Analysis of Single Cell RNA-Seq Data: Data integration and batch effect correction

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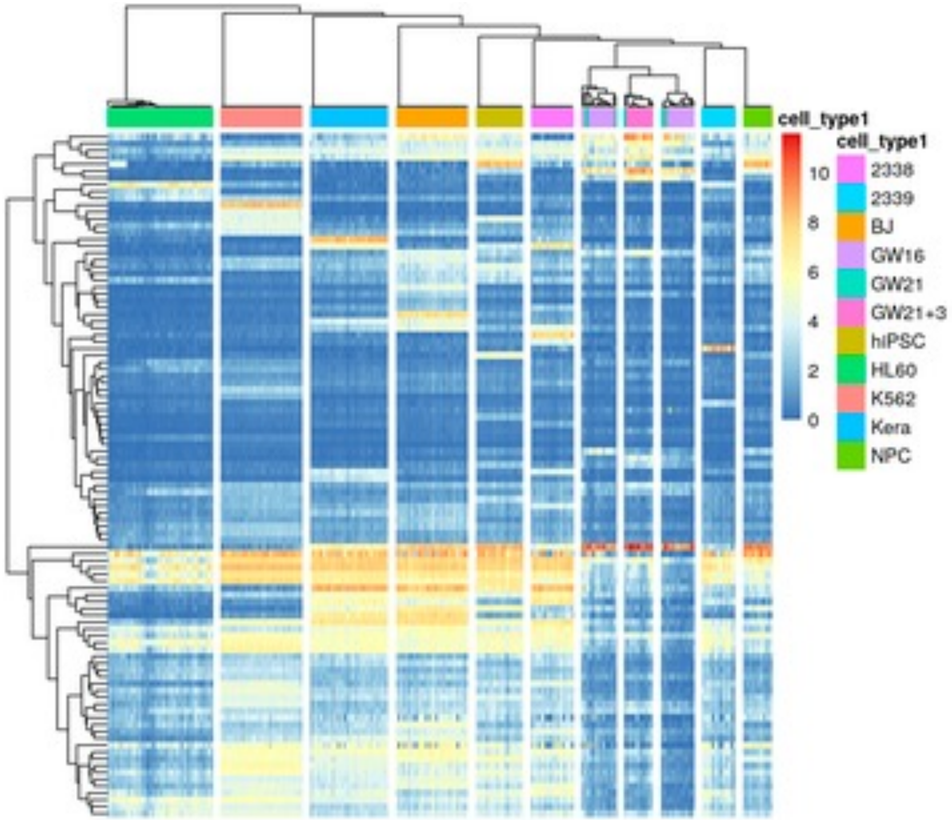
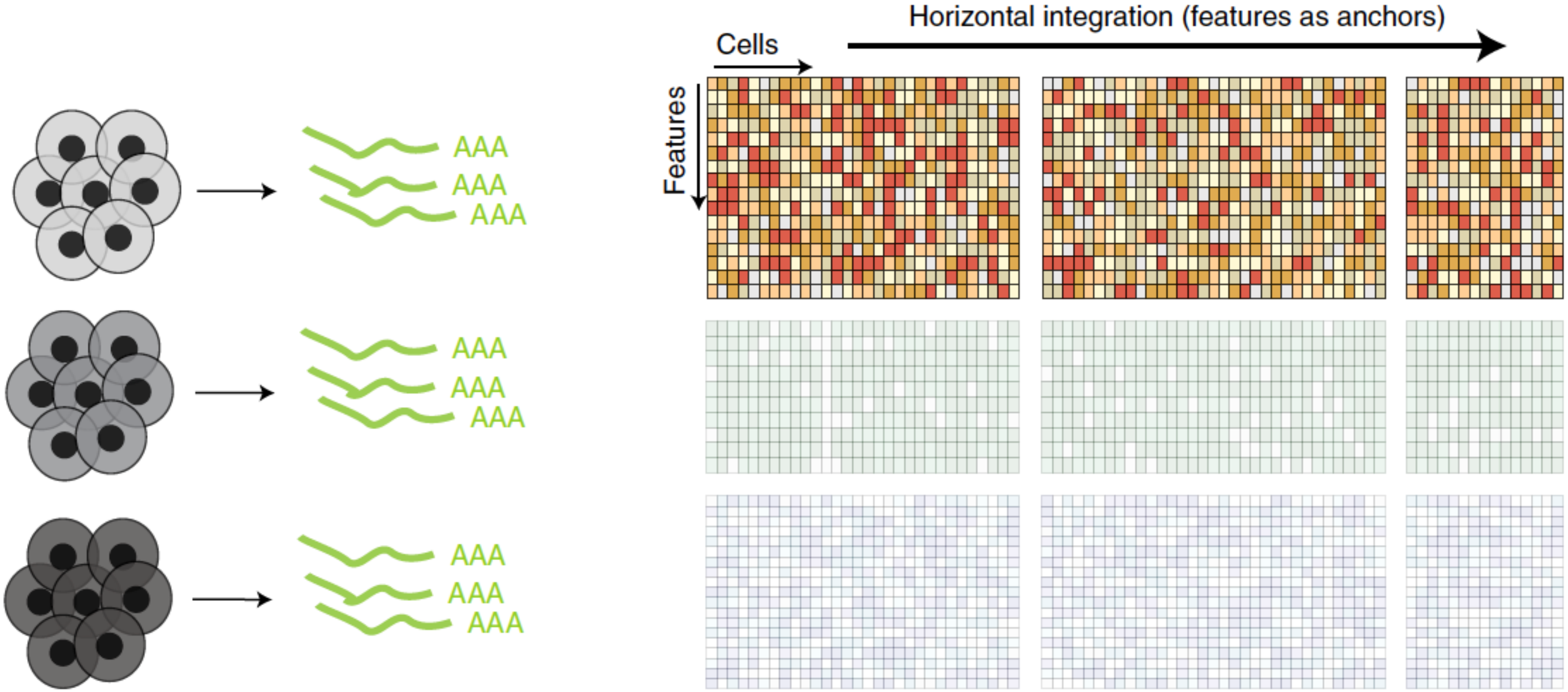
Physalia Courses



Outline: Batch effects and data integration

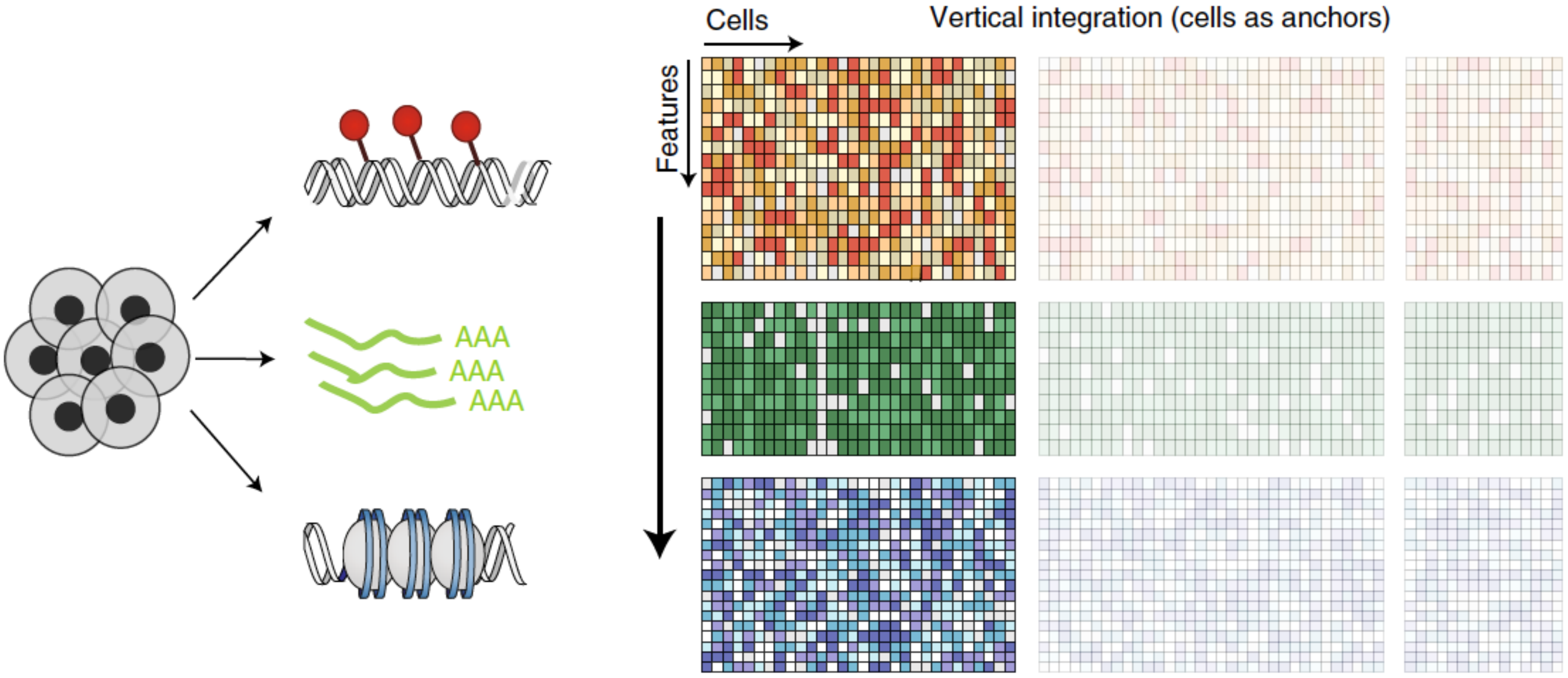
- Types of data integration.
- Where batch effects come from.
- Computational approaches to correct for batch effects and integrate data.
- Making biological comparisons after data integration.

Data integration
Shared features across different batches of cells



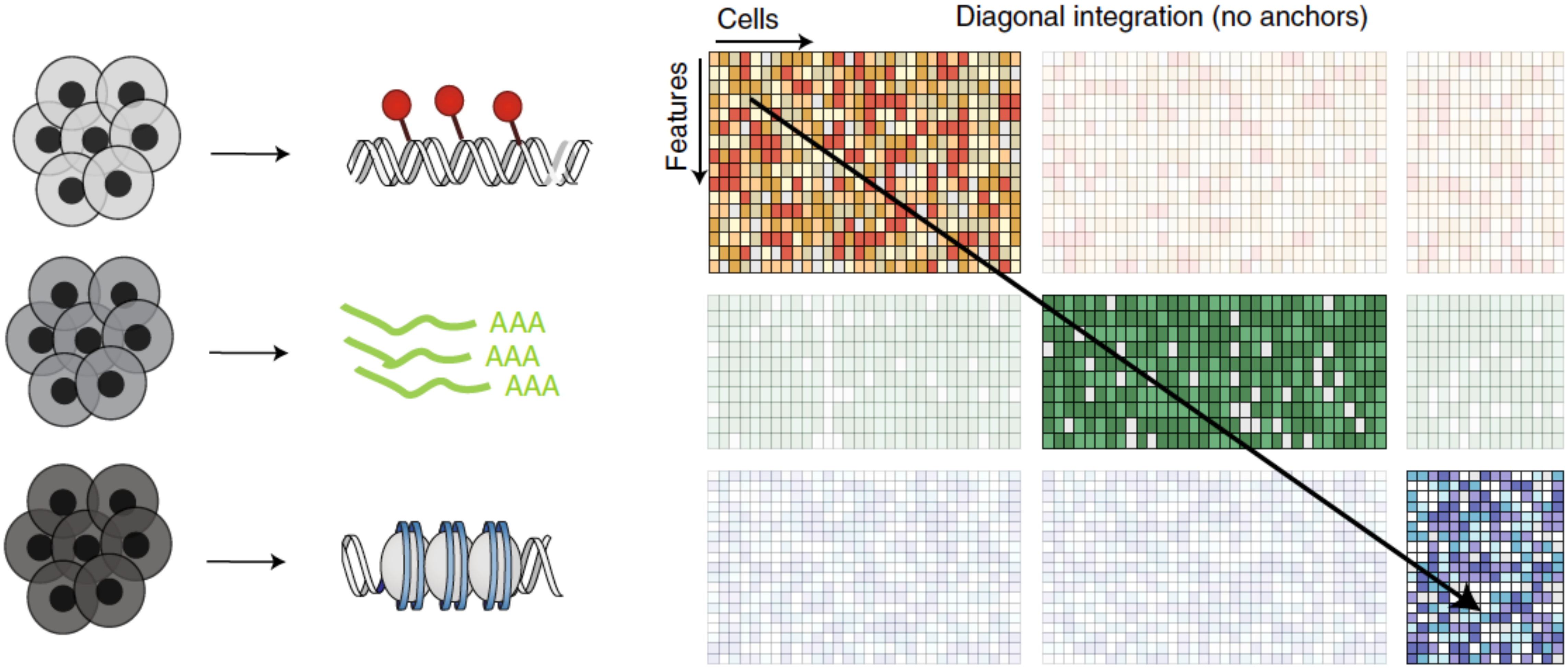
Argelaguet R. et al. (2022) *Nature Methods.*

Data integration
Different features measured from same cells



Argelaguet R. et al. (2022) *Nature Methods.*

Data integration
Different cell batches and features in each experiment

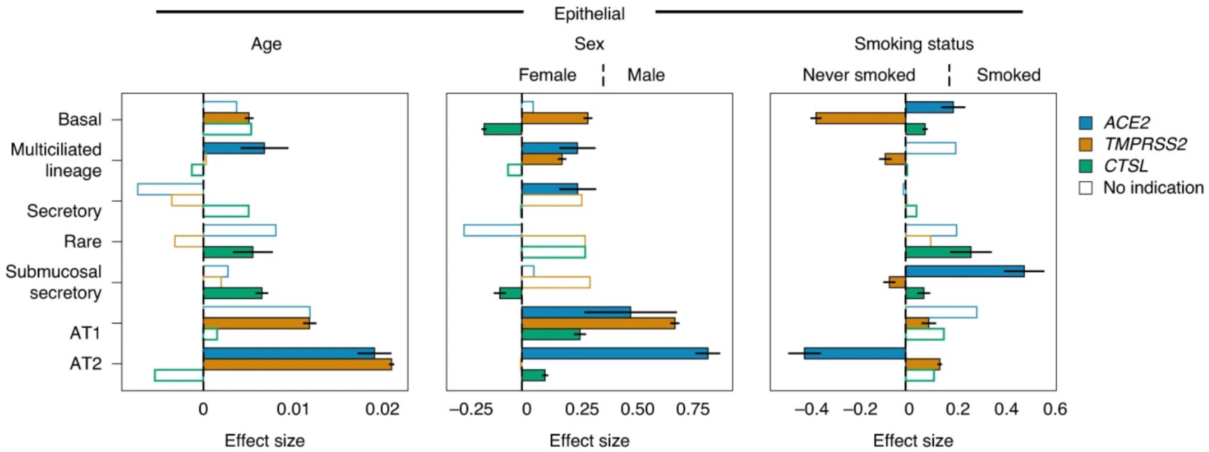


Efremova M. et al. (2020) *Nature Methods.* 17:11-20.

Why perform data integration and batch effect correction?

We often require large cohorts of individuals (*n* > 10) to make biological comparisons (case vs control, mutant vs wildtype etc…).

- As an example, *how do smoking status and age contribute to SARS-CoV-2 entry-related genes in lung cells*?



