STAT 35510
Lecture 8

Spring, 2024

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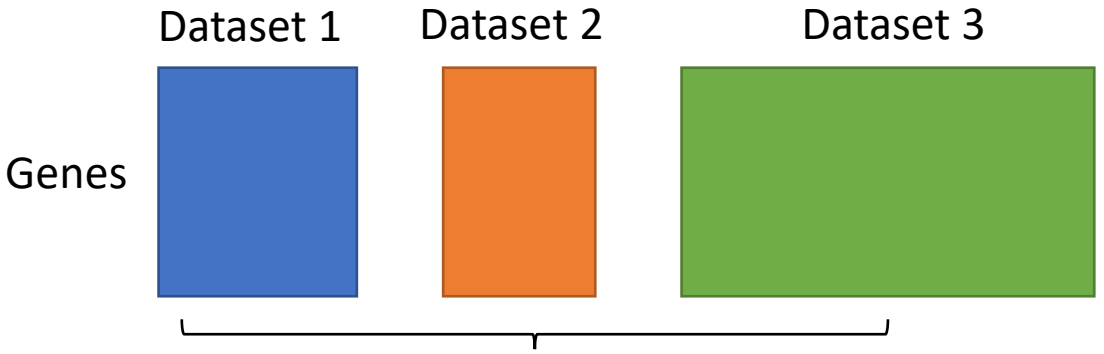
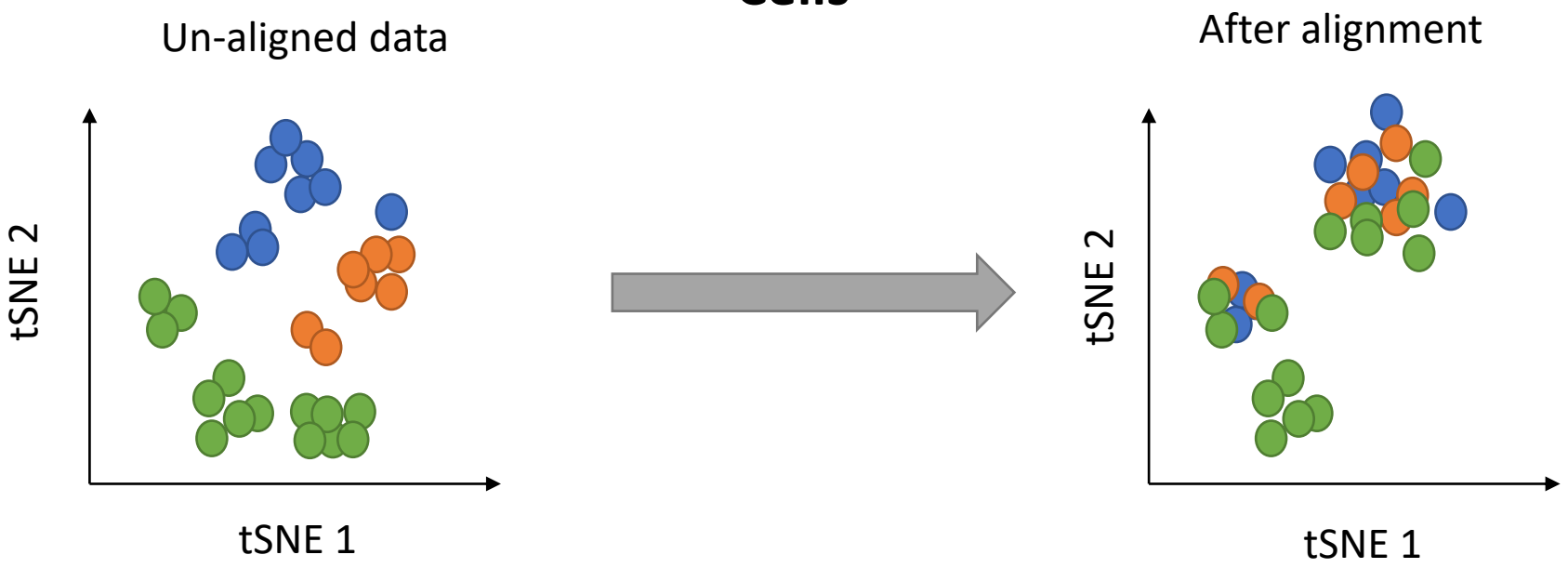
Outline

- scRNA-seq data integration and batch correction
  - Three types of integration for single-cell multi-omics data
  - Factor model-based methods
    - Linear models
    - Variational autoencoders
  - Cell-similarity based methods
  - Comparison between different methods

What is data integration/alignment?



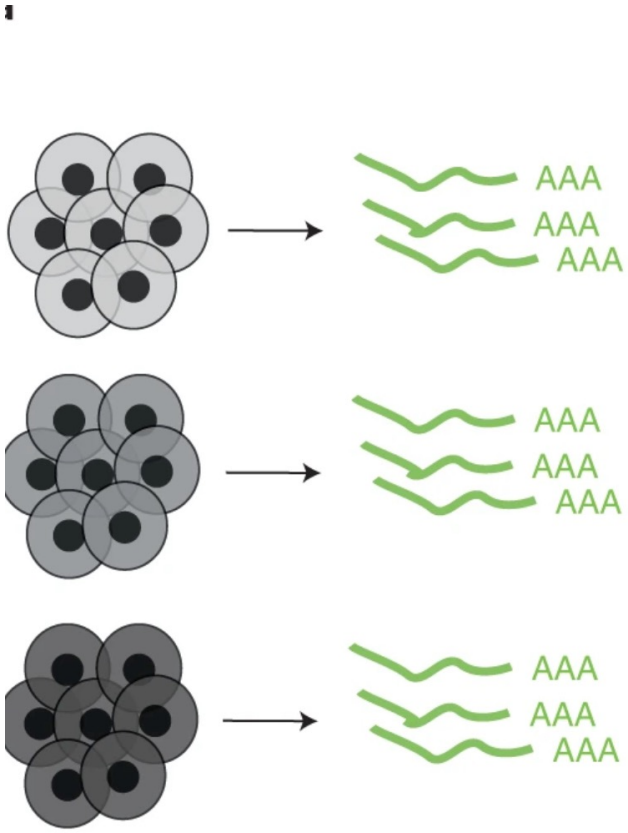


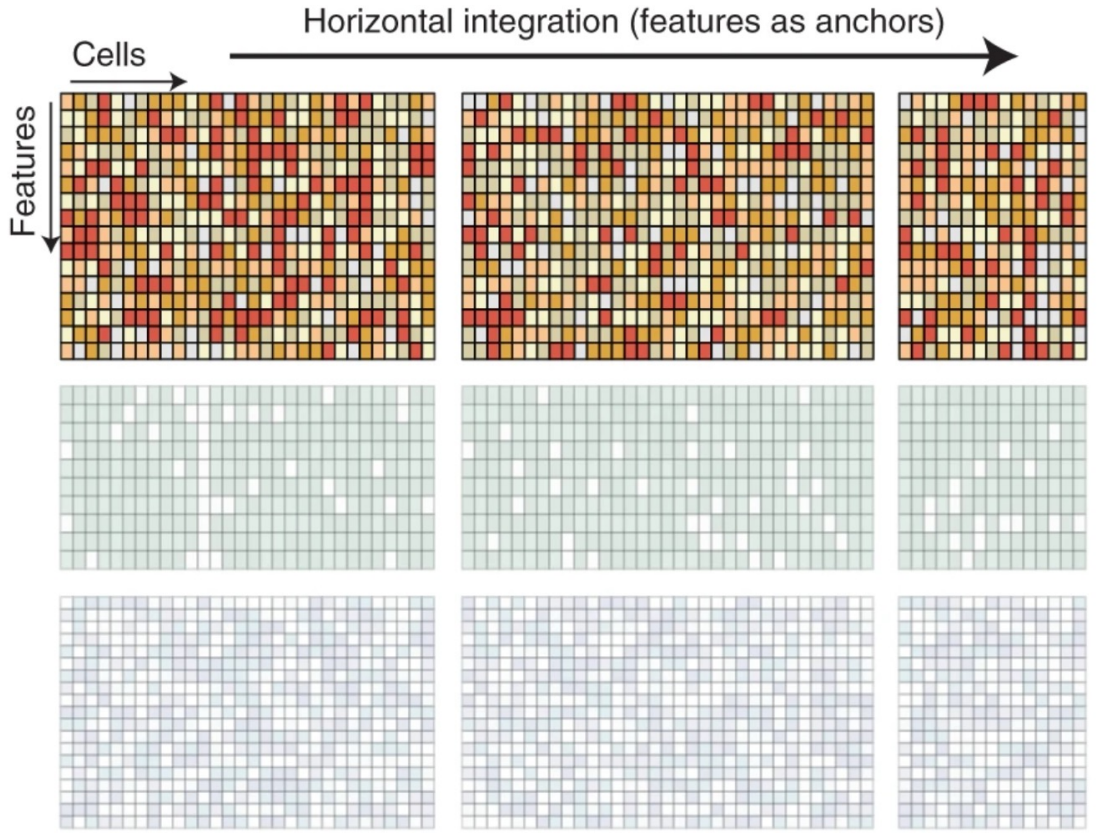
- Data integration may serve as the first step before any down-stream analyses
  - Double dipping: cells are not longer independent anymore after integration
  - Observations are no longer counts, or only obtain low-dimensional features

Three types of data integration for single-cell multiomics data

[Argelaguet et. al., Nature Biotech 2021]

