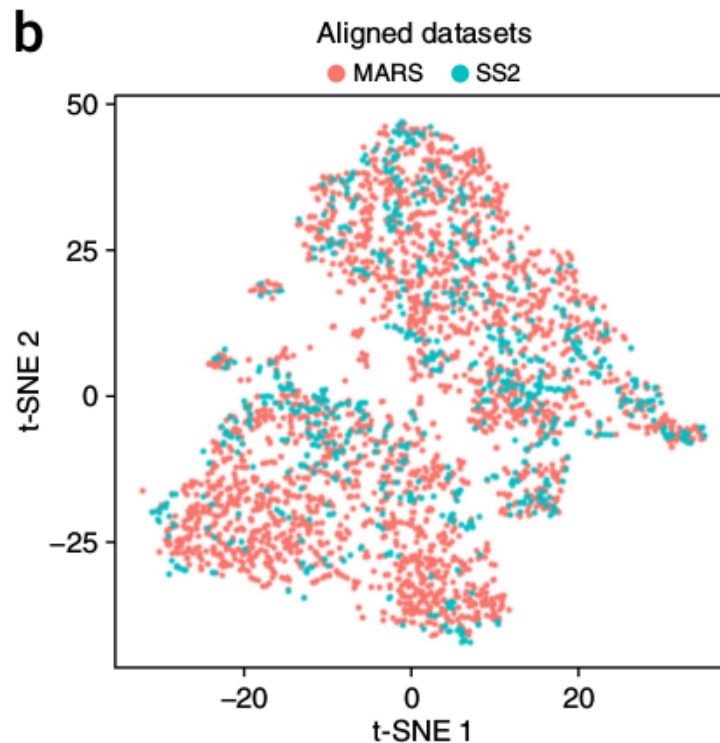
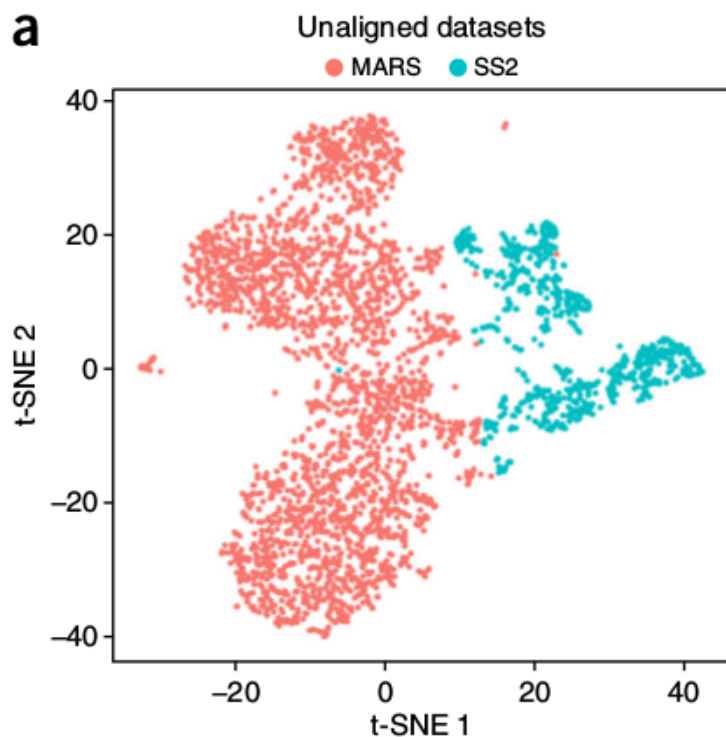


Integration and Batch Correction for Single Cell

Nikolay Oskolkov, Lund University, NBIS SciLifeLab, Sweden
scRNAseq course, 01.04.2025



@NikolayOskolkov

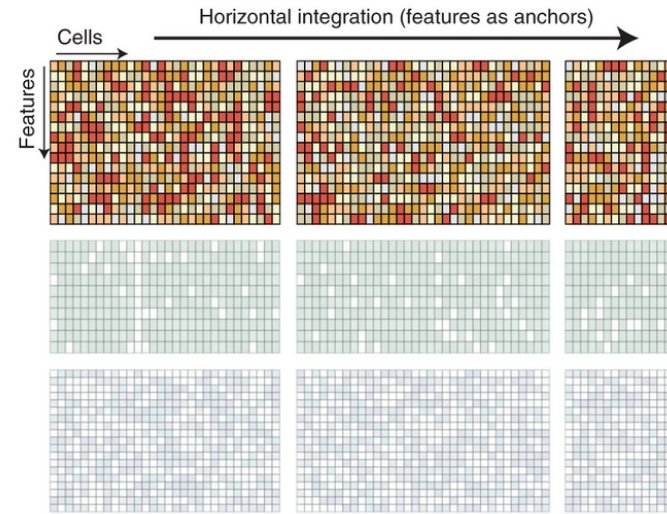
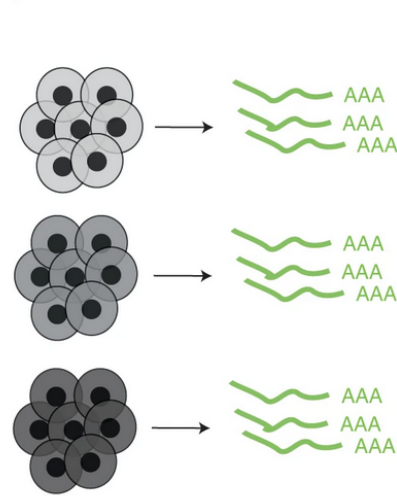


@oskolkov.bsky.social



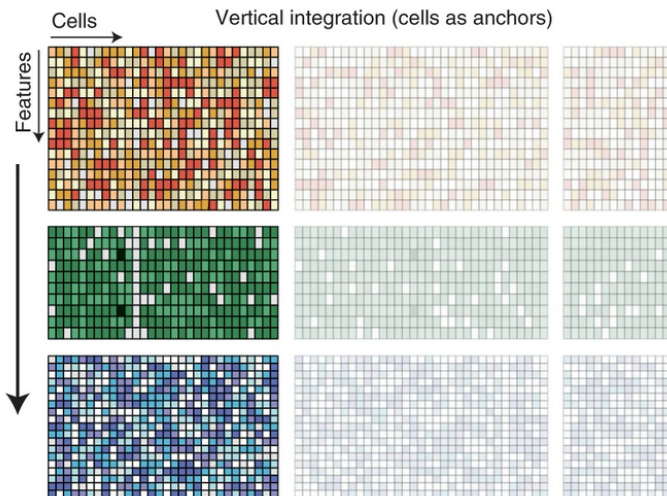
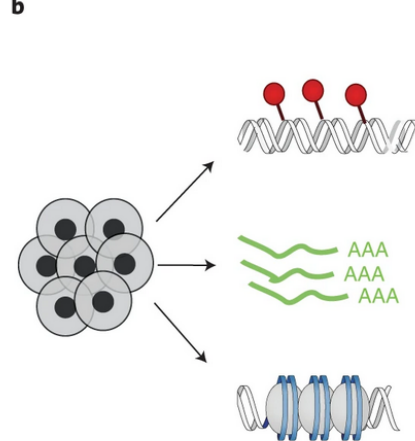
Personal homepage:
<https://nikolay-oskolkov.com>

a



Similar to batch correction problem

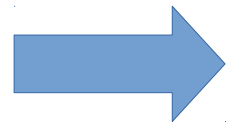
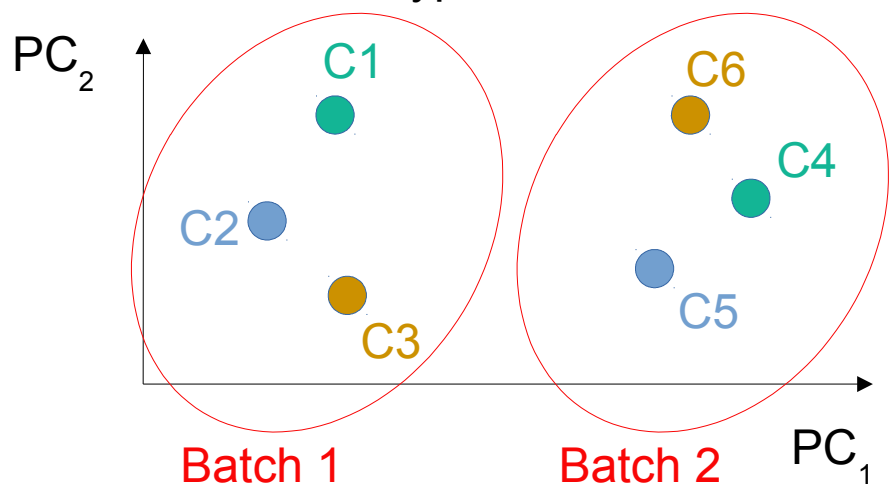
b



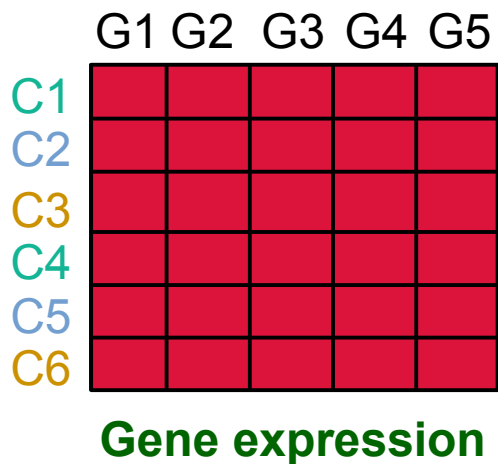
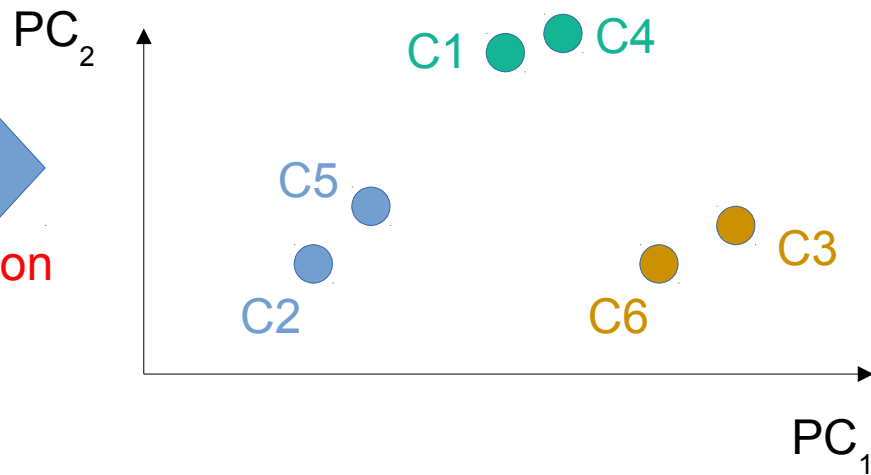
Conceptually difficult problem

Integration across cells (horizontal integration)

3 cell types, 2 batches



integration



Get rid of this variable

$$= \beta_1 * \begin{bmatrix} 1 \\ 1 \\ 1 \\ 2 \\ 2 \\ 2 \end{bmatrix}$$

Batch variable

Keep this variable

$$+ \beta_2 * \begin{bmatrix} 1 \\ 2 \\ 3 \\ 1 \\ 2 \\ 3 \end{bmatrix}$$

Cell type variable

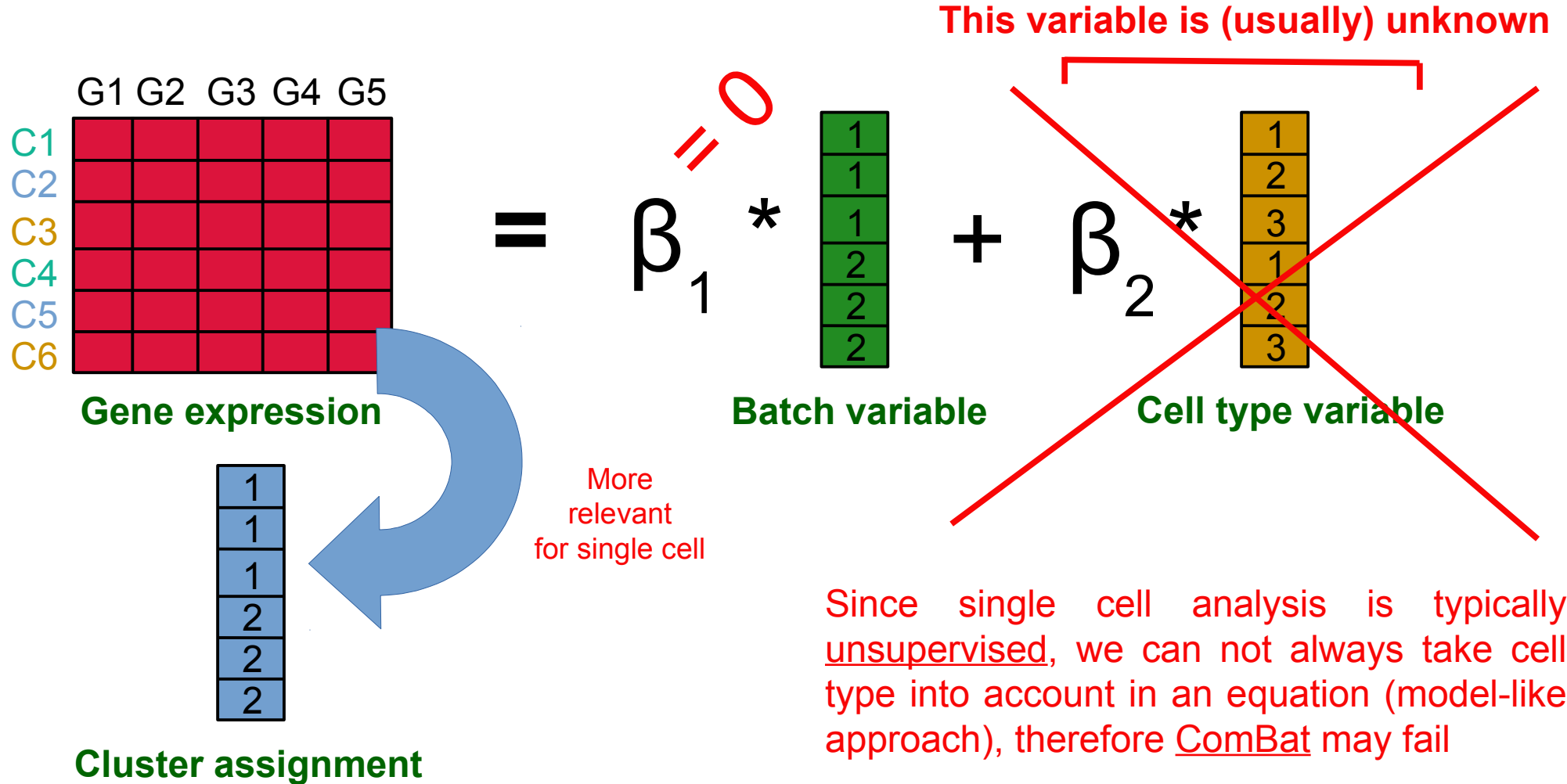
	G1	G2	G3	G4	G5
C1					
C2					
C3					
C4					
C5					
C6					

Gene expression

$$= \beta_1 * \begin{bmatrix} 1 \\ 1 \\ 1 \\ 2 \\ 2 \\ 2 \end{bmatrix} + \beta_2 * \begin{bmatrix} 1 \\ 2 \\ 3 \\ 1 \\ 2 \\ 3 \end{bmatrix}$$

Batch variable Cell type variable

These two variables should be orthogonal



Mapping the Human Body at the Cellular Level

Community generated, multi-omic,
open data processed by standardized pipelines



4.5M
CELLS



33
ORGANS



289
DONORS



28
PROJECTS



81
LABS

Feedback & Support

FIND PROJECTS

Filter projects by attribute e.g. organ, project title.

