



scRNAseq2021

Data integration and batch correction

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Why integrate?

Sources of variance in SC-RNAseq data



Biological:

- Cell Type Heterogeneity
- Genetics
- Cell State/Microenvir.
- GExpr Stochasticity
- Cell Cycle Dynamics
- Transcriptional Bursts
- Oscillations

...

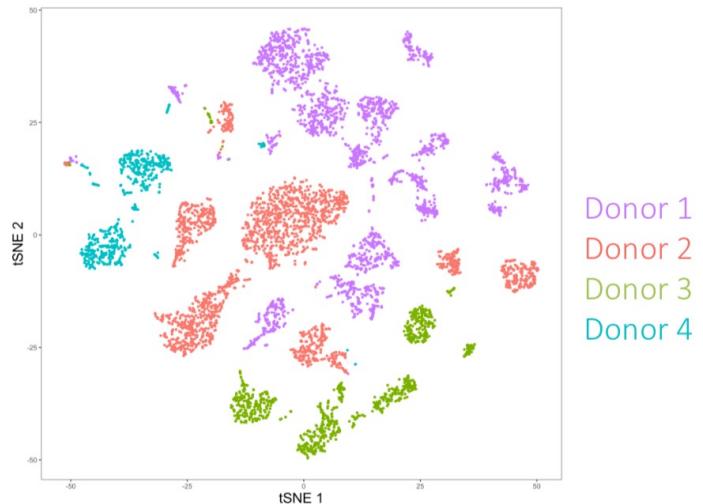
Technical:

- Capture Efficiency
- Amplification Bias
- PCR artifacts
- Contamination
- Cell Doublets
- Cell Damage
- Sampling (Jackpot Effects)

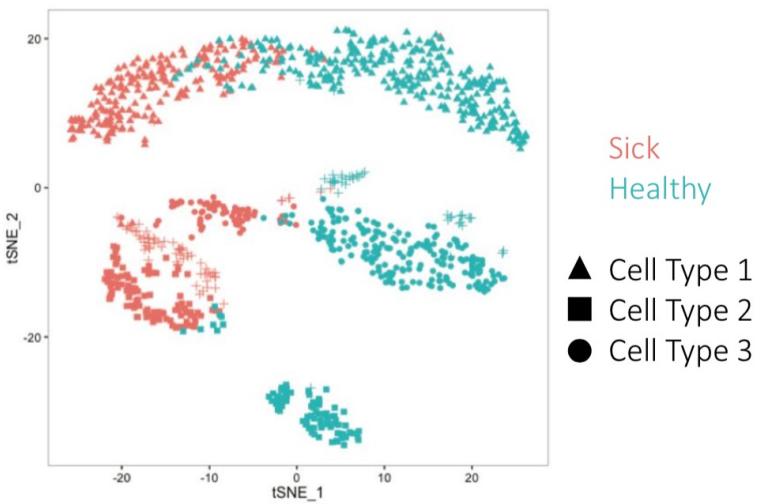
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Why integrate?

Sources of variance in SC-RNAseq data



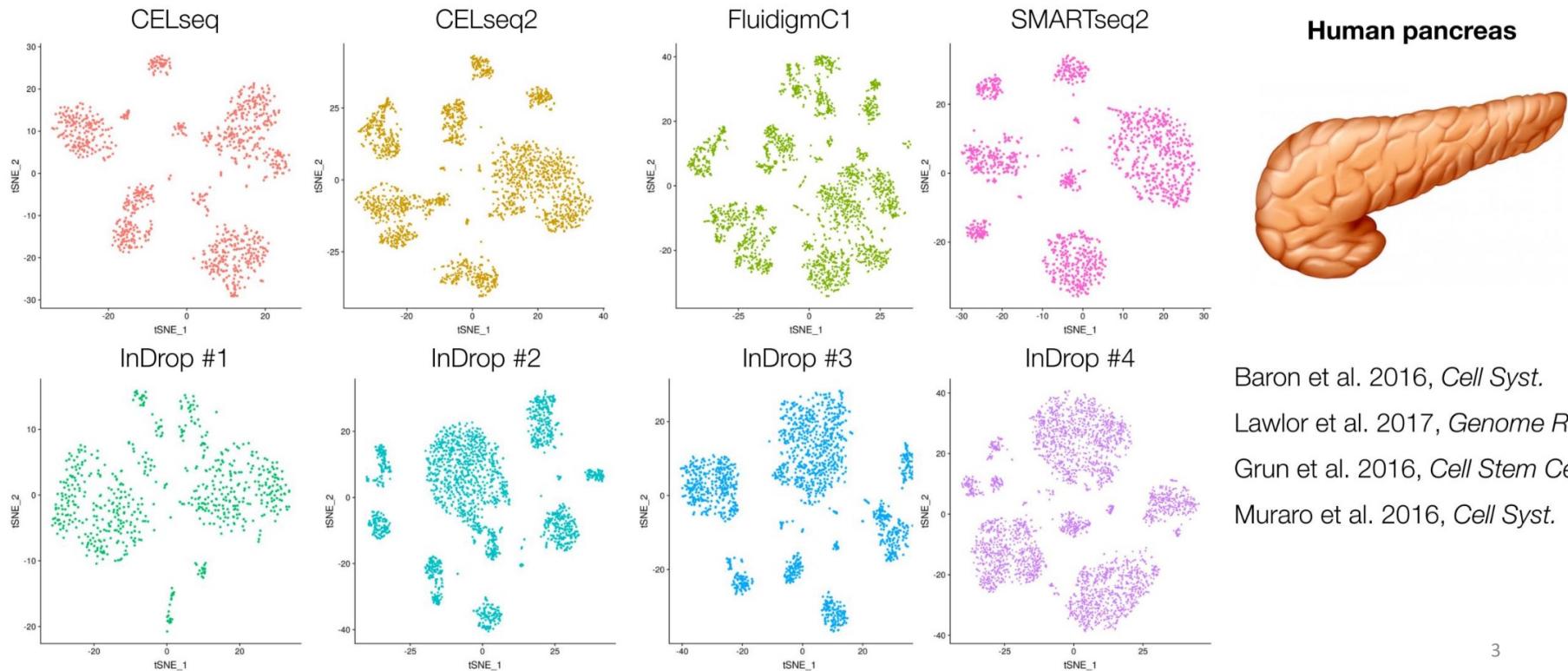
Same tissue from different donors



Cross condition comparisons

Why integrate?