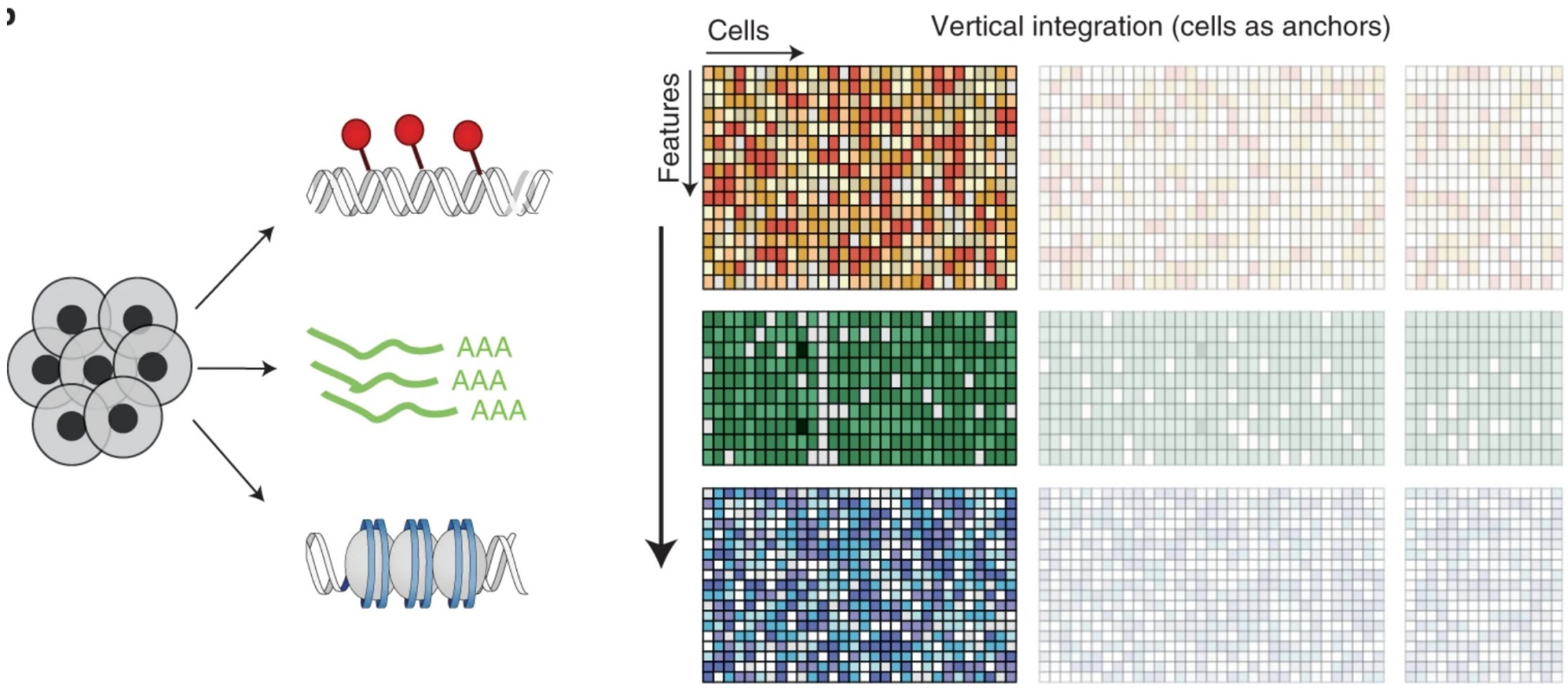
- Same sets of features, different datasets
  - Main challenge: batch effects





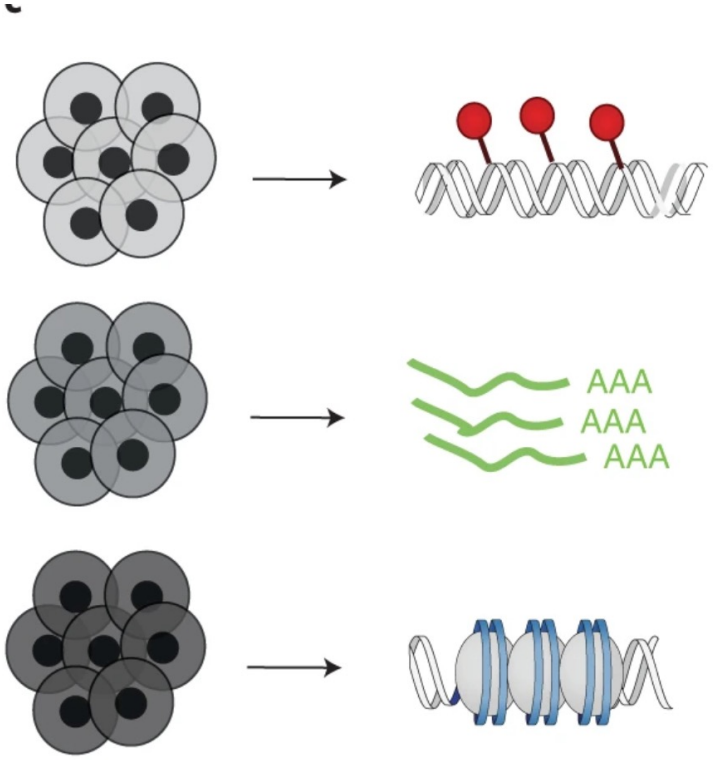
Three types of data integration for single-cell multiomics data

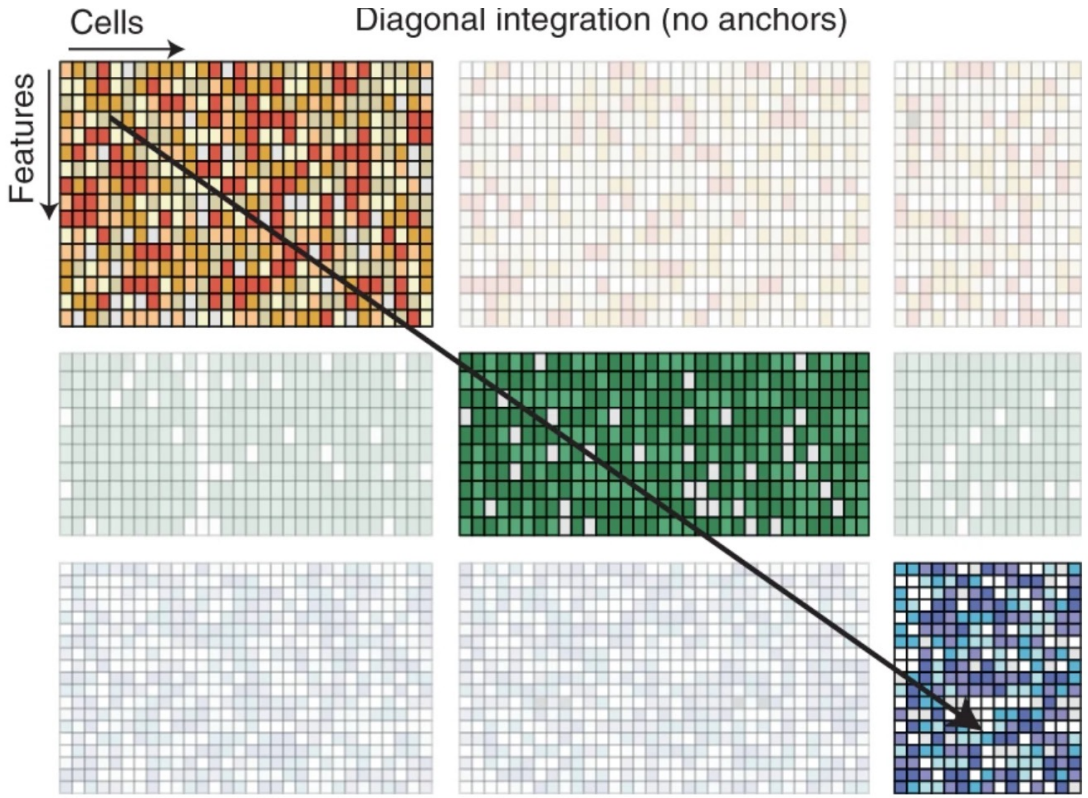
- Same cell, different types of features (multimodal data)
  - Combine different types of features to understand cell-cell similarity
  - Missing modality in some datasets



Three types of data integration for single-cell multiomics data

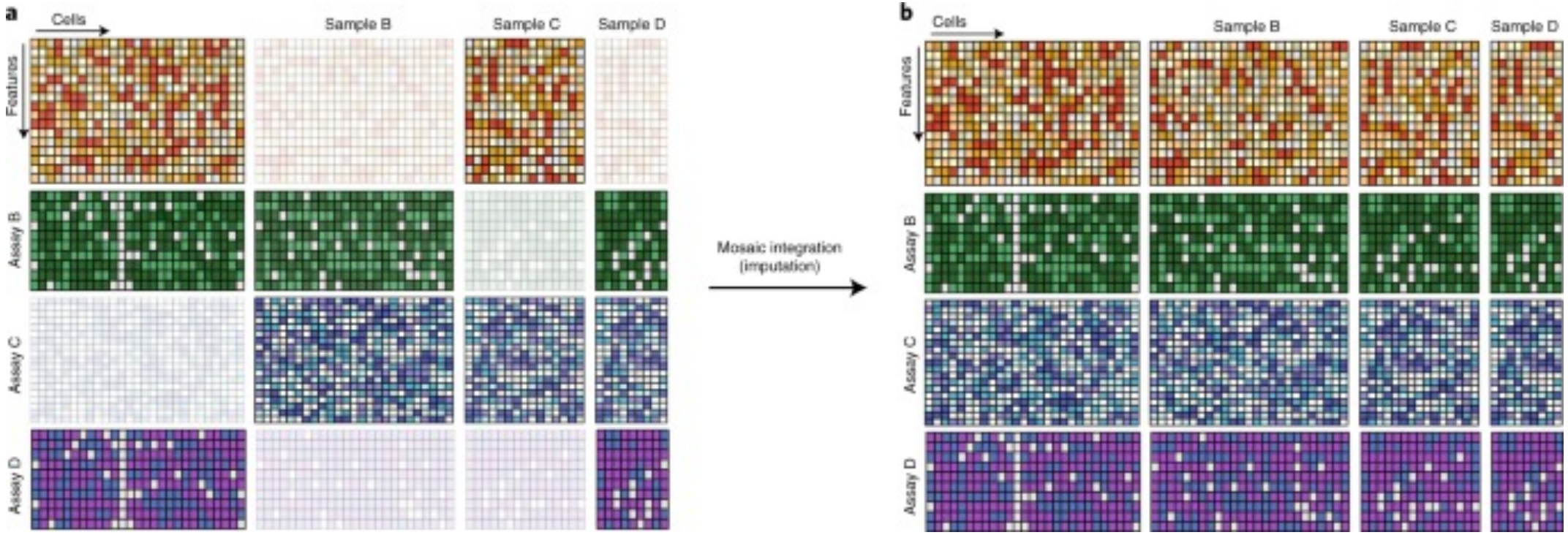
- Different cells, different types of features
  - What is the basis for integration?
    - Extra information about feature connections
    - Use subset of cells with overlapping features as “bridges”





Three types of data integration for single-cell multiomics data

- Mosaic integration between the second and third types



Integration for scRNA-seq data = batch correction?