GO

4.5M Cells

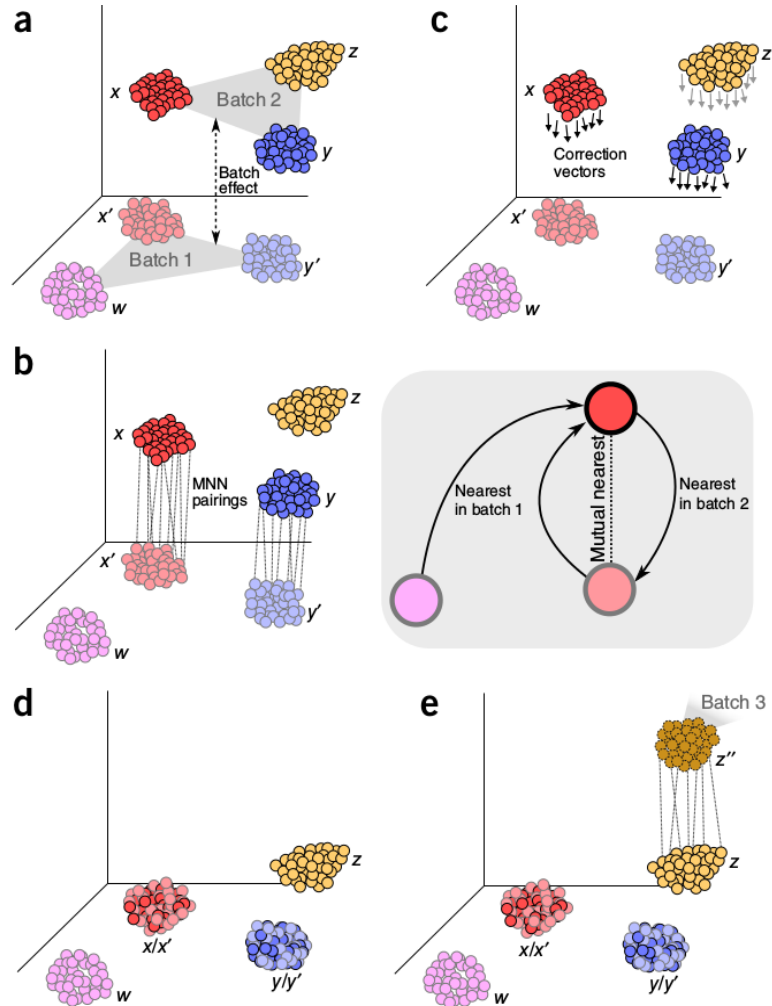
ALL CELLS

Blood

Kidney

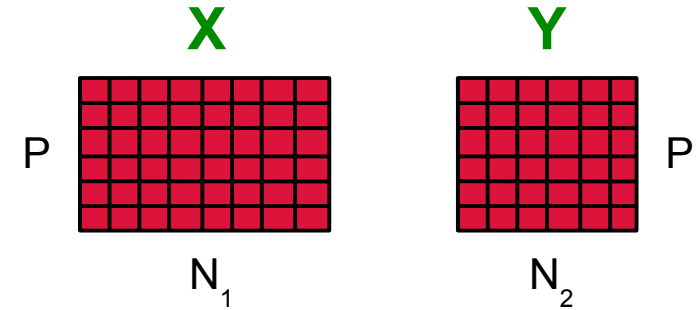
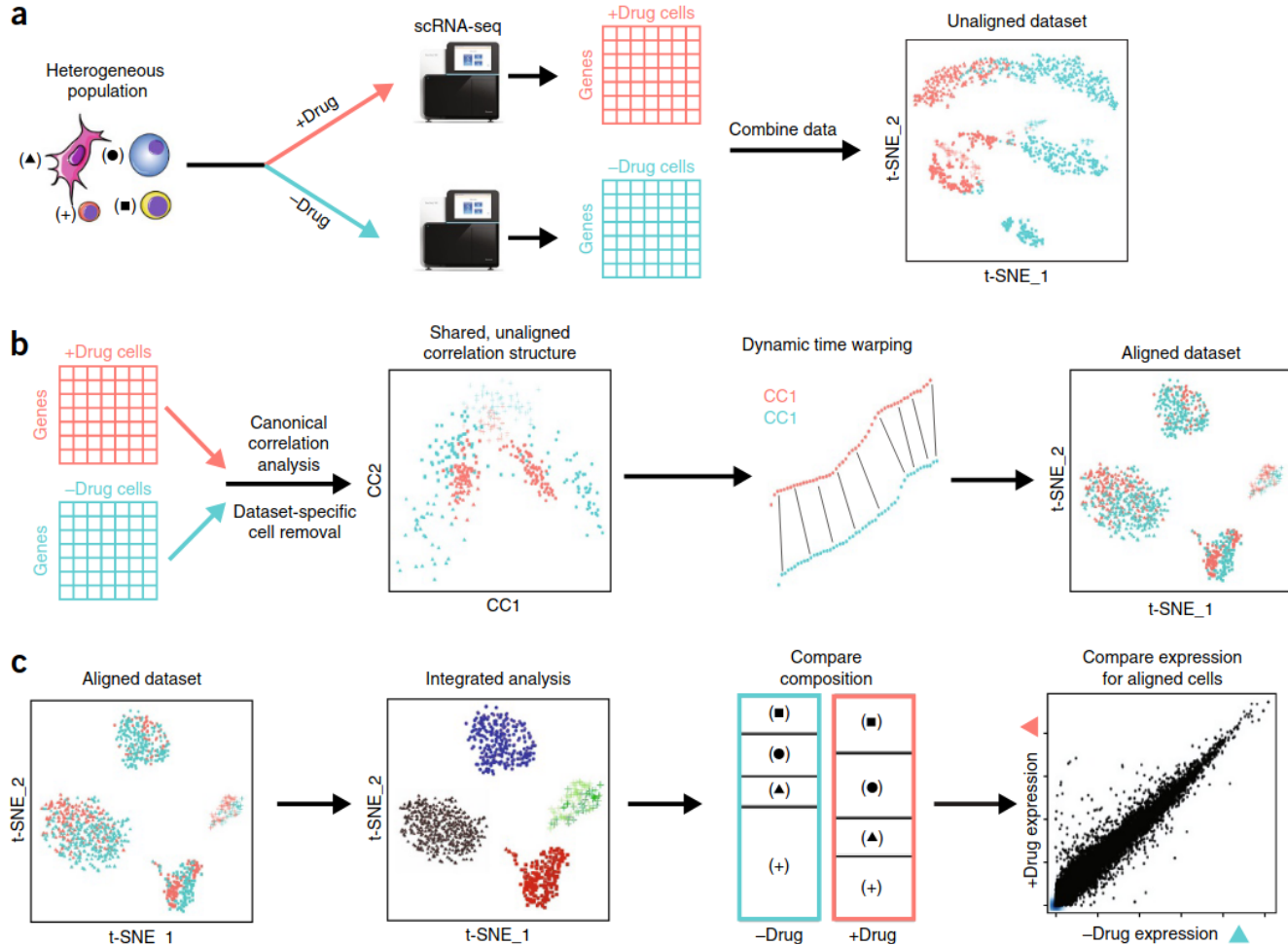


HCA ambition: create a comprehensive Atlas of human cells from all organs / tissues
Data harmonization / integration is one of major challenges of HCA



The next step involves identification of mutual nearest neighbors. Consider an scRNA-seq experiment consisting of two batches 1 and 2. For each cell i_1 in batch 1, we find the k cells in batch 2 with the smallest distances to i_1 , i.e., its k nearest neighbors in batch 2. We do the same for each cell in batch 2 to find its k nearest neighbors in batch 1. If a pair of cells from each batch is contained in each other's set of nearest neighbors, those cells are considered to be mutual nearest neighbors (**Fig. 1**). We interpret these pairs as containing cells that belong to the same cell type or state despite being generated in different batches. Thus, any systematic differences in expression level between cells in MNN pairs should represent the batch effect.

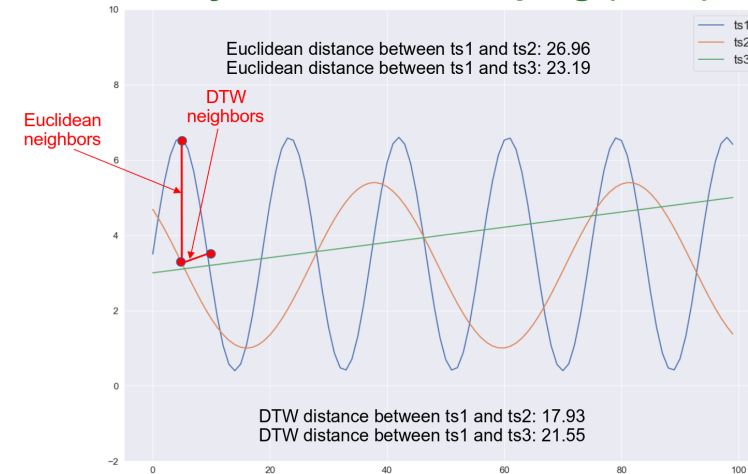
Our use of MNN pairs involves three assumptions: (i) there is at least one cell population that is present in both batches, (ii) the batch effect is almost orthogonal to the biological subspace, and (iii) the batch-effect variation is much smaller than the biological-effect variation between different cell types (more detailed discussion of these assumptions in **Supplementary Note 3**). The biological subspace



$$A = \text{cov}(X, Y) = X * Y^T$$

$$A * u = \lambda * u$$

Dynamic Time Warping (DTW)



Fast, sensitive and accurate integration of single-cell data with Harmony

Ilya Korsunsky^{1,2,3,4}, Nghia Millard^{1,2,3,4}, Jean Fan⁵, Kamil Slowikowski^{1,2,3,4}, Fan Zhang^{1,2,3,4}, Kevin Wei², Yuriy Baglaenko^{1,2,3,4}, Michael Brenner², Po-ru Loh^{1,3,4} and Soumya Raychaudhuri^{1,2,3,4,6*}

The emerging diversity of single-cell RNA-seq datasets allows for the full transcriptional characterization of cell types across a wide variety of biological and clinical conditions. However, it is challenging to analyze them together, particularly when datasets are assayed with different technologies, because biological and technical differences are interspersed. We present Harmony (<https://github.com/immunogenomics/harmony>), an algorithm that projects cells into a shared embedding in which cells group by cell type rather than dataset-specific conditions. Harmony simultaneously accounts for multiple experimental and biological factors. In six analyses, we demonstrate the superior performance of Harmony to previously published algorithms while requiring fewer computational resources. Harmony enables the integration of ~10⁶ cells on a personal computer. We apply Harmony to peripheral blood mononuclear cells from datasets with large experimental differences, five studies of pancreatic islet cells, mouse embryogenesis datasets and the integration of scRNA-seq with spatial transcriptomics data.

Recent technological advances¹ enable unbiased single-cell transcriptional profiling of thousands of cells in one experiment. Projects such as the Human Cell Atlas² (HCA) and Accelerating Medicines Partnership³ exemplify the growing body of reference datasets of primary human tissues. While individual experiments incrementally expand our understanding of cell types, a comprehensive catalog of healthy and diseased cells will require the ability to integrate multiple datasets across donors, studies and technological platforms. Moreover, in translational research, joint analyses across tissues and clinical conditions will be essential to identify disease-expanded populations. Since meaningful biological variation in single-cell RNA-seq datasets from different studies is often confounded by data source⁴, investigators have developed unsupervised multi-dataset integration algorithms^{5–11}. These methods embed cells from diverse experimental conditions and biological contexts into a common reduced dimensional embedding to enable shared cell-type identification across datasets.

Here, we introduce Harmony, an algorithm for robust, scalable and flexible multi-dataset integration to meet four key challenges of unsupervised scRNA-seq joint embedding: scaling to large datasets, identification of both broad populations and fine-grained subpopulations, flexibility to accommodate complex experimental design, and the power to integrate across modalities. We apply Harmony to a diverse range of examples, including cell lines, peripheral blood mononuclear cells (PBMCs) assayed with different technologies, a meta-analysis of pancreatic islet cells from multiple donors and studies, longitudinal samples from mouse embryogenesis, and cross-modality integration of scRNA-seq data with spatial transcriptomics data. Harmony is available as an R package on github (<https://github.com/immunogenomics/harmony>), with functions for standalone and Seurat¹² pipeline analyses.

Results

Harmony iteratively learns a cell-specific linear correction function. Harmony begins with a low-dimensional embedding of cells, such as principal components analysis (PCA) (Supplementary Note 1 and Methods). Using this embedding, Harmony first groups cells into multi-dataset clusters (Fig. 1a). We use soft clustering to assign cells to potentially multiple clusters, to account for smooth transitions between cell states. These clusters serve as surrogate variables, rather than to identify discrete cell types. We developed a new soft k-means clustering algorithm that favors clusters with cells from multiple datasets (Methods). Clusters disproportionately containing cells from a small subset of datasets are penalized by an information theoretic metric. Harmony allows for multiple different penalties to accommodate multiple technical or biological factors, such as different batches and tissue sources. Soft clustering preserves discrete and continuous topologies while avoiding local minima that might result from maximizing representation too quickly across multiple datasets. After clustering, each dataset has cluster-specific centroids (Fig. 1b) that are used to compute cluster-specific linear correction factors (Fig. 1c). Since clusters correspond to cell types and states, cluster-specific correction factors correspond to individual cell-type and cell-state specific correction factors. In this way, Harmony learns a simple linear adjustment function that is sensitive to intrinsic cellular phenotypes. Finally, each cell is assigned a cluster-weighted average of these terms and corrected by its cell-specific linear factor (Fig. 1d). Since each cell may be in multiple clusters, each cell has a potentially unique correction factor. Harmony iterates these four steps until cell cluster assignments are stable.

Quantifying performance in cell-line data. We first assessed Harmony using three carefully controlled datasets, to evaluate

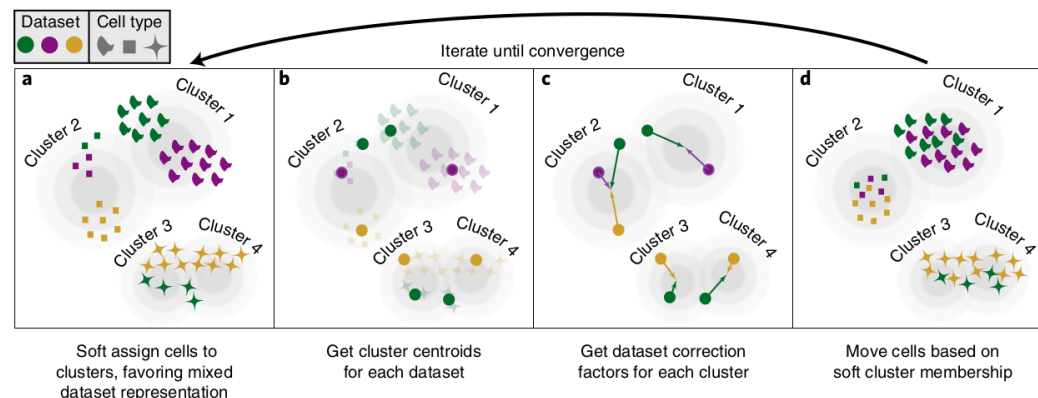


Fig. 1 | Overview of Harmony algorithm. PCA embeds cells into a space with reduced dimensionality. Harmony accepts the cell coordinates in this reduced space and runs an iterative algorithm to adjust for dataset specific effects. **a**, Harmony uses fuzzy clustering to assign each cell to multiple clusters, while a penalty term ensures that the diversity of datasets within each cluster is maximized. **b**, Harmony calculates a global centroid for each cluster, as well as dataset-specific centroids for each cluster. **c**, Within each cluster, Harmony calculates a correction factor for each dataset based on the centroids. **d**, Finally, Harmony corrects each cell with a cell-specific factor: a linear combination of dataset correction factors weighted by the cell's soft cluster assignments made in step **a**. Harmony repeats steps **a** to **d** until convergence. The dependence between cluster assignment and dataset diminishes with each round. Datasets are represented with colors, cell types with different shapes.

Harmony tries to disrupt relation between the cluster assignment and batch variable by penalizing for diversity across batches in its fuzzy k-means algorithm

Objective function for maximum diversity clustering. The full objective function for Harmony's clustering builds on the previous section. In addition to soft assignment regularization, the function below penalizes clusters with low batch-diversity, for all defined batch variables. This penalty, derived in the following section, depends on the cluster and batch identities $\Omega(R, \phi_i) = \sum_{i,k} R_{ki} \log(O_{ki}/E_{ki}) \phi_i$

$$\min_{R,Y} \sum_{i,k} R_{ki} \|Z_i - Y_k\|^2 + \sigma R_{ki} \log R_{ki} + \sigma \theta R_{ki} \log \left(\frac{O_{ki}}{E_{ki}} \right) \phi_i \quad (3)$$

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Efficient integration of heterogeneous single-cell transcriptomes using Scanorama

Brian Hie¹, Bryan Bryson^{2*} and Bonnie Berger^{1,3*}

Integration of single-cell RNA sequencing (scRNA-seq) data from multiple experiments, laboratories and technologies can uncover biological insights, but current methods for scRNA-seq data integration are limited by a requirement for datasets to derive from functionally similar cells. We present Scanorama, an algorithm that identifies and merges the shared cell types among all pairs of datasets and accurately integrates heterogeneous collections of scRNA-seq data. We applied Scanorama to integrate and remove batch effects across 105,476 cells from 26 diverse scRNA-seq experiments representing 9 different technologies. Scanorama is sensitive to subtle temporal changes within the same cell lineage, successfully integrating functionally similar cells across time series data of CD14⁺ monocytes at different stages of differentiation into macrophages. Finally, we show that Scanorama is orders of magnitude faster than existing techniques and can integrate a collection of 1,095,538 cells in just ~9 h.

Individual single-cell RNA sequencing (scRNA-seq) experiments have already been used to discover new cell states and reconstruct cellular differentiation trajectories^{1,2}. Through global efforts such as the Human Cell Atlas³, researchers are now generating large, comprehensive collections of scRNA-seq datasets that profile a diverse range of cellular functions, which promise to enable high-resolution insight into processes underlying fundamental biology and disease. Assembling large, unified reference datasets, however, may be compromised by differences due to experimental batch, sample donor or experimental technology. While recent approaches have shown that it is possible to integrate scRNA-seq studies across multiple experiments^{4,5}, these approaches automatically assume that all datasets share at least one cell type in common⁶ or that the gene expression profiles share largely the same correlation structure across all datasets⁷. These methods are therefore prone to overcorrection, especially when integrating collections of datasets with considerable differences in cellular composition.

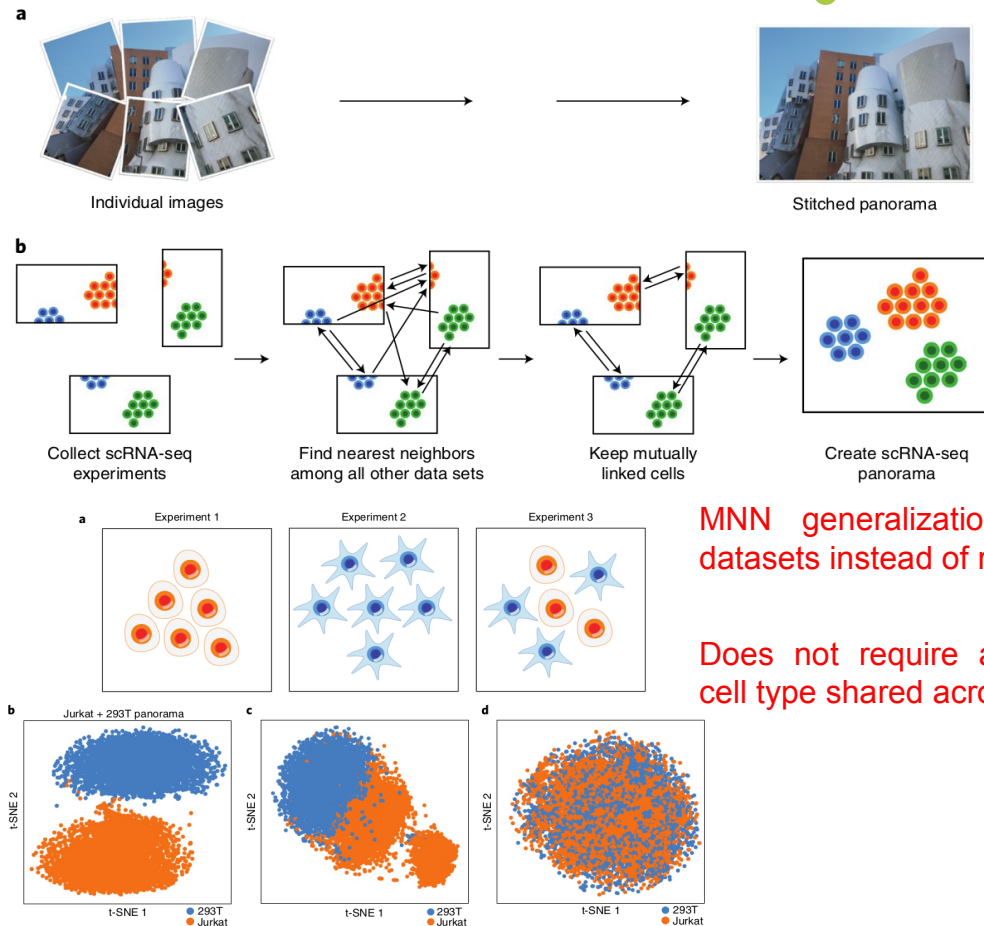
Here, we present Scanorama: a strategy for efficiently integrating multiple scRNA-seq datasets, even when they are composed of heterogeneous transcriptional phenotypes. Our approach is analogous to computer vision algorithms for panorama stitching that identify images with overlapping content and merge these into a larger panorama (Fig. 1a)⁸. Likewise, Scanorama automatically identifies scRNA-seq datasets containing cells with similar transcriptional profiles and can leverage those matches for batch correction and integration (Fig. 1b), without also merging datasets that do not overlap (Methods). Scanorama is robust to different dataset sizes and sources, preserves dataset-specific populations and does not require that all datasets share at least one cell population⁹.

Our approach generalizes mutual nearest-neighbors matching, a technique that finds similar elements between two datasets, to instead find similar elements among many datasets. Originally developed for pattern matching in images¹⁰, finding mutual nearest neighbors has also been used to identify common cell types between two scRNA-seq datasets at a time¹¹. However, to align more than two datasets, existing methods^{12,13} select one dataset as a

reference and successively integrate all other datasets into the reference, one at a time, which may lead to suboptimal results depending on the order in which the datasets are considered (Supplementary Fig. 1). Although Scanorama takes a similar approach when aligning a collection of two datasets, on larger collections of data it is insensitive to order and less vulnerable to overcorrection because it finds matches between all pairs of datasets.

To optimize the process of searching for matching cells among all datasets, we introduce two key procedures. Instead of performing the nearest neighbor search in the high-dimensional gene space, we compress the gene expression profiles of each cell into a low-dimensional embedding using an efficient, randomized singular value decomposition (SVD)¹⁴ of the cell-by-gene expression matrix, which also helps improve the methods' robustness to noise. Further, we use an approximate nearest neighbor search based on hyperplane locality-sensitive hashing¹⁵ and random projection trees¹⁶ to greatly reduce the nearest neighbor query time both asymptotically and in practice (Methods).