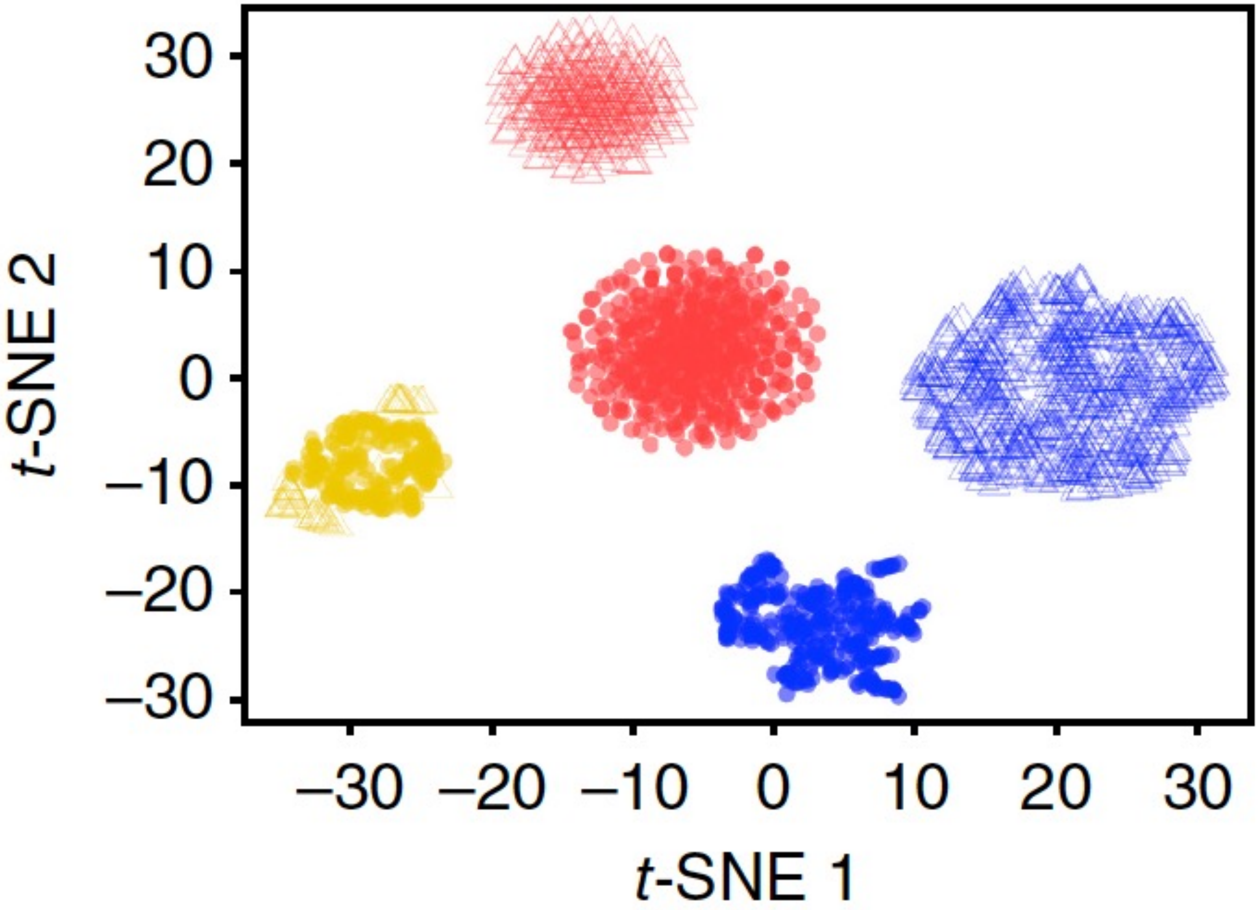
1,320,896 cells from 228 individuals

Challenges

- Technical batch effects confound biological variation of interest (e.g. site and time of collection, experimental protocol used…)
- Different sources of biological variation confound biological variation of interest (e.g. cell cycle, treatment status…)

Muus C. et al. (2021) *Nature Medicine.*

Distinguishing biological effects from technical, batch effects is a difficult problem

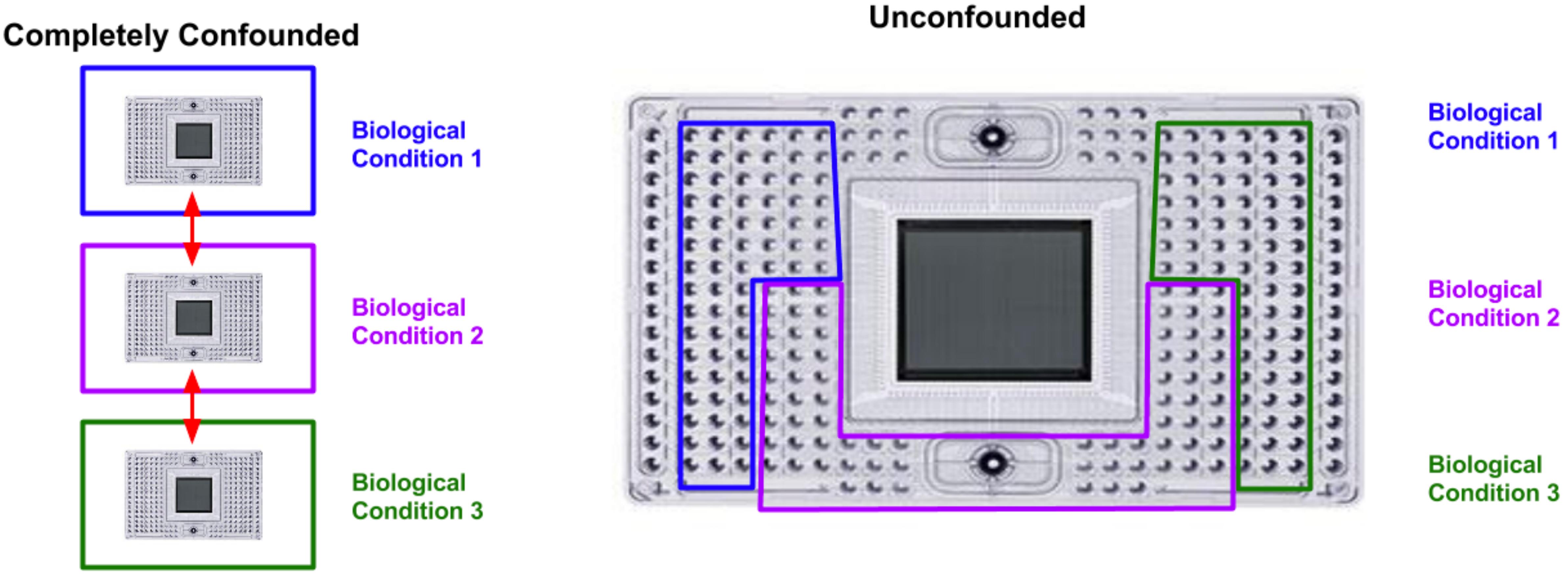


Cells are colored by cell type.

Symbols represent different batches.

Correcting for batch effects allows us to combine datasets and boost biological signal, while reducing technical confounders

The most powerful way to control batch effects is with careful experimental design



Completely Confounded

Biological Condition 1

Biological Condition 2

Biological Condition 3

Unconfounded

Biological Condition 1

Biological Condition 2

Biological Condition 3

Sound experimental design : Replication, Randomization and Blocking

- R. A. Fisher, 1935

https://scrnaseq-course.cog.sanger.ac.uk/website/ideal-scrnaseq-pipeline-as-of-oct-2017.html

Batch effects: technical sources

- Differences in how samples are sequenced
  - sequencing depth and saturation
  - sequencing instrument

Miseq

~ 20M reads total

Nextseq

~ 500M reads total

HiSeq 4000

4 billion reads

A few basic approaches to batch correction

- Down sampling of sequencing reads

- Normalization

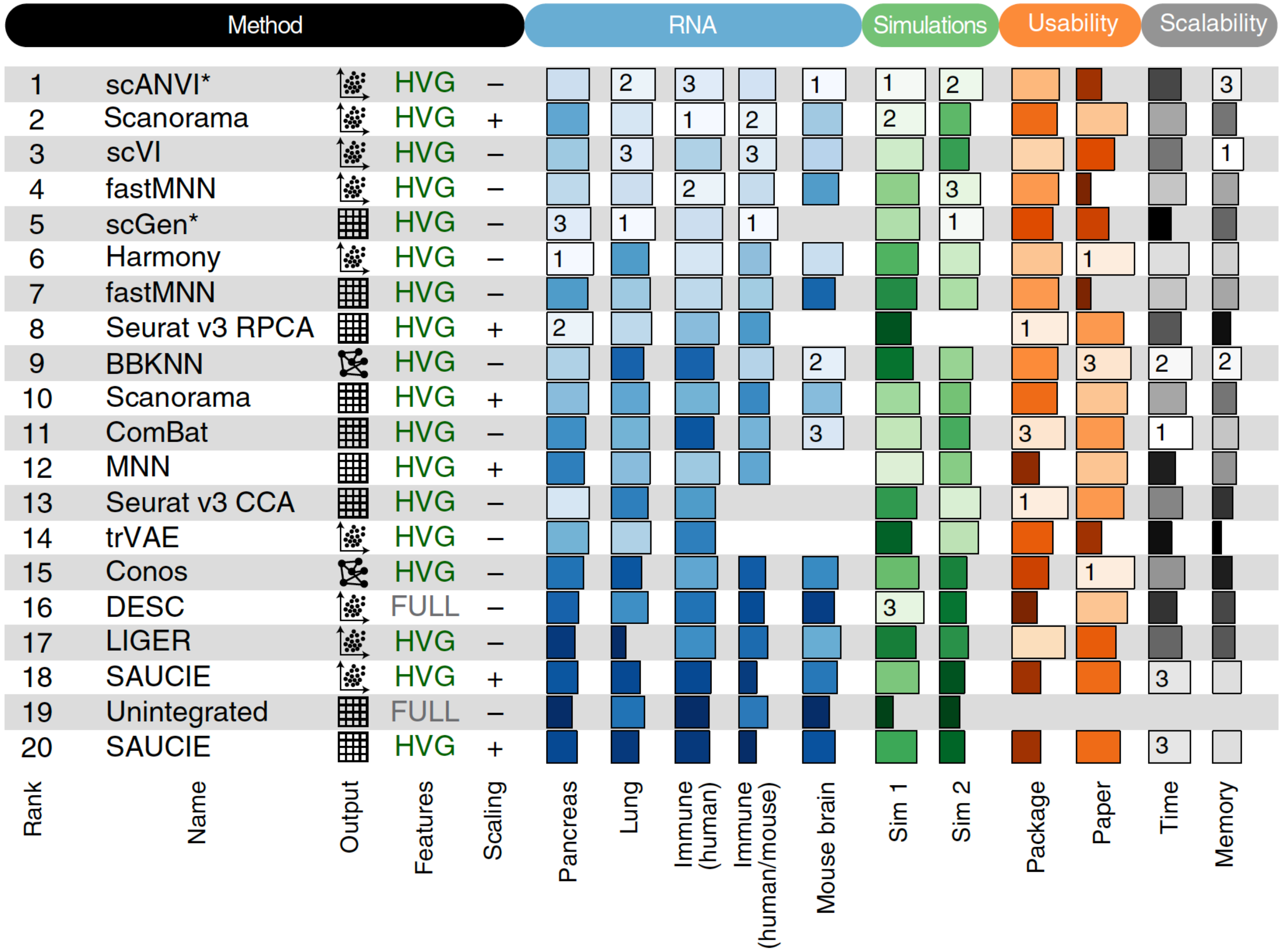
- Using variable genes common to multiple samples

- Removing genes correlated with batch

- Regression of residuals with technical covariates
    - batch id
    - number of UMI per cell
    - number of genes per cell
    - % mitochondrial reads

- ComBat (developed for microarray experiments)

Benchmarking of data integration methods



Method					RNA					Simulations		Usability		Scalability	
Rank	Name	Output	Features	Scaling	Pancreas	Lung	Immune (human)	Immune (human/mouse)	Mouse brain	Sim 1	Sim 2	Package	Paper	Time	Memory
1	scANVI*	⁘	HVG	–	■	2	3	■	1	1	2	■	■	■	3
2	Scanorama	⁘	HVG	+	■	■	1	2	■	2	■	■	■	■	■
3	scVI	⁘	HVG	–	■	3	■	3	■	■	■	■	■	■	1
4	fastMNN	⁘	HVG	–	■	■	2	■	■	■	3	■	■	■	■
5	scGen*	▦	HVG	–	3	1	■	1		■	1	■	■	■	■
6	Harmony	⁘	HVG	–	1	■	■	■	■	■	■	■	1	■	■
7	fastMNN	▦	HVG	–	■	■	■	■	■	■	■	■	■	■	■
8	Seurat v3 RPCA	▦	HVG	+	2	■	■	■		■		1	■	■	■
9	BBKNN	✻	HVG	–	■	■	■	■	2	■	■	■	3	2	2
10	Scanorama	▦	HVG	+	■	■	■	■	■	■	■	■	■	■	■
11	ComBat	▦	HVG	–	■	■	■	■	3	■	■	3	■	1	■
12	MNN	▦	HVG	+	■	■	■	■		■	■	■	■	■	■
13	Seurat v3 CCA	▦	HVG	–	■	■	■			■	■	1	■	■	■
14	trVAE	⁘	HVG	–	■	■	■			■	■	■	■	■	■
15	Conos	✻	HVG	–	■	■	■	■	■	■	■	■	1	■	■
16	DESC	⁘	FULL	–	■	■	■	■	■	3	■	■	■	■	■
17	LIGER	⁘	HVG	–	■	■	■	■	■	■	■	■	■	■	■
18	SAUCIE	⁘	HVG	+	■	■	■	■	■	■	■	■	■	3	■
19	Unintegrated	▦	FULL	–	■	■	■	■	■	■	■				
20	SAUCIE	▦	HVG	+	■	■	■	■	■	■	■	■	■	3	■