Un-alignment between datasets

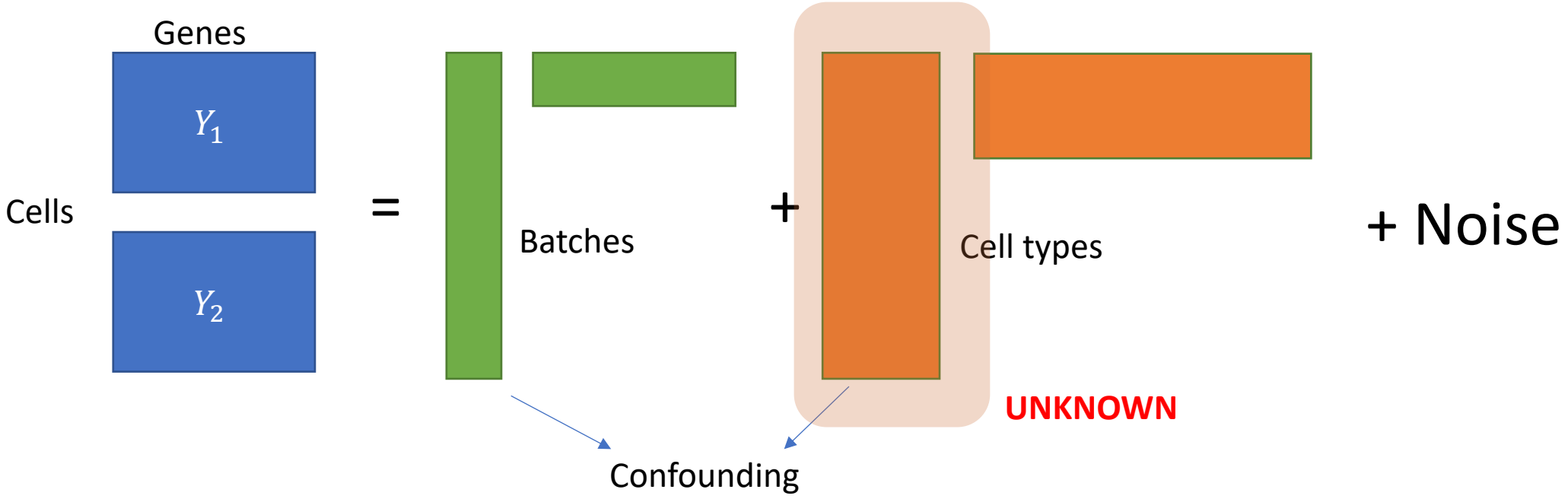
- Biological differences:
  - Different cell population (tissue, individual, species)
  - Different cell types
- Technical differences:
  - batch effects
  - different sequencing depth

- Jointly analyze of multiple datasets
  - Remove batch effects
  - Remove unwanted/not interesting biological differences
    ‘uninteresting’ differences between individuals, species
  - Keep meaningful biological difference between datasets (such as new cell type or true differential expression of cell type marker genes between conditions)

- Challenge: “unknown” Confounding between batches and cell types

Unsupervised Batch Effect Removal

- Confounding between batches and unknown cell types



- Batches can be confounded with other important biological signals

Batch correction with linear model: Limma (Ritchie et. al. , NAR 2015)

- The overall gene expression matrix (mean matrix): $\mu_g \times 1_n^T$
- Batch corrected data: $X - R_s D_s$
- Developed for bulk RNA-seq data where differential testing across conditions is the primary goal
- (D_c^T, D_s^T) needs to be full rank. For scRNA-seq, conditions and batches can be perfectly confounded
- Batches can also be confounded with cell types, trajectories in scRNA-seq

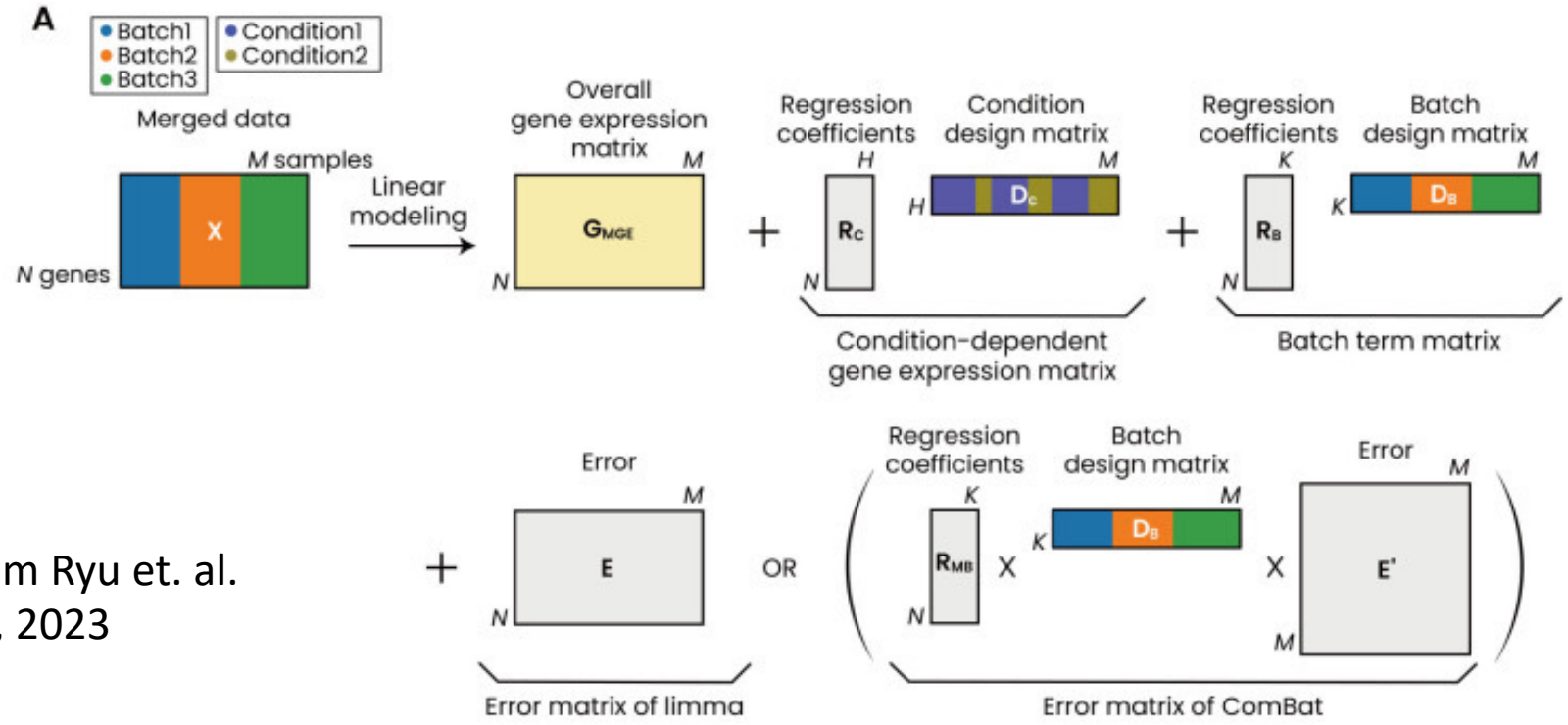


Figure from Ryu et. al.
Mol Cells, 2023

Challenges for batch correction in scRNA-seq

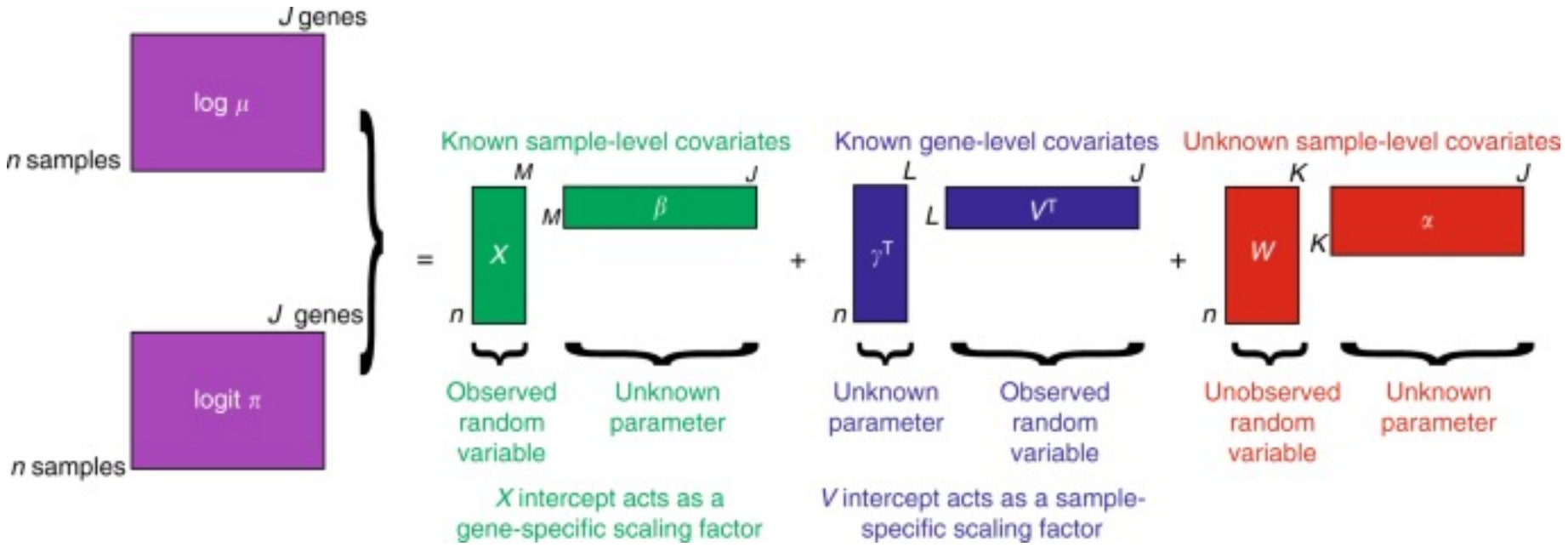
- Batch effects may not be linear

- If Batches are confounded with hidden factors of the data (like clustering structure), then batch effects are not identifiable
  - $Y_{\text{cells}*\text{genes}} = ZV^T + X_{\text{batch}}\beta + \text{error}$, β is not identifiable if the latent factors U and X_{batch} can be arbitrarily correlated

  - One possible identifiability condition: within the same cell type, cells are biologically homogenous across batches
    - If cell types are already known, what is the purpose of integration?
  - Another possible identifiability condition (implicitly assumed in many similarity based methods): $X_{\text{batch}}\beta$ is small compared to ZV^T, similar cells in batch 2 to a cell in batch 1 keep the same with/without batches

- Current batch correction methods tend to overcorrect batches effects (Argelaguet et. al., Nature Biotech 2021). Differential testing between conditions may tend to be conservative after correction