Notably, Scanorama can perform both scRNA-seq dataset integration and (optionally) batch correction. Integration methods (for example, Seurat CCA¹⁷), a previous integrative method based on a canonical correlation analysis (CCA) strategy¹⁸ find lower-dimensional representations of high-dimensional gene expression vectors such that the representations minimize confounding variation (for example, batch effects) with respect to some variation of interest (for example, biological differences among cell types). Batch correction methods (for example, scan MNN¹⁹, a previous batch correction method based on a simpler accumulative mutual nearest-neighbors (MNN) strategy) also remove confounding variation in the original high-dimensional space. Scanorama always performs integration of low-dimensional embeddings but can also perform batch correction if required. Although incurring a greater computational cost, Scanorama makes batch correction feasible for large datasets, enabling a wider array of downstream analyses. For example, differential expression analysis can be performed on batch-corrected gene expression data but not on integrated low-dimensional representations.

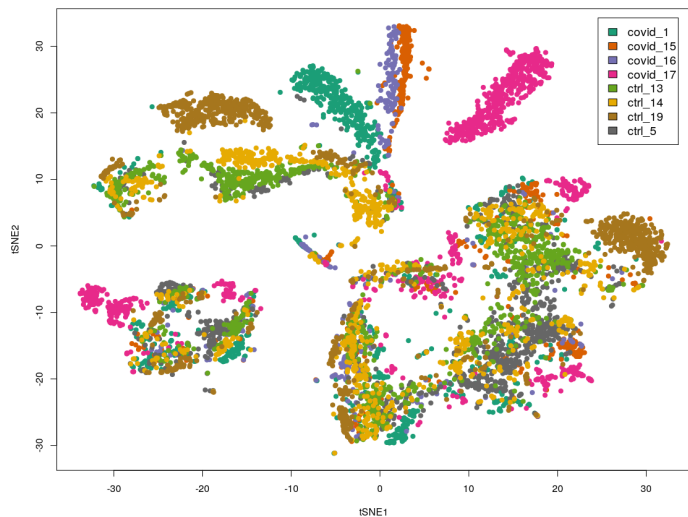


MNN generalization to many datasets instead of ref and query

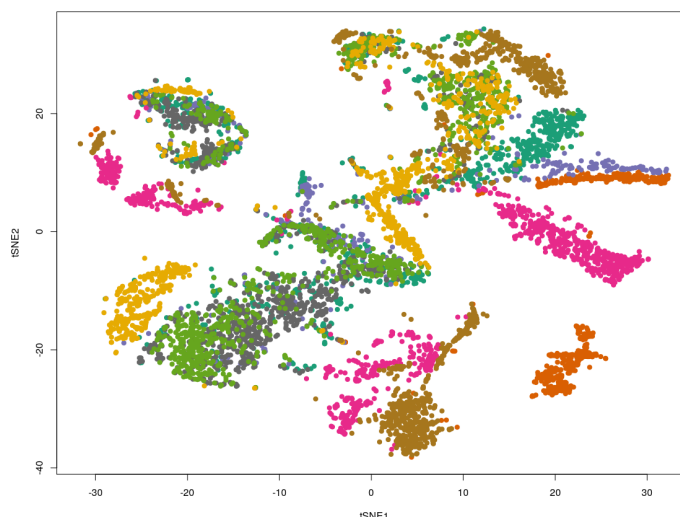
Does not require at least one cell type shared across datasets

Fig. 2 | Scanorama correctly integrates a simple collection of datasets where other methods fail. a, We apply Scanorama to a collection of three datasets²⁰: one entirely of Jurkat cells ($n = 3,257$ cells) (Experiment 1), one entirely of 293T cells ($n = 2,885$ cells) (Experiment 2) and a 50/50 mixture of Jurkat and 293T cells ($n = 3,388$ cells) (Experiment 3). **b**, Our method correctly identifies Jurkat cells (orange) and 293T cells (blue) as two separate clusters. **c, d**, Existing methods for scRNA-seq dataset integration are sensitive to the order in which they consider datasets (see Supplementary Fig. 1) and can incorrectly merge a Jurkat dataset and a 293T dataset together first before subsequently incorporating a 293T/Jurkat mixture, forming clusters that do not correspond to actual cell types: scan MNN corrected (**c**) and Seurat CCA integrated (**d**).

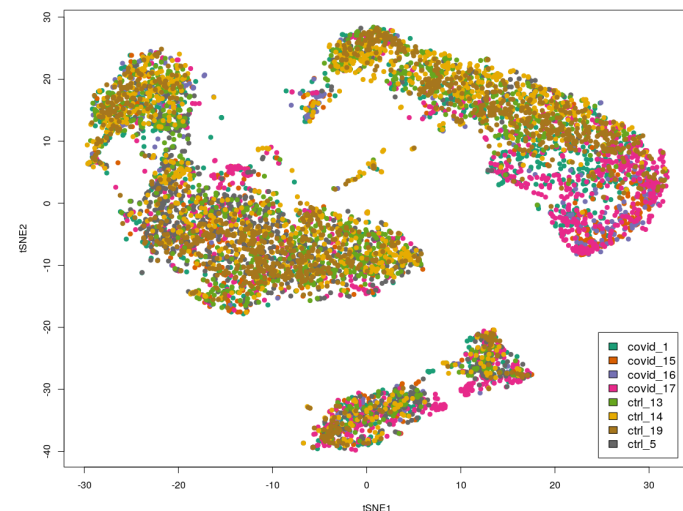
ISNE PLOT



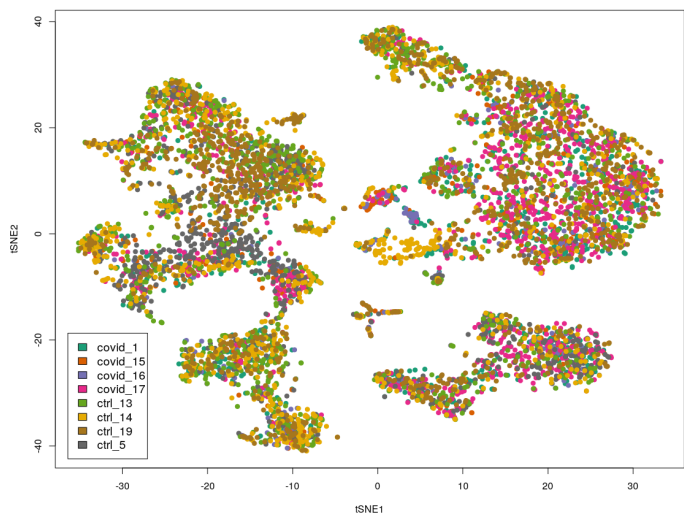
ISNE PLOT: COMBAT CORRECTED



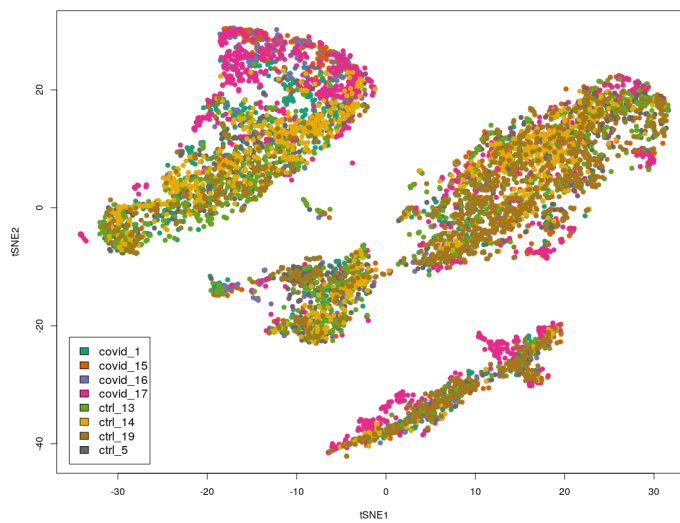
ISNE PLOT: FAST-MNN CORRECTED



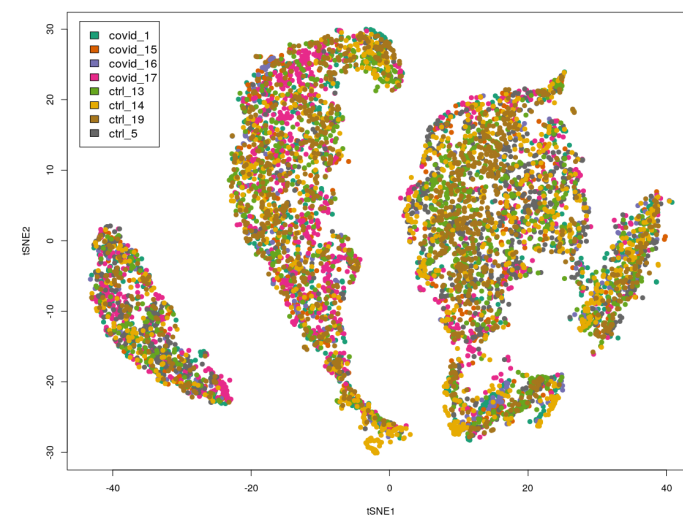
ISNE PLOT: CCA CORRECTED



ISNE PLOT: SCANORAMA CORRECTED



ISNE PLOT: HARMONY CORRECTED



ComBat: low-complexity datasets (subtle batch, few batches, few cell populations)

A recent comparison of classical batch correction methods has revealed that ComBat (Johnson *et al*, 2006) performs well also for single-cell experiments of low-to-medium complexity (Buttner *et al*, 2019). ComBat consists of a linear model of gene expression where the batch contribution is taken into account both in the mean and the variance of the data (Fig 3). Irrespective of computational methods, the best method of batch correction is pre-empting the effect and avoiding it altogether by clever experimental design (Hicks *et al*, 2017). Batch effects can be avoided by pooling cells across experimental conditions and samples. Using strategies such as cell tagging (preprint: Gehring *et al*, 2018), or via genetic variation (Kang *et al*, 2018), it is possible to demultiplex cells that were pooled in the experiment.

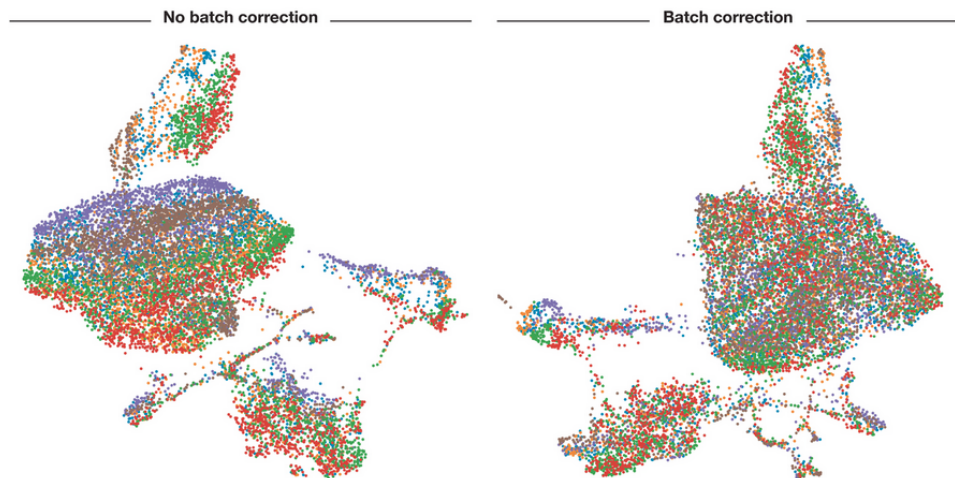


Figure 3 UMAP visualization before and after batch correction

Cells are coloured by sample of origin. Separation of batches is clearly visible before batch correction and less visible afterwards. Batch correction was performed using ComBat on mouse intestinal epithelium data from Haber *et al* (2017).

Luecken MD, Theis FJ. Current best practices in single-cell RNA-seq analysis: a tutorial. *Mol Syst Biol*. 2019 Jun 19;15(6):e8746. doi: 10.15252/msb.20188746

ANALYSIS

NATURE METHODS

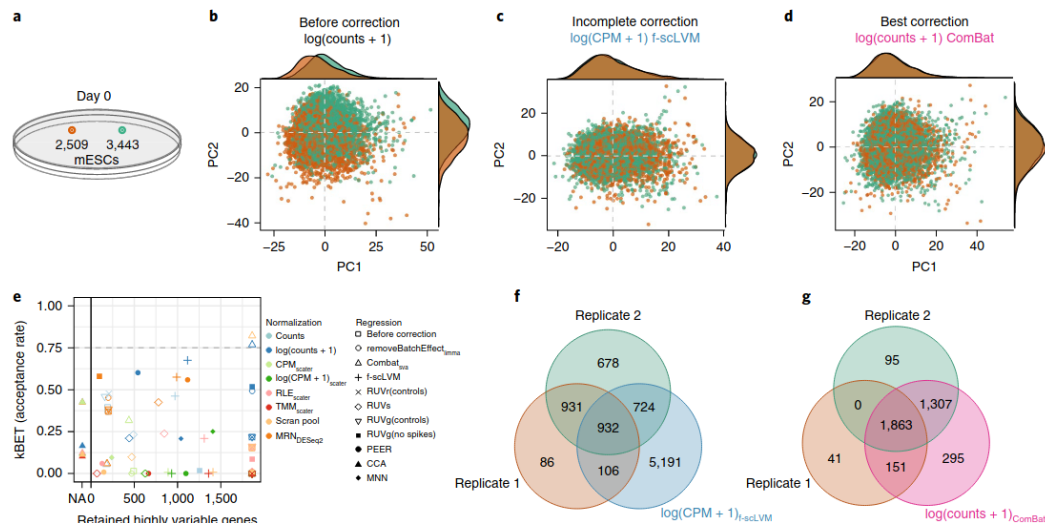


Fig. 3 | ComBat provides the best correction on mESC inDrop technical replicates. **a**, The inDrop protocol provides a large unique-molecular-identifier-count dataset with two technical replicates. **b-d**, PCA plots showing log-normalized counts (**b**), a biology-removing batch removal (f-sLVM on log-transformed CPM; **c**) and a biology-preserving batch removal (ComBat on log-transformed counts; **d**). Density plots on the axes show the frequency of replicates along the PCs. On the basis of visual inspection, the approaches in **c** and **d** appear to work equally well. **e**, Percentage of retained HVGs versus the mean acceptance rate (1 - rejection rate, from $n=100$ kBET runs) for all combinations of normalizations and batch-regression approaches. Seurat's CCA alignment batch-corrects data only in a latent space generated by manifold learning, and thus we could not compute HVGs for it. **f,g**, HVGs per replicate before correction and for the whole dataset after batch correction. HVGs in each replicate are computed on $\log(\text{counts} + 1)$ values. f-sLVM (**f**) retained 932 HVGs but had a high false positive rate, whereas ComBat (**g**) captured all HVGs with a low false positive rate.

Büttner M, Miao Z, Wolf FA, Teichmann SA, Theis FJ. A test metric for assessing single-cell RNA-seq batch correction. *Nat Methods*. 2019 Jan;16(1):43-49. doi: 10.1038/s41592-018-0254-1

RESEARCH

Open Access

A benchmark of batch-effect correction methods for single-cell RNA sequencing data

Hoa Thi Nhu Tran[†], Kok Siong Ang[†], Marion Chevrier[†], Xiaomeng Zhang[†], Nicole Yee Shin Lee, Michelle Goh and Jinmiao Chen[†]✉



Abstract

Background: Large-scale single-cell transcriptomic datasets generated using different technologies contain batch-specific systematic variations that present a challenge to batch-effect removal and data integration. With continued growth expected in scRNA-seq data, achieving effective batch integration with available computational resources is crucial. Here, we perform an in-depth benchmark study on available batch correction methods to determine the most suitable method for batch-effect removal.

Results: We compare 14 methods in terms of computational runtime, the ability to handle large datasets, and batch-effect correction efficacy while preserving cell type purity. Five scenarios are designed for the study: identical cell types with different technologies, non-identical cell types, multiple batches, big data, and simulated data. Performance is evaluated using four benchmarking metrics including kBET, LISI, ASW, and ARI. We also investigate the use of batch-corrected data to study differential gene expression.

Conclusion: Based on our results, Harmony, LIGER, and Seurat 3 are the recommended methods for batch integration. Due to its significantly shorter runtime, Harmony is recommended as the first method to try, with the other methods as viable alternatives.

Keywords: Single-cell RNA-seq, Batch correction, Batch effect, Integration, Differential gene expression

Introduction

Technological advances in the recent years have increased our ability to generate high-throughput single-cell gene expression data. Single-cell data is often compiled from multiple experiments with differences in capturing times, handling personnel, reagent lots, equipments, and even technology platforms. These differences lead to large variations or batch effects in the data, and can confound biological variations of interest during data integration. As such, effective batch-effect removal is essential. Batch

developed for microarray data batch correction such as ComBat [1] and limma [2] have been employed on single-cell RNA-seq (scRNA-seq) data. However, single-cell experiments suffer from “drop out” events due to the stochasticity of gene expression, or failure in RNA capture or amplification during sequencing [3]. This has prompted efforts to develop workflows to handle data with such characteristics [4–6].

A popular and successful approach, pioneered by Haghverdi et al. [5], identifies cell mappings between

nature | methods

ANALYSIS

https://doi.org/10.1038/s41592-021-01336-8



OPEN

Benchmarking atlas-level data integration in single-cell genomics

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Single-cell atlases often include samples that span locations, laboratories and conditions, leading to complex, nested batch effects in data. Thus, joint analysis of atlas datasets requires reliable data integration. To guide integration method choice, we benchmarked 68 method and preprocessing combinations on 85 batches of gene expression, chromatin accessibility and simulation data from 23 publications, altogether representing >1.2 million cells distributed in 13 atlas-level integration tasks. We evaluated methods according to scalability, usability and their ability to remove batch effects while retaining biological variation using 14 evaluation metrics. We show that highly variable gene selection improves the performance of data integration methods, whereas scaling pushes methods to prioritize batch removal over conservation of biological variation. Overall, scANVI, Scanorama, scVI and scGen perform well, particularly on complex integration tasks, while single-cell ATAC-seq integration performance is strongly affected by choice of feature space. Our freely available Python module and benchmarking pipeline can identify optimal data integration methods for new data, benchmark new methods and improve method development.

The complexity of single-cell omics datasets is increasing. Current datasets often include many samples¹, generated across multiple conditions², with the involvement of multiple laboratories³. Such complexity, which is common in reference atlas initiatives such as the Human Cell Atlas⁴, creates inevitable batch effects. Therefore, the development of data integration methods that overcome the complex, nonlinear, nested batch effects in these data has become a priority: a grand challenge in single-cell RNA-seq data analysis^{5,6}.

Batch effects represent unwanted technical variation in data that result from handling cells in distinct batches. These effects can arise from variations in sequencing depth, sequencing lanes, read length, plates or flow cells, protocol, experimental laboratories, sample acquisition and handling, sample composition, reagents or media and/or sampling time. Furthermore, biological factors such as tissues, spatial locations, species, time points or inter-individual variation can also be regarded as a batch effect.