Building a cell atlas: 8 maps of the human pancreas



Baron et al. 2016, *Cell Syst.*

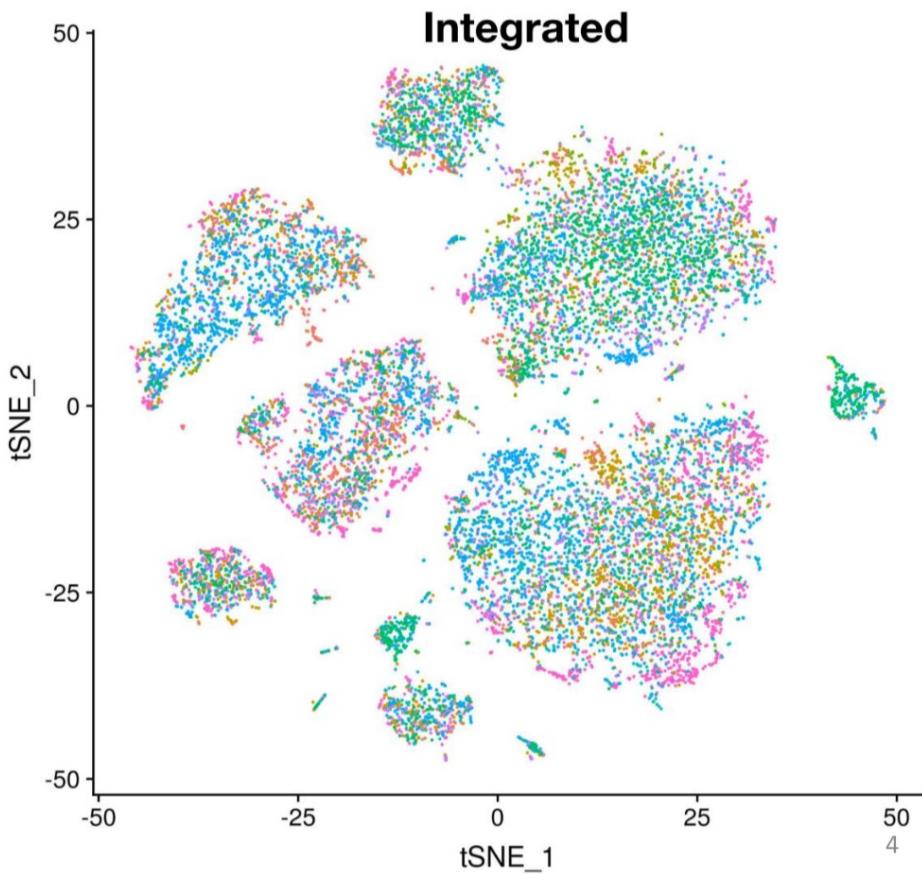
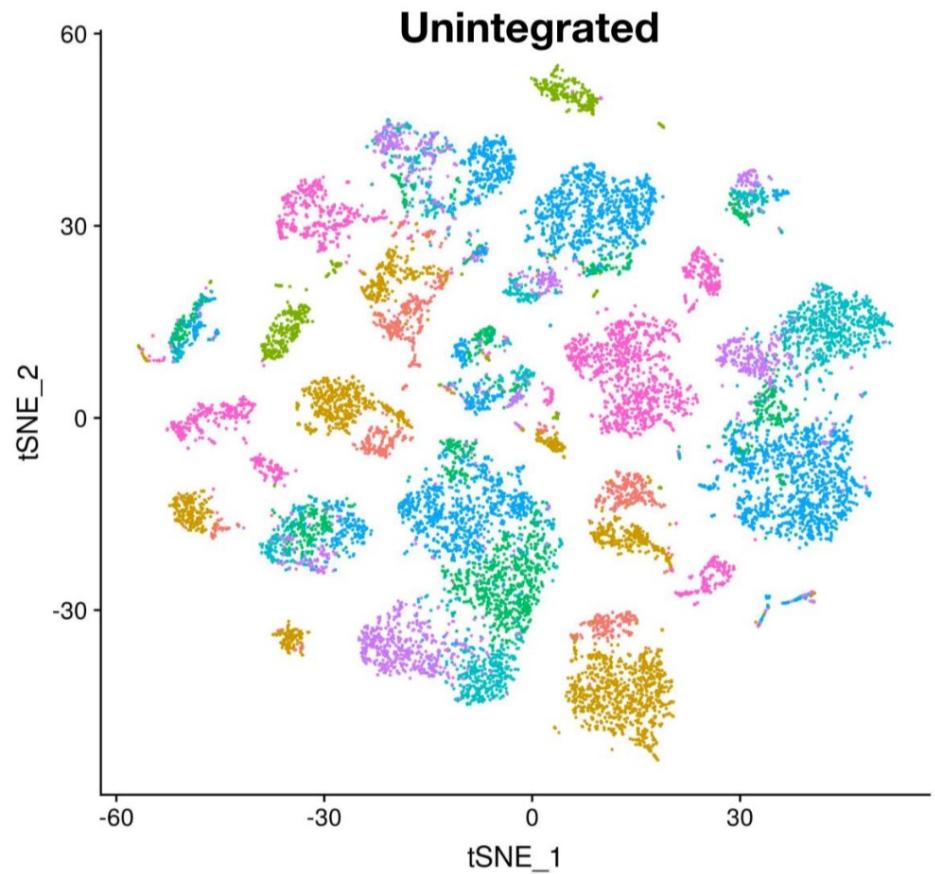
Lawlor et al. 2017, *Genome Res.*