Output

▦ Genes

⁘ Embedding

✻ Graph

Scaling

+ Scaled

– Unscaled

Ranking

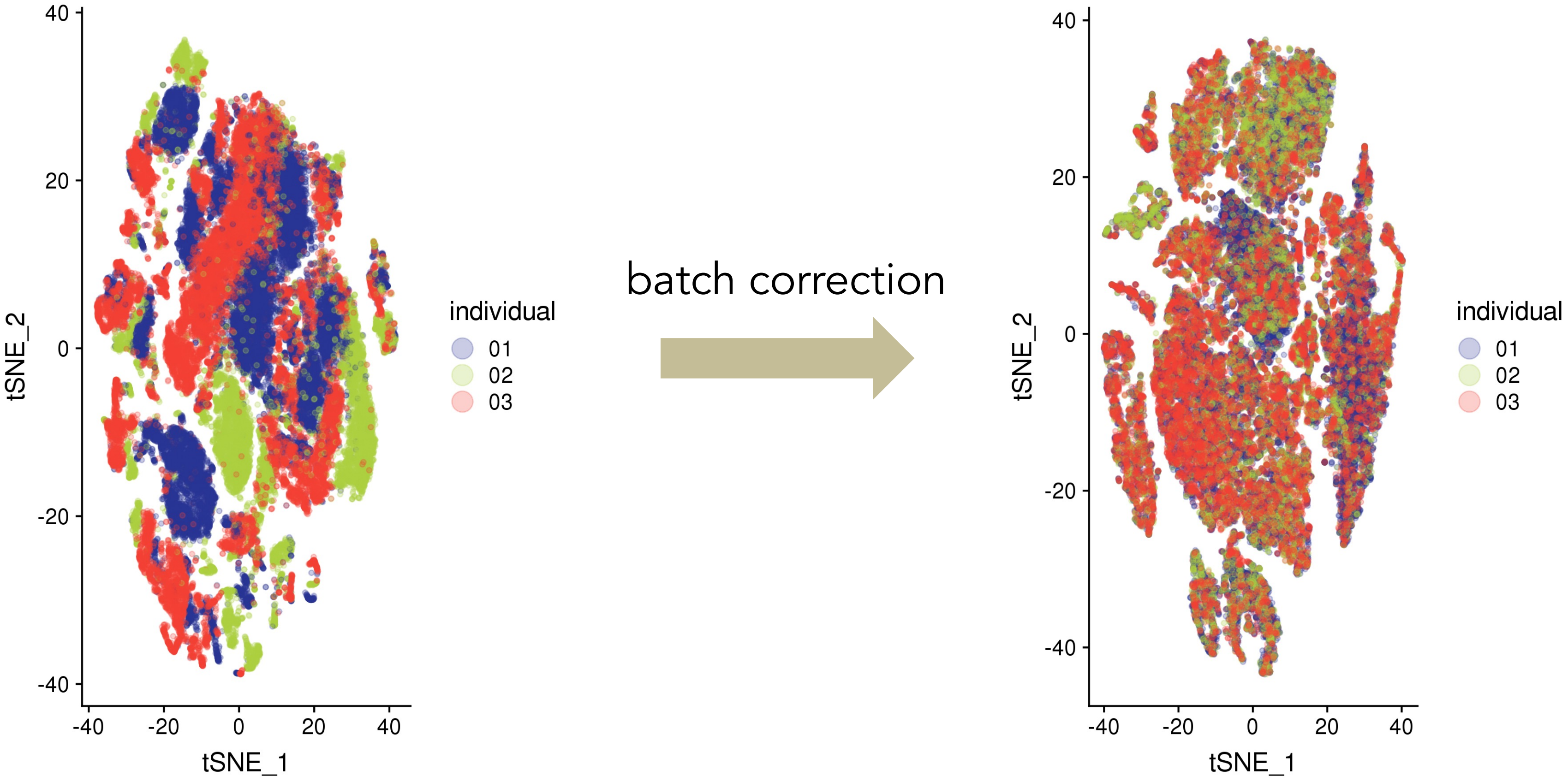
↑ 1

20

Luecken M. D. et al. (2022) *Nature Methods.*

Batch correction and data modality integration

Batch effects often arise when patient samples are analyzed together



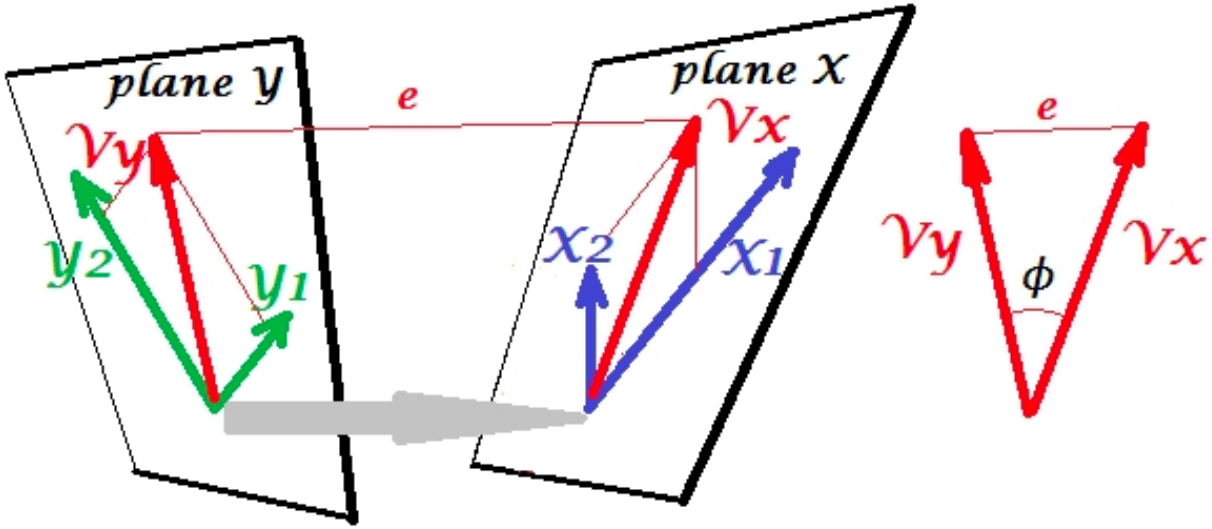
batch correction

Batch correction and data modality integration

- Seurat v3
- LIGER (Linked Inference of Genomic Experimental Relationships)
- Conos (Clustering on Network of Samples), Harmony
- scVI (deep learning approach)

Canonical Correlation Analysis (CCA)

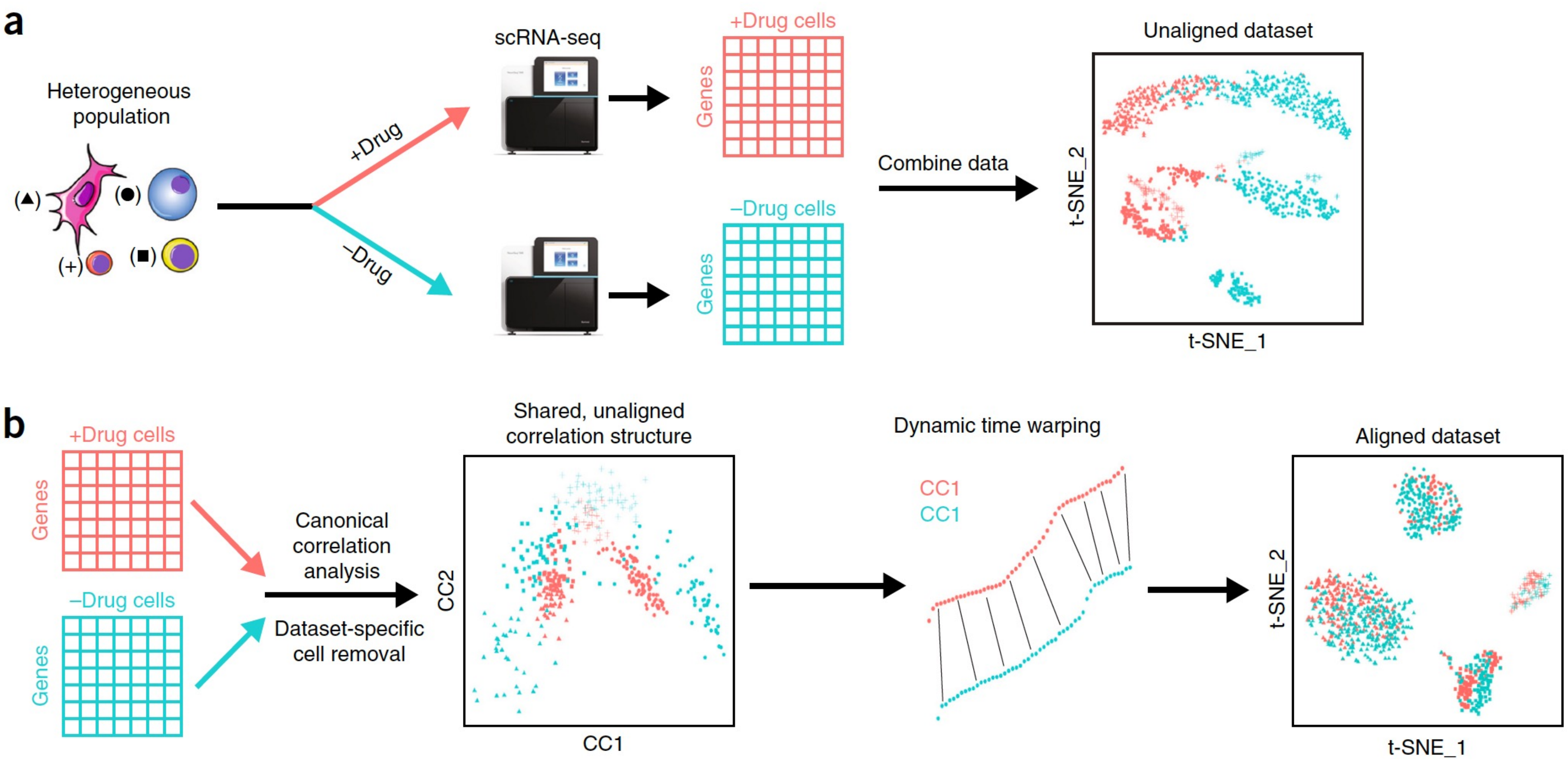
- CCA finds the linear combinations of variables across two datasets that are maximally correlated with one another.
  - The first pair of canonical variables maximizes the correlation across datasets.
  - The second pair of canonical variables maximizes the correlation subject to the constraint of not being correlated with the first pair, and so on.



- Goals of CCA
  - Similar to Principal Components Analysis (PCA)
  - Dimensional reduction: explain covariation between datasets with a small number of linear combinations of variables

https://www.mathworks.com
https://github.com/mhaghighat/ccaFuse

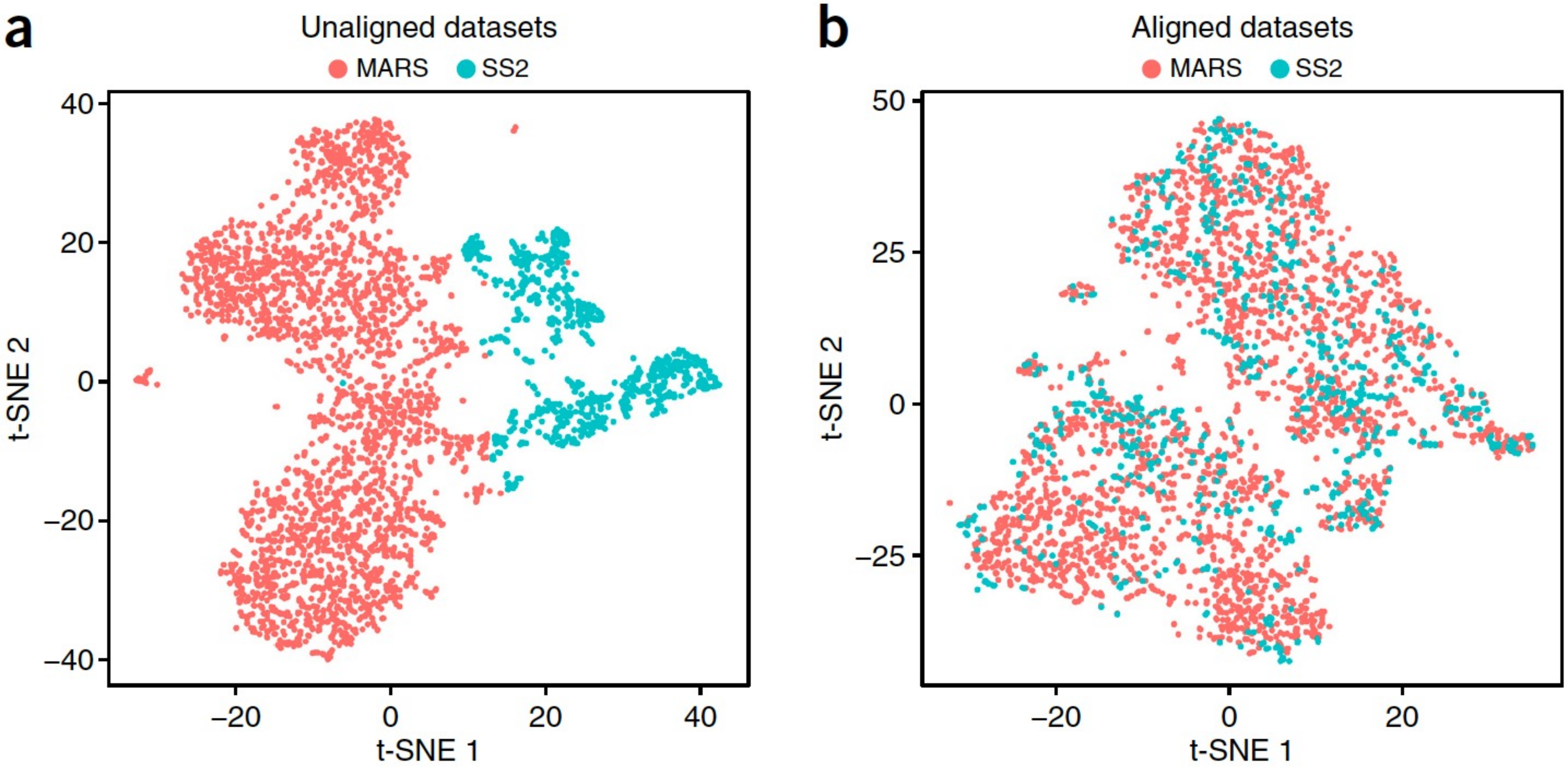
Canonical Correlation Analysis (CCA)



"Effectively, we treat the data sets as multiple measurements of a gene–gene covariance structure, and search for patterns that are common to the data sets."

Butler, A, et al. *Nature Biotechnology* 36.5 (2018): 411.

Canonical Correlation Analysis (CCA)



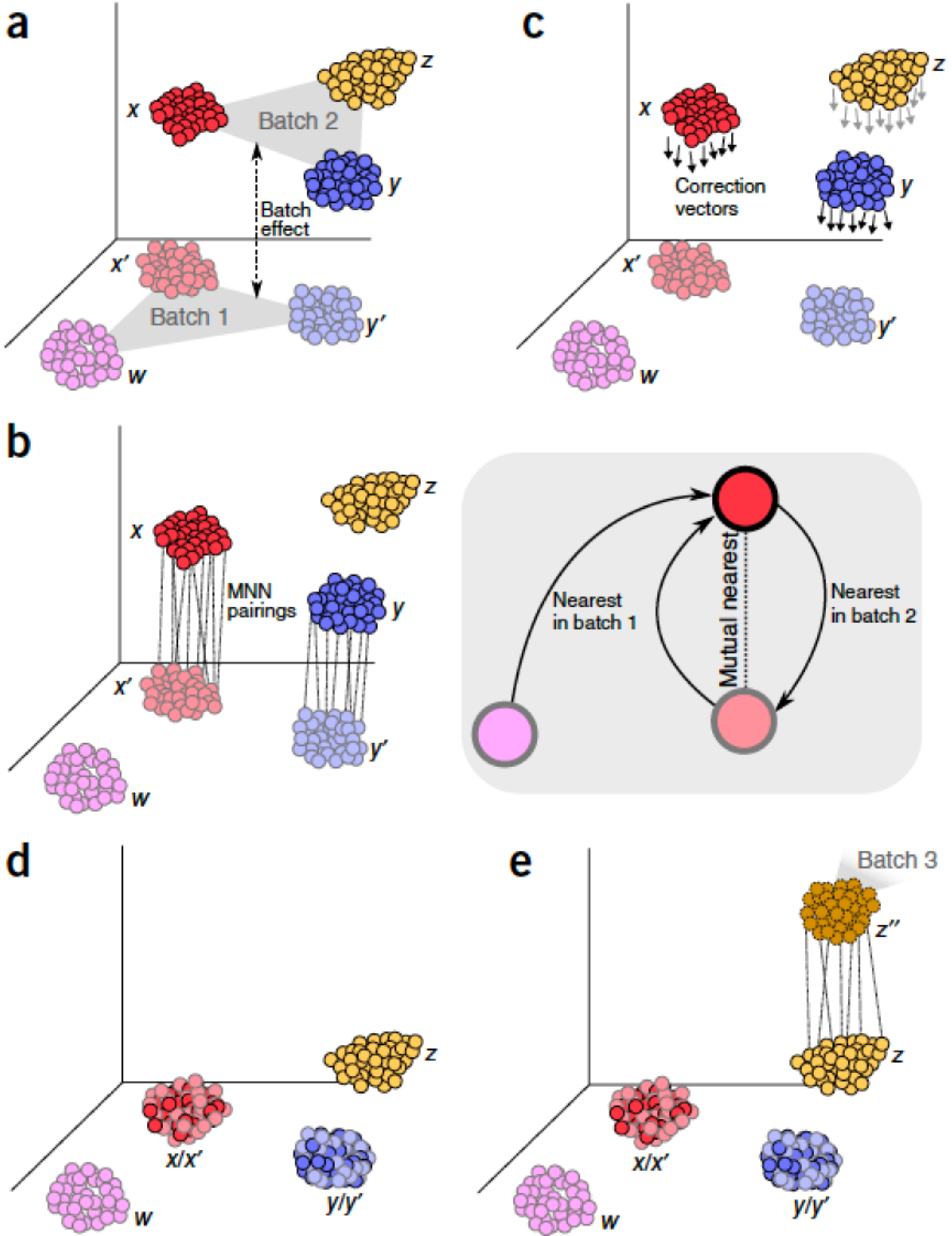
Assessing the performance of batch correction

"For every cell, we calculate how many of its k nearest-neighbors belong to the same data set and average this over all cells. If the data sets are well-aligned, we would expect that each cells' nearest neighbors would be evenly shared across all data sets."

Butler, A, et al. *Nature Biotechnology* 36.5 (2018): 411.