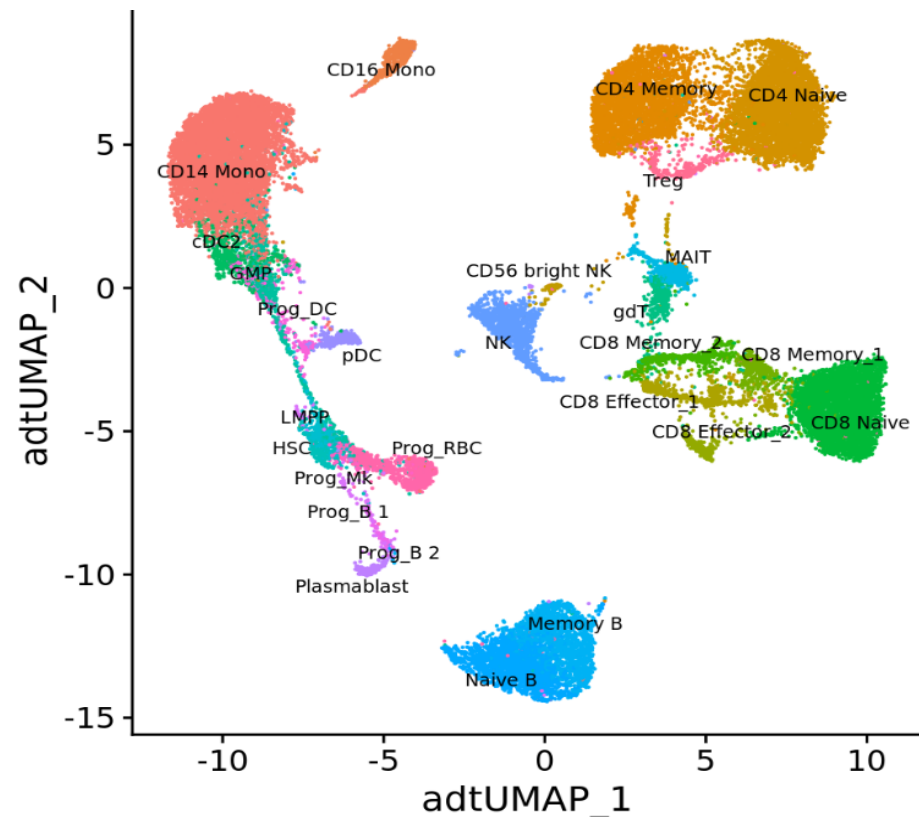
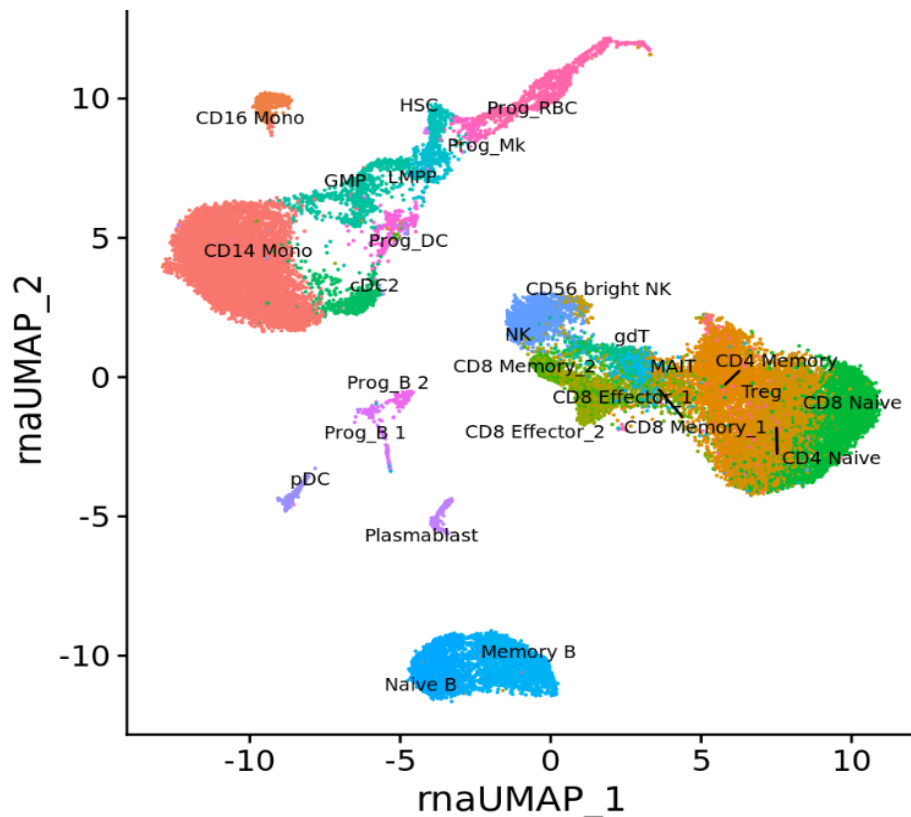
A single-cell data integration method aims to combine high-throughput sequencing datasets or samples to produce a self-consistent version of the data for downstream analysis⁷. Batch-integrated cellular profiles are represented as an integrated graph, a joint embedding or a corrected feature matrix.

Currently, at least 49 integration methods for scRNA-seq data are available⁸ (as of November 2020, Supplementary Table 1). In the absence of objective metrics, subjective opinions based on visualizations of integrated data will determine method evaluation. Benchmarking integration methods facilitates this process to provide an unbiased guide to method choice.

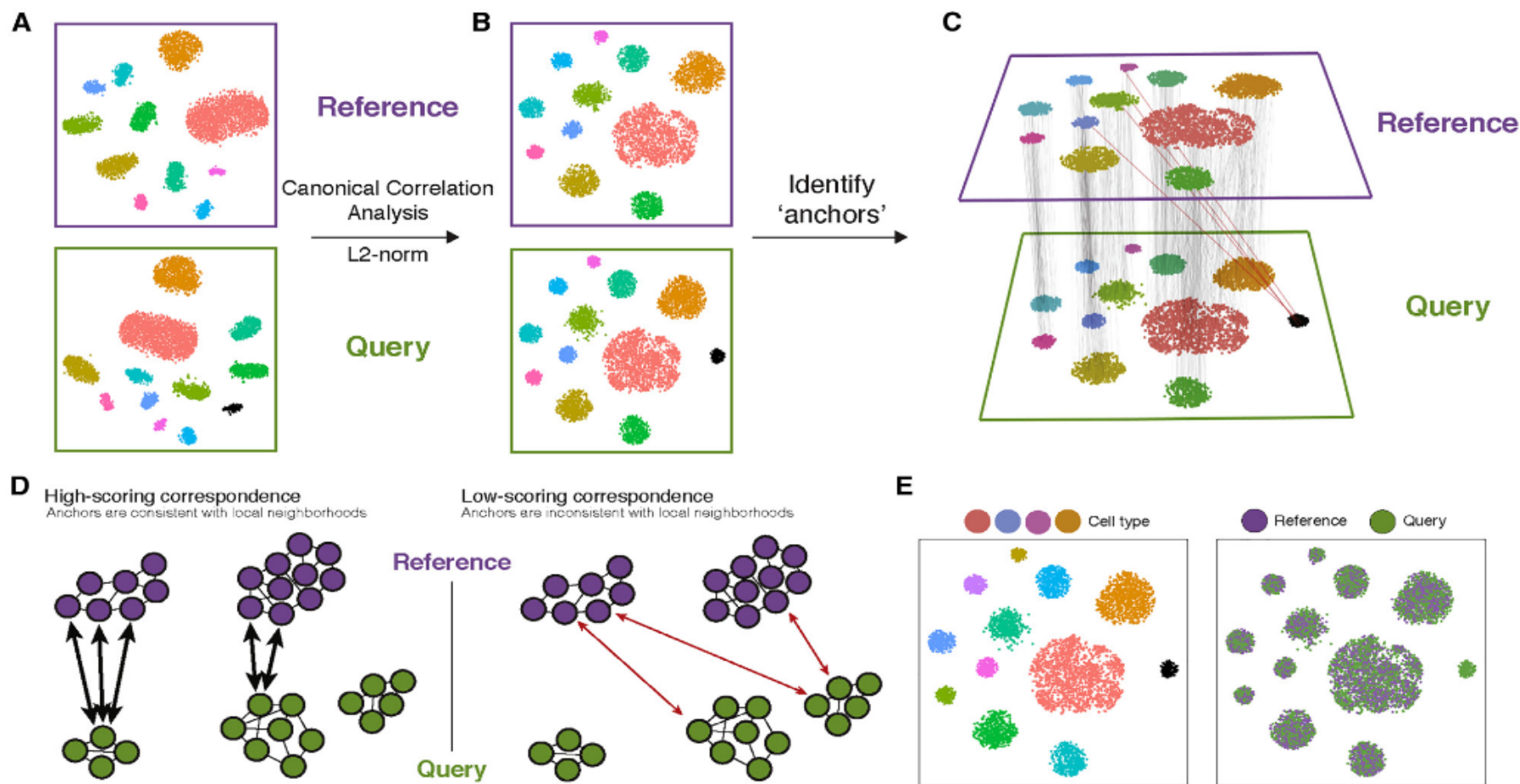
compare different output options such as corrected features or joint embeddings, finding that ComBat¹¹ or the linear, principal component analysis (PCA)-based, Harmony method⁴ outperformed more complex, nonlinear, methods.

Here, we present a benchmarking study of data integration methods in complex integration tasks, such as tissue or organ atlases. Specifically, we benchmarked 16 popular data integration tools on 13 data integration tasks consisting of up to 23 batches and 1 million cells, for both scRNA- and single-cell ATAC-seq (scRNA-seq and scATAC-seq) data. We selected 12 single-cell data integration tools: mutual nearest neighbors (MNN)¹² and its extension FastMNN¹³, Seurat v3 (CCA and RPCA)¹⁴, scVI¹⁵ and its extension to an annotation framework (scANVI¹⁶), Scanorama¹⁶, batch-balanced *k* nearest neighbors (BBKNN)¹⁷, LIGER¹⁸, Conos¹⁹, SAUCIE²⁰ and Harmony⁴; one bulk data integration tool (ComBat²¹); a method for clustering with batch removal (DESC²²) and two perturbation modeling tools developed previously by one of the authors (trVAE²³ and scGen²⁴). Moreover, we use 14 metrics to evaluate the integration methods on their ability to remove batch effects while conserving biological variation. We focus in particular on assessing the conservation of biological variation beyond cell identity labels via new integration metrics on trajectories or cell-cycle variation. We find that Scanorama and scVI perform well, particularly on complex integration tasks. If cell annotations are available, scGen and scANVI outperform most other methods across tasks, and Harmony and LIGER are effective for scATAC-seq data integration on window and peak feature spaces.

Integration across features (vertical integration)



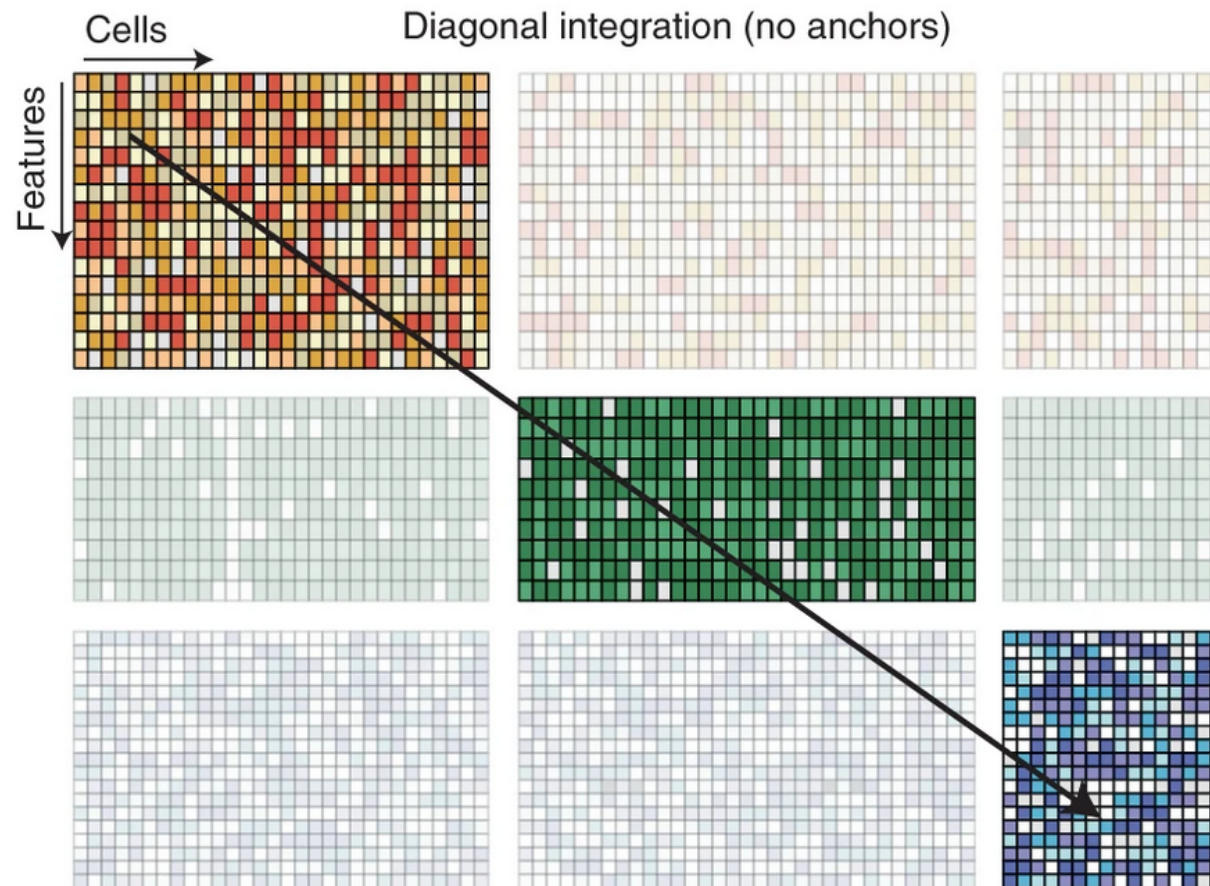
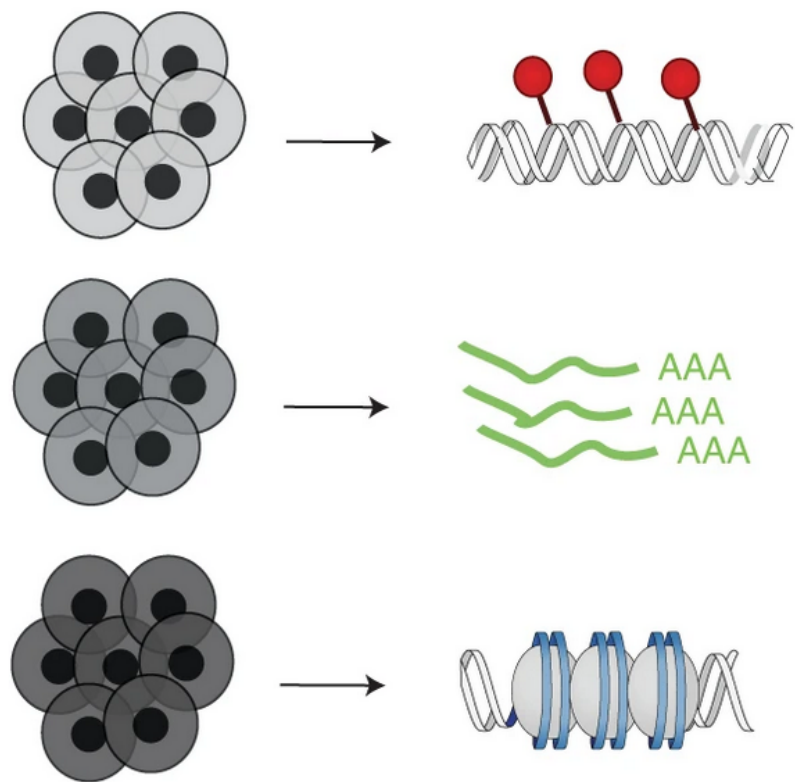
“Despite tremendous functional diversity, distinct populations of T cells such as effector, regulatory, $\gamma\delta$, and mucosal associated invariant T (MAIT), often cannot be effectively separated by scRNA-seq alone, even when using the most sensitive and cutting-edge technologies”

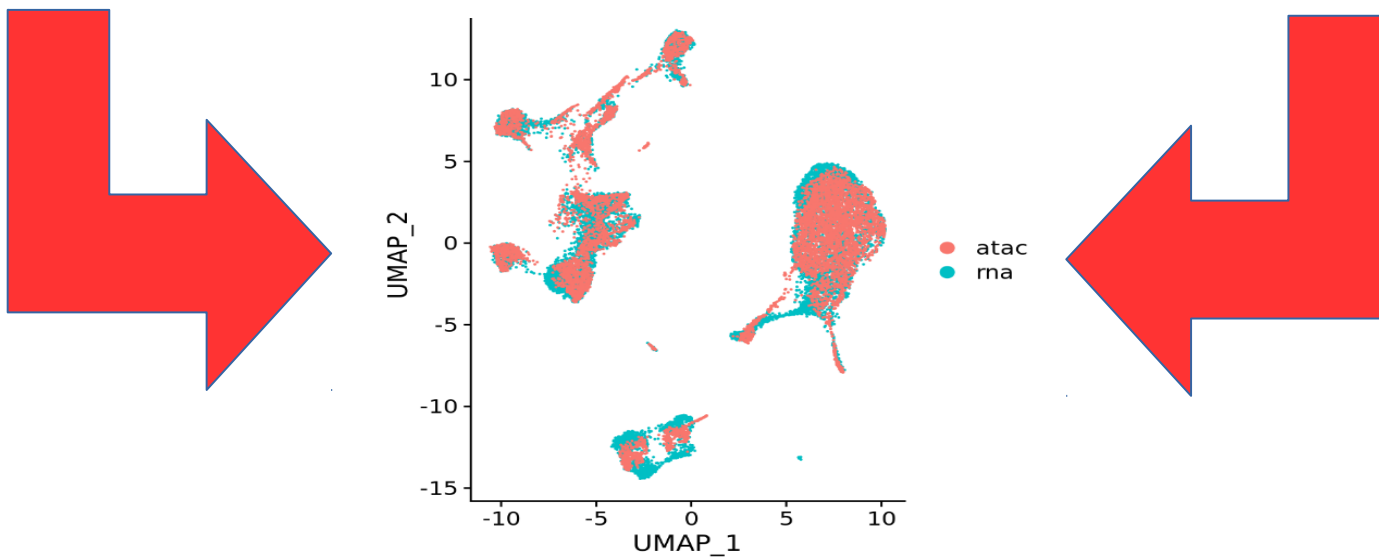
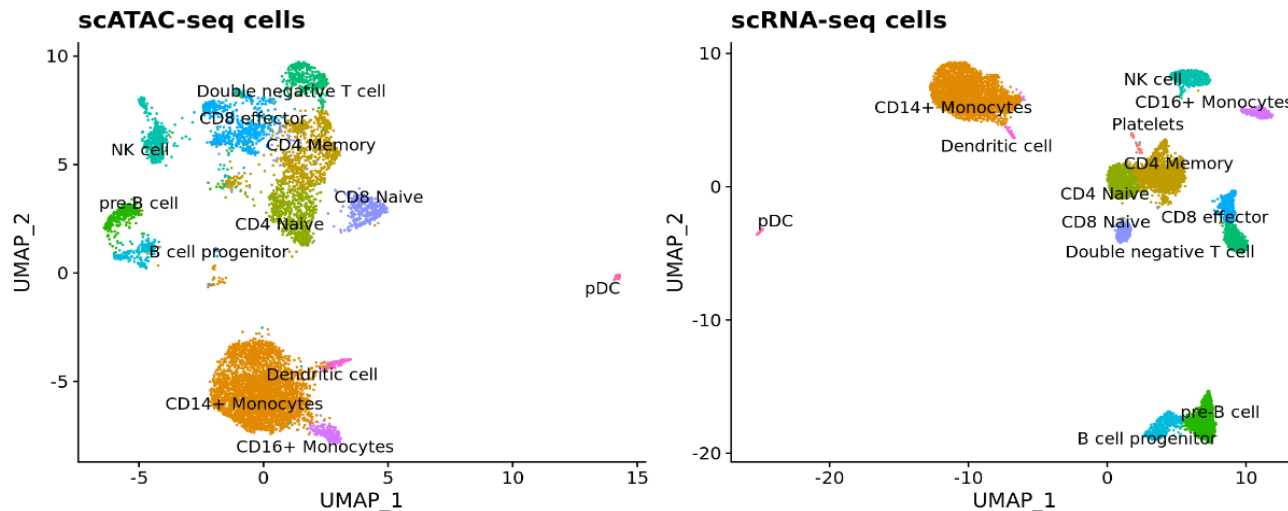