Grun et al. 2016, *Cell Stem Cell*

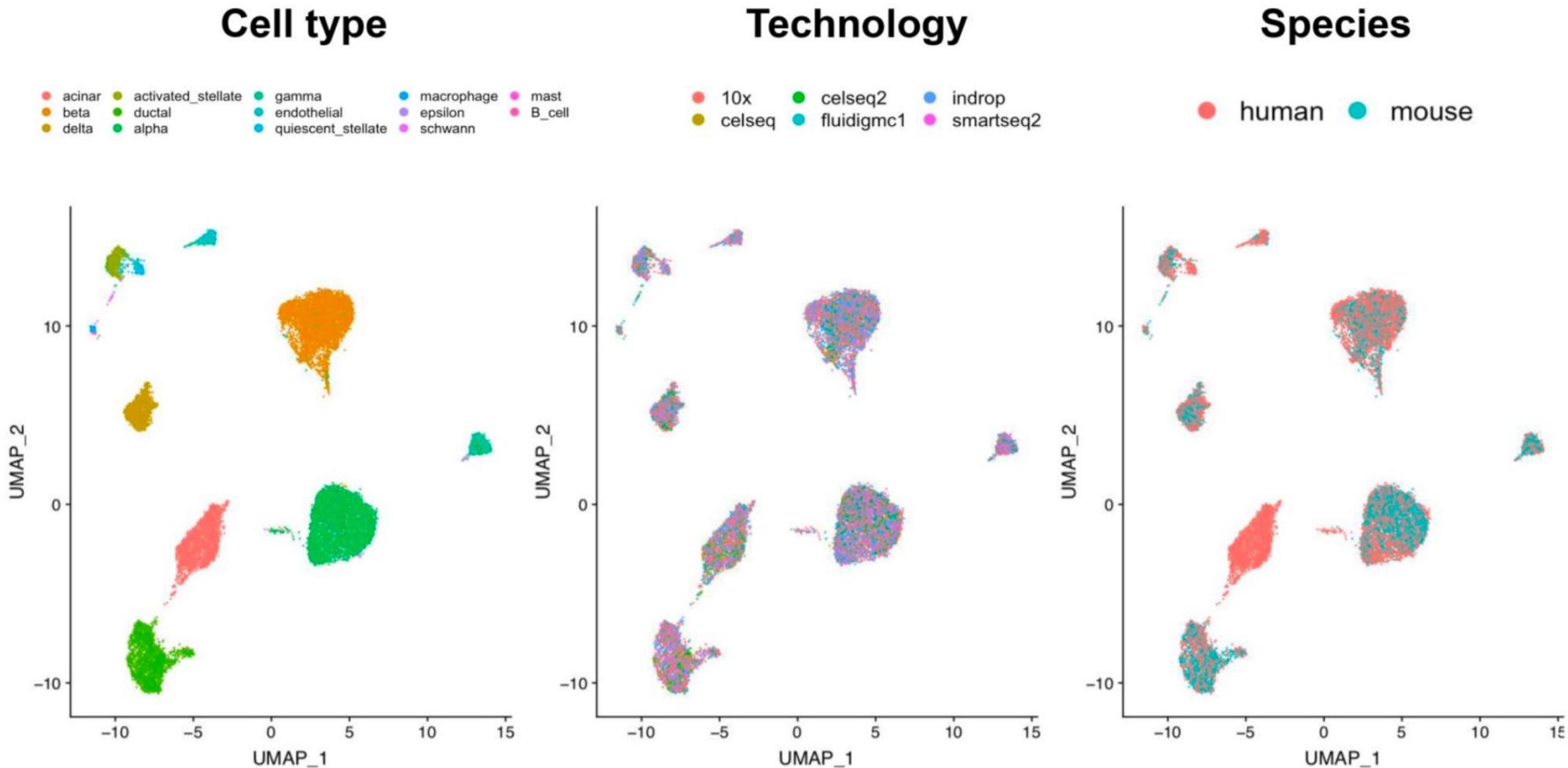
Muraro et al. 2016, *Cell Syst.*

Why integrate?