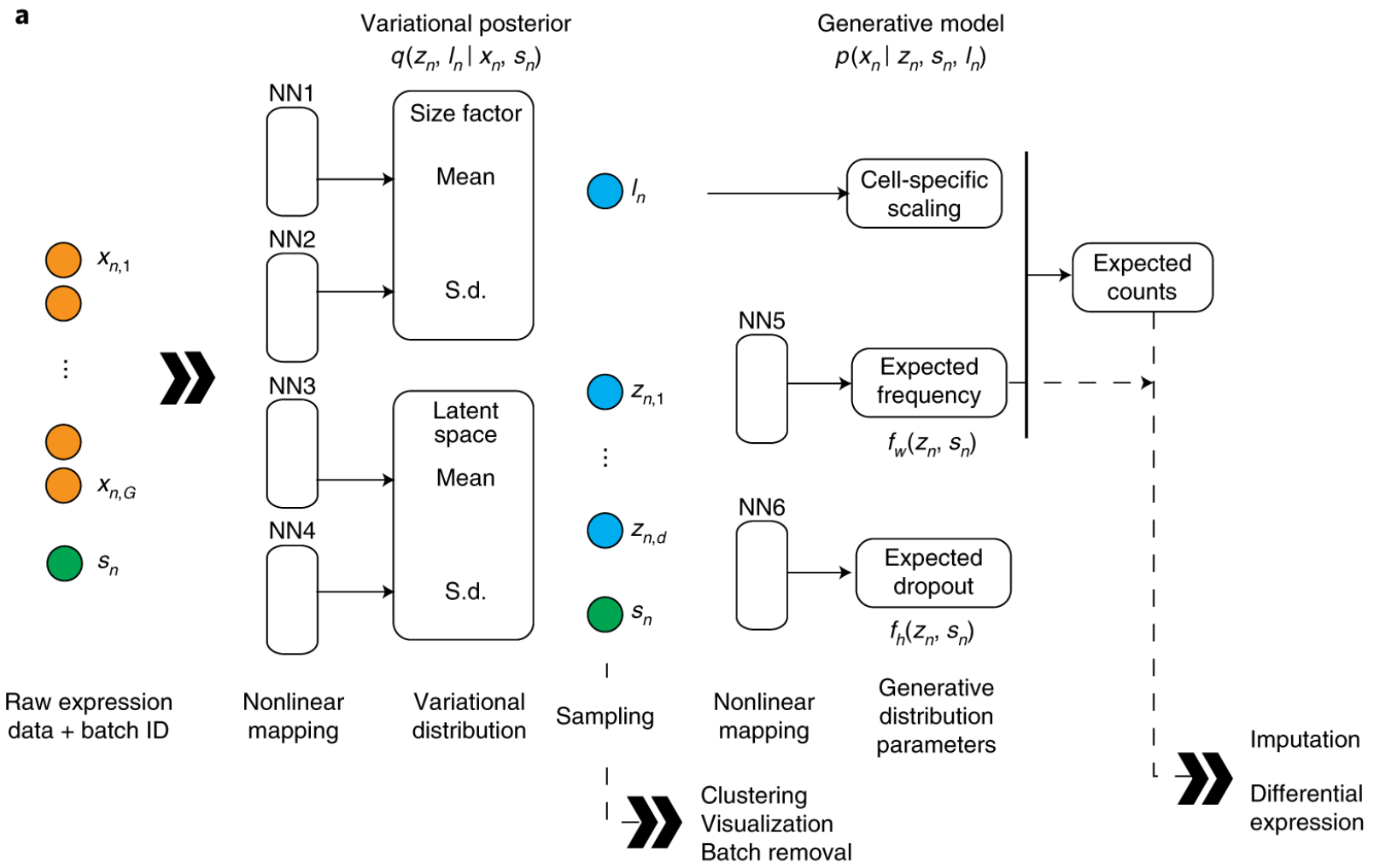
ZINB-WaVE (Risso et. al., Nature Comm 2018)



- The batches are sample-level covariates
- Gene-level covariates can include gene features such as gene length and GC content
- W is the batch adjusted latent representation of cells
- Implicitly assume that the latent factors and batches are uncorrelated
  - They used an L2 penalization on W in the loss function
  - The algorithm does not force latent factors and batches to be uncorrelated

- Assume that the observed counts follow ZINB model

scVI (Lopez et. al. Nature Methods 2018)



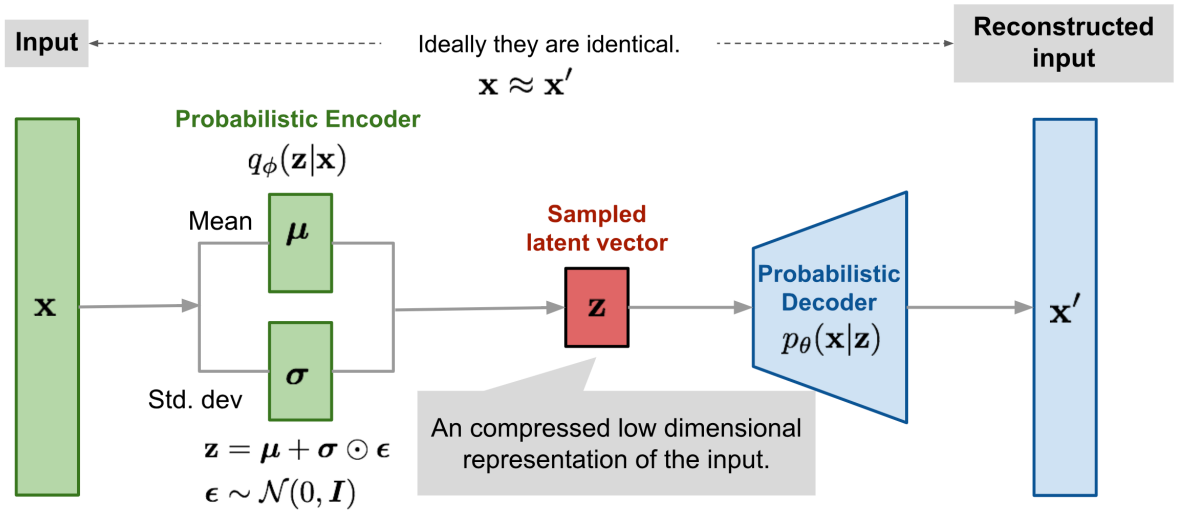
- Use variational autoencoder (next page)
- Main feature: add batch information as extra nodes in both the input and bottleneck layer

- Implicitly assumes that latent factors Z and X_{batch} are uncorrelated as X_{batch} is fixed and Z has prior $Z \sim N(0, I)$

- Under linear model $Y_{\text{cells}*\text{genes}} = UV^T + X_{\text{batch}}\beta + E$ estimated Z and X_{batch} are uncorrelated

- Estimated Z can be correlated with X_{batch} in scVI because of using VAE

Details of scVI model



Variational autoencoder

- Assume that the latent variables $Z \sim N(0, I)$
- Approximate the posterior of Z given input data by Gaussian distribution
- Encoder: posterior mean and variance of Z as non-linear functions of input data
- Decoder: non-linear mapping from Z to the observed data
- Generalization of linear probabilistic factor model to nonlinear probabilistic factor models

- scVI assumes a ZINB model on the observed data
  - Both posterior distributions of Z and mapping from Z to the observed data depend on the batches

Final output of scVI

- Use latent factors for visualization and clustering
- Use output layer for denoising (imputation)

$$
\begin{aligned}
z_n &\sim \text{Normal}\,(0, I) \\
\ell_n &\sim \text{log normal}\,\left(\ell_\mu, \ell_\sigma^2\right) \\
\rho_n &= f_w\left(z_n, s_n\right) \\
w_{ng} &\sim \text{Gamma}\,\left(\rho_n^g, \theta\right) \\
y_{ng} &\sim \text{Poisson}\,\left(\ell_n w_{ng}\right) \\
h_{ng} &\sim \text{Bernoulli}\,\left(f_h^g\left(z_n, s_n\right)\right) \\
x_{ng} &= \begin{cases} y_{ng} \,\text{if}\, h_{ng} = 0 \\ 0 \,\text{otherwise} \end{cases}
\end{aligned}
$$

scGen (Lotfollahi et. al. Nature Methods, 2019)

- Originally designed to perturbation prediction but can also be used for batch correction
- scGen also used VAE, not sure if batches are inputs in the VAE as in scVI
- Batch correction is done to each cell type separately
  - Requires cell type information as input data (may not be applicable in practice)
  - In the latent space, for each cell type, calculate

$$\delta = \text{avg}(z_{\text{condition}=1}) - \text{avg}(z_{\text{condition}=0})$$

    - Add δ to the corresponding latent vectors so cells within the same cell type are mixed
    - Get the corrected gene expression matrix
    - Sounds like applying Limma on the latent space for each cell type separately