Mutual Nearest Neighbors

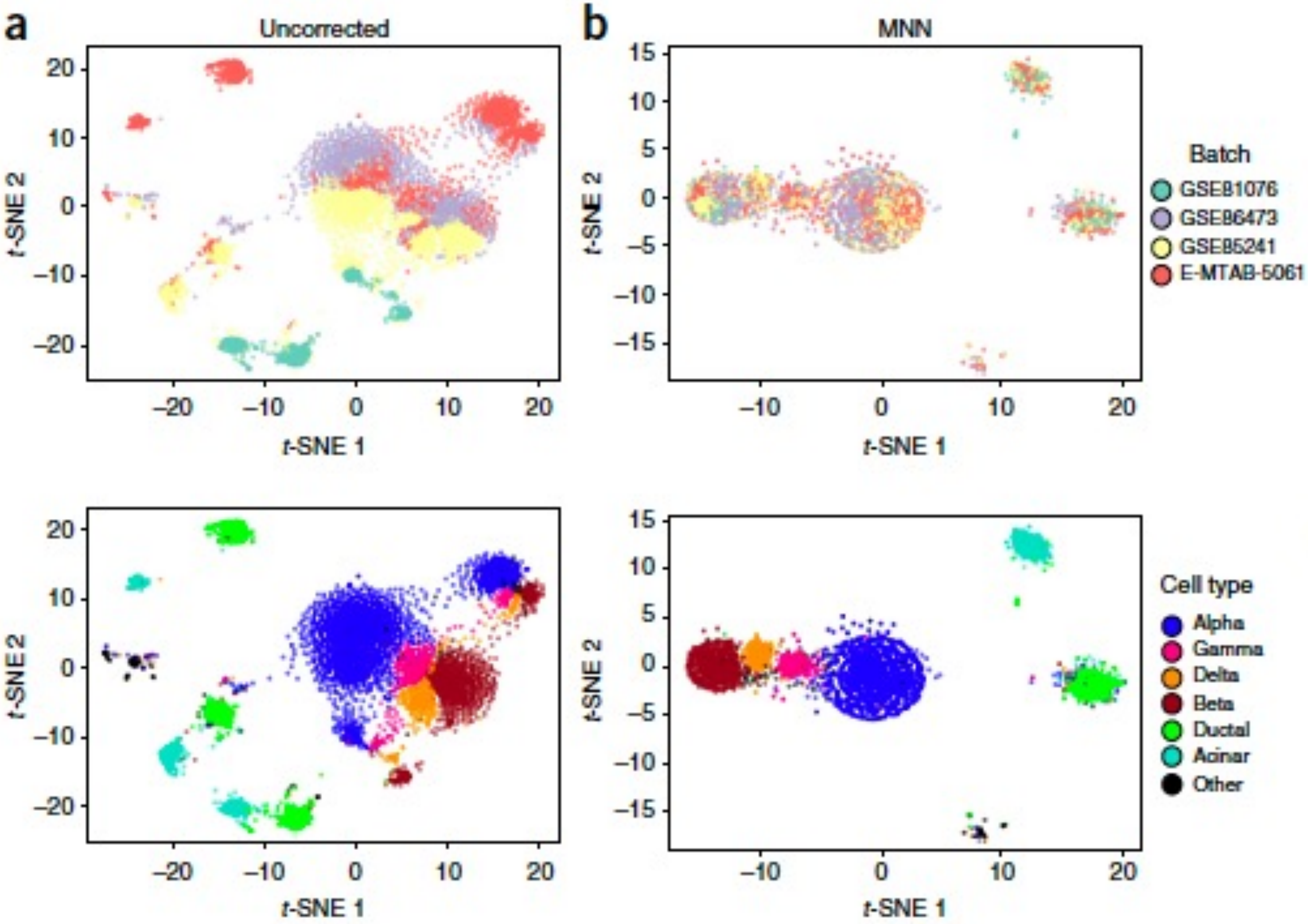
“If a pair of cells from each batch is contained in each other’s set of nearest neighbors, those cells are considered to be mutual nearest neighbors. We interpret these pairs as containing cells that belong to the same cell type or state despite being generated in different batches. Thus, any systematic differences in expression level between cells in MNN pairs should represent the batch effect.”



Haghverdi, L, et al. *Nature biotechnology* 36.5 (2018): 421.

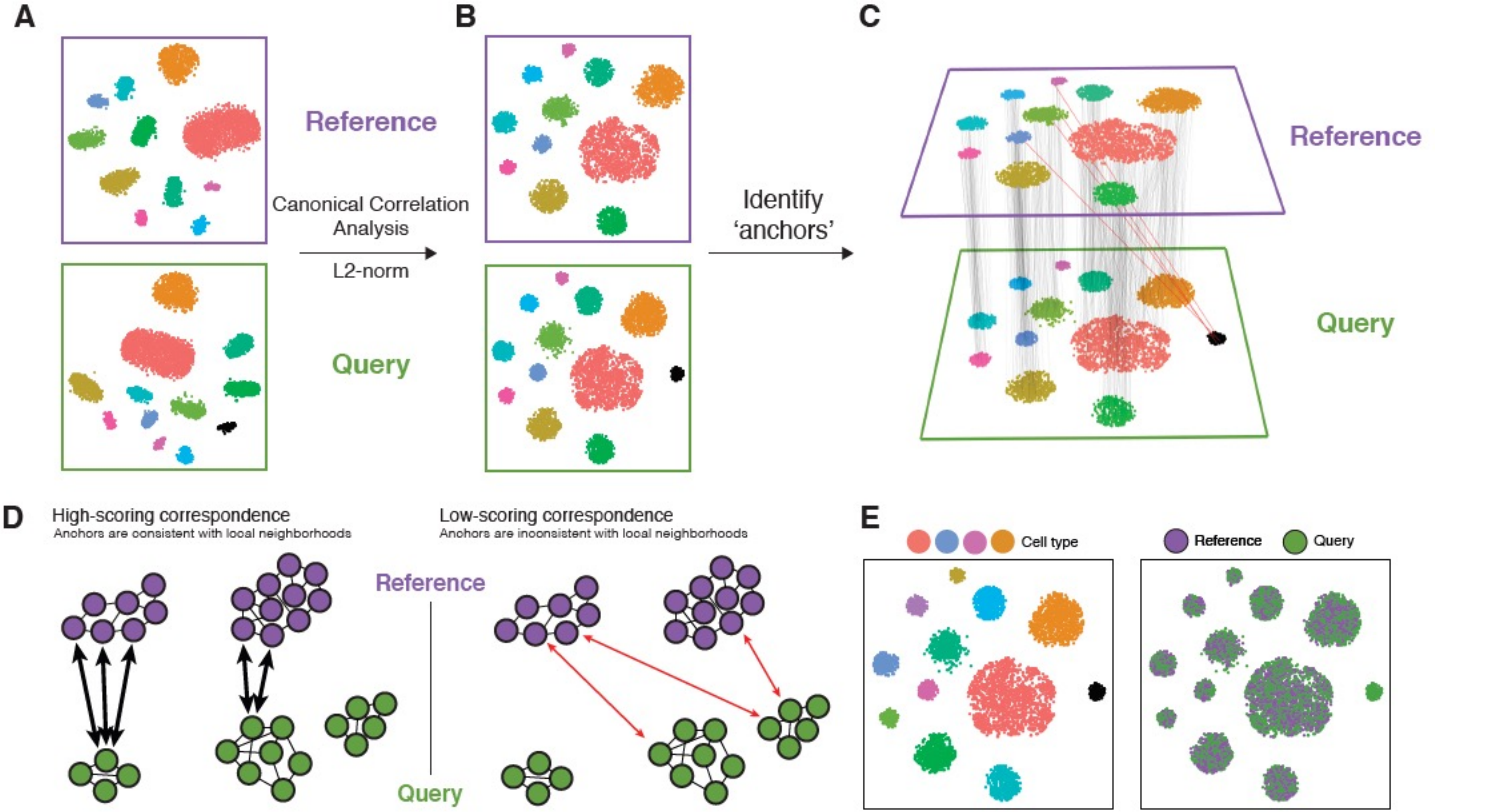
Mutual Nearest Neighbors

4 pancreas datasets



Haghverdi, L, et al. *Nature Biotechnology* (2018).

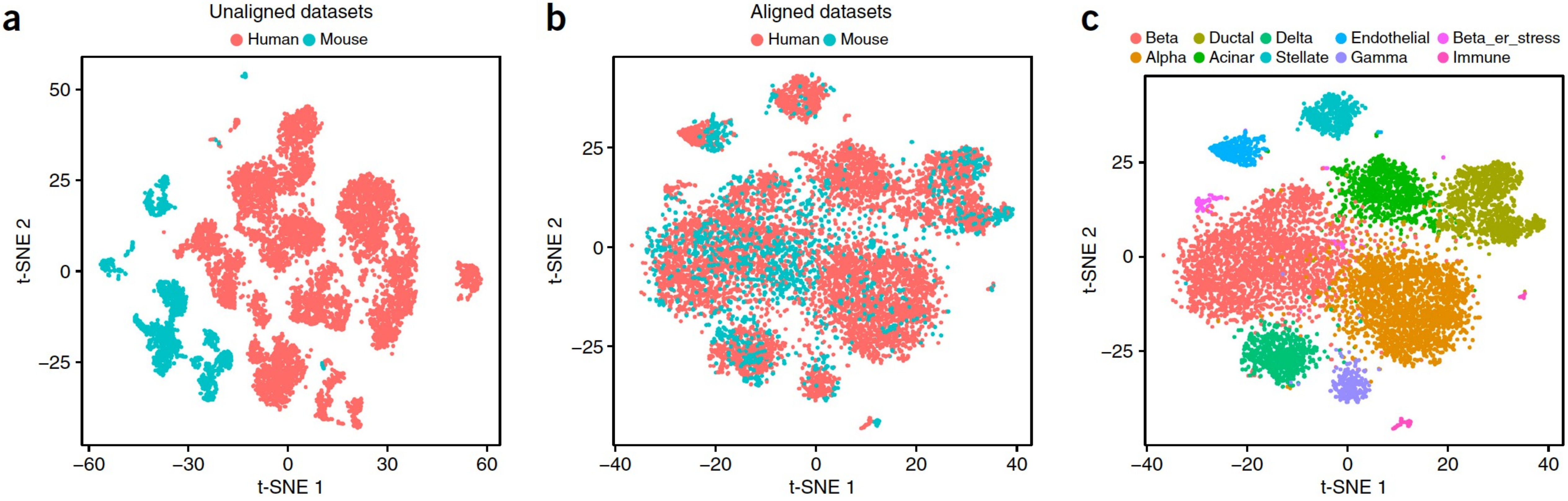
Combining CCA and Mutual Nearest Neighbors (Seurat v3)


A
B
C
Reference
Canonical Correlation Analysis
L2-norm
Query
Identify 'anchors'
Reference
Query
D
High-scoring correspondence
Anchors are consistent with local neighborhoods
Low-scoring correspondence
Anchors are inconsistent with local neighborhoods
Reference
Query
E
Cell type
Reference
Query

Stuart et al. *Cell* (2019).

Combining CCA and Mutual Nearest Neighbors (Seurat v3)

Human and mouse pancreas datasets

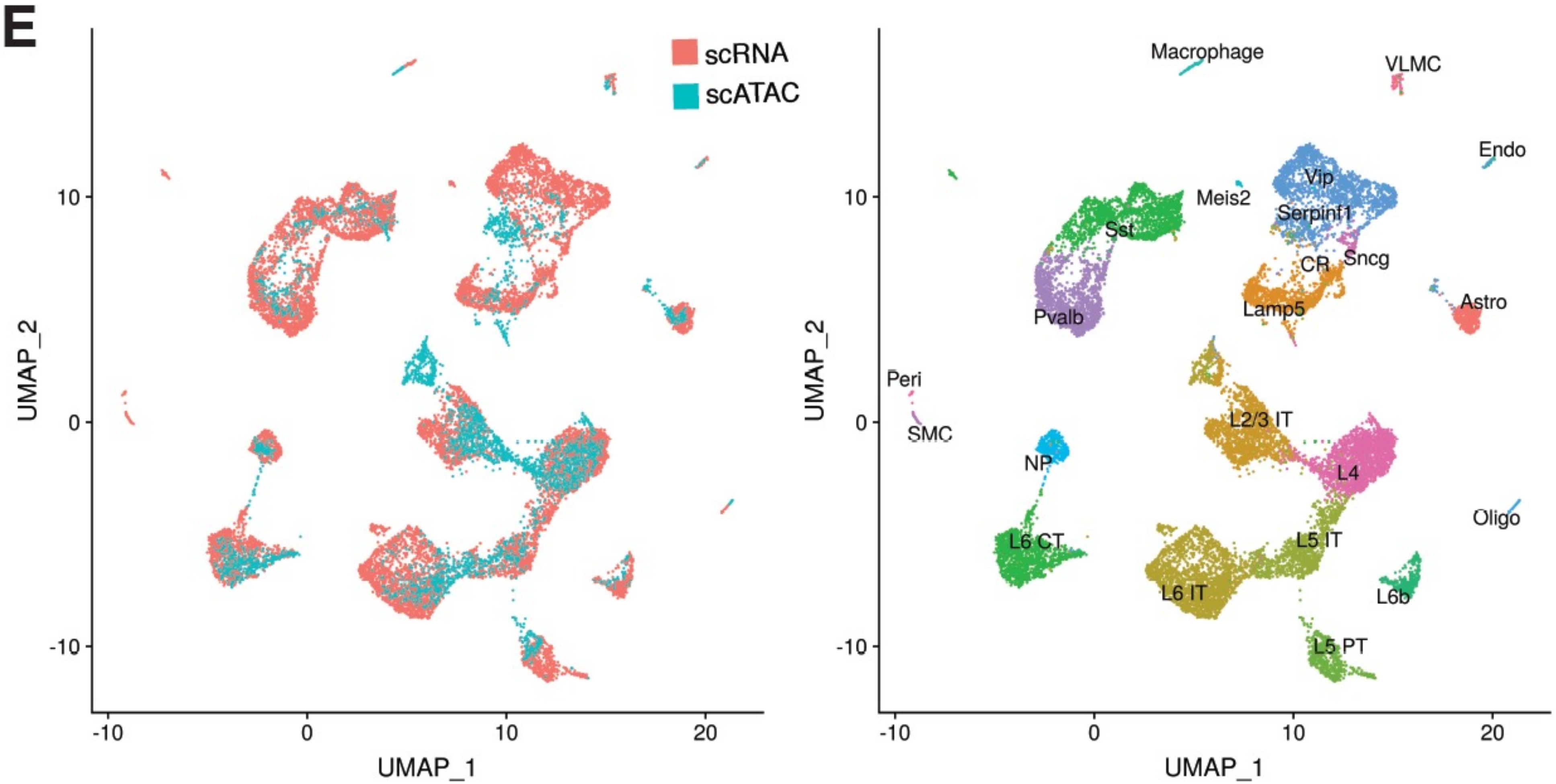


Stuart et al. *Cell* (2019).