Building a cell atlas: 8 maps of the human pancreas



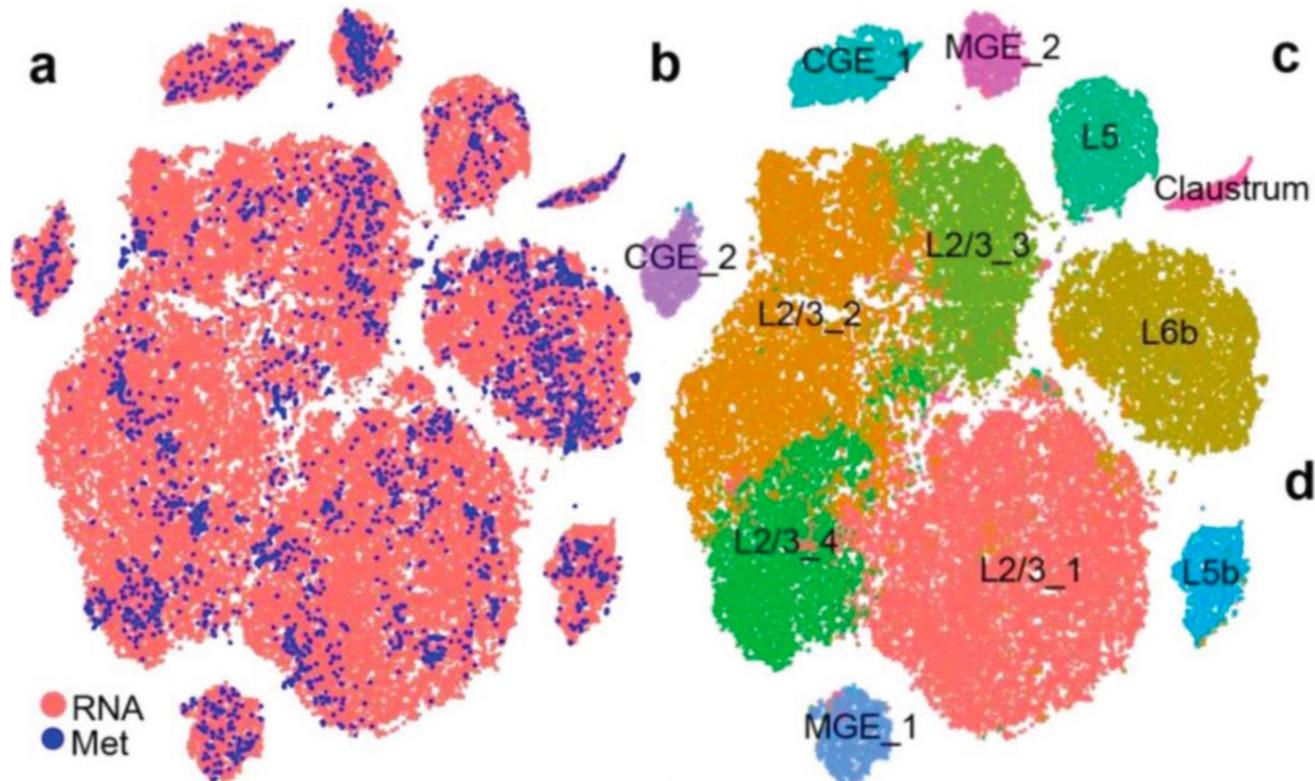
Integration across modalities



Retinal bipolar datasets: 51K cells, 6 technologies, 2 Species

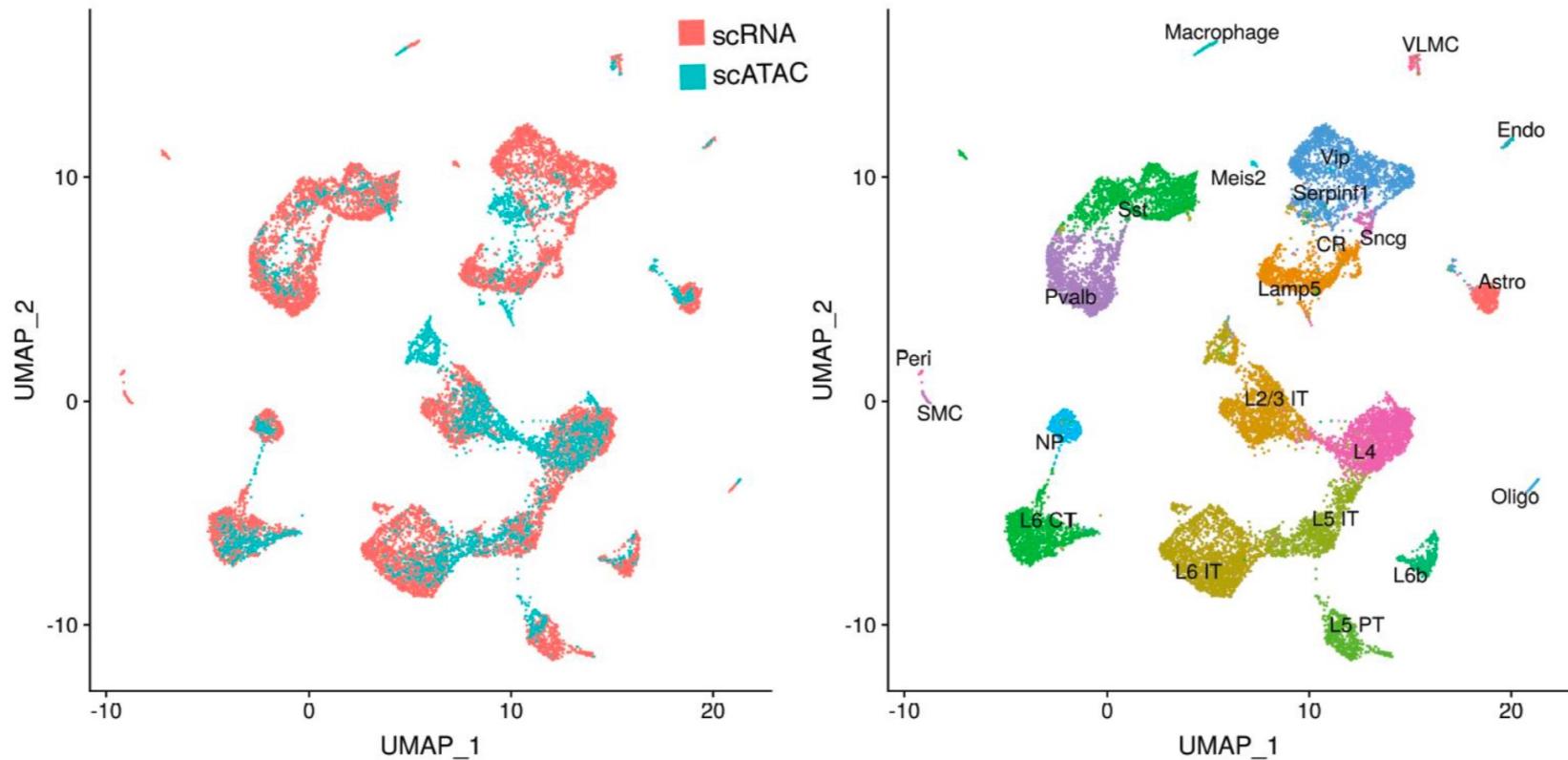
Integration across modalities

RNA-seq and methylation



Integration across modalities

RNA-seq and ATAC-seq

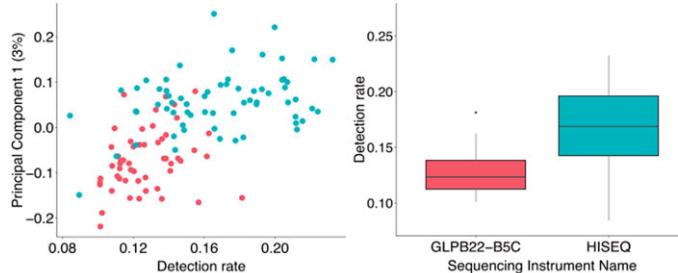


Confounders and batch effects

1. Technical variability

- Changes in sample quality/processing
- Library prep or sequencing technology
- ‘Experimental reality’

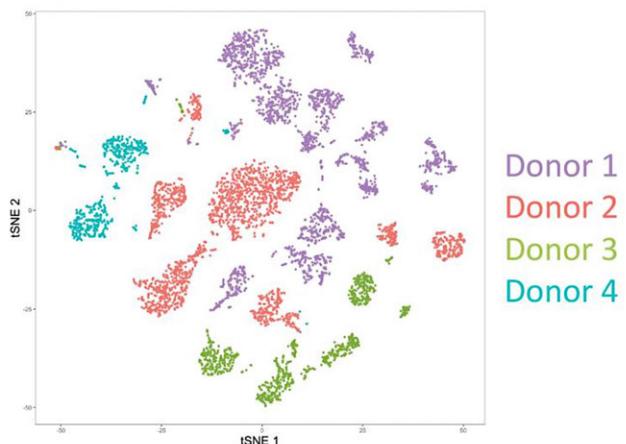
Technical ‘batch effects’ confound downstream analysis



2. Biological variability

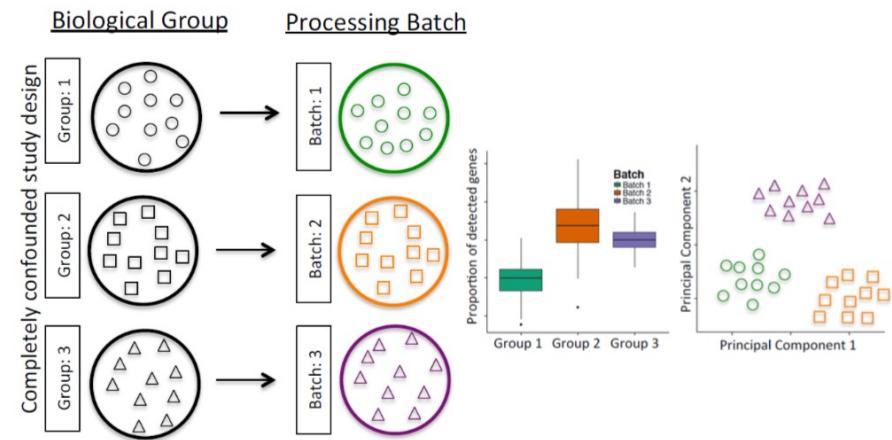
- Patient differences
- Environmental/genetic perturbation
- Evolution! (cross-species analysis)

Biological ‘covariates’ confound comparisons of scRNA-seq data



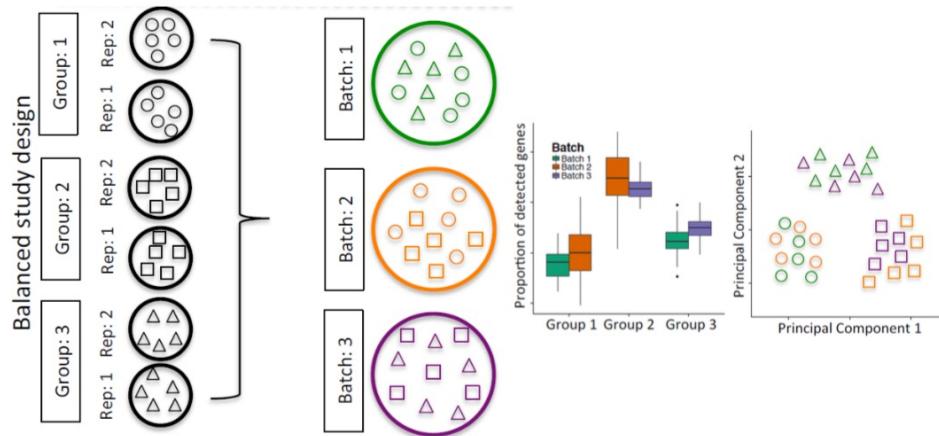
Confounders and batch effects

Confounded design



Don't design your experiment like this!!!