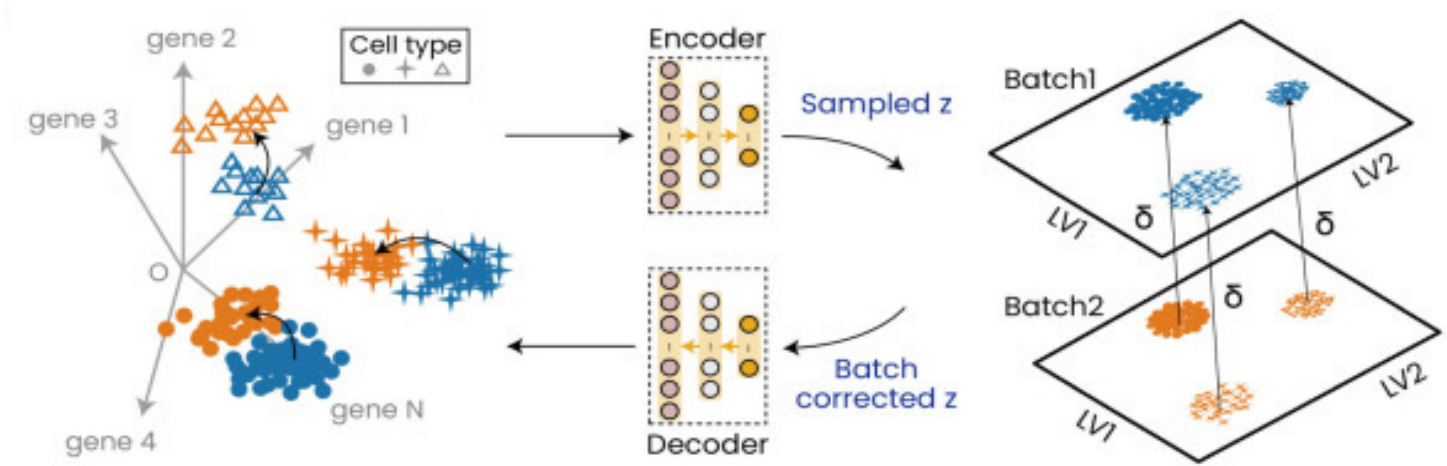


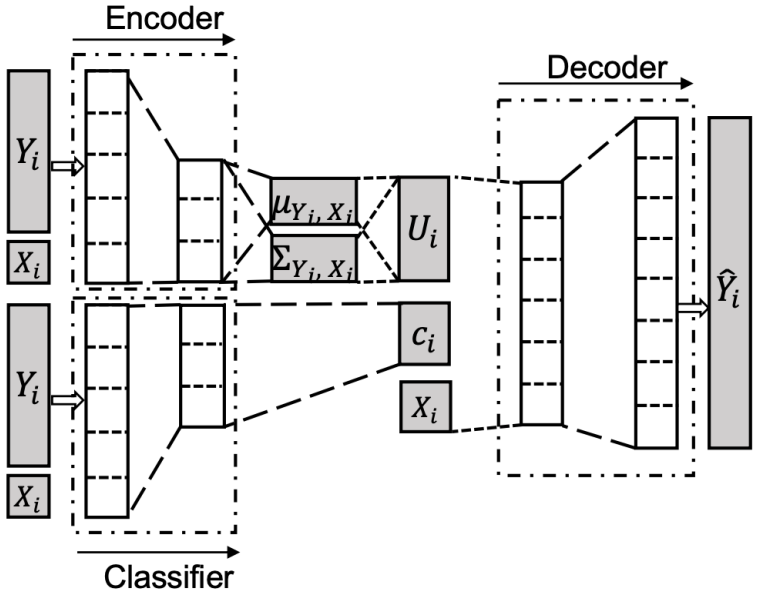
Figure from Ryu et. al. Mol Cells, 2023

scANVI (Xu et. al. Mol Syst Biol, 2021)

- Perform automatic cell type annotation by integrating cells with cell type labels and cells without cell type labels

- Other assumptions are the same as scVI
  - More complicated encoders for representing posterior distribution of the latent space
    - Cells without labels: posterior distribution of Z given observed data and batch
    - Cells with labels: posterior distribution of Z given observed data, batch and cell type

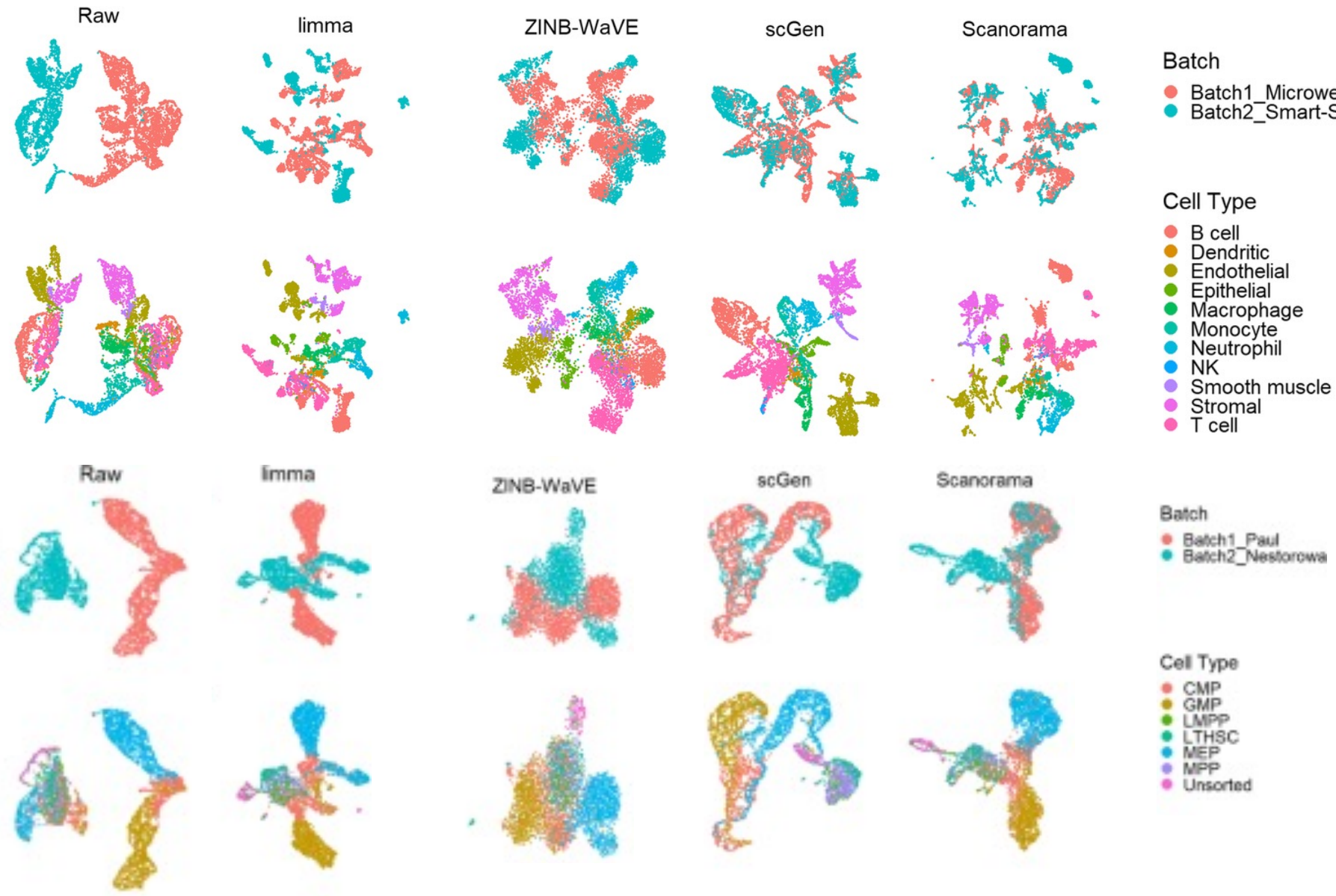
    - Specific algorithm and neural network design:
      - Details not provided, claim following Kingma et. al., NeulPS 2014
      - A guess of the neural network constructure based on (Kingma et. al., NeulPS 2014) model M2
- What if there are unseen cell types in the unlabeled data?
  - Common problem in automatic cell type annotation as discussed earlier



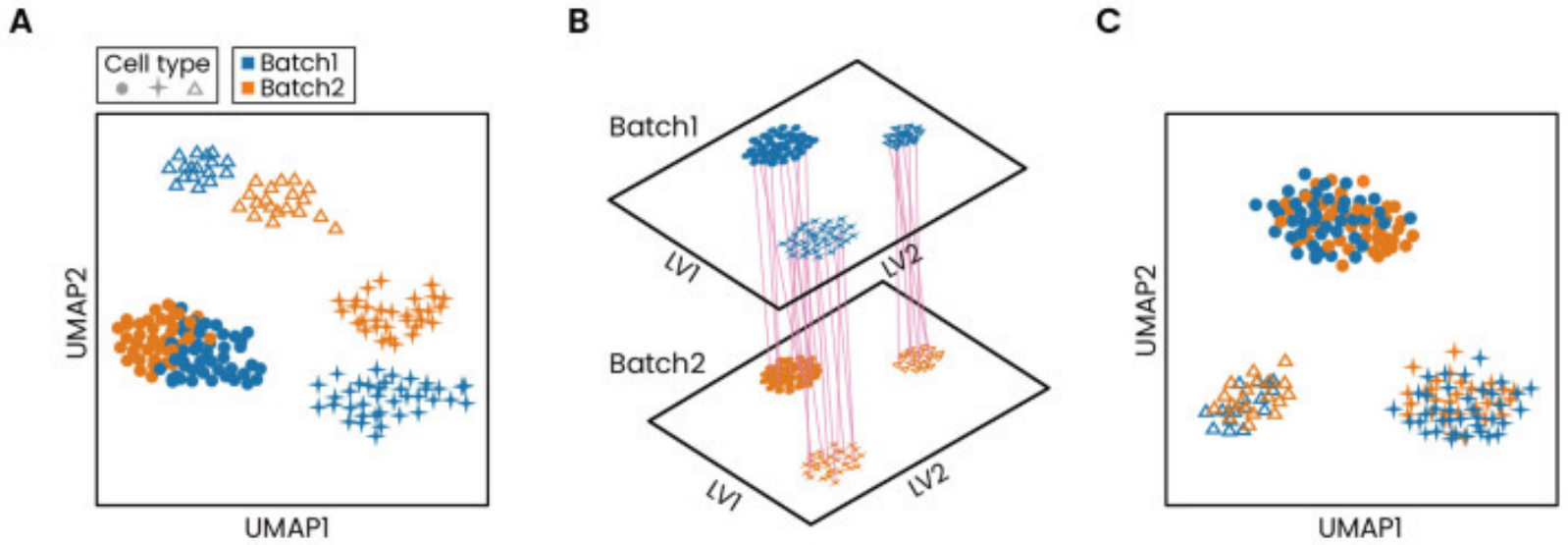
Some benchmarking results (Tran et. al. Genome Biology 2018)

Identical cell types, different technologies

Non identical cell types



Similarity-based batch correction methods



Common steps:

- Project the merged datasets onto a low-dimensional space
- Identify similar cells (pairs of cells) between batches
- Correction: correct batch effects so that cells pairs are together on the low-dimensional space

- Batch correction is only performed on low-dimensional space
  - Previous factor-model-based methods provide batch corrected gene expressions

MNNcorrect / fast MNN (Hadhverdi L. et. al., Nature Biotech, 2018)