Classifying nuclei from a single cell ATAC-seq experiment using single cell RNA-seq data as a reference

Integrating data modalities

14,249 cells from scRNA-seq and 2,548 cells from scATAC-seq



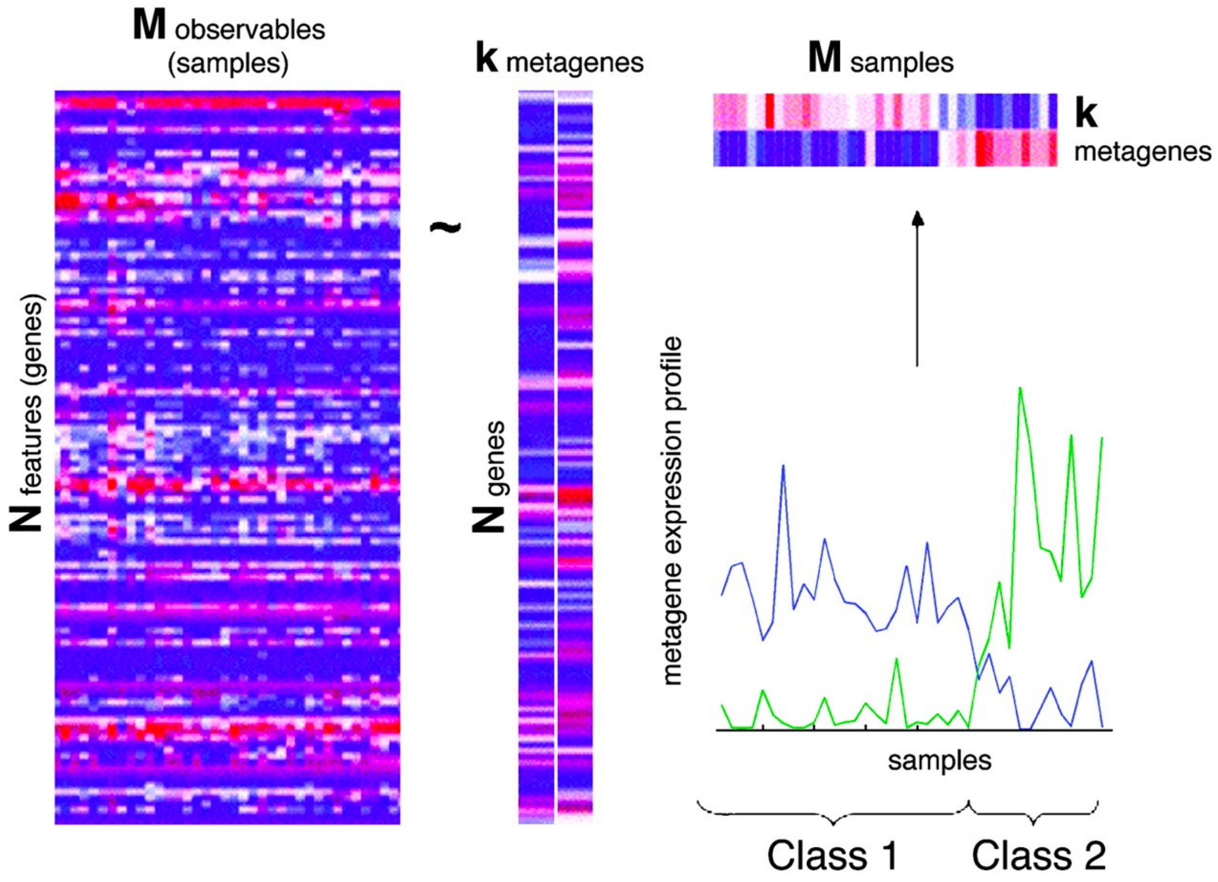
Stuart et al. *Cell* (2019).

Batch correction and data modality integration

Nonnegative Matrix Factorization (NMF)

A (rank M) ~ W H (rank k=2)

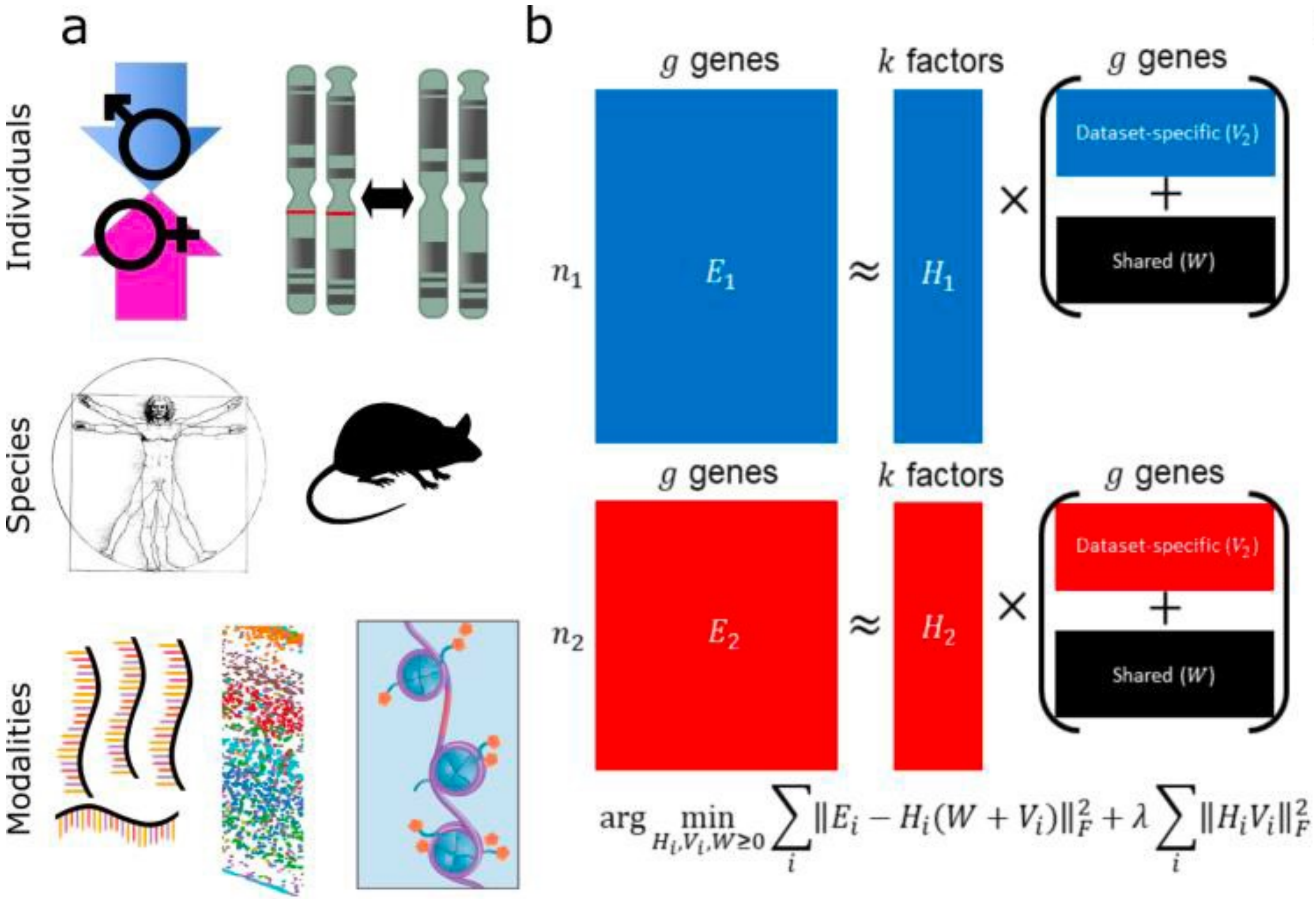
"Our goal is to find a small number of metagenes, each defined as a positive linear combination of the *N* genes. We can then approximate the gene expression pattern of samples as positive linear combinations of these metagenes. Mathematically, this corresponds to factoring matrix *A* into two matrices with positive entries, *A ~ WH*."



Brunet J. et *al*. (2004) *PNAS*..

Batch correction and data modality integration using LIGER

LIGER implements non-negative matrix factorization



$$\arg \min_{H_i, V_i, W \geq 0} \sum_i \| E_i - H_i(W + V_i) \|_F^2 + \lambda \sum_i \| H_i V_i \|_F^2$$

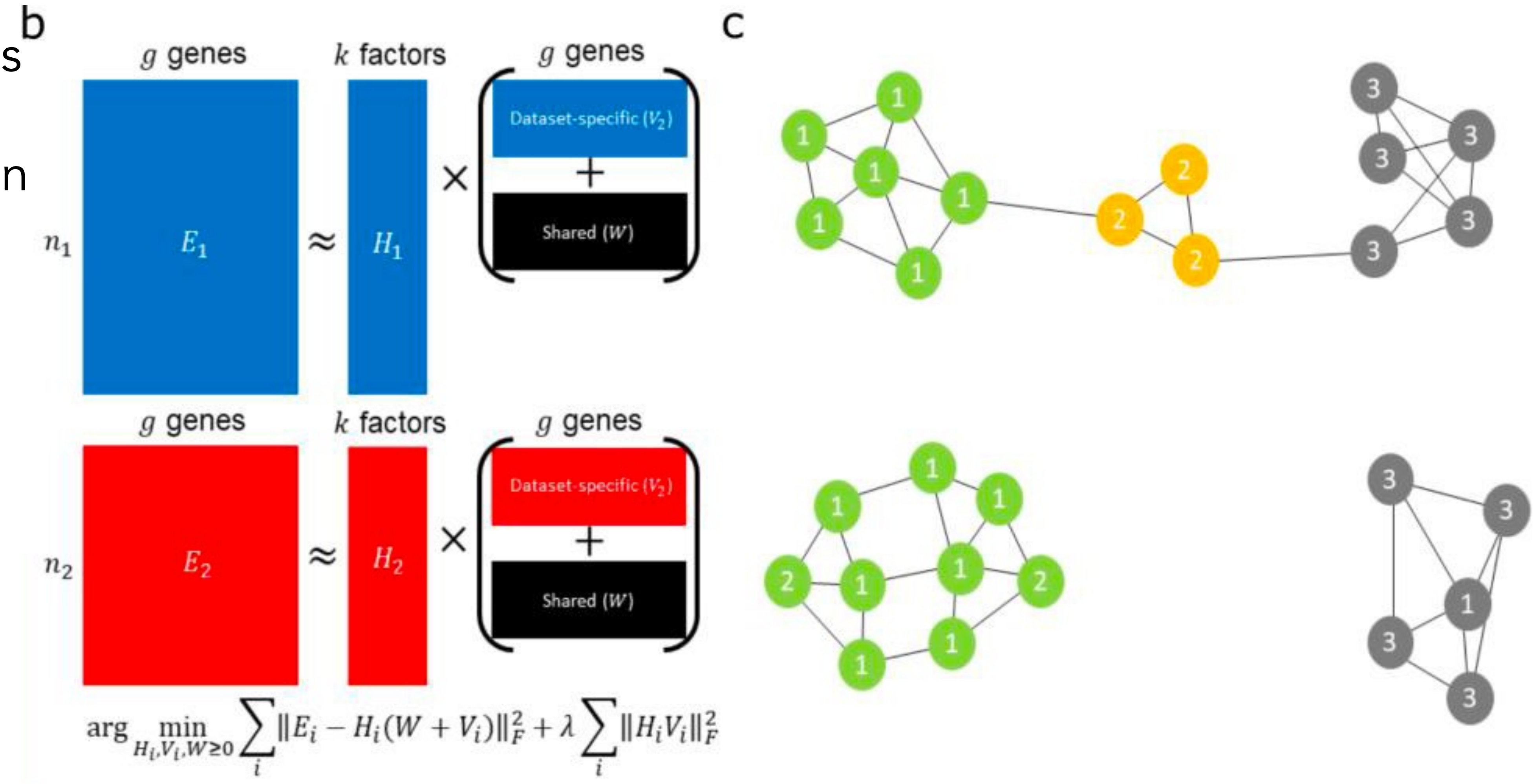
Heterogeneous single cell datasets

Integrative nonnegative matrix factorization with dataset-specific factors

Welch, et al. *Cell* (2019).

Batch correction and data modality integration using LIGER

LIGER implements non-negative matrix factorization



$$\arg\min_{H_i,V_i,W\geq 0}\sum_i \|E_i - H_i(W+V_i)\|_F^2 + \lambda\sum_i \|H_iV_i\|_F^2$$

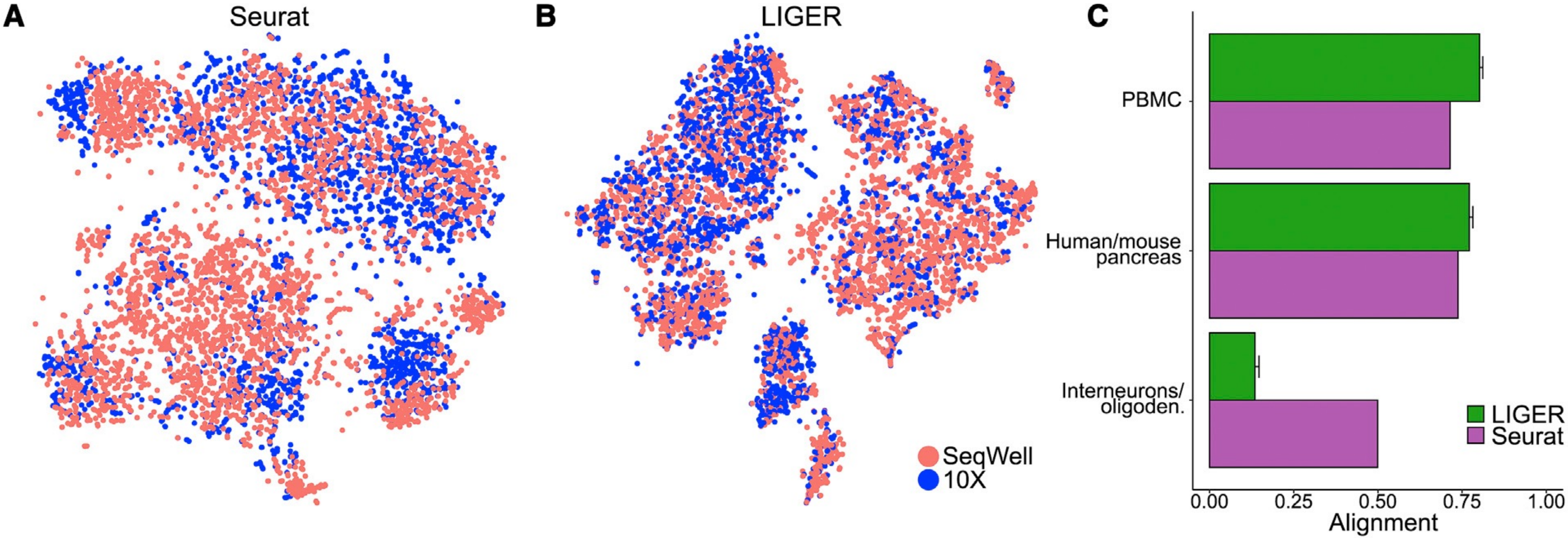
Integrative nonnegative matrix factorization with dataset-specific factors

Joint clustering using shared factor neighborhood graph

Welch, et al. *Cell* (2019).

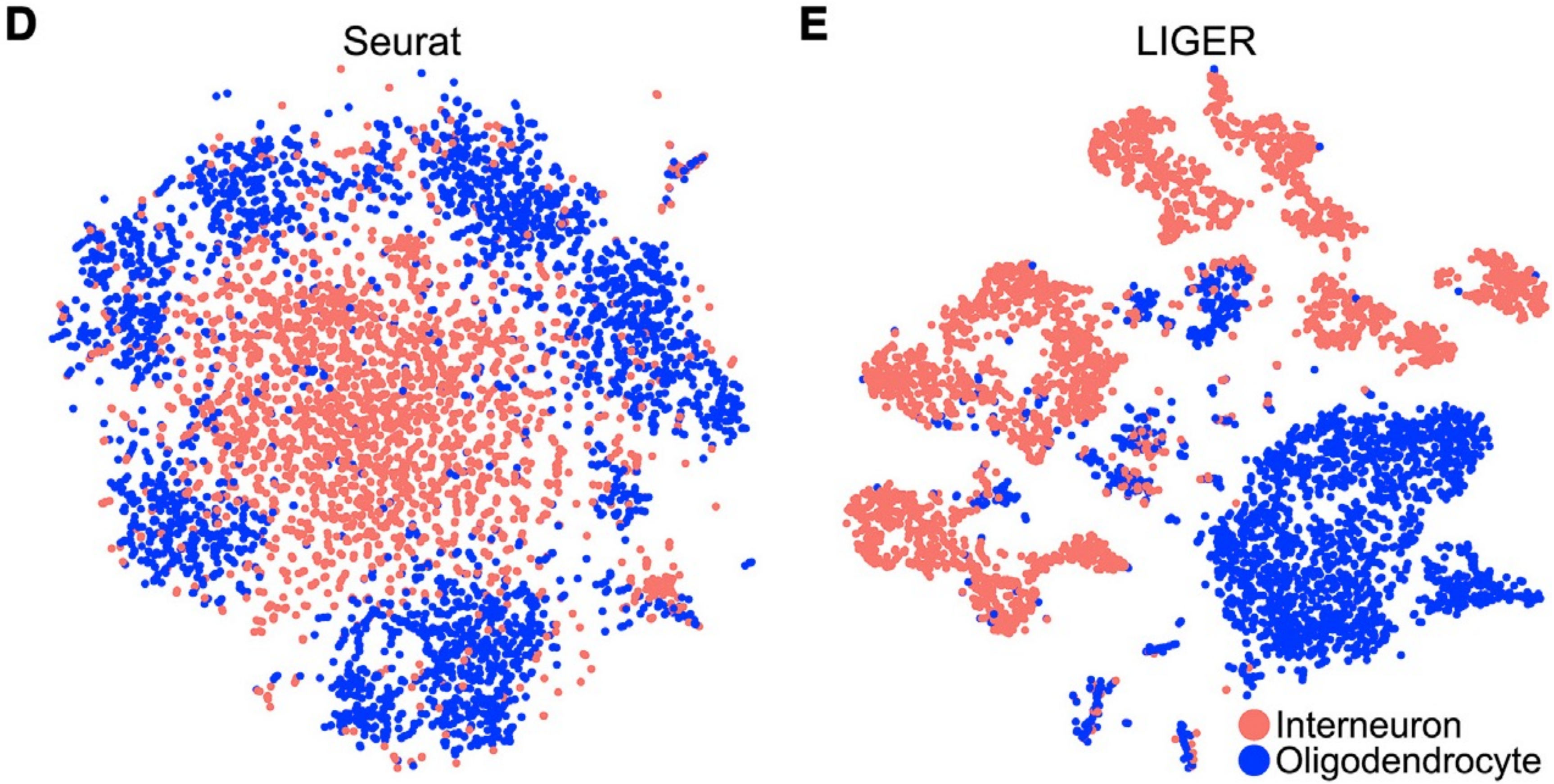
Integrating blood cell datasets using LIGER

Higher alignment score = better data integration



Welch, et al. *Cell* (2019).