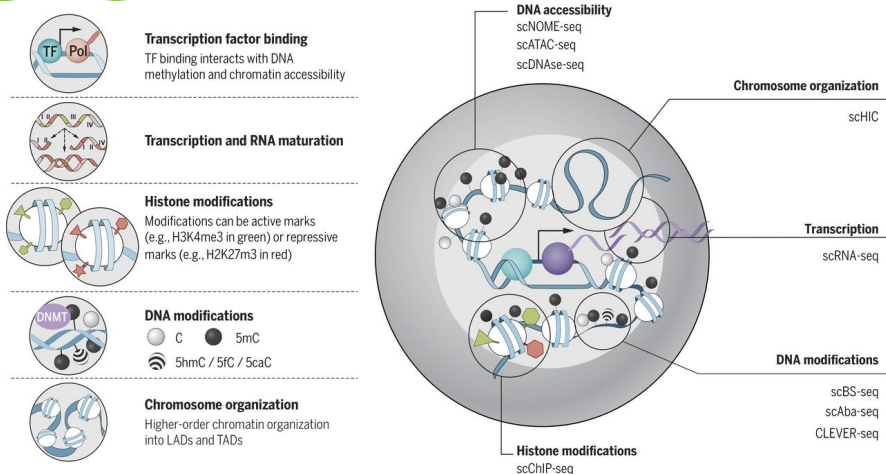
Not the same biological cells but scATAC peaks can be assigned to genes, so the feature names are the same

Seurat “transfer anchors across Omics” as an example of diagonal integration

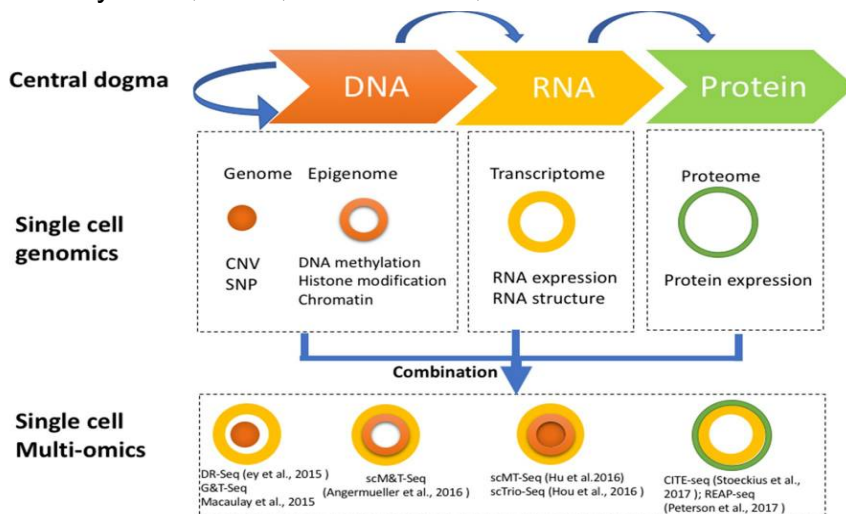
c



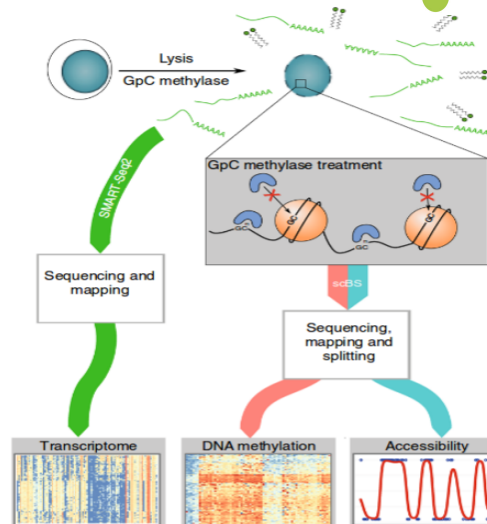




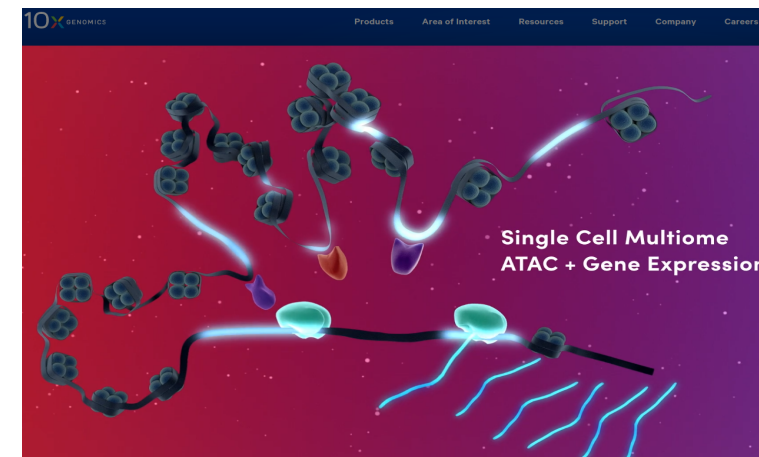
Kelsey et al., 2017, Science 358, 69-75



Hu et al., 2018, Frontier in Cell and Developmental Biology 6, 1-13

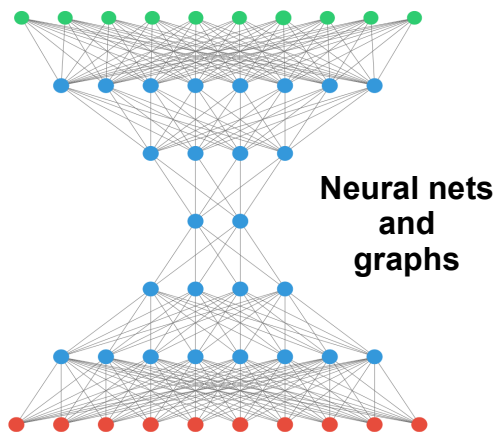


Clark et al., 2018, Nature Communications 9, 781

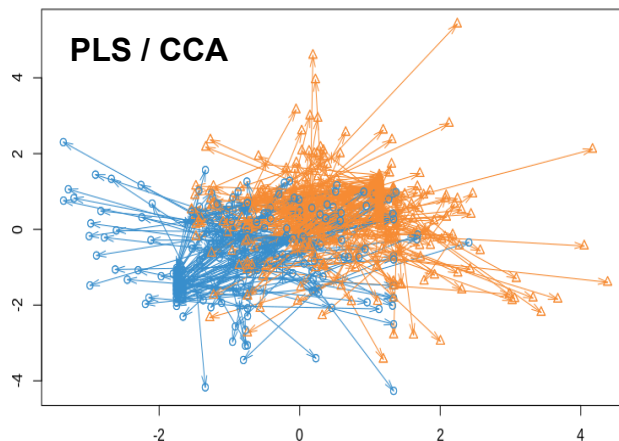


10X Genomics Multiome ATAC + Gene Expression

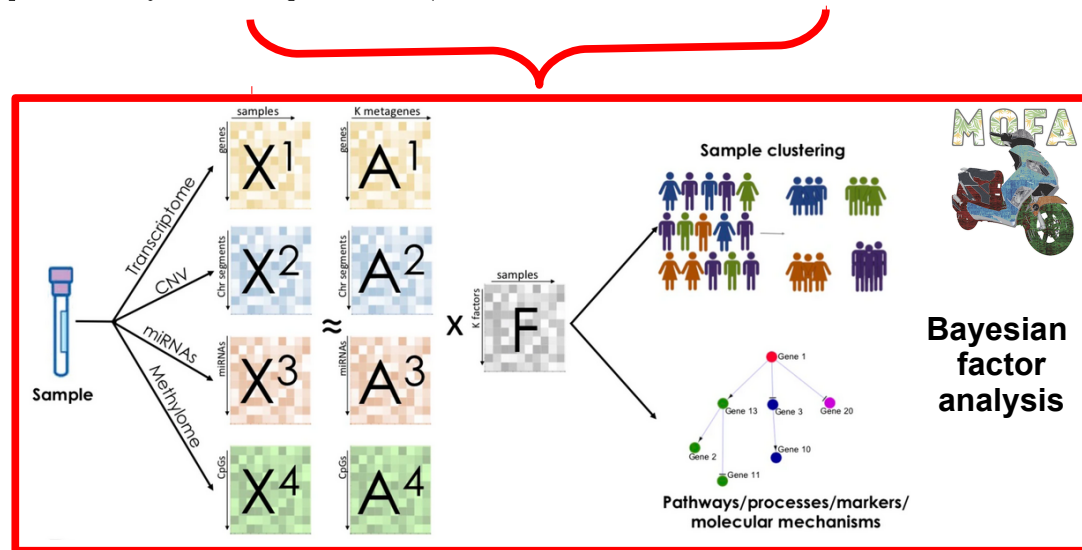
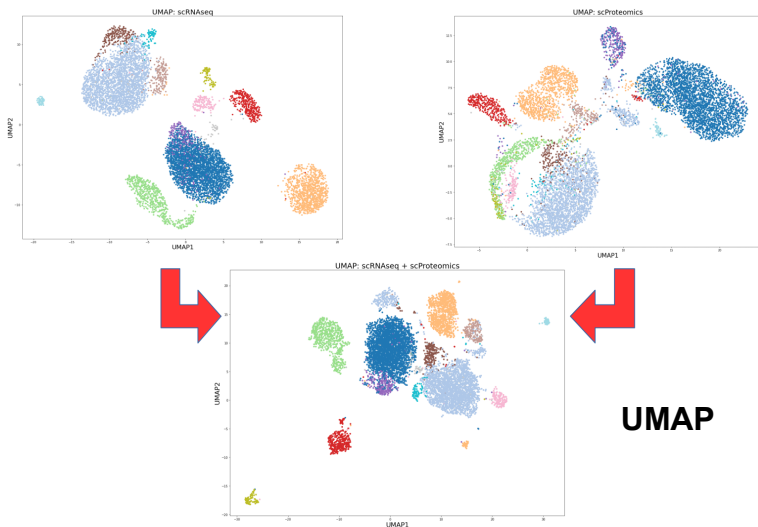
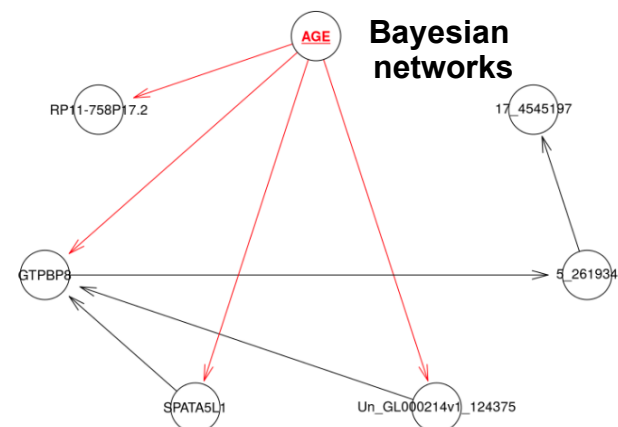
Convert to common space



Extract common variation



Combine via Bayes rule



Method



molecular
systems
biology

Multi-Omics Factor Analysis—a framework for unsupervised integration of multi-omics data sets

Ricard Argelaguet^{1,†} , Britta Velten^{2,†} , Damien Arno¹ , Sascha Dietrich³ , Thorsten Zenz^{3,4,5} , John C Marion^{1,6,7} , Florian Buettner^{1,8,*} , Wolfgang Huber^{2,**} & Oliver Stegle^{1,2,***}

Abstract

Multi-omics studies promise the improved characterization of biological processes across molecular layers. However, methods for the unsupervised integration of the resulting heterogeneous data sets are lacking. We present Multi-Omics Factor Analysis (MOFA), a computational method for discovering the principal sources of variation in multi-omics data sets. MOFA infers a set of (hidden) factors that capture biological and technical sources of variability. It disentangles axes of heterogeneity that are shared across multiple modalities and those specific to individual data modalities. The learnt factors enable a variety of downstream analyses, including identification of sample subgroups, data imputation and the detection of outlier samples. We applied MOFA to a cohort of 200 patient samples of chronic lymphocytic leukaemia, profiled for somatic mutations, RNA expression, DNA methylation and *ex vivo* drug responses. MOFA identified major dimensions of disease heterogeneity, including immunoglobulin heavy-chain variable region status, trisomy of chromosome 12 and previously underappreciated drivers, such as response to oxidative stress. In a second application, we used MOFA to analyse single-cell multi-omics data, identifying coordinated transcriptional and epigenetic changes along cell differentiation.

Keywords data integration; dimensionality reduction; multi-omics; personalized medicine; single-cell omics
Subject Categories Computational Biology; Genome-Scale & Integrative Biology; Methods & Resources
DOI 10.1525/msb.2017.8124 | Received 27 November 2017 | Revised 28 May 2018 | Accepted 29 May 2018
Mol Syst Biol. (2018) 14: e8124

Introduction

Technological advances increasingly enable multiple biological layers to be probed in parallel, ranging from genome, epigenome, transcriptome, proteome and metabolome to phenome profiling (Hasin *et al.*, 2017). Integrative analyses that use information across these data modalities promise to deliver more comprehensive insights into the biological systems under study. Motivated by this, multi-omics profiling is increasingly applied across biological domains, including cancer biology (Gerstung *et al.*, 2015; Iorio *et al.*, 2016; Mertins *et al.*, 2016; Cancer Genome Atlas Research Network, 2017), regulatory genomics (Chen *et al.*, 2016), microbiology (Kim *et al.*, 2016) or host-pathogen biology (Soderholm *et al.*, 2016). Most recent technological advances have also enabled performing multi-omics analyses at the single-cell level (Macaulay *et al.*, 2015; Angermueller *et al.*, 2016; Guo *et al.*, 2017; Clark *et al.*, 2018; Colomé-Tatché & Theis, 2018). A common aim of such applications is to characterize heterogeneity between samples, as manifested in one or several of the data modalities (Ritchie *et al.*, 2015). Multi-omics profiling is particularly appealing if the relevant axes of variation are not known *a priori*, and hence may be missed by studies that consider a single data modality or targeted approaches.

A basic strategy for the integration of omics data is testing for marginal associations between different data modalities. A prominent example is molecular quantitative trait locus mapping, where large numbers of association tests are performed between individual genetic variants and gene expression levels (GTEx Consortium, 2015) or epigenetic marks (Chen *et al.*, 2016). While eminently useful for variant annotation, such association studies are inherently *local* and do not provide a coherent global map of the molecular differences between samples. A second strategy is the use of kernel- or graph-based methods to combine different

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Method | [Open Access](#) | [Published: 11 May 2020](#)

MOFA+: a statistical framework for comprehensive integration of multi-modal single-cell data

Ricard Argelaguet , Damien Arno, Danila Bredikhin, Yonatan Deloro, Britta Velten, John C. Marion & Oliver Stegle

[Genome Biology](#) 21, Article number: 111 (2020) | [Cite this article](#)

30k Accesses | 127 Citations | 119 Altmetric | [Metrics](#)

Abstract

Technological advances have enabled the profiling of multiple molecular layers at single-cell resolution, assaying cells from multiple samples or conditions. Consequently, there is a growing need for computational strategies to analyze data from complex experimental designs that include multiple data modalities and multiple groups of samples. We present Multi-Omics Factor Analysis v2 (MOFA+), a statistical framework for the comprehensive and scalable integration of single-cell multi-modal data. MOFA+ reconstructs a low-dimensional representation of the data using computationally efficient variational inference and supports flexible sparsity constraints, allowing to jointly model variation across multiple sample groups and data modalities.

Background

Single-cell methods have provided unprecedented opportunities to assay cellular heterogeneity. This is particularly important for studying complex biological processes, including the immune system, embryonic development, and cancer [1,2,3,4].

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Sections

Figures

References

[Abstract](#)

[Background](#)

[Results](#)

[Discussion](#)

[Conclusions](#)

[Methods](#)

[Availability of data and materials](#)

[References](#)

[Acknowledgements](#)

[Funding](#)

[Author information](#)

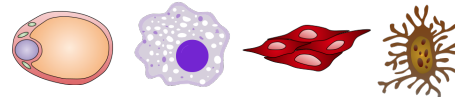
[Ethics declarations](#)

[Additional information](#)

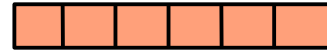
[Supplementary information](#)

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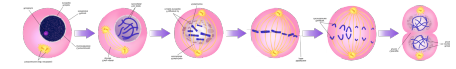
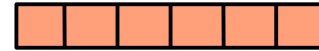
[About this article](#)