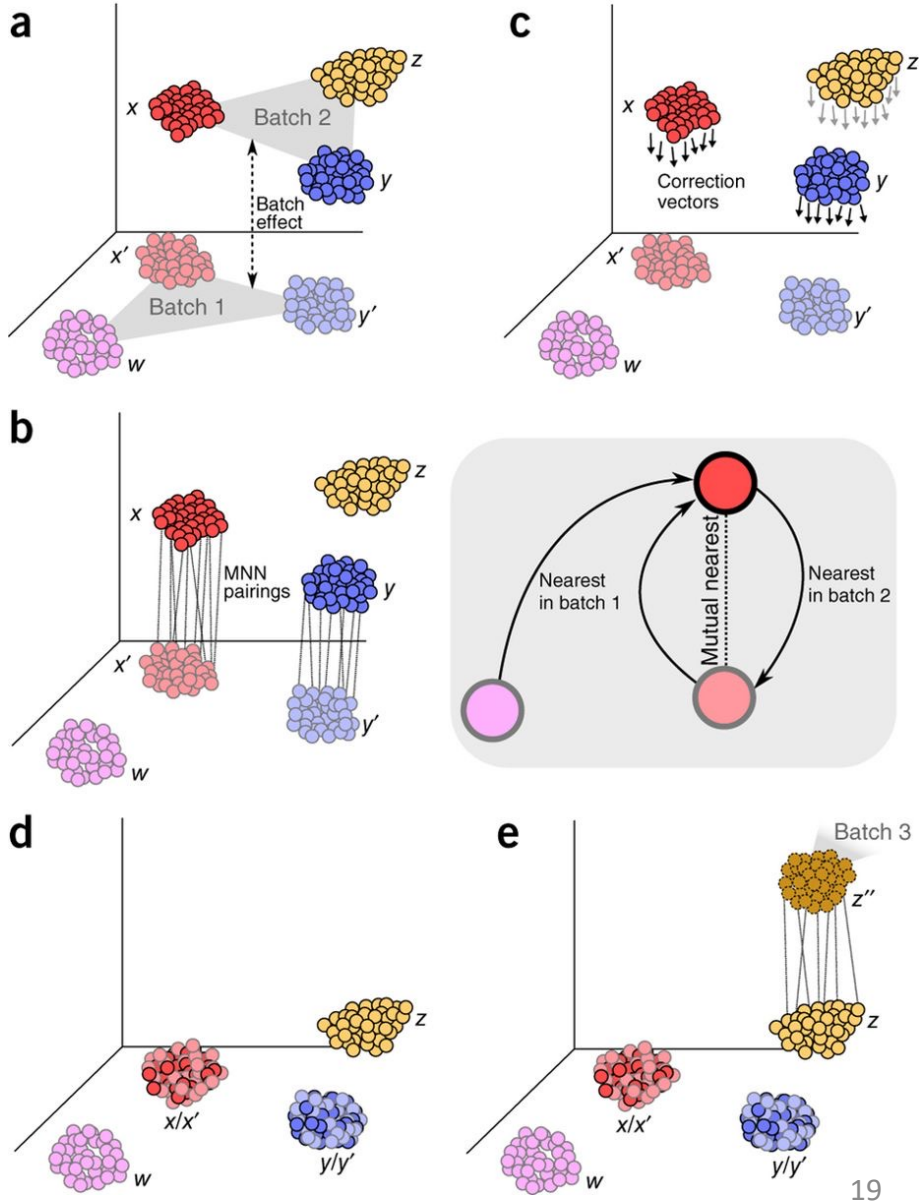
- Steps:

a) Measure cell similarity (Euclidean distance after normalization)

b) Find paired cells from two batches
   - Identify KNN of each cell in batch 1 (2) in the other batch 2 (1)
   - Keep the pair of cells if the they are both KNN of each other

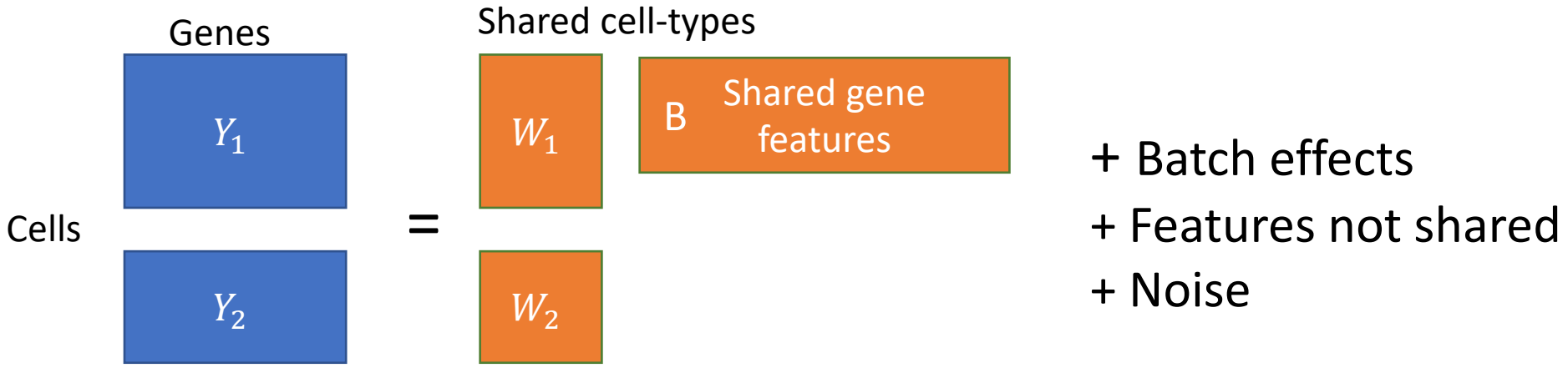
c) Batch correction:
   - Compute pair-specific differences
   - Use Gaussian kernel smoothing (weights) to compute the correction vector of each cell
     - The cell-specific correction vector is a weighted average of the pair-specific correction vectors

- Critical assumption
  - Batch effects are relatively small



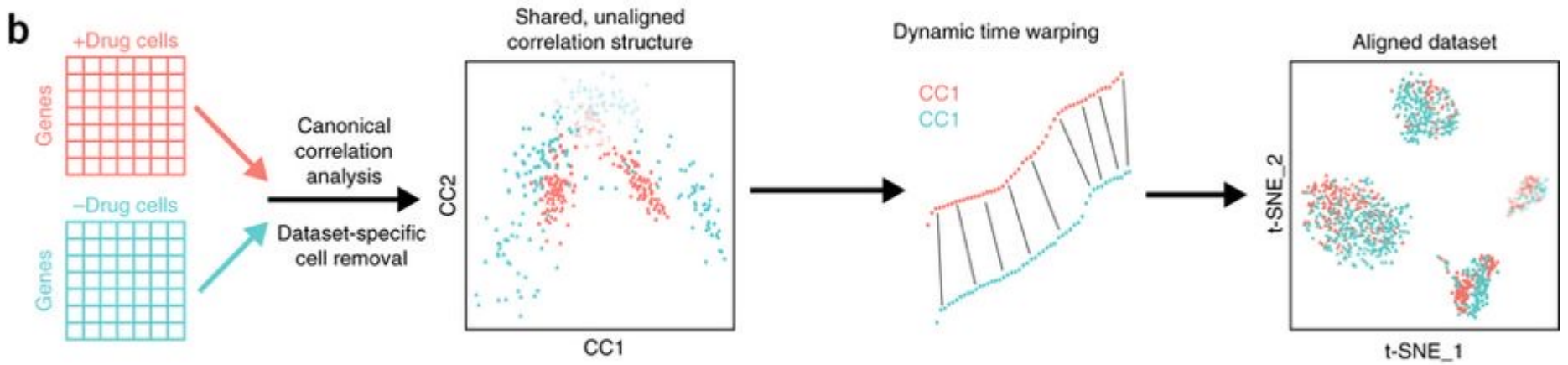
Use Canonical correlation analysis (CCA) for scRNA-seq alignment

- CCA: originally used to find best combination of two sets of variables that have largest correlation
  - For scRNA-seq, treat each cell as a “feature”, each gene as an “observation”
  - Compute weighted combination of cells within each batch so that the combined cells have best correlation between the two batches
  - Essentially solving SVD of $Y_1^T Y_2$
    - Left and right eigenvectors of $Y_1^T Y_2$ are estimates of W_1 and W_2
  - Treat CCA as a dimension reduction step that minimize the effect of batches



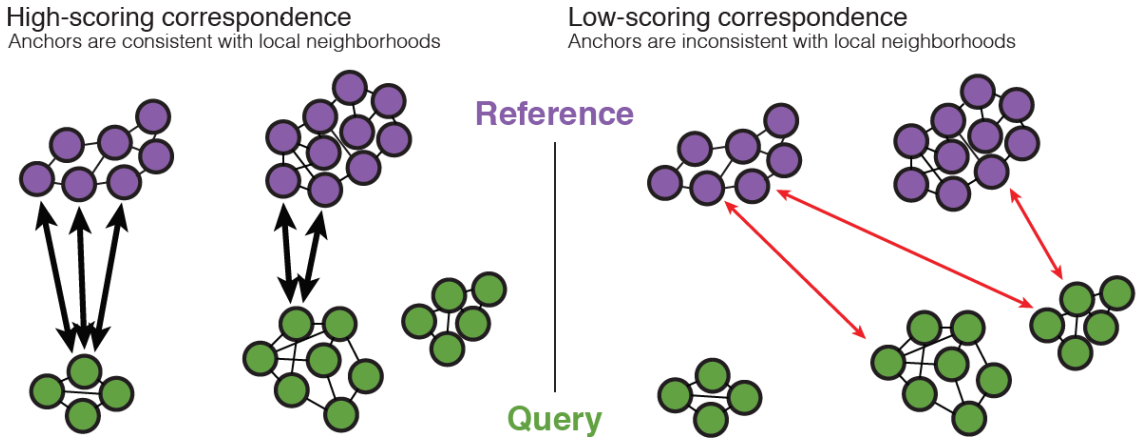
Seurat CCA

- MultiCCA (v1) (Butler et. al. Nature Biotech 2018) further uses dynamic time wrapping to further align the CC vectors to remove remaining batch effects



- They later have developed multiCCA (v2) which is hybrid between multiCCA (v1) and MNNcorrect

MultiCCA v2 (Stuart et. al. Cell, 2019)

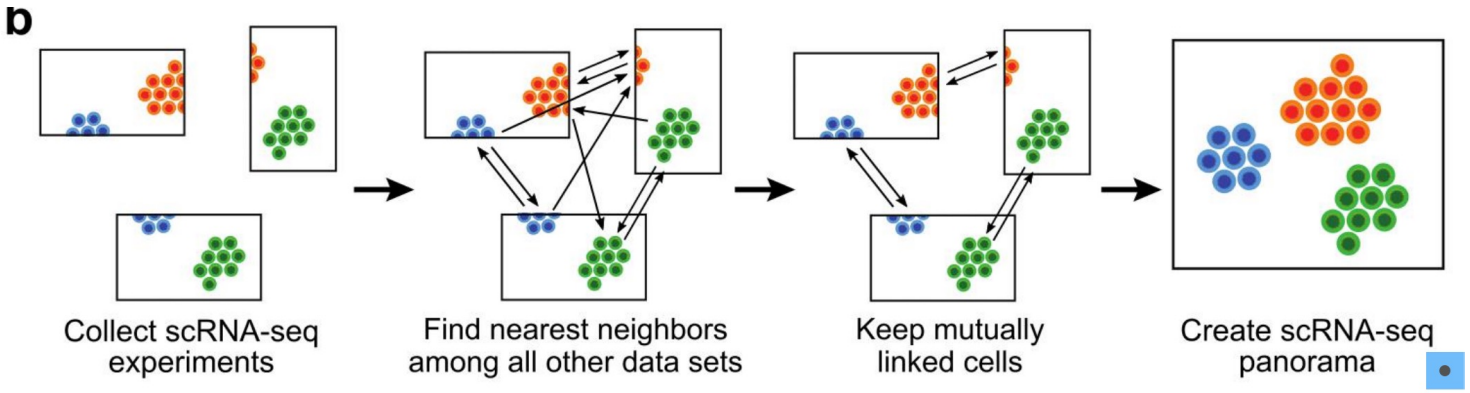


- Steps
  - CCA as in MultiCCA V1 to project both datasets into lower dimensions
    - PCA may amplify differences between two datasets and focus on variation directions that are unique to one dataset
  - Identify anchor cells using MNN
    - Give each cell an anchor score
      - Check MNN also in the original space to improve robustness
      - **Anchors scoring**: find consistency of KNNs within each dataset and with other datasets
      - **Anchor weighting W**: a matrix of anchors by cells in Y_2
        - Weights depend on cell-cell distance, only use k nearest anchors
      - **Alignment**: $\hat{Y}_2 = Y_2 + (Y_{1,\mathrm{A}} - Y_{2,A})W$
      - Multiple datasets: align sequentially
  - Label transfer and feature imputation