Not confounded design



Good experimental design *does not remove batch effects*, it prevents them from biasing your results.

Integration methods overview

Batch correction methods

Regression-based correction:

- Regression via GLM
- ComBat (doi.org/10.1093/biostatistics/kxj037)
- RUVseq (10.1038/nbt.2931)

Joint dimensionality reduction:

- common PCA / CPCCA (doi.org/10.1006/jmva.2000.1908)
- contrastive PCA / cPCA (<https://doi.org/10.1038/s41467-018-04608-8>)
- LIGER (<https://doi.org/10.1101/459891>)
- zinb-wave (10.1038/s41467-017-02554-5)
- scMerge (<https://doi.org/10.1073/pnas.1820006116>)
- Harmony (<https://doi.org/10.1101/461954>)

Graph-based joint clustering:

- MNНcorrect (<https://doi.org/10.1038/nbt.4091>)
- Conos (<https://doi.org/10.1101/460246>)

Joint dimensionality reduction + Graph-based joint clustering

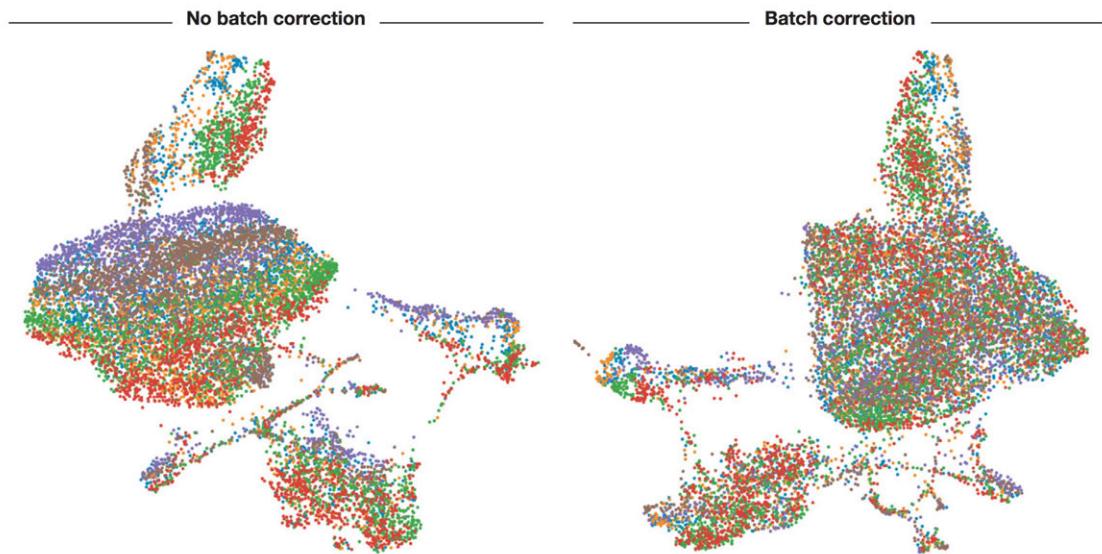
- CCA + anchors (Seurat v3) (<https://doi.org/10.1101/460147>)
- CCA + dynamic time warping (Seurat v2) (<https://doi.org/10.1038/nbt.4096>)
- Scanorama (<https://doi.org/10.1101/371179>)
- fastMMN (<https://doi.org/10.1038/nbt.4091>)

And many many others

ComBat

ComBat

- Uses empirical Bayes regression on shared gene factors
- Works well on simpler small-medium datasets
- All datasets need to be similar in cell type composition
- Will fail in large datasets with complex mixture of cell type



Major issues of regression-based batch correction methods:

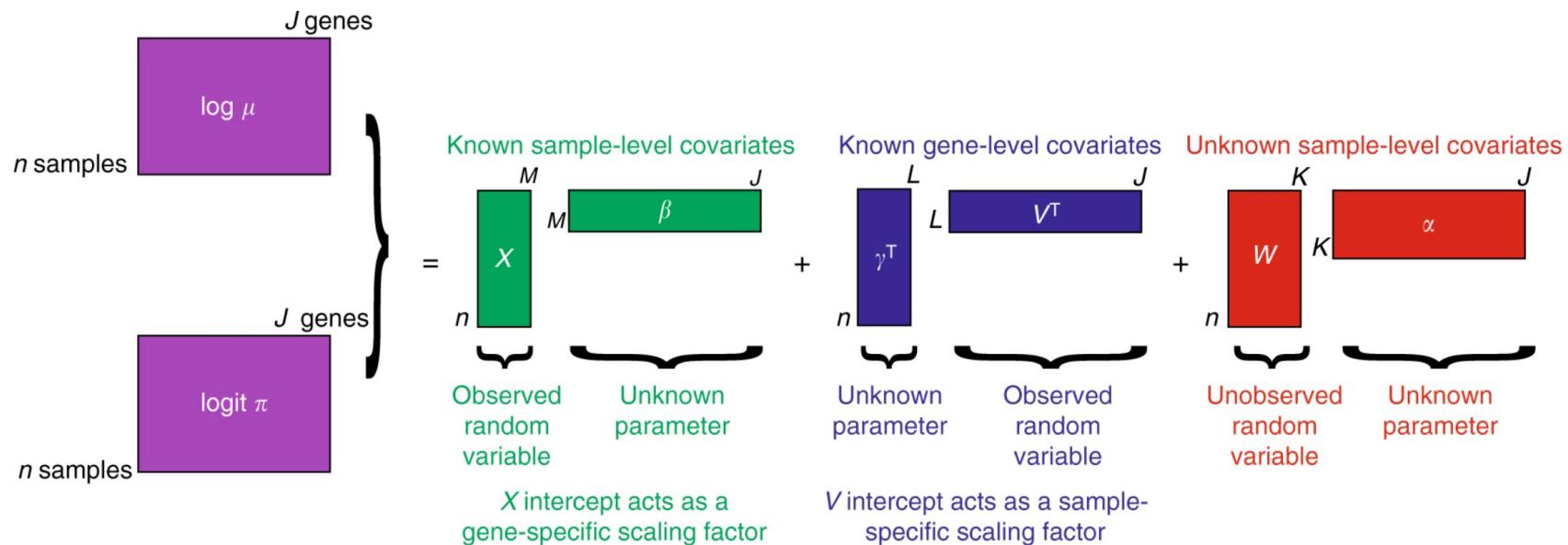
- `limma::removeBatchEffect()`
- `seurat::ScaleData()` #using the regression parameter
- `sva::combat()`
- `batchelor::rescaleBatches()`