CELL TYPE



CELL CYCLE



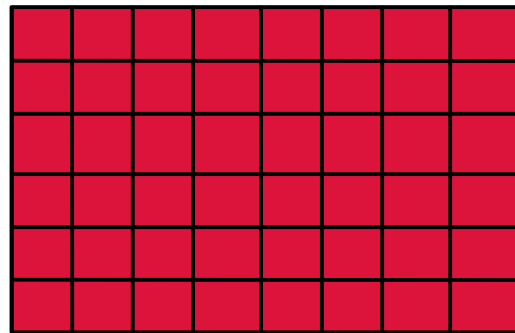
L_1

L_2

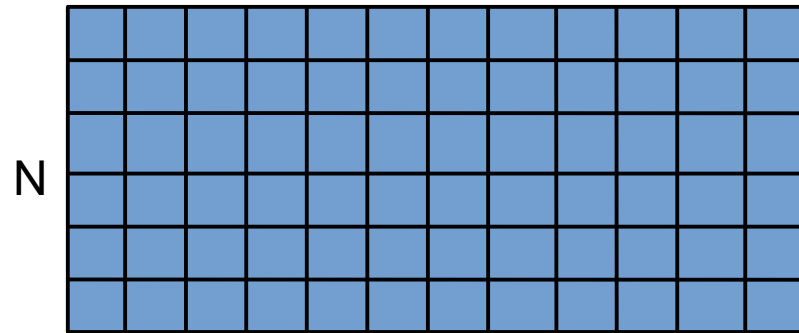
P_1

P_2

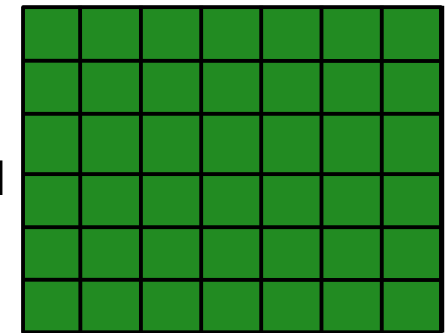
P_3



Gene expression



Methylation



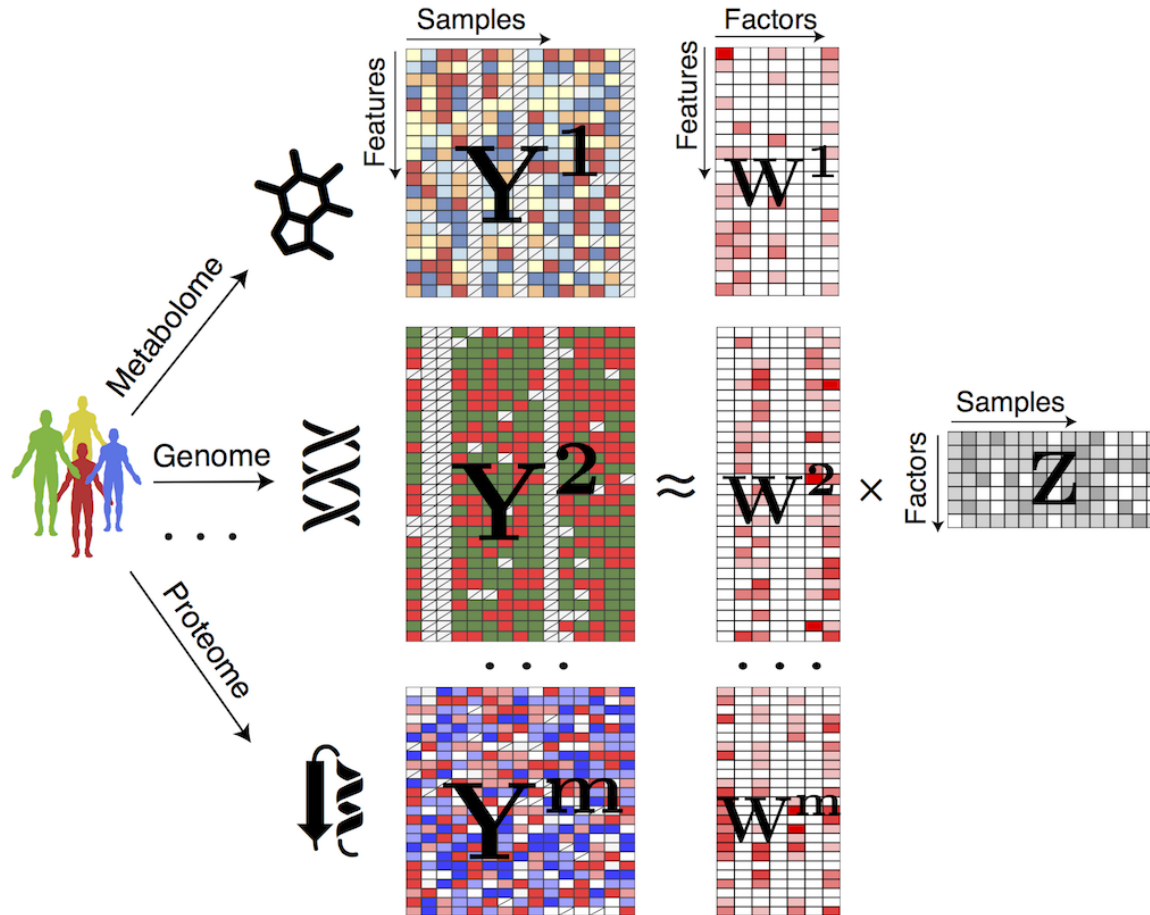
Genetic mutation

N

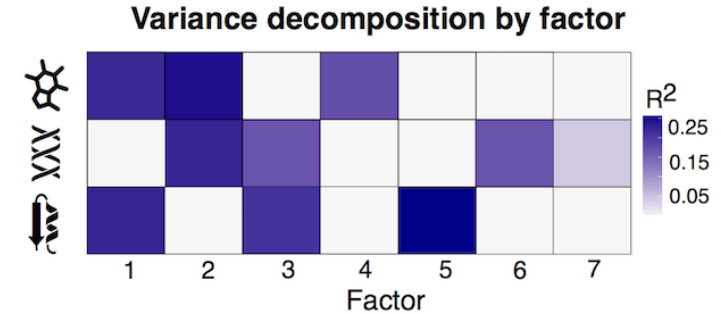
N

N

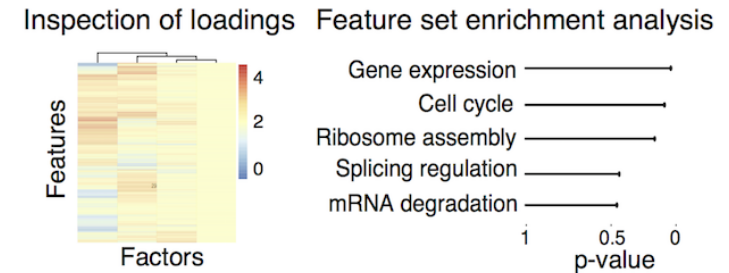
Step 1: train a MOFA model



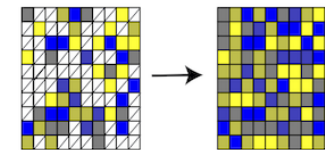
Step 2: downstream analysis



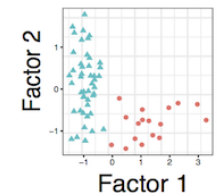
Annotation of factors



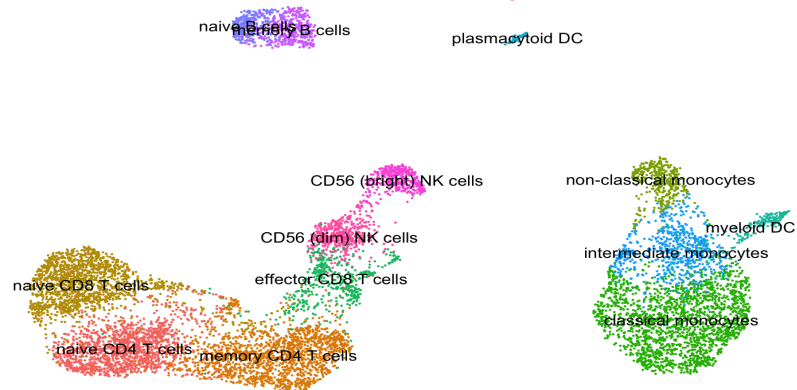
Imputation of missing values



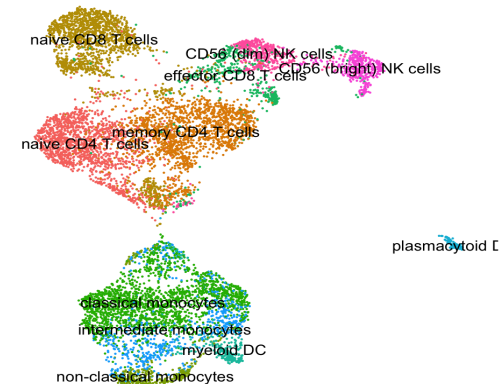
Inspection of factors



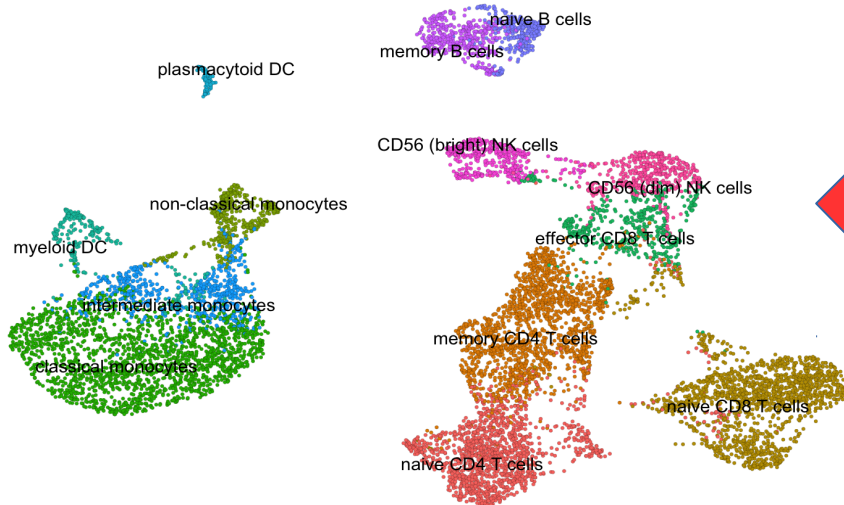
scRNAseq



scATACseq



scRNAseq + scATACseq





ARTICLE

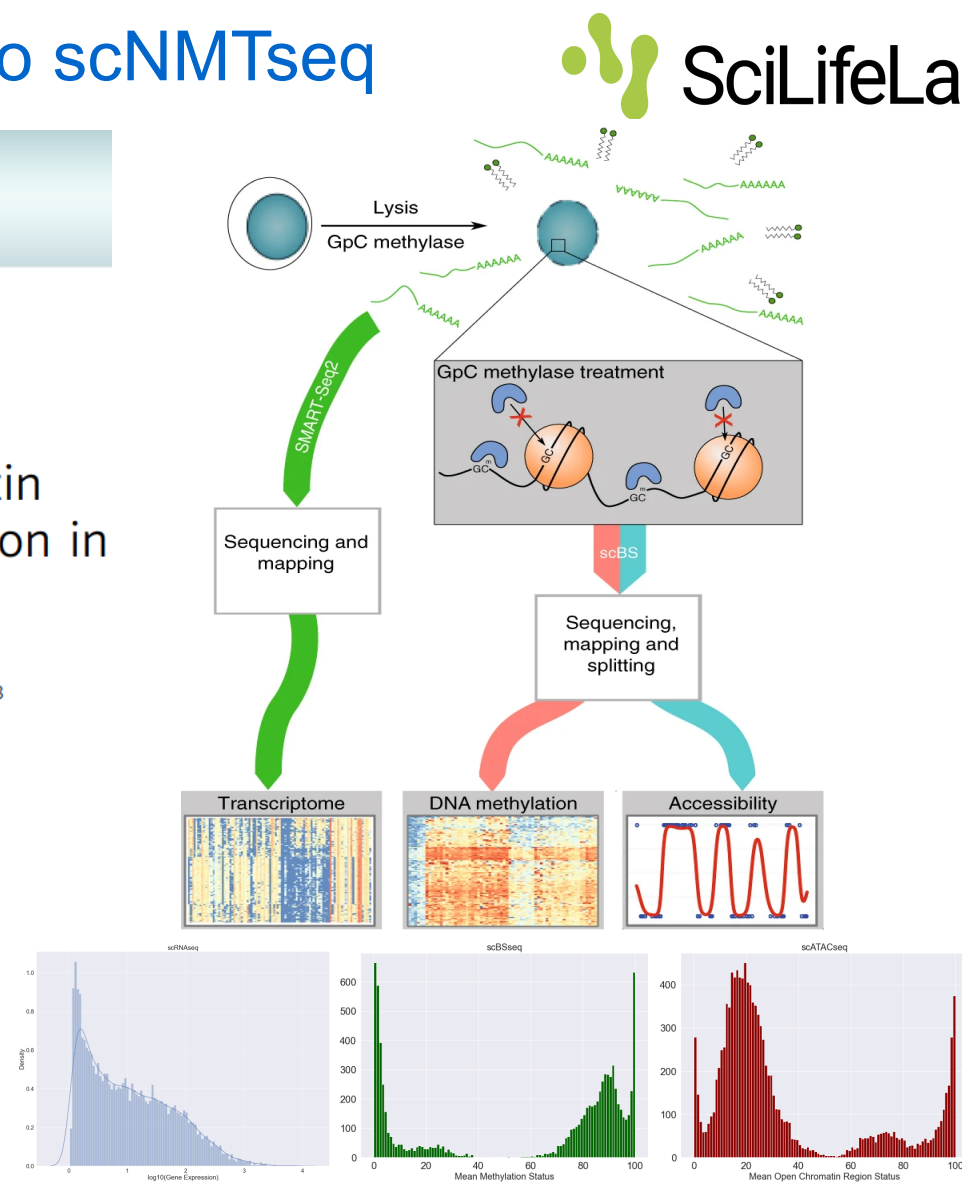
DOI: 10.1038/s41467-018-03149-4

OPEN

scNMT-seq enables joint profiling of chromatin accessibility DNA methylation and transcription in single cells

Stephen J. Clark¹, Ricard Argelaguet^{2,3}, Chantiriolnt-Andreas Kapourani⁴, Thomas M. Stubbs¹, Heather J. Lee^{1,5,6}, Celia Alda-Catalinas¹, Felix Krueger⁷, Guido Sanguinetti⁴, Gavin Kelsey^{1,8}, John C. Marioni^{2,3,5}, Oliver Stegle², Wolf Reik^{1,5,8}

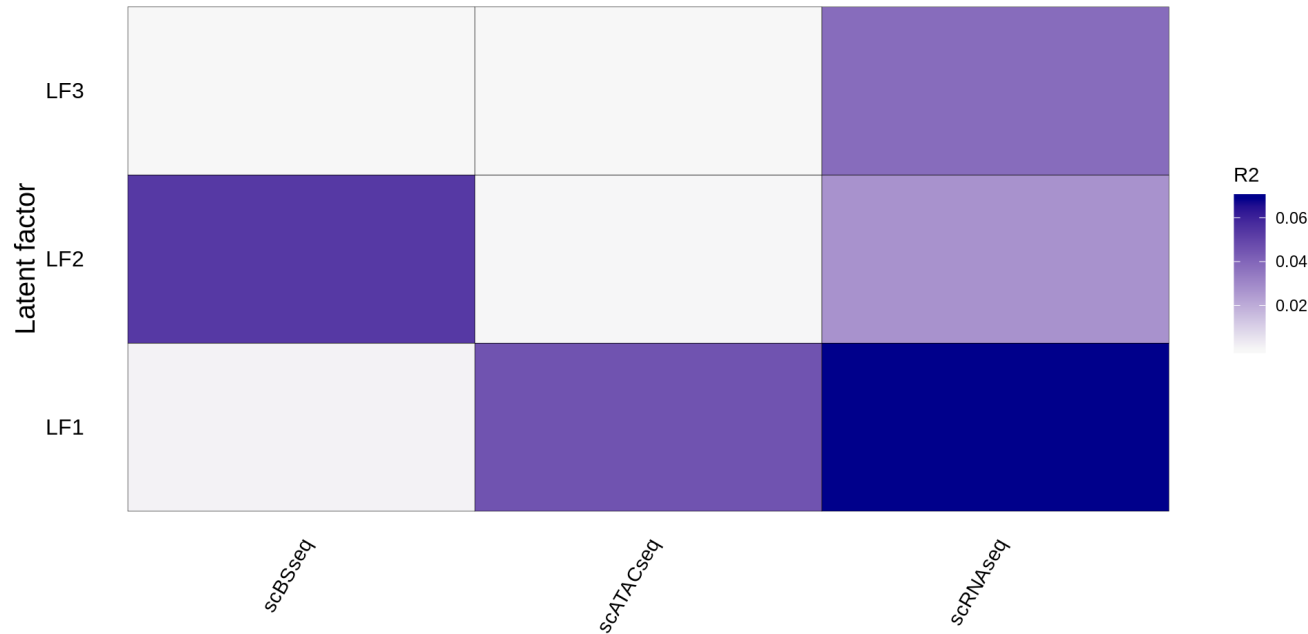
Parallel single-cell sequencing protocols represent powerful methods for investigating regulatory relationships, including epigenome-transcriptome interactions. Here, we report a single-cell method for parallel chromatin accessibility, DNA methylation and transcriptome profiling. scNMT-seq (single-cell nucleosome, methylation and transcription sequencing) uses a GpC methyltransferase to label open chromatin followed by bisulfite and RNA sequencing. We validate scNMT-seq by applying it to differentiating mouse embryonic stem cells, finding links between all three molecular layers and revealing dynamic coupling between epigenomic layers during differentiation.



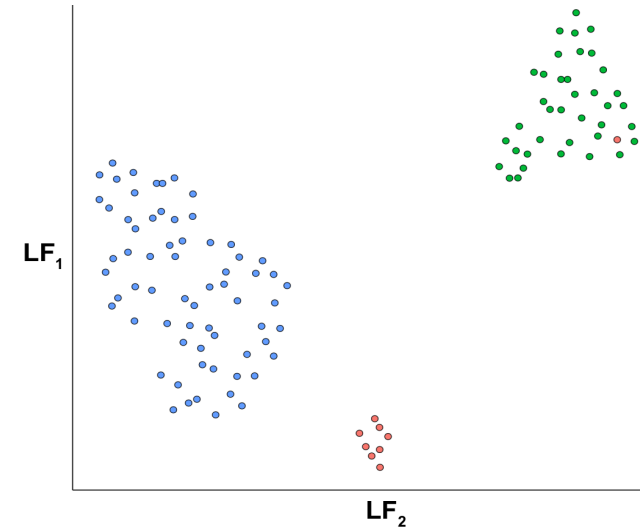
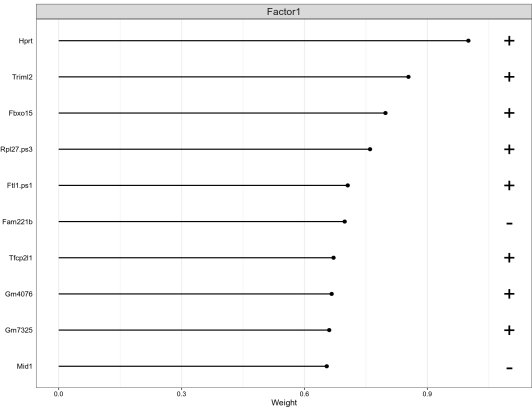
Total variance explained per view

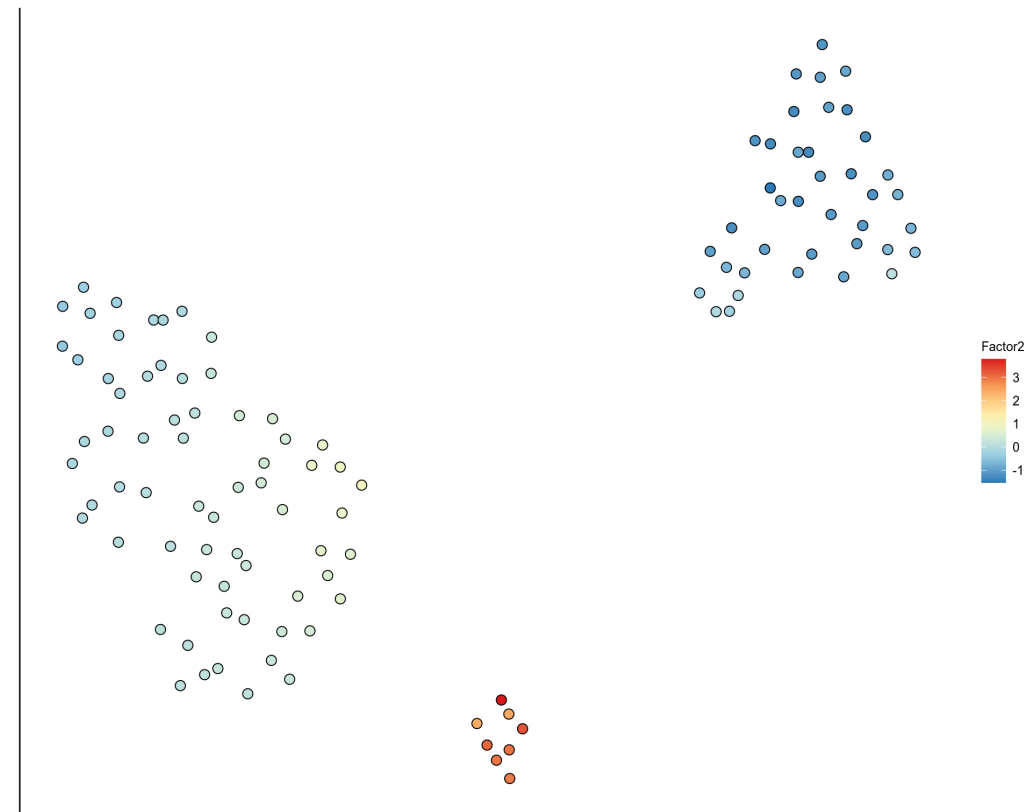
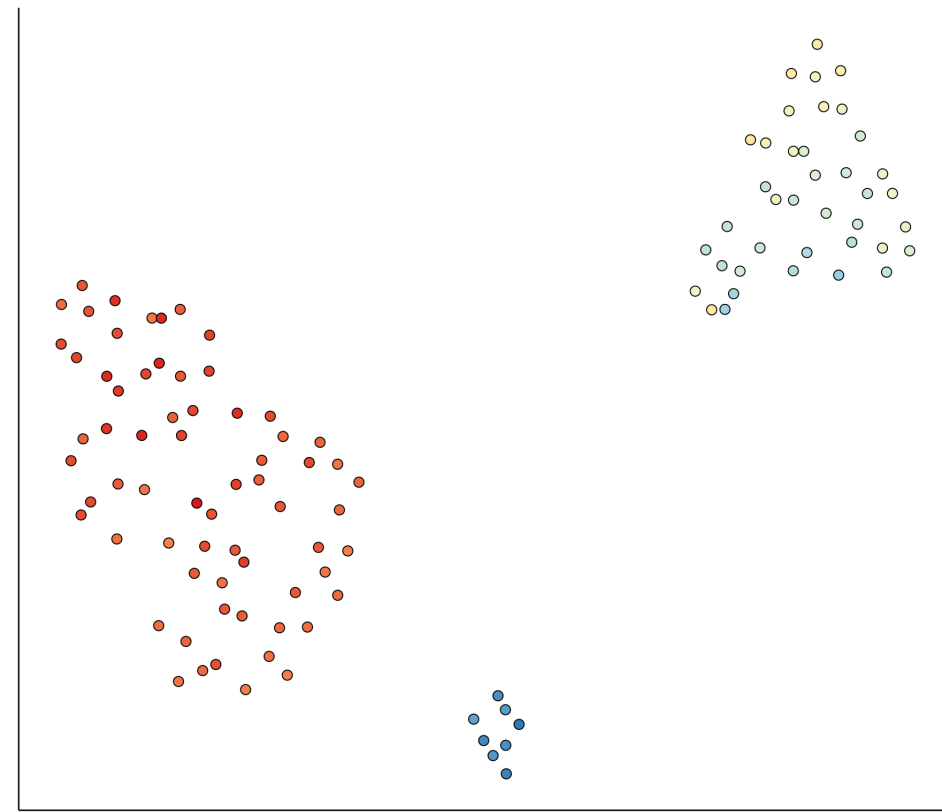