Scanorama (Hie et. al. , Nature Biotech 2019)

Main advantage: computationally fast MNN

- Find KNN of a cell in one dataset from all other datasets
  - To reduce computational cost in finding KNN by approximation with random projection trees to make computational cost less than $O(kn)$
- Anchor cells: keep a pair of cells if they are KNN to each other
  - Computational cost reduce from $O(k^2 n_1 n_2)$ to $O(k \min(n_1, n_2))$
- Batch correction from anchors using Gaussian kernel smoothing weights same as MNNcorrect/fastMNN
- Scanorama performs better than MNNcorrect/fastMNN in benchmarking studies
  - Only methodological difference between Scanorama and MNNcorrect/fastMNN seems to be the dimension reduction first step before finding KNN



Harmony (Korsunsky et. al. Nature Methods, 2019)

Steps

- PCA
- Iteratively perform
  - Soft k-means clustering
    - Penalize clusters that has less batch diversity

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

$$\text{s.t.} \; \forall_i \forall_k \; R_{ki} > 0, \forall_i \sum_{k=1}^{K} R_{ki} = 1$$

$O \in [0,1]^{K \times B}$ The observed co-occurence matrix of cells in clusters (rows) and batches (columns).

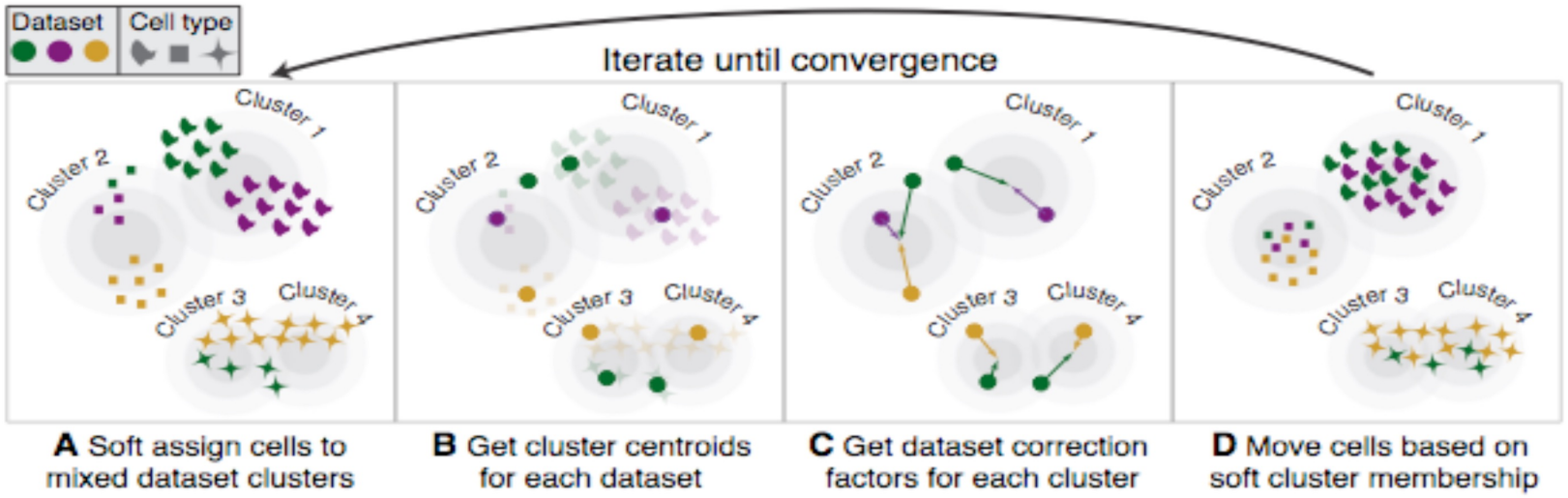
$E \in [0,1]^{K \times B}$ The expected co-occurence matrix of cells in clusters and batches, under the assumption of independence between cluster and batch assignment.

$Y \in [0,1]^{d \times K}$ Cluster centroid locations in the *k*-means clustering algorithm.

Harmony (Korsunsky et. al. Nature Methods, 2019)

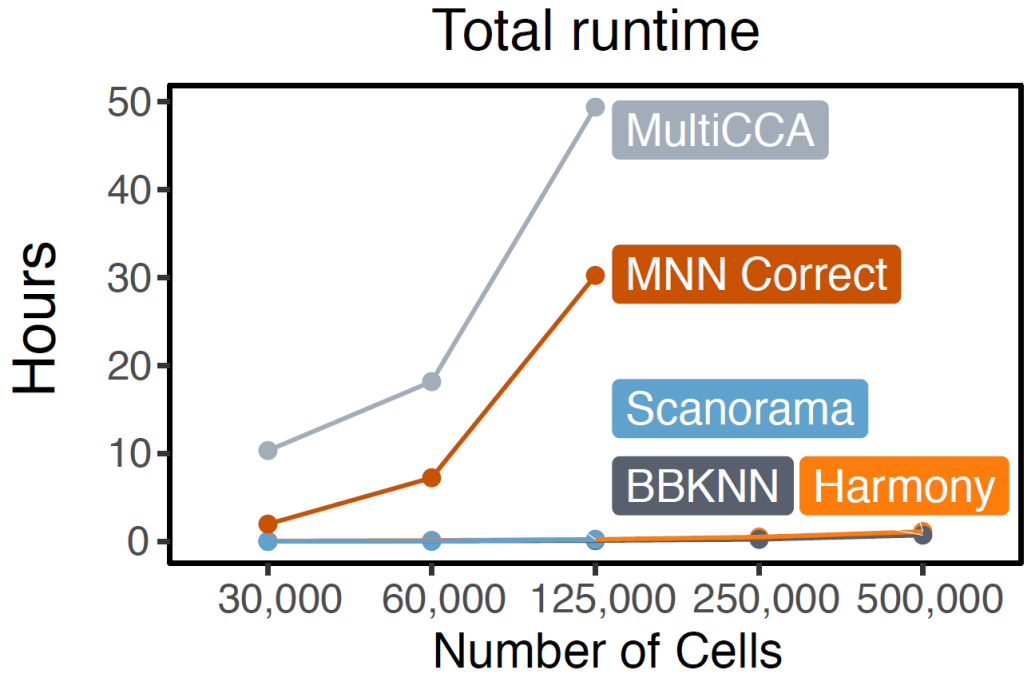
Steps

- PCA
- Iteratively perform
  - Soft k-means clustering
    - Penalize clusters that has less batch diversity
    - Goal: make cells of the same cell type in each cluster
  - Mixture of experts model for correction
    - Compute cluster-specific batch correction by linear regression
      - Assume that the mean of each cell within each cluster linearly depend on the batch information
    - Move cells in each cluster by subtracting the batch and cluster specific mean effect



Comparison of computational costs

A



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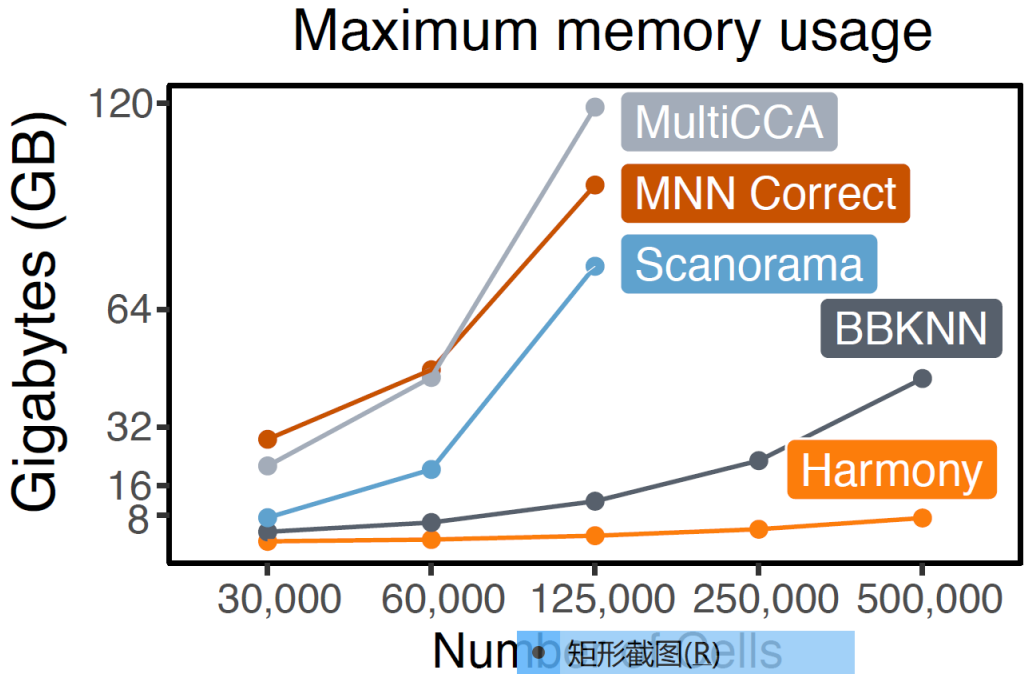