1. Do not account for differences in population composition
2. Assume batch effect is additive
3. Prone to overcorrection (in cases of partial confounding)

zinb-wave

Zimwave

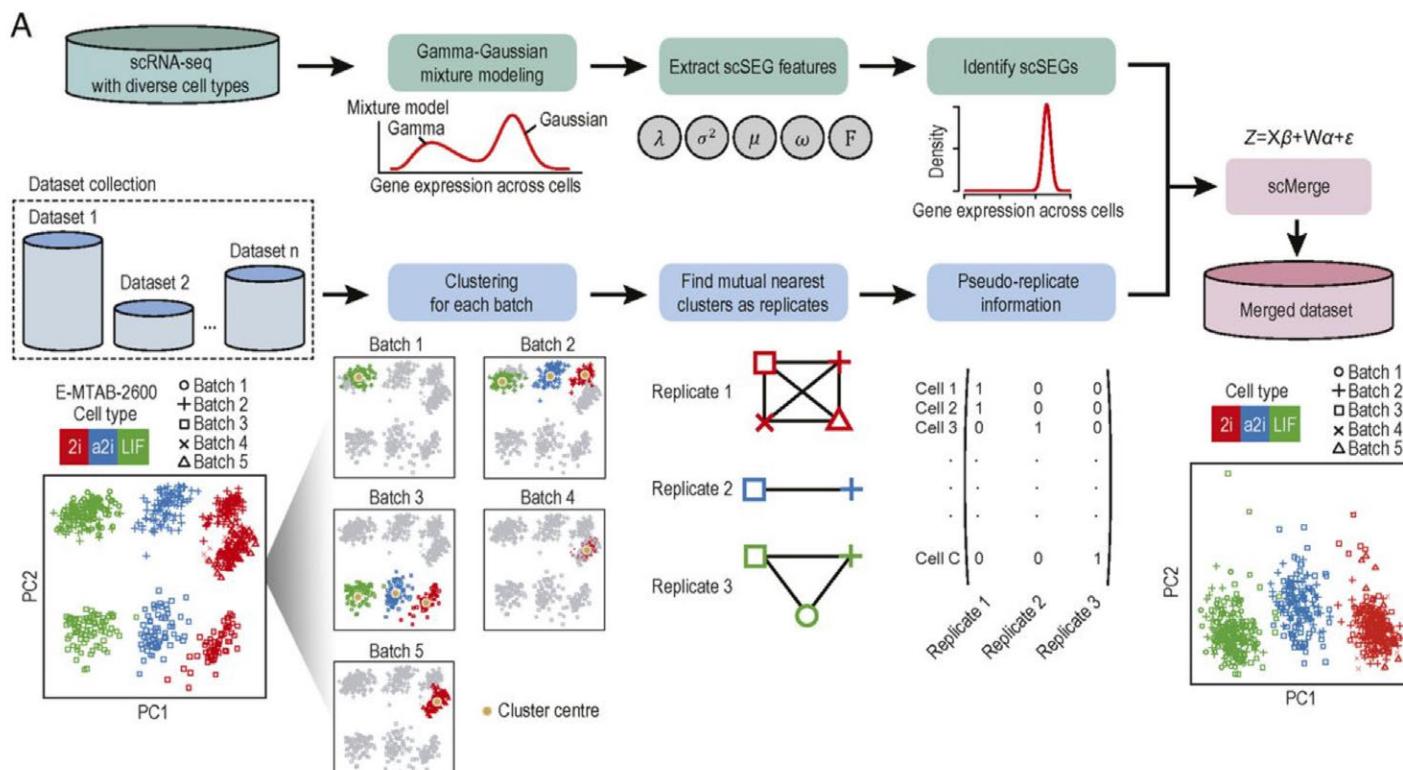
- Applies a matrix factorization model to accommodate both gene and cell covariates
- Similar to ZIFA (zero-inflated factor analysis)
- **Works well on simpler small-medium datasets**
- **It will be slow on large datasets**



scMerge

scMerge

- Identifies single-cell stably expressed genes (scSEGs)
- Uses a fast implementation of RUV-seq to scale other genes based on the scSEGs
- Works well on simpler small-medium datasets**
- It will be slow on large datasets**

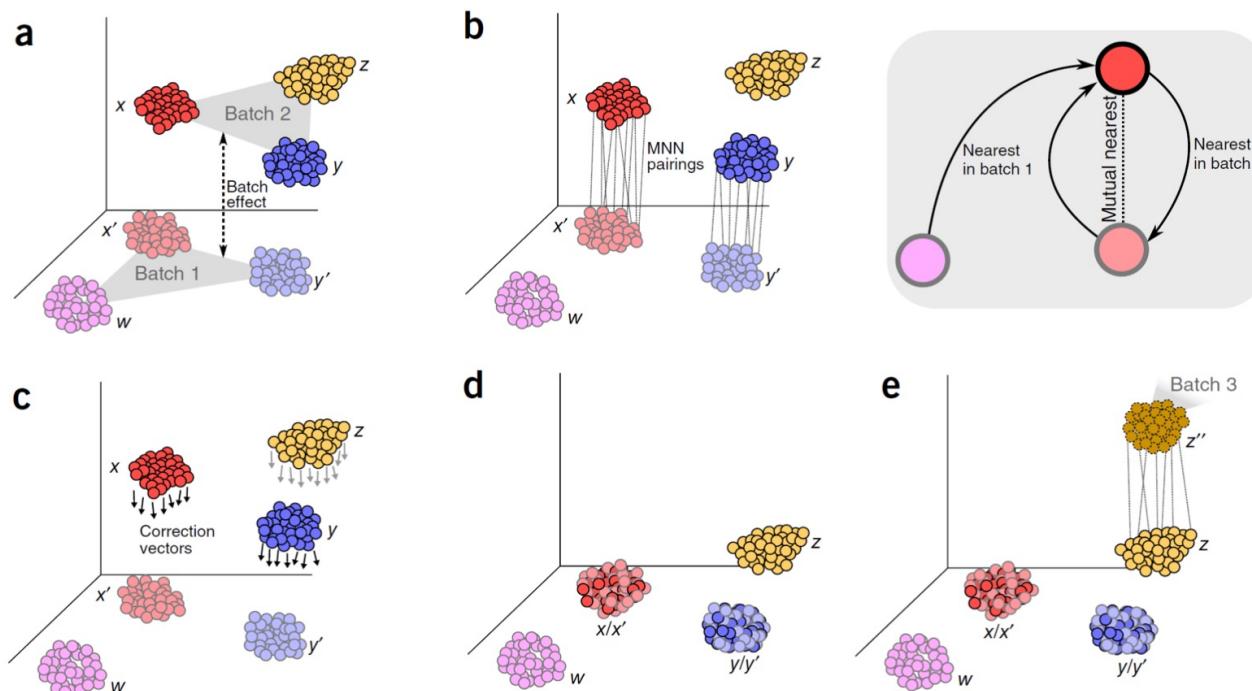


Mutual Nearest Neighbors (MNN)

THE “turning point” method

Mutual Nearest Neighbors (MNN)