Variance explained per factor

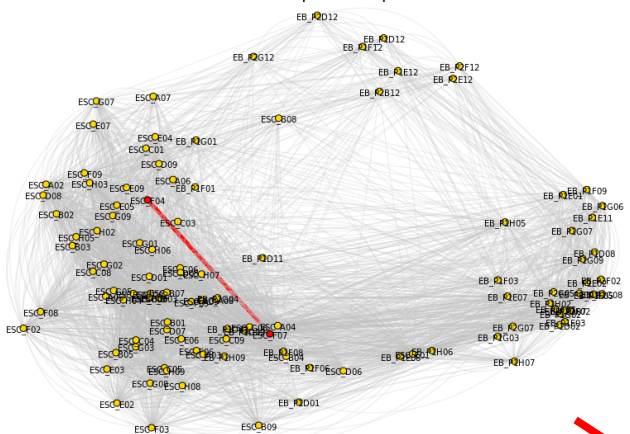


$$R_{m,k}^2 = 1 - \left(\sum_{n,d} y_{nd}^m - z_{nk} w_{kd}^m - \mu_d^m \right)^2 / \left(\sum_{n,d} y_{nd}^m - \mu_d^m \right)^2$$



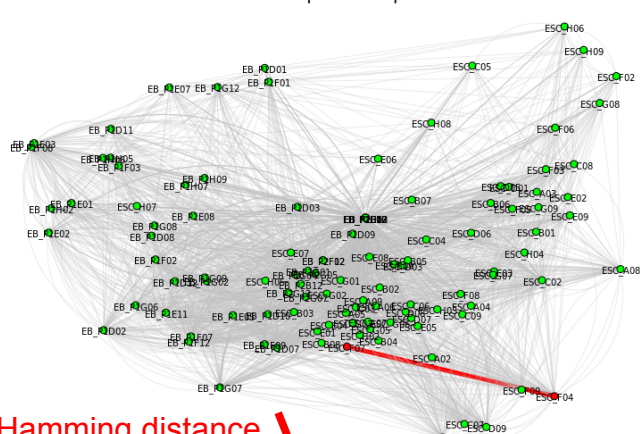


scRNAseq KNN Graph



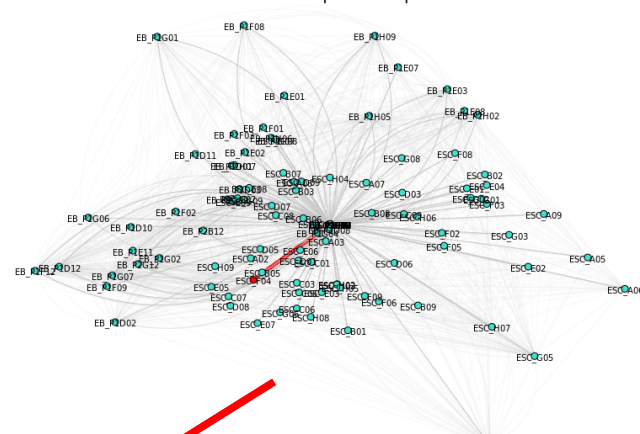
Euclidean distance

scBSseq KNN Graph



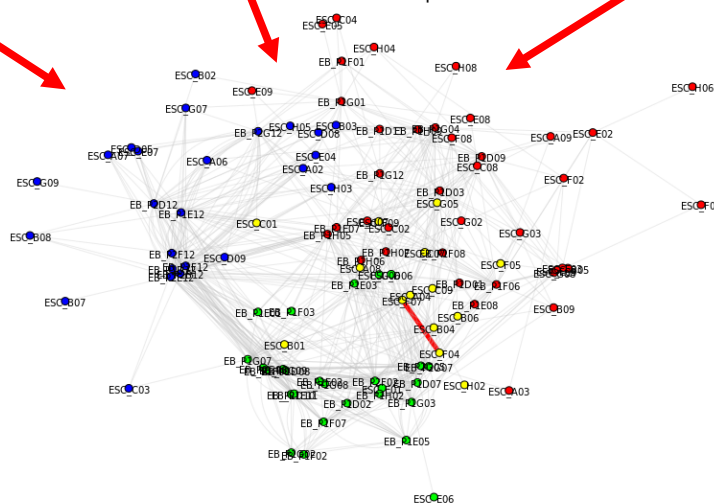
Hamming distance

scATACseq KNN Graph



Hamming distance

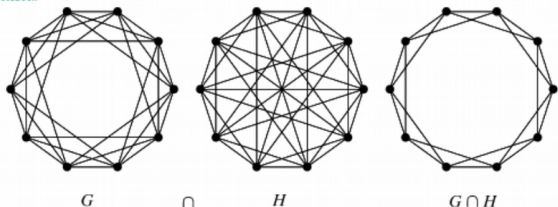
Consensus Graph



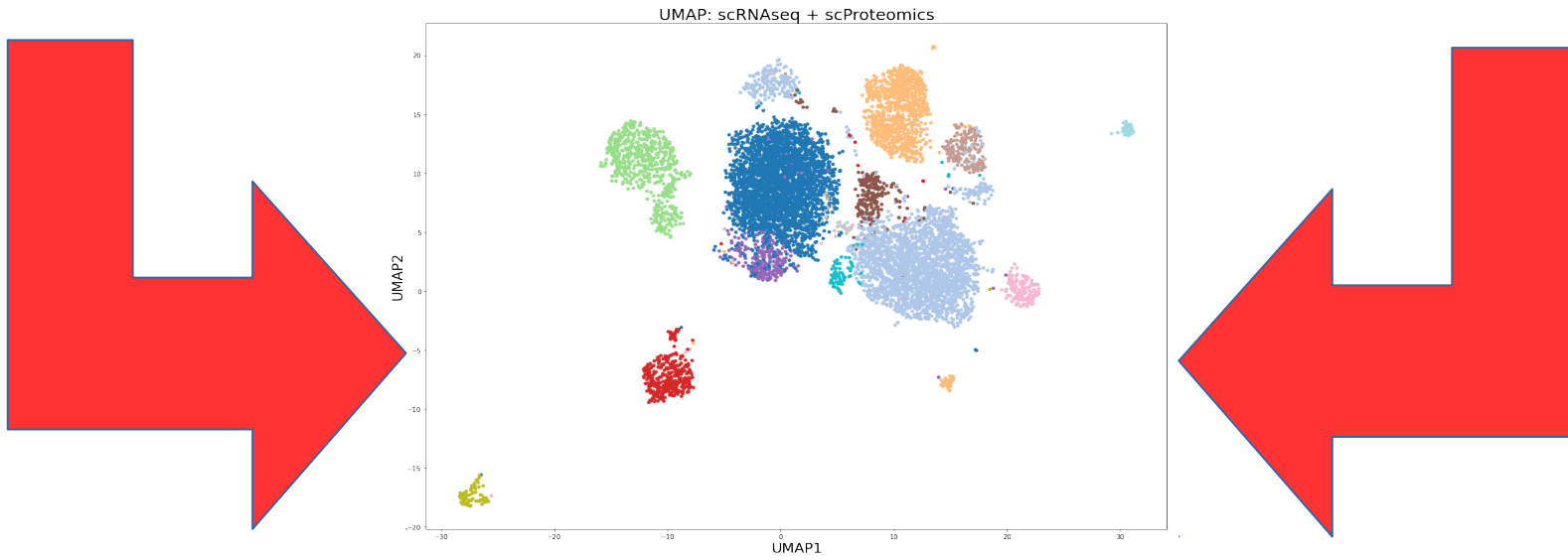
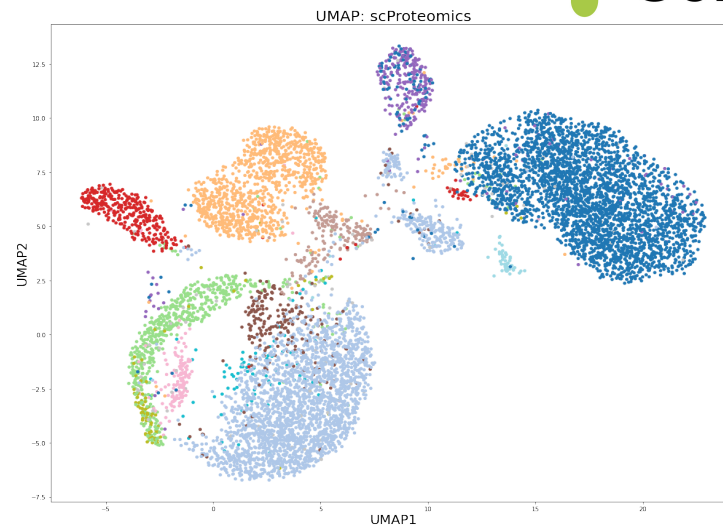
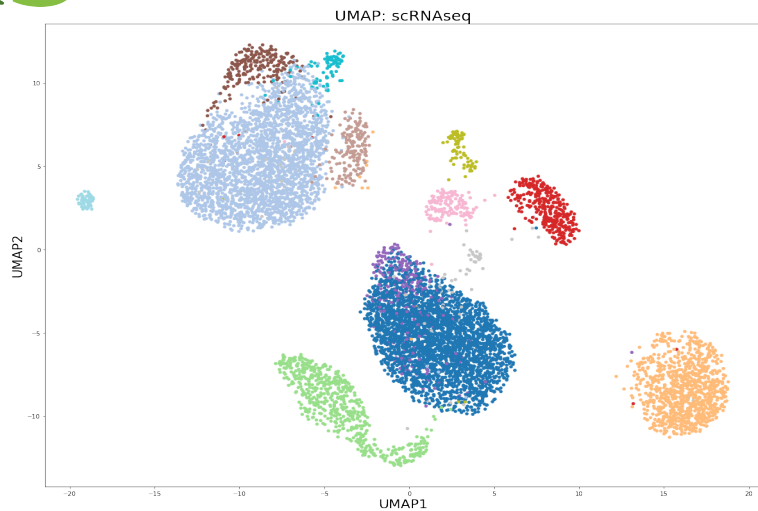
Keep edges consistently present across the Omics

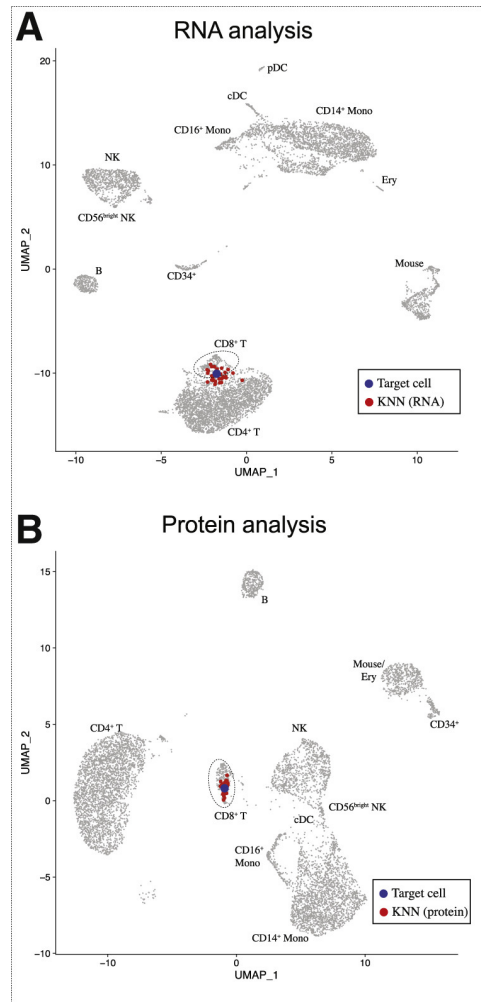
Graph Intersection

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Wolfram Notebook

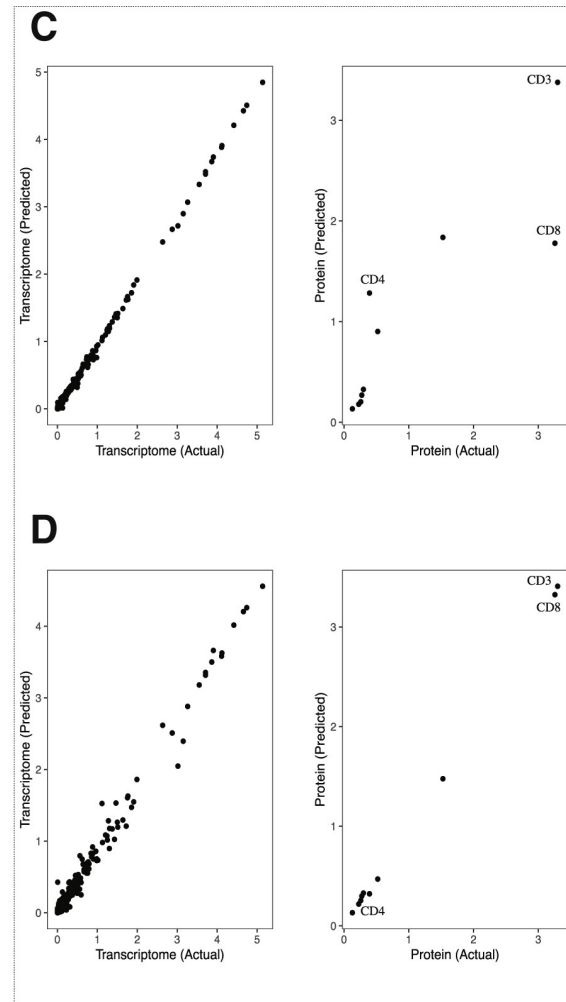


Let S be a set and $F = \{S_1, \dots, S_p\}$ a nonempty family of distinct nonempty subsets of S whose union is $\bigcup_{i=1}^p S_i = S$. The intersection graph of F is denoted $\Omega(F)$ and defined by $V(\Omega(F)) = F$, with S_i and S_j adjacent whenever $i \neq j$ and $S_i \cap S_j \neq \emptyset$. Then a graph G is an intersection graph on S if there exists a family F of subsets for which G and $\Omega(F)$ are isomorphic graphs (Harary 1994, p. 19). Graph intersections can be computed in the [Wolfram Language](#) using `GraphIntersection[g, h]`.

