript writing and figure generation: ZC and QK; Manuscript review: QK and ML.

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SUPPLEMENTARY MATERIAL

Supplementary Table S1. Statistical evaluation results of mouse embryonic brain datasets before data integration

Variation Analysis	*n_batch*	*n_sample*	*F*	*p* value	*F* ref (2, 3116)
	5	81181	20433.7507	0.	2.3720
K-S Test	*n_sample*		*k-s* stat		*p* value
batch0-1	16673		0.6706		0.
batch0-2	20986		0.5892		0.
batch0-3	24791		0.6569		0.
batch0-4	28064		0.7920		0.
batch1-2	31437		0.3494		0.
batch1-3	35242		0.2209		0.
batch1-4	38515		0.6507		0.
batch2-3	39555		0.1641		0.
batch2-4	42828		0.7101		0.
batch3-4	46633		0.7073		0.
Cramer's Test	Pearson Correlation Coefficient		Cramer's V Coeffient		
	0.9775		0.9005		

Supplementary Table S2. Biological variance evaluation results of mouse olfactory bulb dataset before data integration

Batch/Domain Estimate score	*n_batch*	*n_sample*	train size	accept rate
	3	3119	2183	0
k-BET score	chi mean	95% *p* value	accept rate	reject rate
	29.8851	0	0	1
Local inverse Simpson's index		*iLISI*	*cLISI*	F1 score
		0.0034	0.0888	0.0067
Silhouette score		*iSS*	*cSS*	F1 score
		0.7842	0.4911	0.2999

Supplementary Table S3. The summary evaluation of mouse olfactory dataset

	Raw	spatiAlign	Harmony	BBKNN
k-BET score	0	0.6916	0.0901	0.1828
95% *p* value	0	0.2870	0.0228	0.0546
BatchEval score	0.1022	0.7035	0.5256	0.3305
Conclusion	This dataset has batch effect and requires further processing and recommend "spatiAlign". More details of 'spatiAlign' can be found in 'https://github.com/STOmics/Spatialign.git'.			

REFERENCES

1. Hao Y, Hao S, Andersen-Nissen E *et al*: **Integrated analysis of multimodal single-cell**

data. *Cell* 2021, **184**(13):3573-3587. e3529.

2. Johnson WE, Li C, Rabinovic A: **Adjusting batch effects in microarray expression data using empirical Bayes methods**. *Biostatistics* 2007, **8**(1):118-127.

3. Haghverdi L, Lun AT, Morgan MD *et al*: **Batch effects in single-cell RNA-sequencing data are corrected by matching mutual nearest neighbors**. *Nature biotechnology* 2018, **36**(5):421-427.

4. Finak G, McDavid A, Yajima M *et al*: **MAST: a flexible statistical framework for assessing transcriptional changes and characterizing heterogeneity in single-cell RNA sequencing data**. *Genome biology* 2015, **16**(1):1-13.

5. Love MI, Huber W, Anders S: **Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2**. *Genome biology* 2014, **15**(12):1-21.

6. Ritchie ME, Phipson B, Wu D *et al*: **limma powers differential expression analyses for RNA-sequencing and microarray studies**. *Nucleic acids research* 2015, **43**(7):e47-e47.

7. Zhang C, Liu L, Zhang Y *et al*: **spatiAlign: An Unsupervised Contrastive Learning Model for Data Integration of Spatially Resolved Transcriptomics**. *bioRxiv* 2023.

8. Liu W, Liao X, Luo Z *et al*: **Probabilistic embedding, clustering, and alignment for integrating spatial transcriptomics data with PRECAST**. *Nature Communications* 2023, **14**(1):296.

9. Büttner M, Miao Z, Wolf FA *et al*: **A test metric for assessing single-cell RNA-seq batch correction**. *Nature methods* 2019, **16**(1):43-49.

10. Korsunsky I, Millard N, Fan J *et al*: **Fast, sensitive and accurate integration of single-cell data with Harmony**. *Nature methods* 2019, **16**(12):1289-1296.

11. Manimaran S, Selby HM, Okrah K *et al*: **BatchQC: interactive software for evaluating sample and batch effects in genomic data**. *Bioinformatics* 2016, **32**(24):3836-3838.

12. Polański K, Young MD, Miao Z *et al*: **BBKNN: fast batch alignment of single cell transcriptomes**. *Bioinformatics* 2020, **36**(3):964-965.

13. Chen A, Liao S, Cheng M *et al*: **Spatiotemporal transcriptomic atlas of mouse organogenesis using DNA nanoball-patterned arrays**. *Cell* 2022, **185**(10):1777-1792 e1721.

14. Stahl PL, Salmen F, Vickovic S *et al*: **Visualization and analysis of gene expression in**

tissue sections by spatial transcriptomics. *Science* 2016, **353**(6294):78-82.

15. MacFarland TW, Yates JM, MacFarland TW *et al*: **Kruskal–Wallis H-test for oneway analysis of variance (ANOVA) by ranks**. *Introduction to nonparametric statistics for the biological sciences using R* 2016:177-211.

16. Massey Jr FJ: **The Kolmogorov-Smirnov test for goodness of fit**. *Journal of the American statistical Association* 1951, **46**(253):68-78.

17. Cramér H: **Mathematical methods of statistics**, vol. 26: Princeton university press; 1999.

18. McInnes L, Healy J, Melville J: **Umap: Uniform manifold approximation and projection for dimension reduction**. *arXiv preprint arXiv:180203426* 2018.

19. Tsai D-M, Yang C-H: **A quantile–quantile plot based pattern matching for defect detection**. *Pattern Recognition Letters* 2005, **26**(13):1948-1962.

20. Lin T-Y, Goyal P, Girshick R *et al*: **Focal loss for dense object detection**. In: *Proceedings of the IEEE international conference on computer vision: 2017*; 2017: 2980-2988.