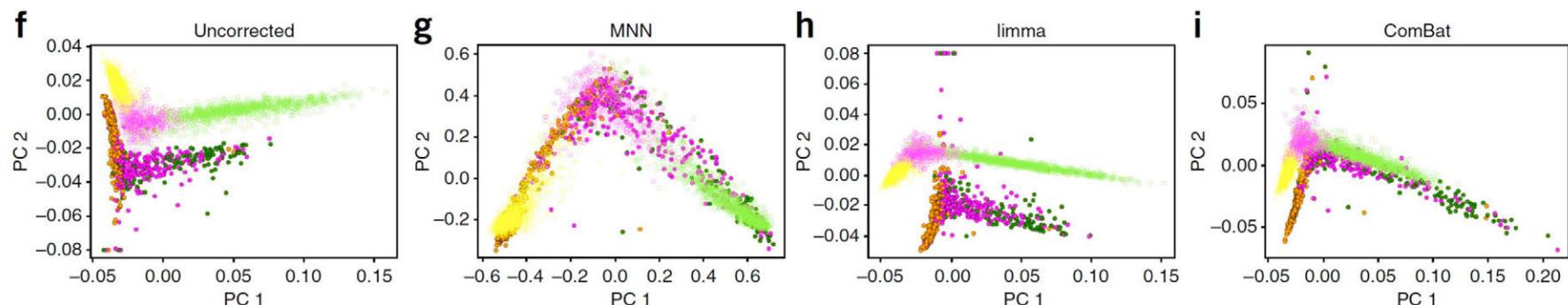
- Dimensionality reduction via multibatch PCA with all datasets
- Find K-NN across datasets
- Compute merging vectors
- **It scales well on large datasets**



Mutual Nearest Neighbors (MNN)

Model assumptions

1. There is at least one cell population that is present in both batches,
2. The batch effect is almost orthogonal to the biological subspace, and
3. Batch effect variation is much smaller than the biological effect variation between different cell types



SMART-seq2

- MEP
- GMP
- CMP

MARS-seq

- MEP
- GMP
- CMP

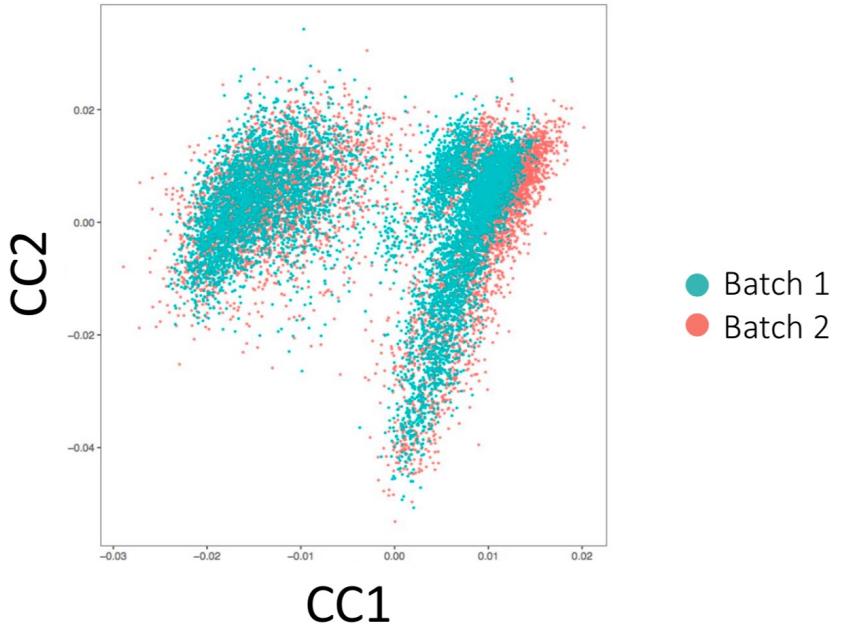
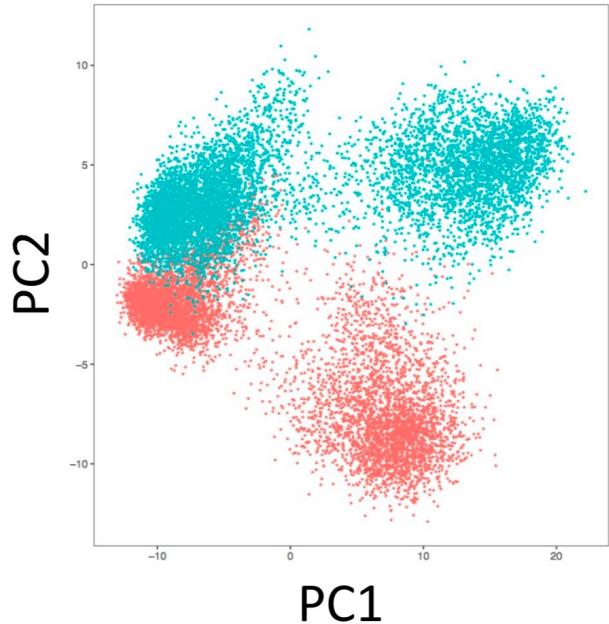
MEPs: megakaryocyte–erythrocyte progenitors
 GMPs: granulocyte–monocyte progenitors
 CMPs: common myeloid progenitors

CCA + anchors (Seurat v3)

How CCA works?