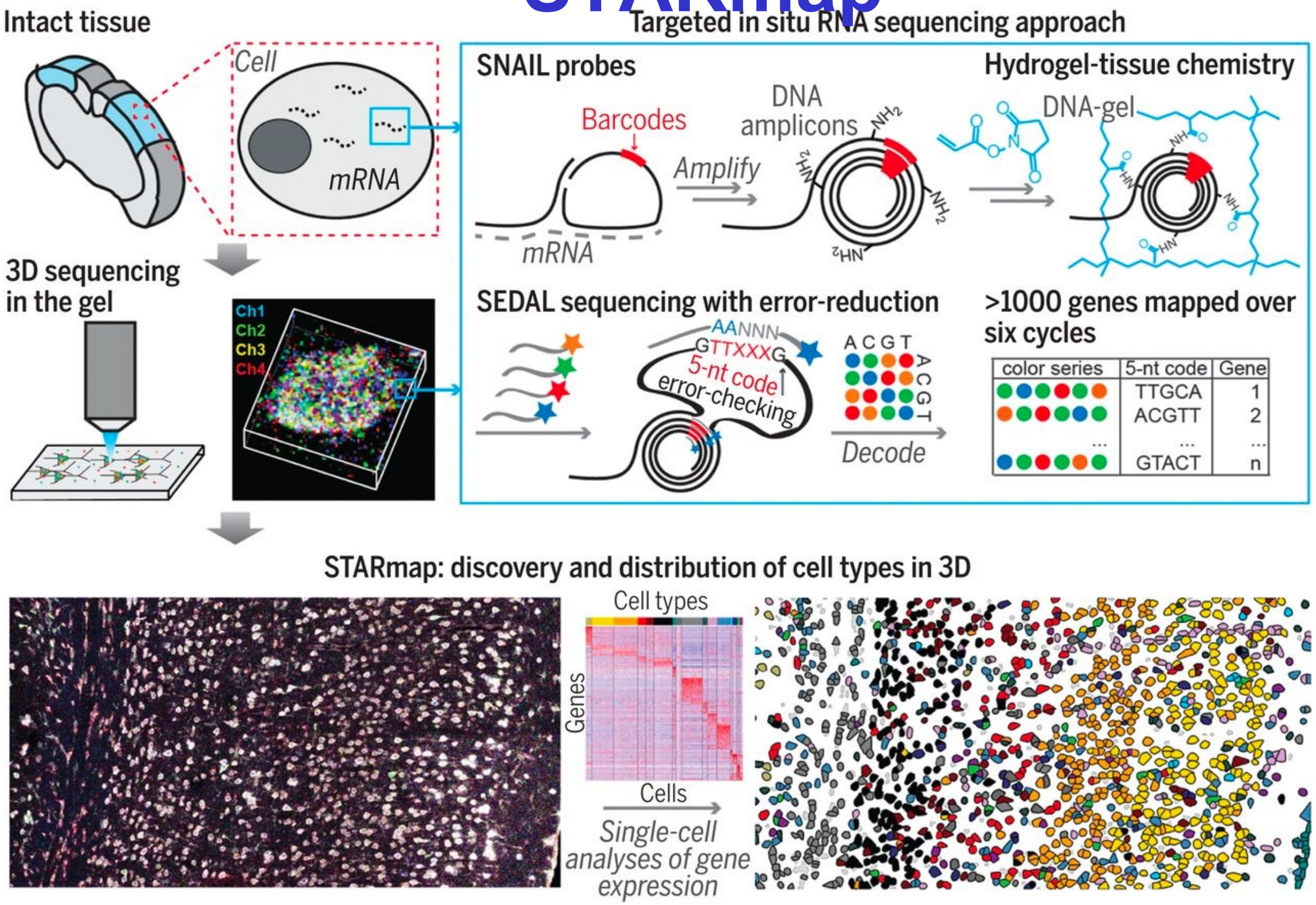
Integrating blood cell datasets using LIGER



Ideally, divergent cell types should not cluster together after batch correction.

Welch, et al. *Cell* (2019).

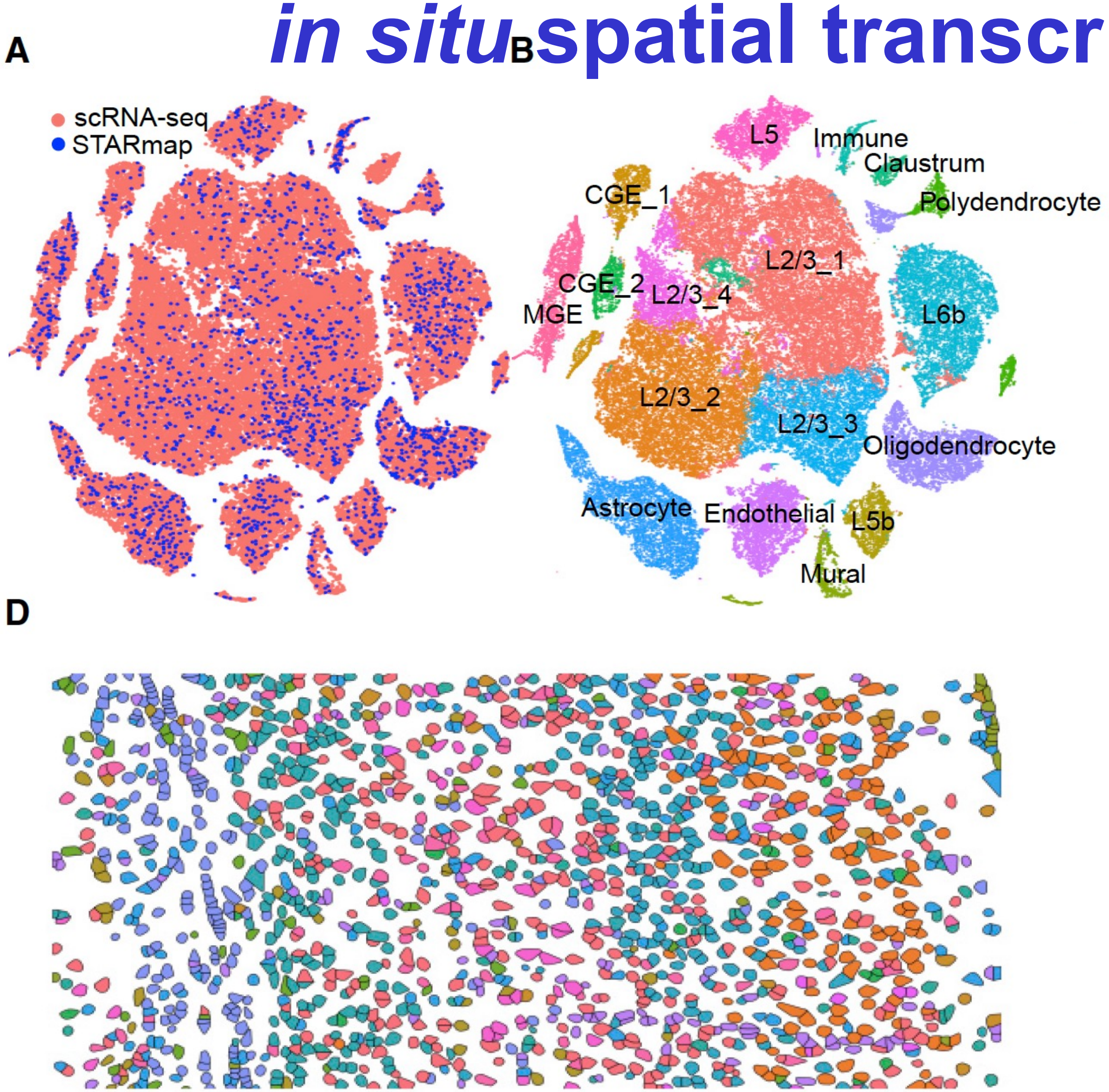
In situ spatial transcriptomic data in mouse frontal cortex
STARmap


Intact tissue
Cell
mRNA
Targeted in situ RNA sequencing approach
SNAIL probes
Barcodes
mRNA
Amplify
DNA amplicons
NH_2
Hydrogel-tissue chemistry
DNA-gel
3D sequencing in the gel
Ch1
Ch2
Ch3
Ch4
SEDAL sequencing with error-reduction
AANNN
GTTXXXG
5-nt code
error-checking
A C G T
Decode
>1000 genes mapped over six cycles
STARmap: discovery and distribution of cell types in 3D
Cell types
Genes
Cells
Single-cell analyses of gene expression

color series	5-nt code	Gene
🟢🔵🟢🔴🟢🟠	TTGCA	1
🟠🟢🔴🟢🔵🟢	ACGTT	2
...	...	...
🔵🟢🔴🟢🟠🟢	GTACT	n

Wang, X. et al. Science (2018).

Using LIGER to integrate single-cell transcriptomic and *in situ* spatial transcriptomic data

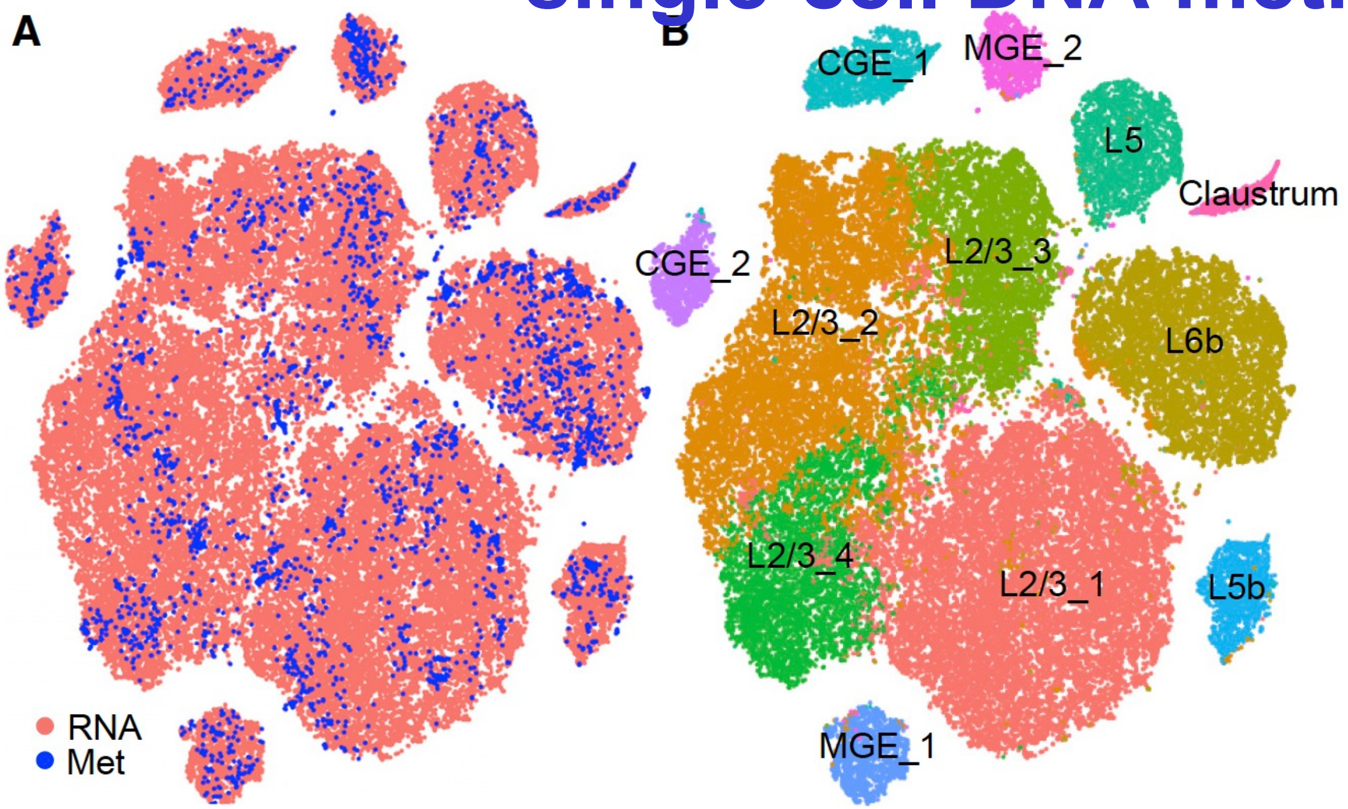


71,000 cells from scRNA-seq
2,500 cells from STARmap

What are advantages of integrated analysis of these 2 datasets?

Welch, et al. *Cell* (2019).

Using LIGER to integrate single-cell transcriptomic and single-cell DNA methylation data



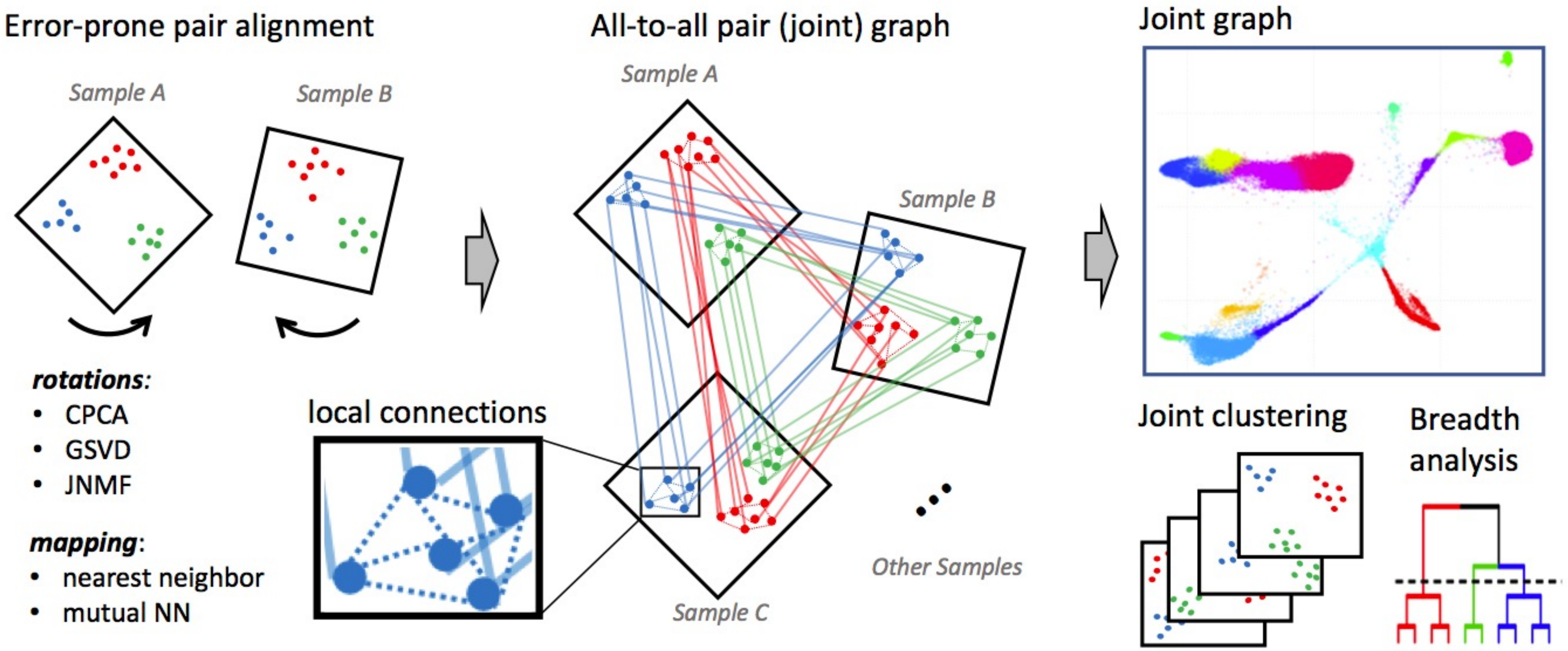
56,000 cells from scRNA-seq
3,000 cells from DNA methylation

"We reasoned that, because gene body methylation is generally anticorrelated with gene expression, reversing the direction of the methylation signal would allow joint analysis."