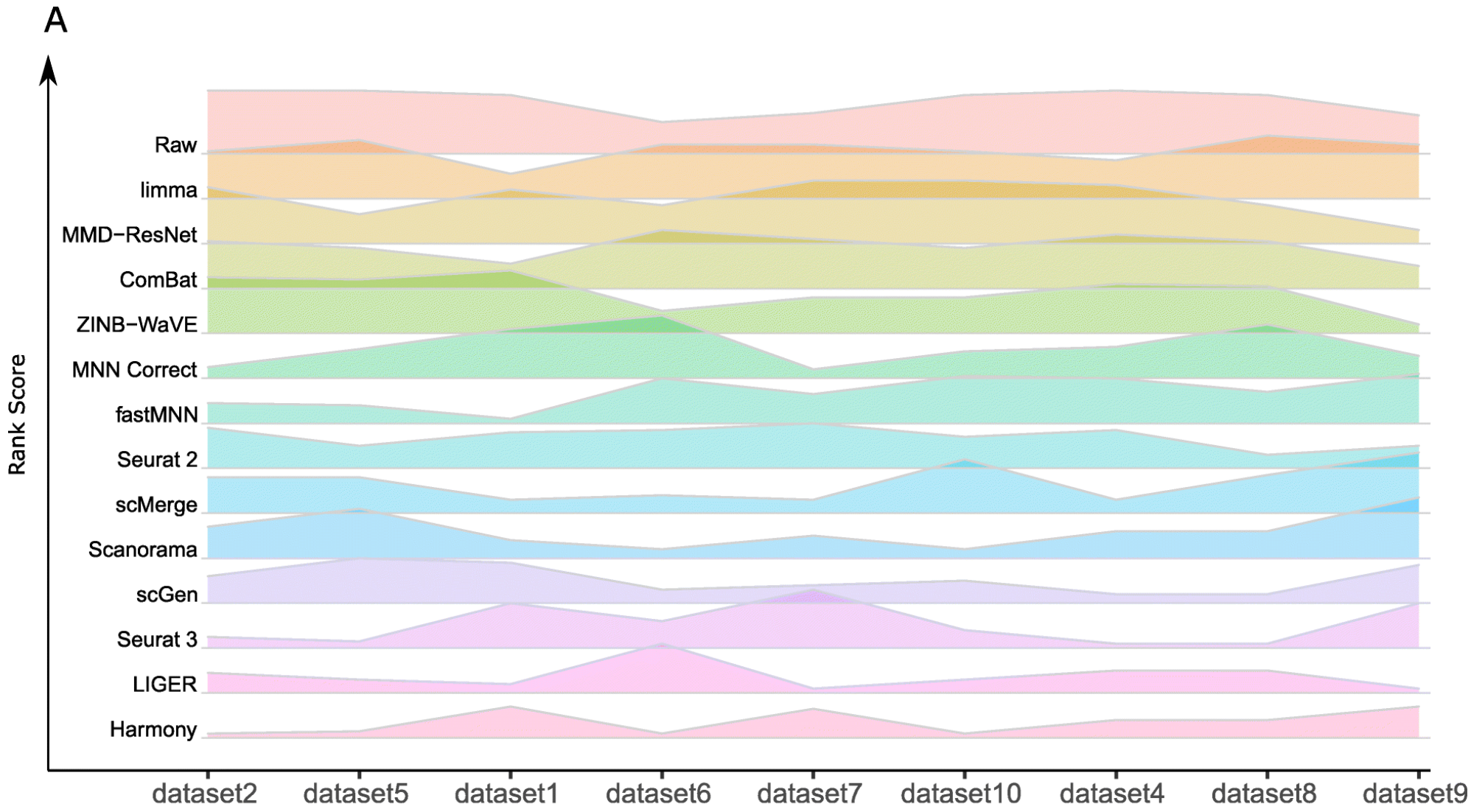
Summary

- Factor-model-based methods and cell-similarity based methods seem to be based on two different sets of assumptions on the batch effects
  - Factor-model-based: batch information and latent factors are nearly orthogonal to each other
  - Cell-similarity based: batch effect is very small compared to biological signals
  - The two assumptions seem quite different -> what is the consequence on performance?

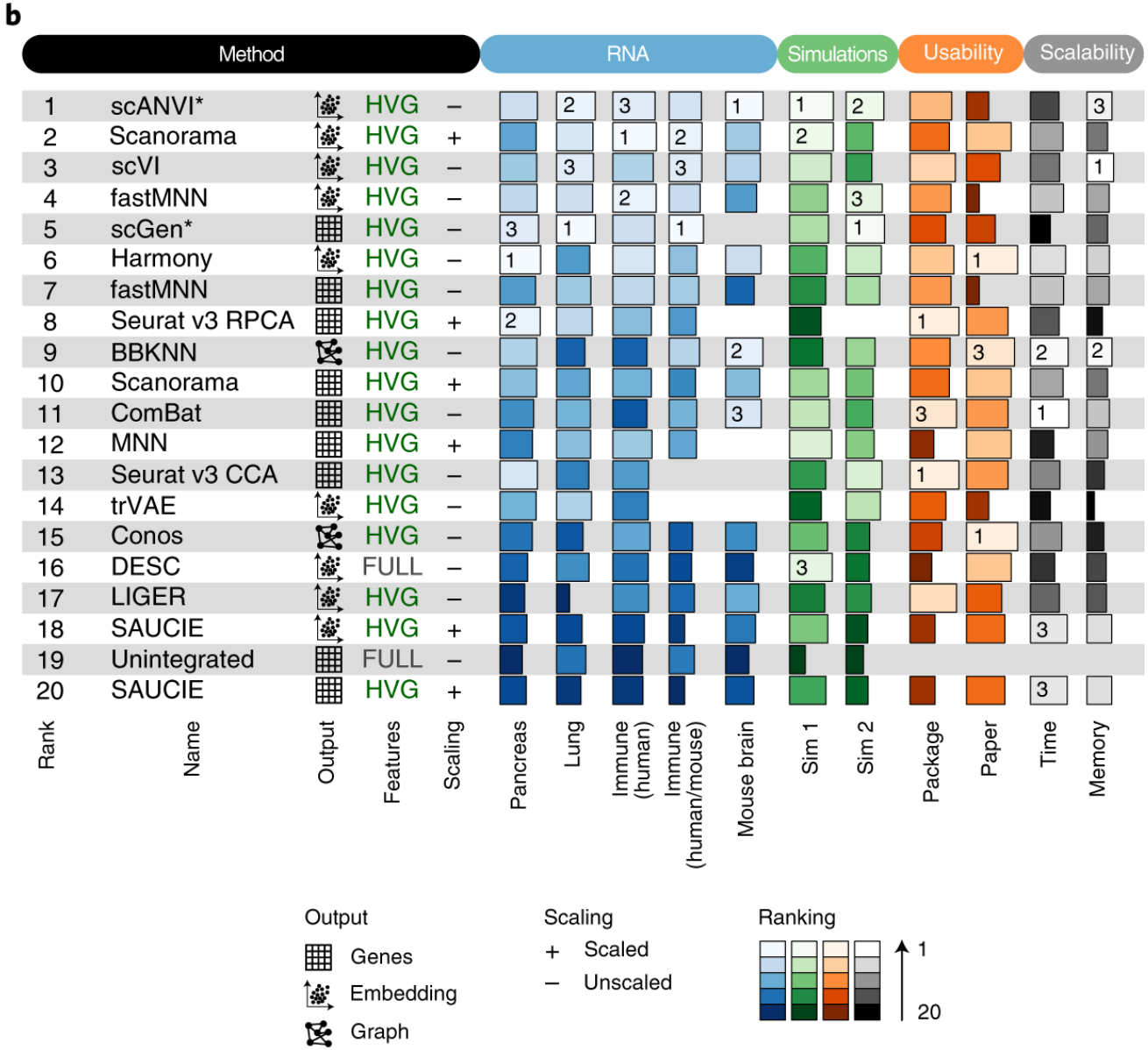
- Factor-model-based methods can provide batch corrected gene expression matrix
  - May introduce false positives in down-stream differential testing

- Performance: Without using additional cell type information, cell-similarity based methods perform slightly better but the two strategies seem comparable

Benchmarking results (Tran et. al. Genome Biology 2020)



Benchmarking results (Luecken et. al. Nature Methods 2022)



Related papers

- Argelaguet, R., Cuomo, A. S., Stegle, O., & Marioni, J. C. (2021). Computational principles and challenges in single-cell data integration. Nature biotechnology, 39(10), 1202-1215.
- Ritchie, M. E., Phipson, B., Wu, D. I., Hu, Y., Law, C. W., Shi, W., & Smyth, G. K. (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic acids research, 43(7), e47-e47.
- Risso, D., Perraudeau, F., Gribkova, S., Dudoit, S., & Vert, J. P. (2018). A general and flexible method for signal extraction from single-cell RNA-seq data. Nature communications, 9(1), 284.
- Lopez, R., Regier, J., Cole, M. B., Jordan, M. I., & Yosef, N. (2018). Deep generative modeling for single-cell transcriptomics. Nature methods, 15(12), 1053-1058.
- Lotfollahi, M., Wolf, F. A., & Theis, F. J. (2019). scGen predicts single-cell perturbation responses. Nature methods, 16(8), 715-721.
- Xu, C., Lopez, R., Mehlman, E., Regier, J., Jordan, M. I., & Yosef, N. (2021). Probabilistic harmonization and annotation of single-cell transcriptomics data with deep generative models. Molecular systems biology, 17(1), e9620.
- Tran, H. T. N., Ang, K. S., Chevrier, M., Zhang, X., Lee, N. Y. S., Goh, M., & Chen, J. (2020). A benchmark of batch-effect correction methods for single-cell RNA sequencing data. *Genome biology*, *21*, 1-32.
- Haghverdi, L., Lun, A. T., Morgan, M. D., & Marioni, J. C. (2018). Batch effects in single-cell RNA-sequencing data are corrected by matching mutual nearest neighbors. *Nature biotechnology*, *36*(5), 421-427.
- Butler, A., Hoffman, P., Smibert, P., Papalexi, E., & Satija, R. (2018). Integrating single-cell transcriptomic data across different conditions, technologies, and species. *Nature biotechnology*, *36*(5), 411-420.
- Stuart, T., Butler, A., Hoffman, P., Hafemeister, C., Papalexi, E., Mauck, W. M., ... & Satija, R. (2019). Comprehensive integration of single-cell data. *cell*, *177*(7), 1888-1902.
- Hie, B., Bryson, B., & Berger, B. (2019). Efficient integration of heterogeneous single-cell transcriptomes using Scanorama. *Nature biotechnology*, *37*(6), 685-691.
- Korsunsky, I., Millard, N., Fan, J., Slowikowski, K., Zhang, F., Wei, K., ... & Raychaudhuri, S. (2019). Fast, sensitive and accurate integration of single-cell data with Harmony. *Nature methods*, *16*(12), 1289-1296.
- Luecken, M. D., Büttner, M., Chaichoompu, K., Danese, A., Interlandi, M., Müller, M. F., ... & Theis, F. J. (2022). Benchmarking atlas-level data integration in single-cell genomics. *Nature methods*, *19*(1), 41-50.