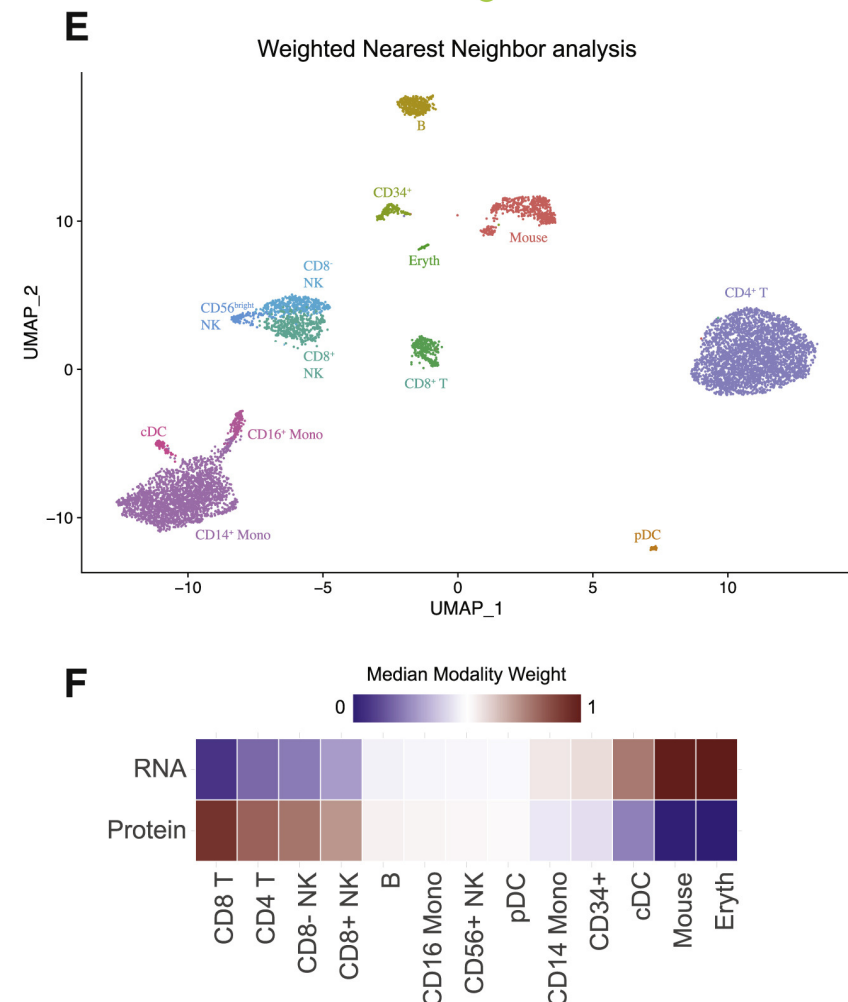


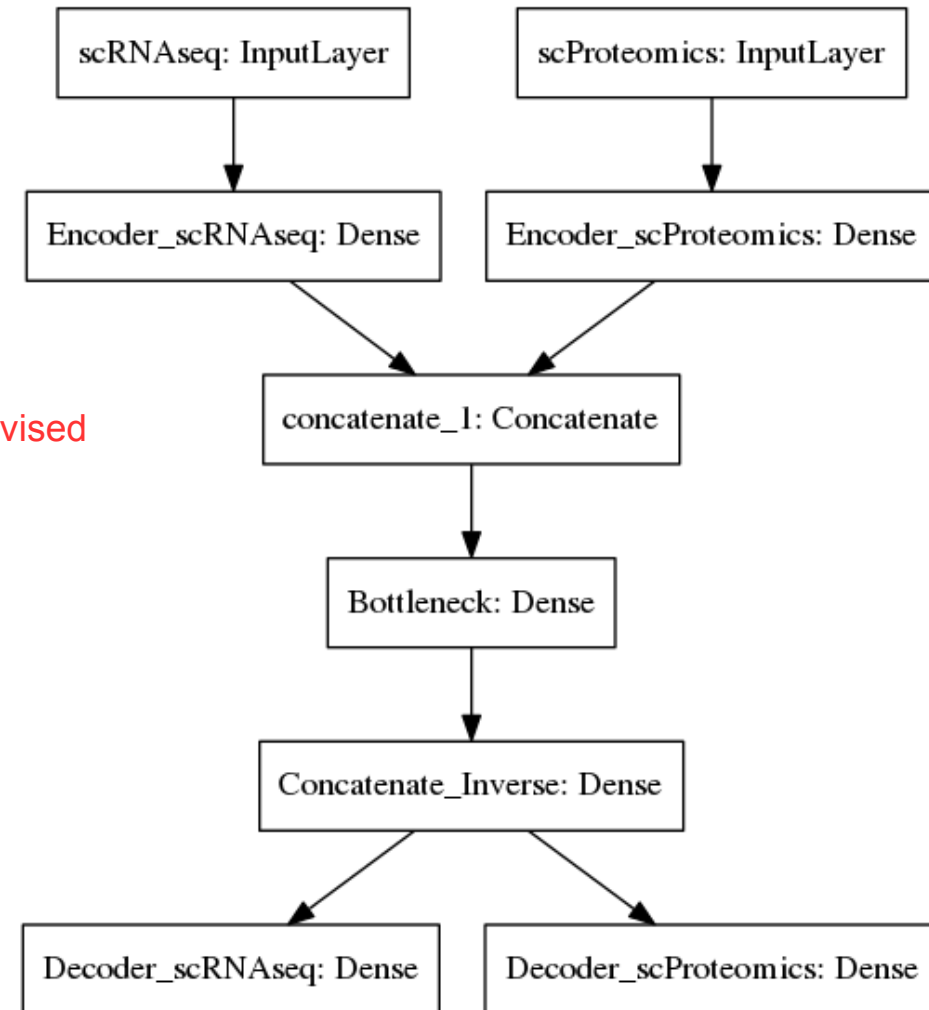
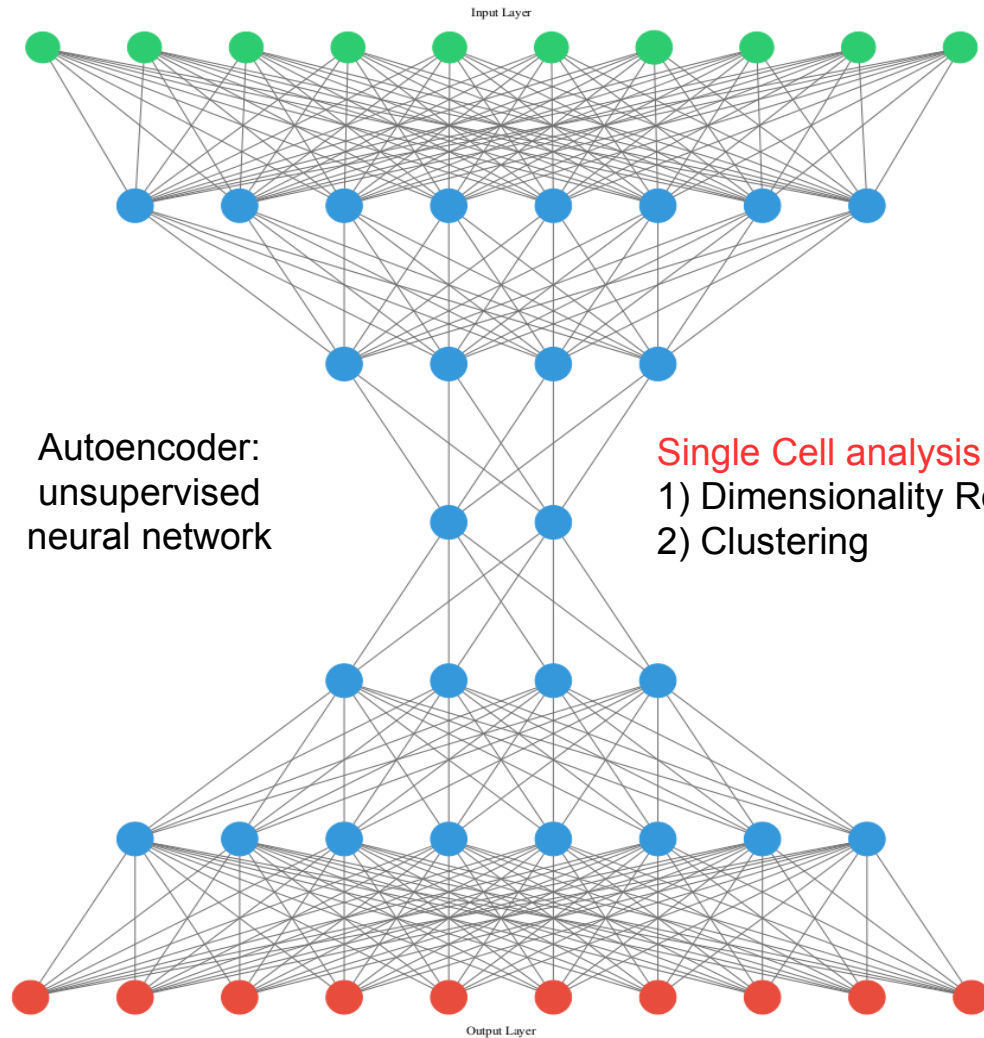


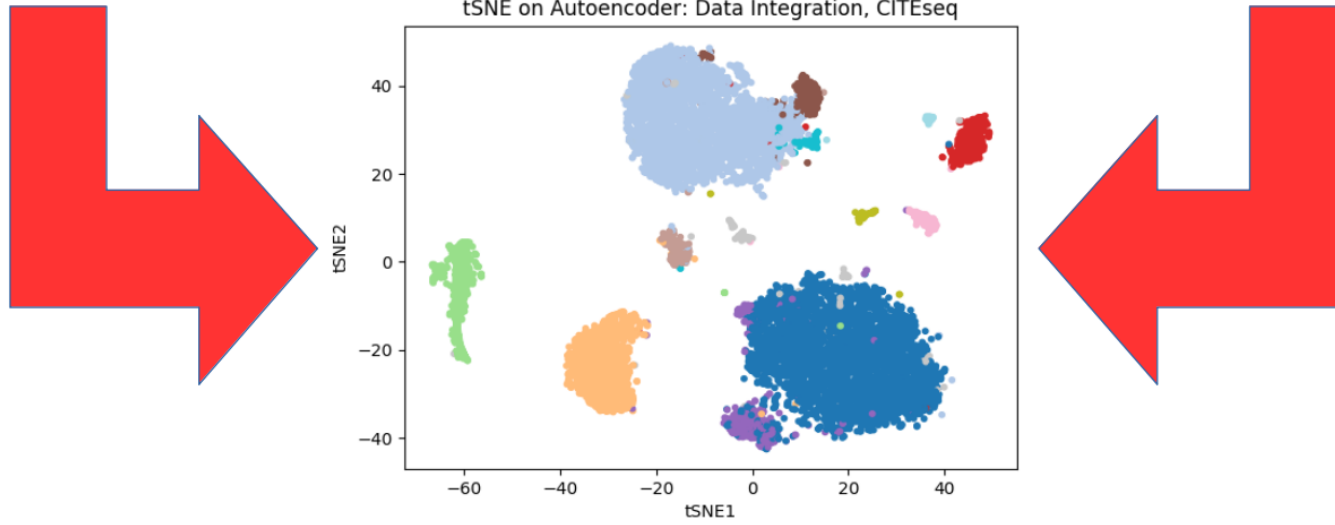
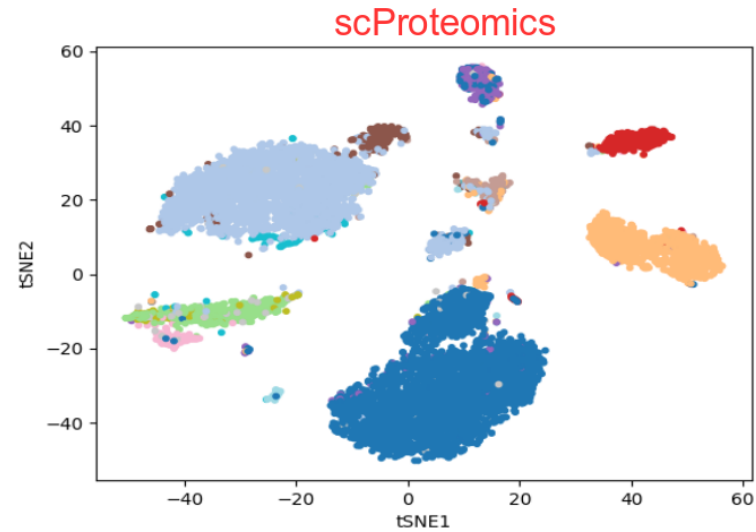
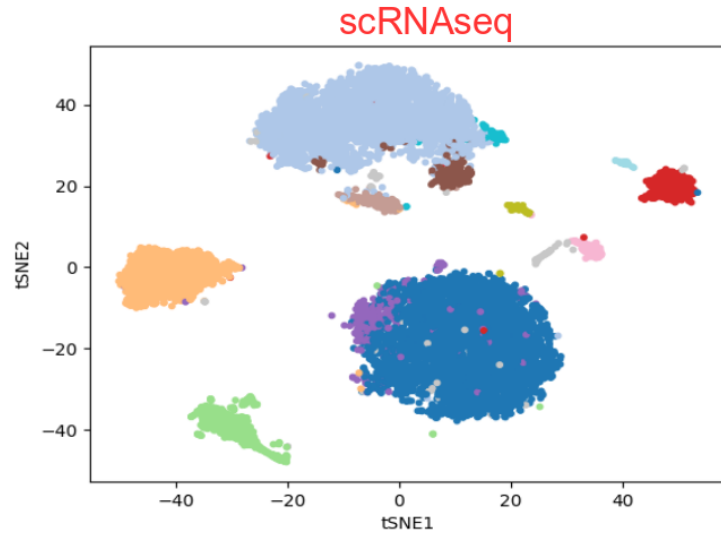
Predict cell from
RNA neighbors



Predict cell from
protein neighbors







Deep generative modeling for single-cell transcriptomics

Romain Lopez¹, Jeffrey Regier¹, Michael B. Cole², Michael I. Jordan^{1,3} and Nir Yosef^{1,4,5*}

Single-cell transcriptome measurements can reveal unexplored biological diversity, but they suffer from technical noise and bias that must be modeled to account for the resulting uncertainty in downstream analyses. Here we introduce single-cell variational inference (scVI), a ready-to-use scalable framework for the probabilistic representation and analysis of gene expression in single cells (<https://github.com/YosefLab/scVI>). scVI uses stochastic optimization and deep neural networks to aggregate information across similar cells and genes and to approximate the distributions that underlie observed expression values, while accounting for batch effects and limited sensitivity. We used scVI for a range of fundamental analysis tasks including batch correction, visualization, clustering, and differential expression, and achieved high accuracy for each task.

nature methods

Article

<https://doi.org/10.1038/s41592-024-02201-0>

scGPT: toward building a foundation model for single-cell multi-omics using generative AI

Received: 12 July 2023

Accepted: 30 January 2024

Published online: 26 February 2024

Check for updates

Haotian Cui^{1,2,3,8}, Chloe Wang^{1,2,3,8}, Hassaan Maan^{1,3,4}, Kuan Pang^{2,3}, Fengning Luo^{2,3}, Nan Duan⁵ & Bo Wang^{1,2,3,4,6,7}✉

Generative pretrained models have achieved remarkable success in various domains such as language and computer vision. Specifically, the



ARTICLE

DOI: [10.1038/s41467-018-04368-5](https://doi.org/10.1038/s41467-018-04368-5)

OPEN

Interpretable dimensionality reduction of single cell transcriptome data with deep generative models

Jiarui Ding^{1,2,3,4}, Anne Condon¹ & Sohrab P. Shah^{1,2,3,5}

Huang et al. *Genome Biology* (2023) 24:259
<https://doi.org/10.1186/s13059-023-03100-x>

Genome Biology

RESEARCH

Open Access

Evaluation of deep learning-based feature selection for single-cell RNA sequencing data analysis

Hao Huang^{1,2,3}, Chunlei Liu^{1,3}, Manoj M. Wagle^{1,2,3} and Pengyi Yang^{1,2,3,4*}✉

Biomedicine & Pharmacotherapy 165 (2023) 115077



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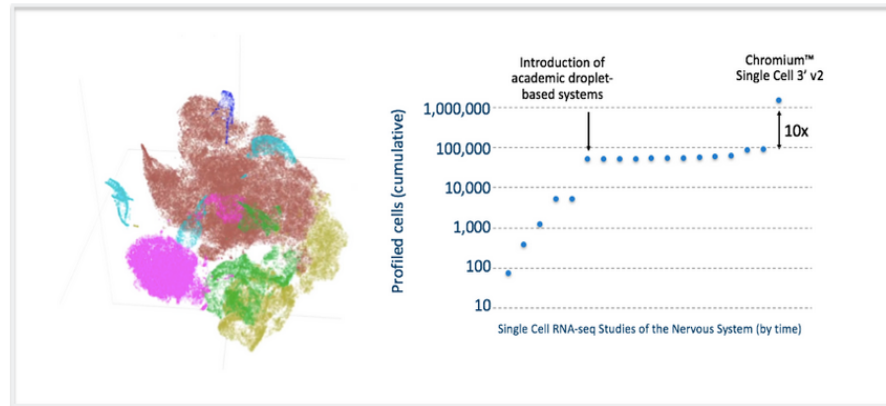
Review

Deep learning applications in single-cell genomics and transcriptomics data analysis

Nafiseh Erfanian^a, A. Ali Heydari^{b,c}, Adib Miraki Feriz^a, Pablo Iañez^d, Afshin Derakhshani^e, Mohammad Ghasemigol^f, Mohsen Farahpour^g, Seyyed Mohammad Razavi^g, Saeed Nasserhi^h, Hossein Safarpour^{h,i}, Amirhossein Sahebkar^{i,j,k,l,m,n,*}

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Our 1.3 million single cell dataset is ready to download



POSTED BY: [grace-10x](#) on Feb 21, 2017 at 2:28 PM

At ASHG last year, we announced our 1.3 Million Brain Cell Dataset, which is, to date, the largest dataset published in the single cell RNA-sequencing (scRNA-seq) field. Using the Chromium™ Single Cell 3' Solution (v2 Chemistry), we were able to sequence and profile 1,308,421 individual cells from embryonic mice brains. Read more in our application note [Transcriptional Profiling of 1.3 Million Brain Cells with the Chromium™ Single Cell 3' Solution](#).



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Current Opinion in
Systems Biology

Single cells make big data: New challenges and opportunities in transcriptomics

Philipp Angerer¹, Lukas Simon¹, Sophie Tritschler¹,
F. Alexander Wolf¹, David Fischer¹ and Fabian J. Theis^{1,2}

Abstract

Recent technological advances have enabled unprecedented insight into transcriptomics at the level of single cells. Single cell transcriptomics enables the measurement of transcriptomic information of thousands of single cells in a single experiment. The volume and complexity of resulting data make it a paradigm of big data. Consequently, the field is presented with new scientific and, in particular, analytical challenges where currently no scalable solutions exist. At the same time, exciting opportunities arise from increased resolution of single-cell RNA sequencing data and improved statistical power of ever growing datasets. Big single cell RNA sequencing data promises valuable insights into cellular heterogeneity which may significantly improve our understanding of biology and human disease. This review focuses on single cell transcriptomics and highlights the inherent opportunities and challenges in the context of big data analytics.

Addresses

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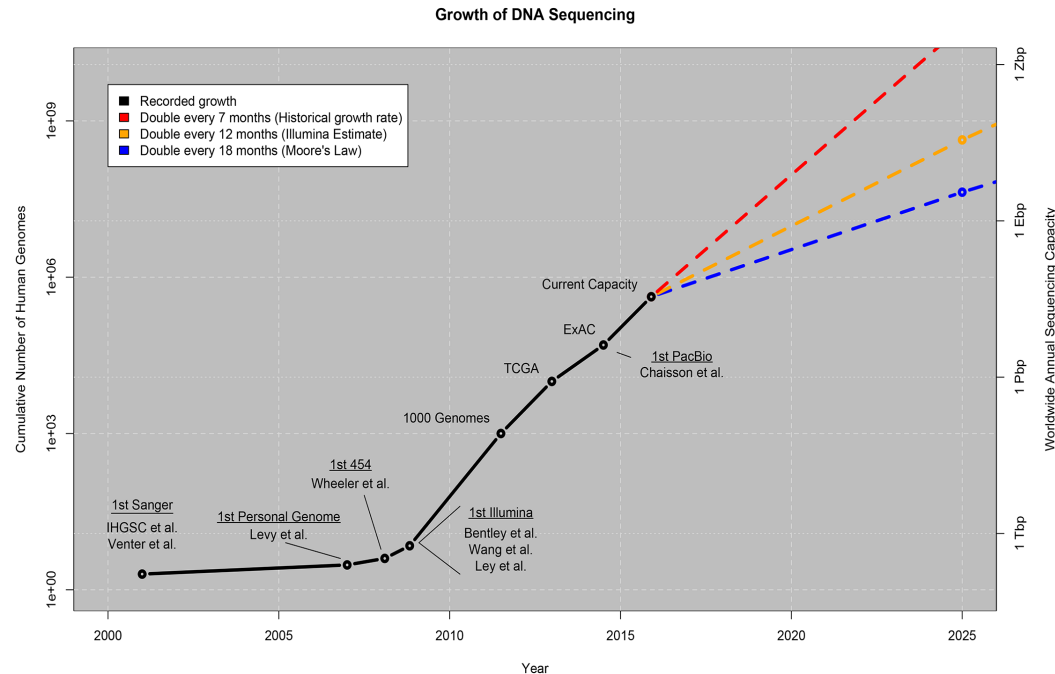
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volume — the amount of data, velocity — the required processing speed, veracity — trustworthiness and availability, and variety — necessary model complexity [2]. The traditional scientific big data field is astronomy because of the huge *volume* of image data produced by telescopes with a high daily *velocity* [3]. Big data has also reached biology, mainly driven through the advent of next generation sequencing technology. For biologists, assessing *veracity* through statistical means is nothing new.

Recent technological advances now allow the profiling of single cells at a *variety* of omic layers (genomes, epigenomes, transcriptomes and proteomes) at an unprecedented level of resolution [4]. Single cell transcriptomics (SCT) entails the profiling of all messenger RNAs present in a single cell and constitutes the most widely-used sc profiling technology [4]. Unlike bulk RNA-seq profiling where sequencing libraries are generated from thousands of cells, scRNA-seq technologies isolate single cells and generate cell-specific sequencing libraries (e.g. Fluidigm [5]) mark RNA content with a cell-specific molecular barcode [6–9]. Both approaches generate gene expression estimates at the single cell level [10]. SCT enables, for the first time, the measurement of the transcriptomic information of thousands, and up to millions of single cells, in a single experiment [7]. The complexity of SCT data coupled with the massive volume inherent to next generation sequencing data makes it a paradigm of big data.

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This review comes from a themed issue on **Big data acquisition and**



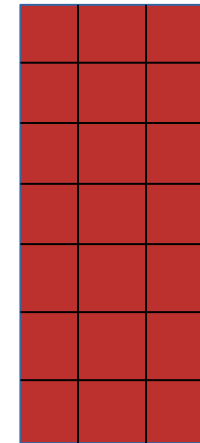
Stephens et al., (2015). Big Data: Astronomical or Genomical? PLoS Biology 13(7)

Potential Big Data in Life Sciences:

- Microscopy imaging (well known, AI widely used)
- Single cell Omics (novel type of data for AI)
- Metagenomics (possibly, not high-dimensional)
- Genomics (possibly, sequence is an observation)
- Epidemiology (population level data)

Genomics / WGS: Little Data

$$N_1 \sim 10^3$$

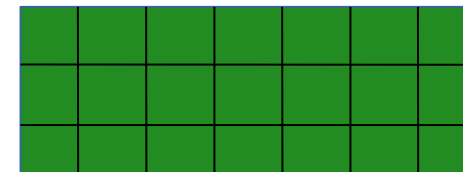


$$P_1 \sim 10^6$$

$$N_1 * P_1 = N_2 * P_2 = 10^9$$

scRNAseq: Big Data

$$N_2 \sim 10^6$$



$$P_2 \sim 10^3$$



*Knut och Alice
Wallenbergs
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