Welch, et al. *Cell* (2019).

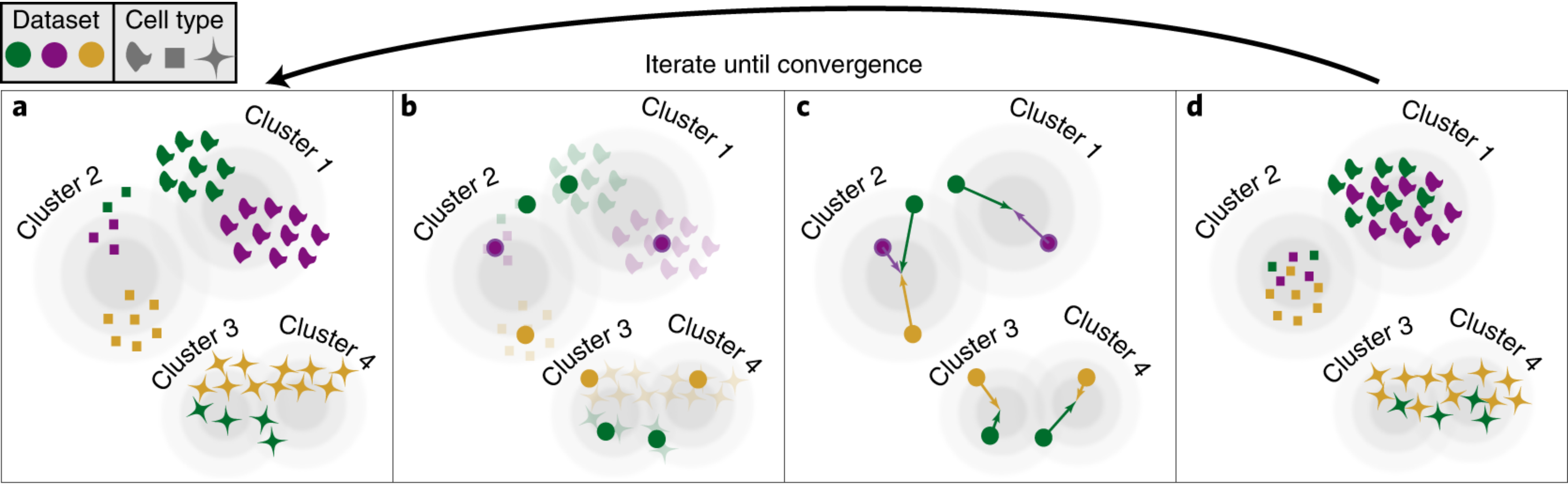
Batch correction and data modality integration using Conos

Conos (Clustering on Network of Samples)



Barkas N., et al. *Nature Methods* (2019).

Batch correction and data modality integration using Harmony



Soft assign cells to clusters, favoring mixed dataset representation

Get cluster centroids for each dataset

Get dataset correction factors for each cluster

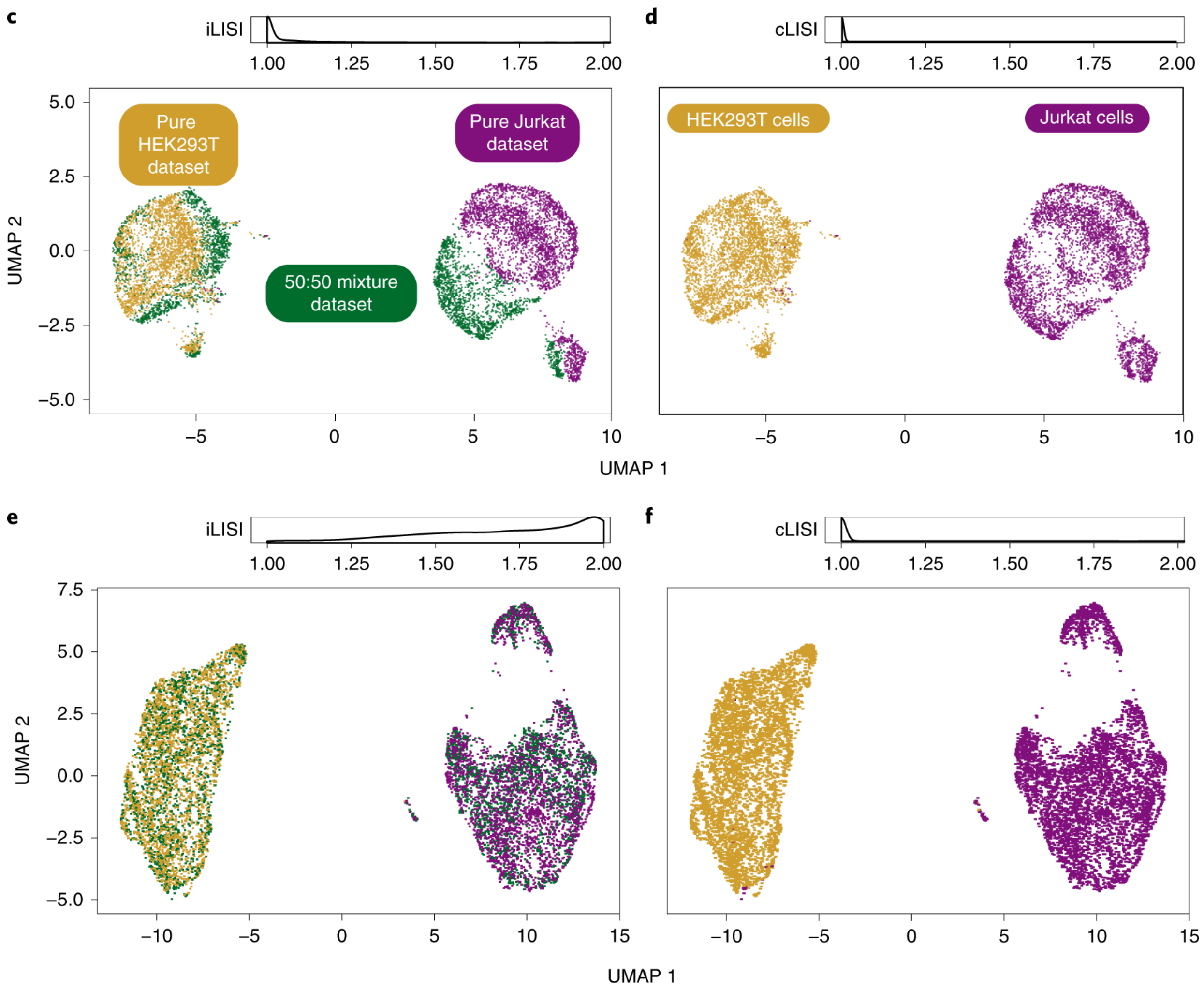
Move cells based on soft cluster membership

Korsunksy I. et al. *Nature Methods* (2019).

Harmony integrates different datasets while maintaining cell type differences

Before integration

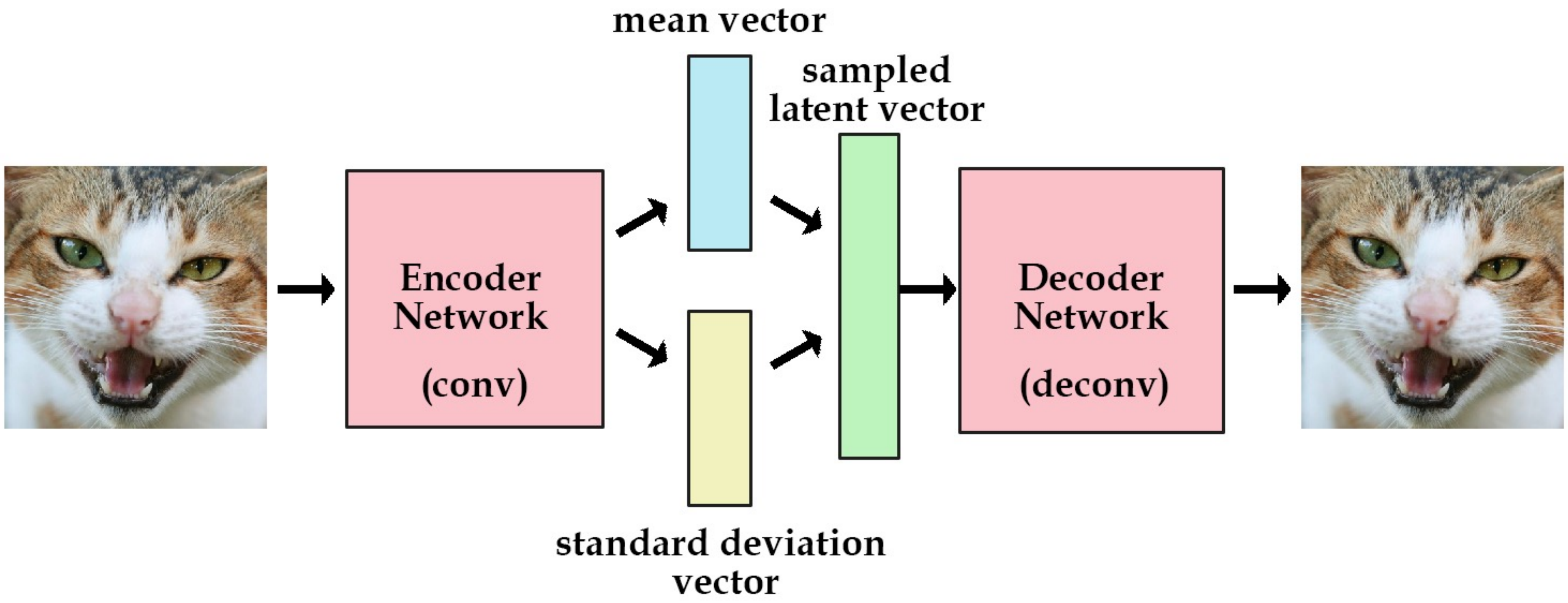
After integration



Korsunksy I. et al. *Nature Methods* (2019).

Batch correction with deep learning approaches

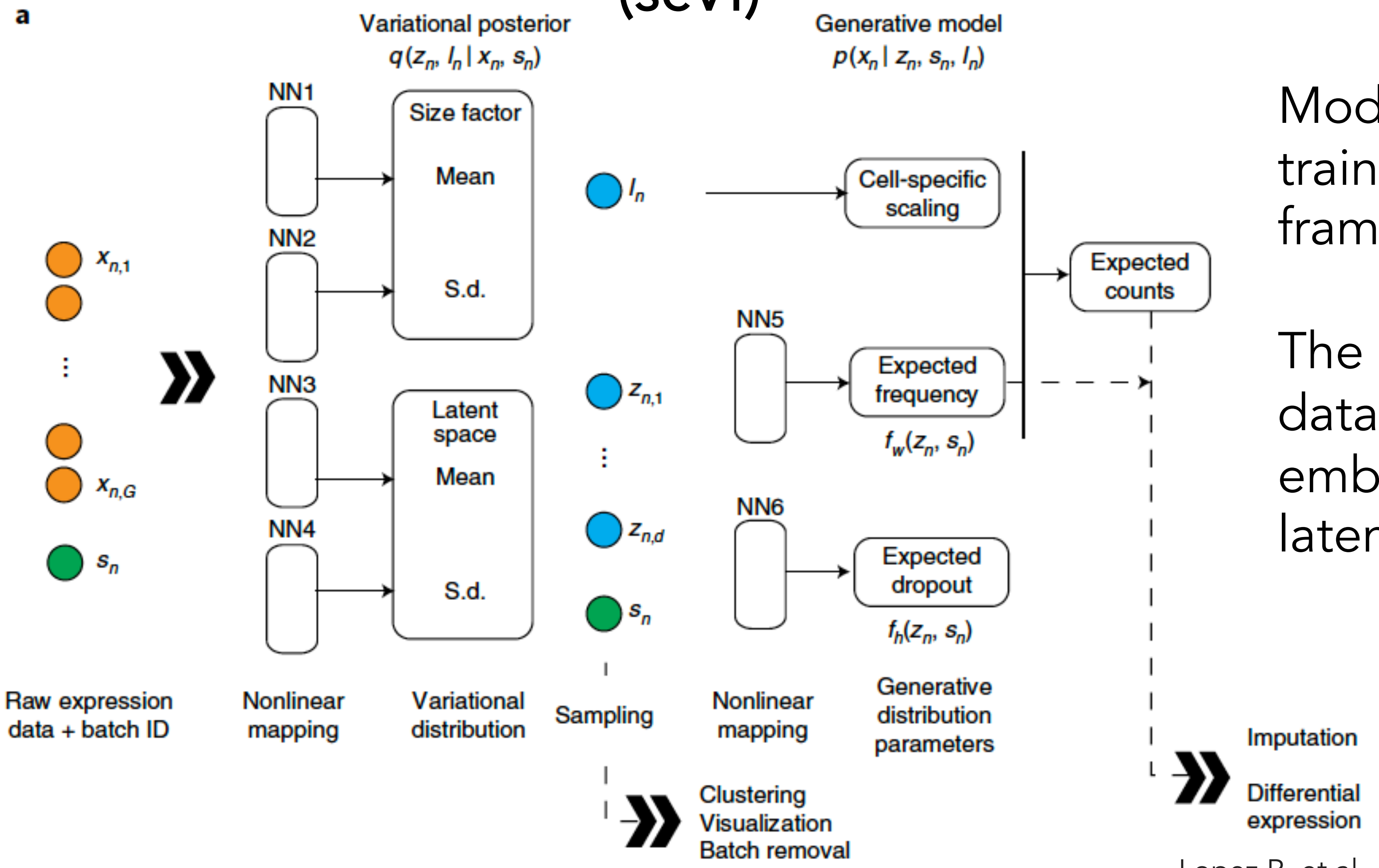
Autoencoders and variational autoencoders are popular frameworks for embedding single cell genomics datasets.



http://kvfrans.com/variational-autoencoders-explained/

Batch correction with deep learning approaches

single-cell variation inference (scVI)



Models follow a train/validate/test framework

The "integrated data" is the embedding in the latent space.

Lopez R. et al. Nature Methods (2018).

Comparison of data integration methods

a

	Considerations	scANVI	Scanorama embed	scVI	FastMNN embed	scGen	Harmony	FastMNN gene	Seurat v3 RPCA	BBKNN	Scanorama gene	ComBat	MNN	Seurat v3 CCA	trVAE	Conos	DESC	LIGER	SAUCIE embed	SAUCIE gene
Input	Programming language	🐍	🐍	🐍	R	🐍	R	R	R	🐍	🐍	🐍/R	🐍/R	R	🐍	R	🐍	R	🐍	🐍
	Method runs without additional information	✗				✗														
Scib results	Consistent top performer	✓	✓	✓		✓														
	Top method on small/ simple tasks		✓		✓	✓	✓													
	Top method on large/ complex tasks	✓	✓	✓		✓														
	Top method on ATAC data	—		—			✓											✓		
Task details	Integrates strong batch effects	✓	—	—		✓			—	—				—						
	Top method for recovery cell states or modules	✓	✓								✓	✓	✓							
	Confounding of bio and batch variance	✓	—			✓														
	Top method for trajectories	—	✓	—	✓	✓														
	Method deals with varying compositions											✗								
Speed	Fast method for quick results									✓		✓								
	Scales well to large datasets on CPU	✓	—	✓						✓	—								✓	✓
	Method has GPU support	✓		✓		✓									✓		✓		✓	✓
	Scales well to feature spaces beyond genes														✓	✓				
Output	Method shows corrected expression					✓		✓	✓		✓	✓	✓	✓						✓
	Method gives relative cell embeddings									✗						✗				

✓ Fulfills the criterion

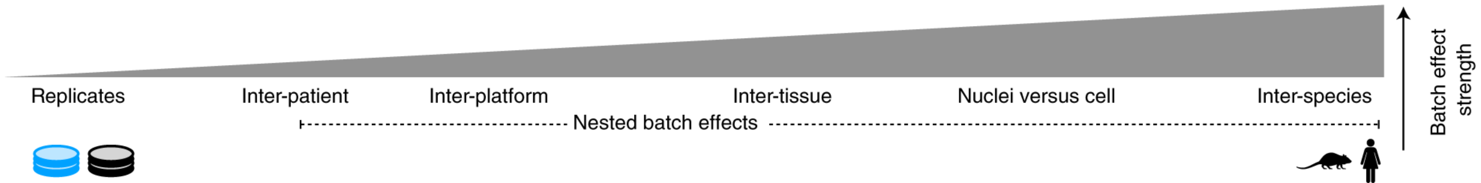
— Partial fulfillment of criterion

✗ Does not fulfill criterion

🐍 Python

R R

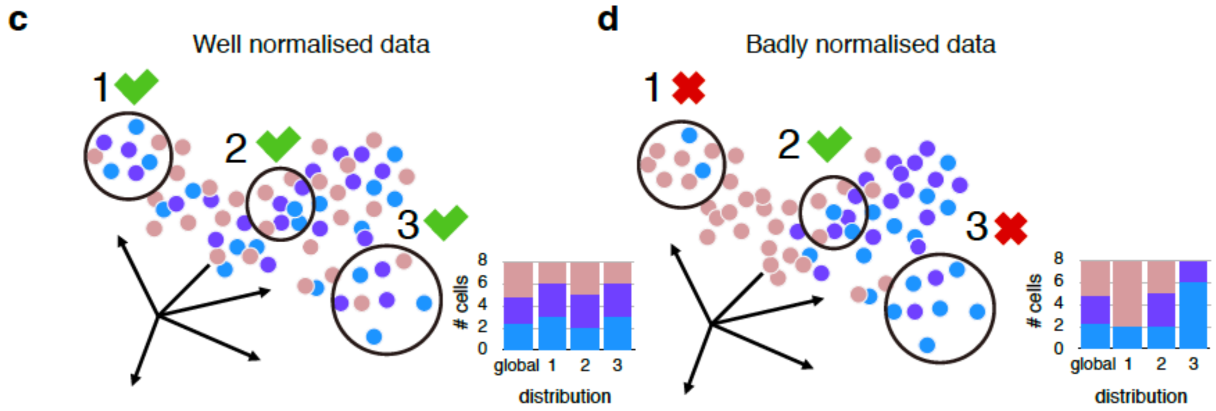
b



Luecken M. D. et al. (2022) *Nature Methods*.

Methods to assess performance of batch correction

- Entropy of batch mixing
- kBET - k-nearest neighbor batch effect test



- Silhouette coefficient
  - cells of the same cell type are close together and far from other cells of a different type
- Adjusted rand index
  - do batch labels and cluster labels agree with one another?
- Biological significance

Luecken M. D. et al. (2022) *Nature Methods*.

Common assumptions during batch correction

- At least one cell population is found shared in both datasets.

- The batch effect and the biological differences do not overlap with one another (ie orthogonal).

- The magnitude and variation of the biological effect you care about is greater than that of the batch effect.

Will batch correction remove biological differences in single cell transcriptomic data? (ChatGPT response)

Batch correction in single-cell transcriptomics is designed to remove technical variations that arise due to differences in the experimental procedures such as sample preparation, sequencing, and data analysis. The goal of batch correction is to reduce technical noise in the data and allow biological differences between cells to be better distinguished.

However, batch correction can potentially remove some biological differences in the data, particularly if the biological differences are correlated with the batch effect. For example, if cells from different batches are isolated from different tissues or at different stages of development, then the batch correction algorithm may inadvertently adjust the expression levels of genes that are differentially expressed between these groups.

Therefore, it is important to carefully evaluate the results of batch correction and to consider the biological context of the data. One approach is to compare the results of batch-corrected data with uncorrected data and to ensure that the biological differences identified in the uncorrected data are still present after batch correction. It is also important to choose an appropriate batch correction method that is suitable for the specific experimental design and to use quality control metrics to ensure that the batch correction has not removed important biological information.

Recent helpful articles on data integration methods

Argelaguet, Ricard, et al. "Computational principles and challenges in single-cell data integration." *Nature biotechnology* (2021).

- Review

Luecken, Malte D., et al. "Benchmarking atlas-level data integration in single-cell genomics." *Nature methods* (2022).

- Methods comparison

Tran, Hoa Thi Nhu, et al. "A benchmark of batch-effect correction methods for single-cell RNA sequencing data." *Genome biology* (2020).

- Methods comparison

Once data is integrated, how do we compare across biological conditions?

When detecting gene differentially expressed across different biological conditions (e.g. case vs control, treated vs untreated, etc…), what is the replicate? A single cell? A sample?

Treating a single cell as a replicate will lead to false discoveries. Strongly consider using pseudobulk models or mixed effect models when comparing samples across conditions.

- Crowell, Helena L., et al. "Muscat detects subpopulation-specific state transitions from multi-sample multi-condition single-cell transcriptomics data." *Nature communications* (2020).
- Squair, Jordan W., et al. "Confronting false discoveries in single-cell differential expression." *Nature communications* (2021).
- https://www.nxn.se/valent/2019/2/15/handling-confounded-samples-for-differential-expression-in-scrna-seq-experiments

Remember: go back to normalized (non batch-corrected) gene expression values for DE analyses! Add covariates for your batches in the DE statistical model.

Dylan Rausch
@dylanrausch

🧐🤔 #singlecell #Bioinformatics

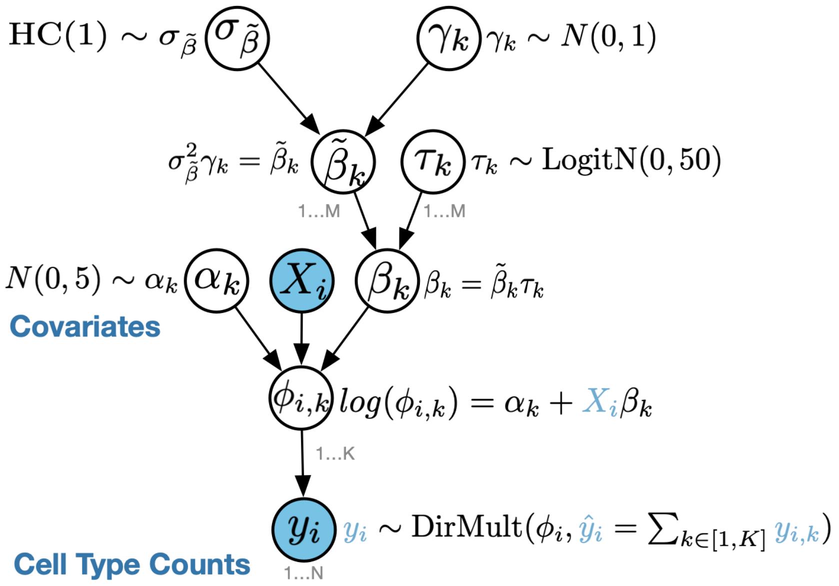
IM RUNNING DIFFERENTIAL EXPRESSION ON SINGLE CELL DATA
YOU'RE NOT TREATING CELLS AS REPLICATES, RIGHT?
YOU'RE NOT TREATING CELLS AS REPLICATES, RIGHT?
imgflip.com

Testing for significant changes in cell type abundance across biological conditions

Assume we have 3 cell types (A, B, C) in a treated and untreated sample. If the frequency of cell type A increases in the treated sample due to the treatment, what will happen to the frequencies of cell types B and C?

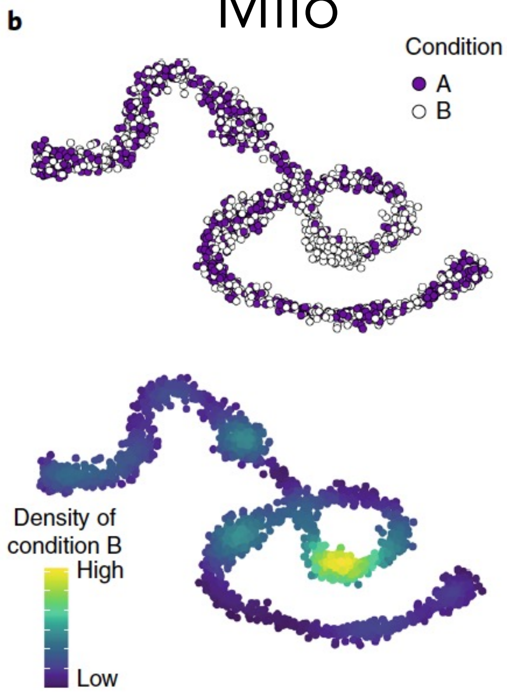
The compositional nature of cell type abundance data means the abundances of cell type A, cell type B, and cell type C are not independent of one other. Their frequencies must always sum to 1.

scCODA



$\mathrm{HC}(1) \sim \sigma_{\tilde{\beta}}$
$\sigma_{\tilde{\beta}}$
γ_k
$\gamma_k \sim N(0,1)$
$\sigma^2_{\tilde{\beta}}\gamma_k = \tilde{\beta}_k$
$\tilde{\beta}_k$
τ_k
$\tau_k \sim \mathrm{LogitN}(0,50)$
1...M
1...M
$N(0,5) \sim \alpha_k$
α_k
X_i
β_k
$\beta_k = \tilde{\beta}_k\tau_k$
Covariates
$\phi_{i,k}$
$log(\phi_{i,k}) = \alpha_k + X_i\beta_k$
1...K
y_i
$y_i \sim \mathrm{DirMult}(\phi_i, \hat{y}_i = \sum_{k\in[1,K]} y_{i,k})$
Cell Type Counts
1...N

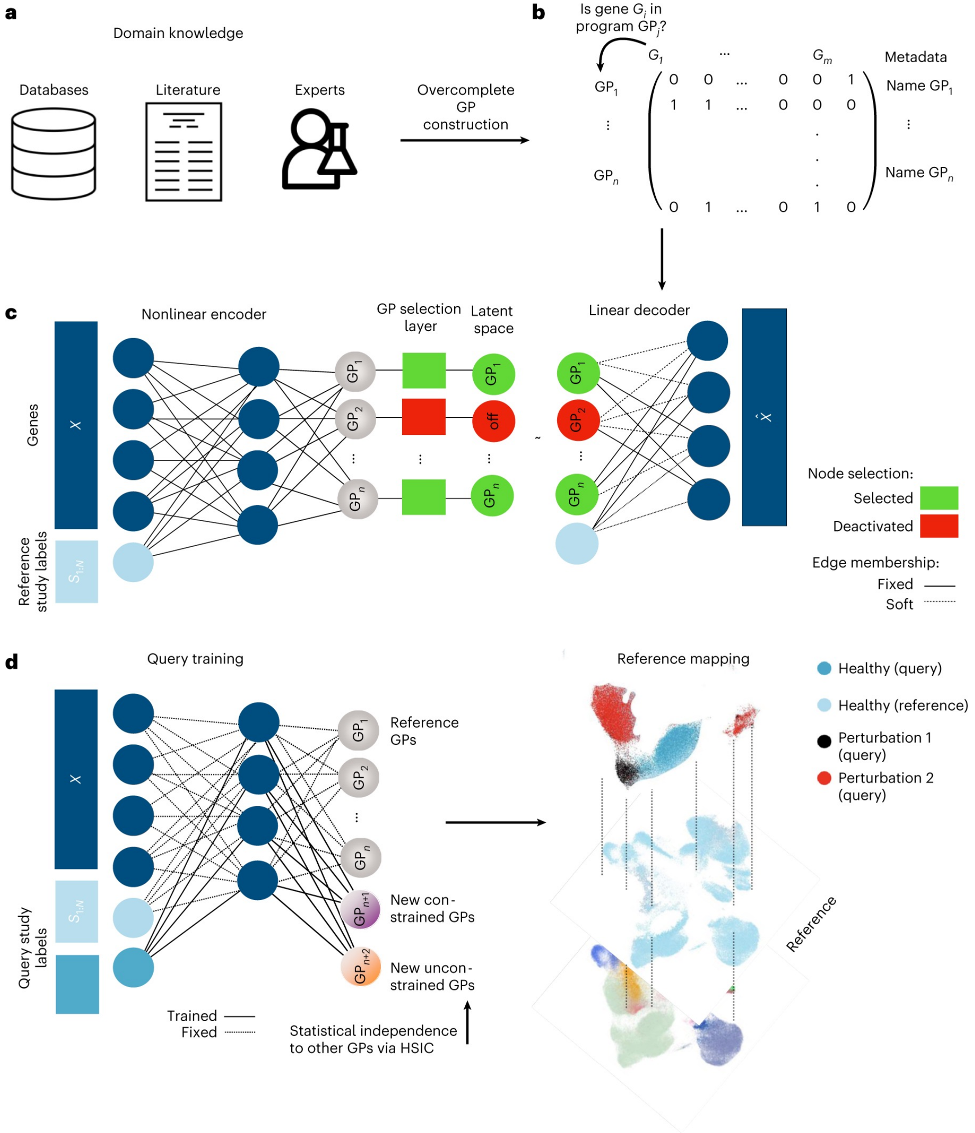
Milo



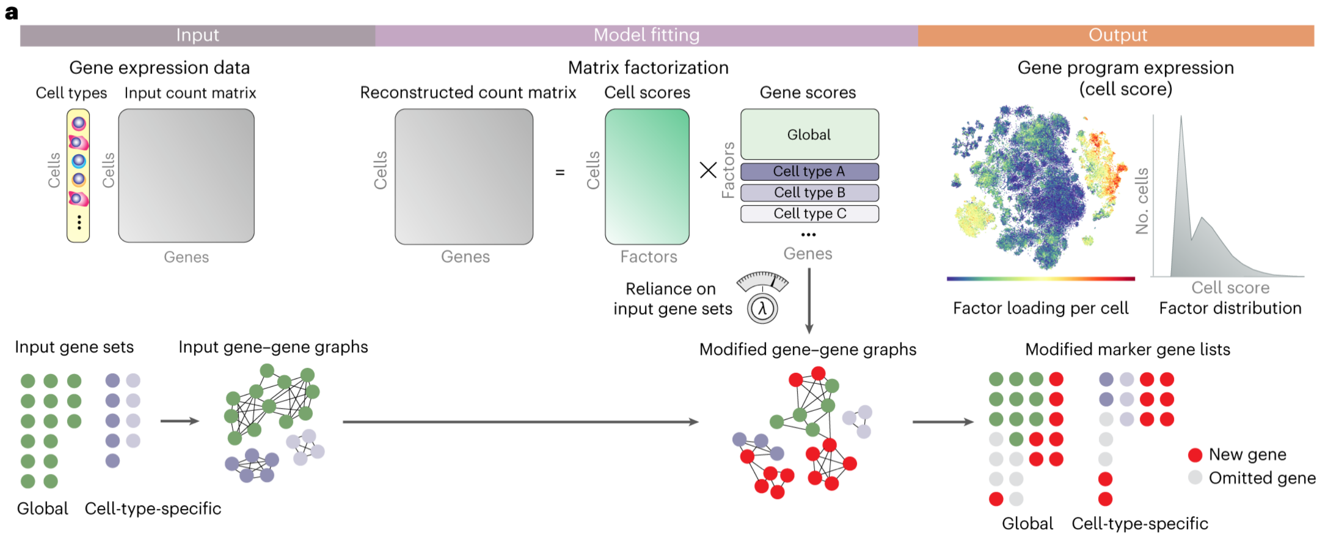
Büttner et al., *Nature Communications*. (2021).
Dann et al., *Nature Biotech*. (2022).

Integrating datasets and mapping to gene programs

ExpiMap (deep learning)



Spectra (matrix factorization)



Lotfollahi M. et al., *Nature Methods*. (2023).
Kunes R. Z. et al., *Nature Biotechnology*. (2023).