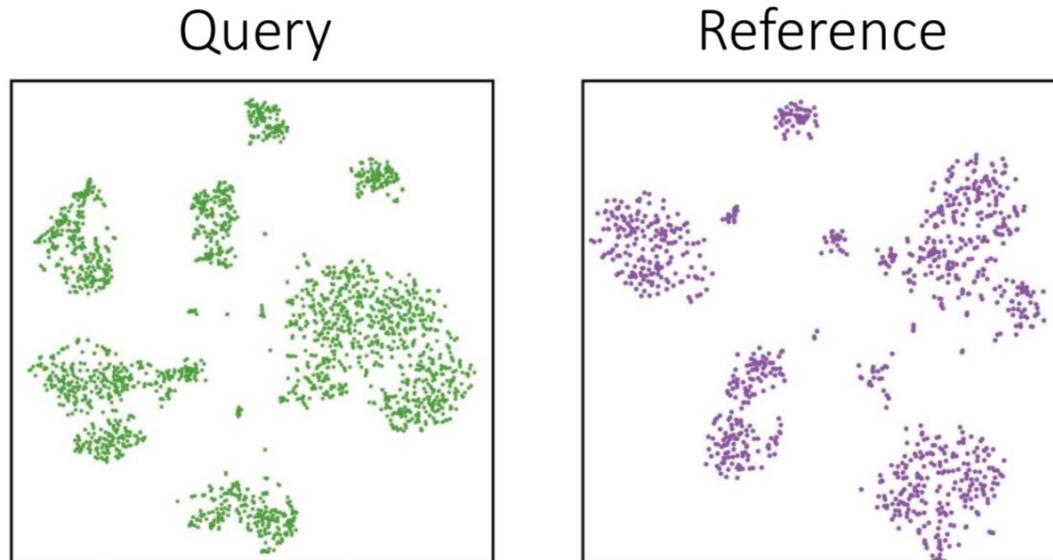
Canonical correlation analysis

CCA captures correlated sources of variation between two datasets



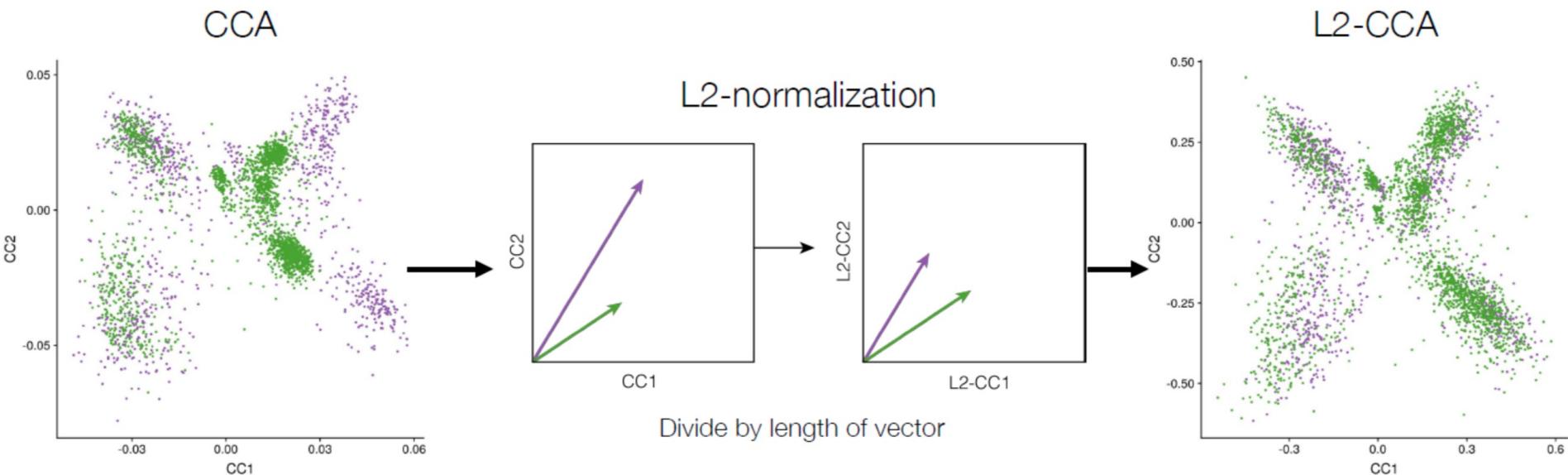
CCA + anchors (Seurat v3)

1. Find corresponding cells across datasets
2. Compute a data adjustment based on correspondences between cells
3. Apply the adjustment



Mutual Nearest Neighbors (MNN)

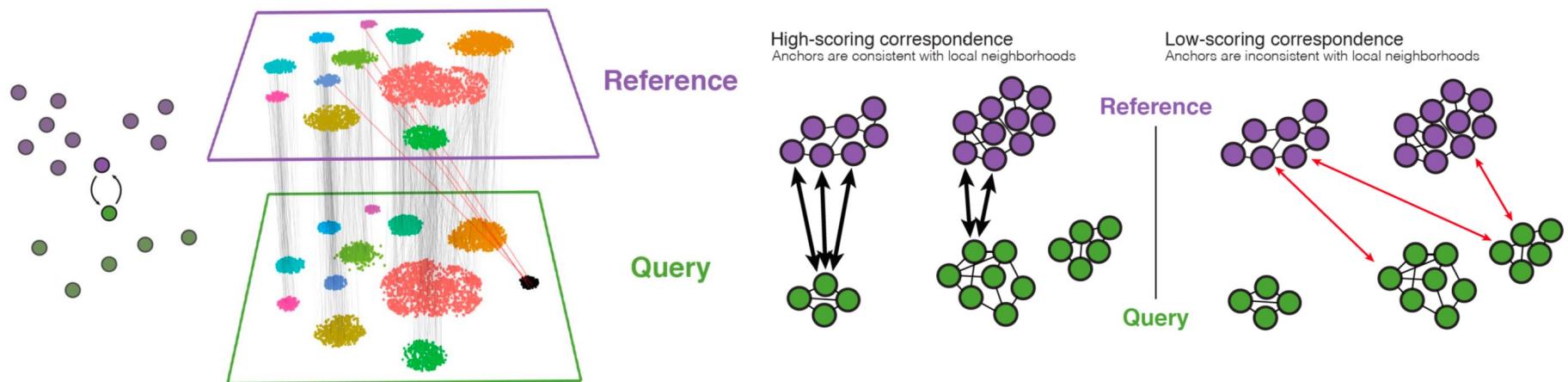
L2-normalization corrects for differences in scale



Finding corresponding cells

Anchors: mutual nearest neighbours (MNN)

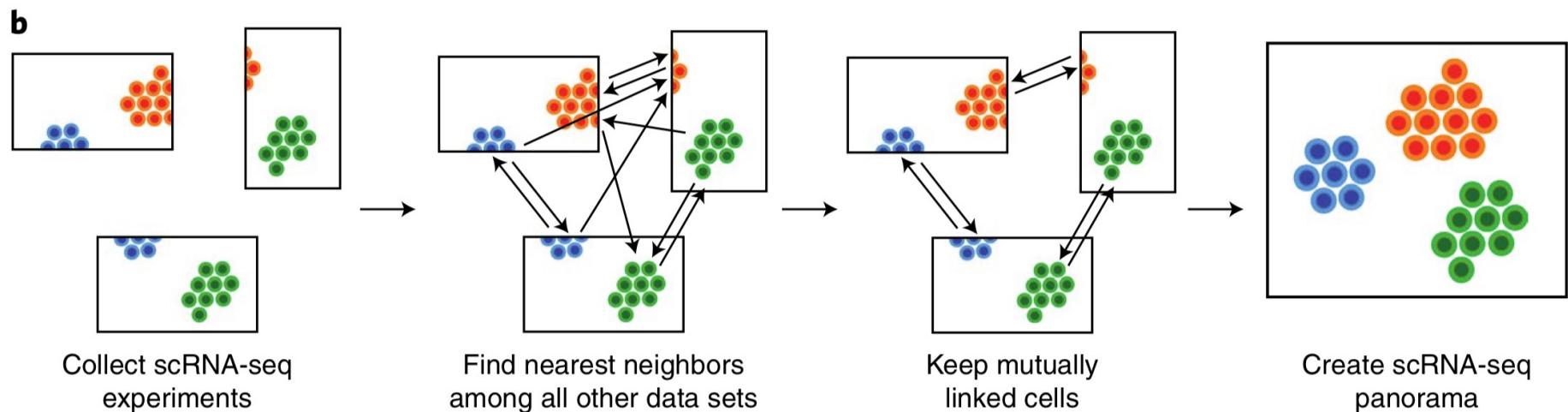
- It scales well on large datasets



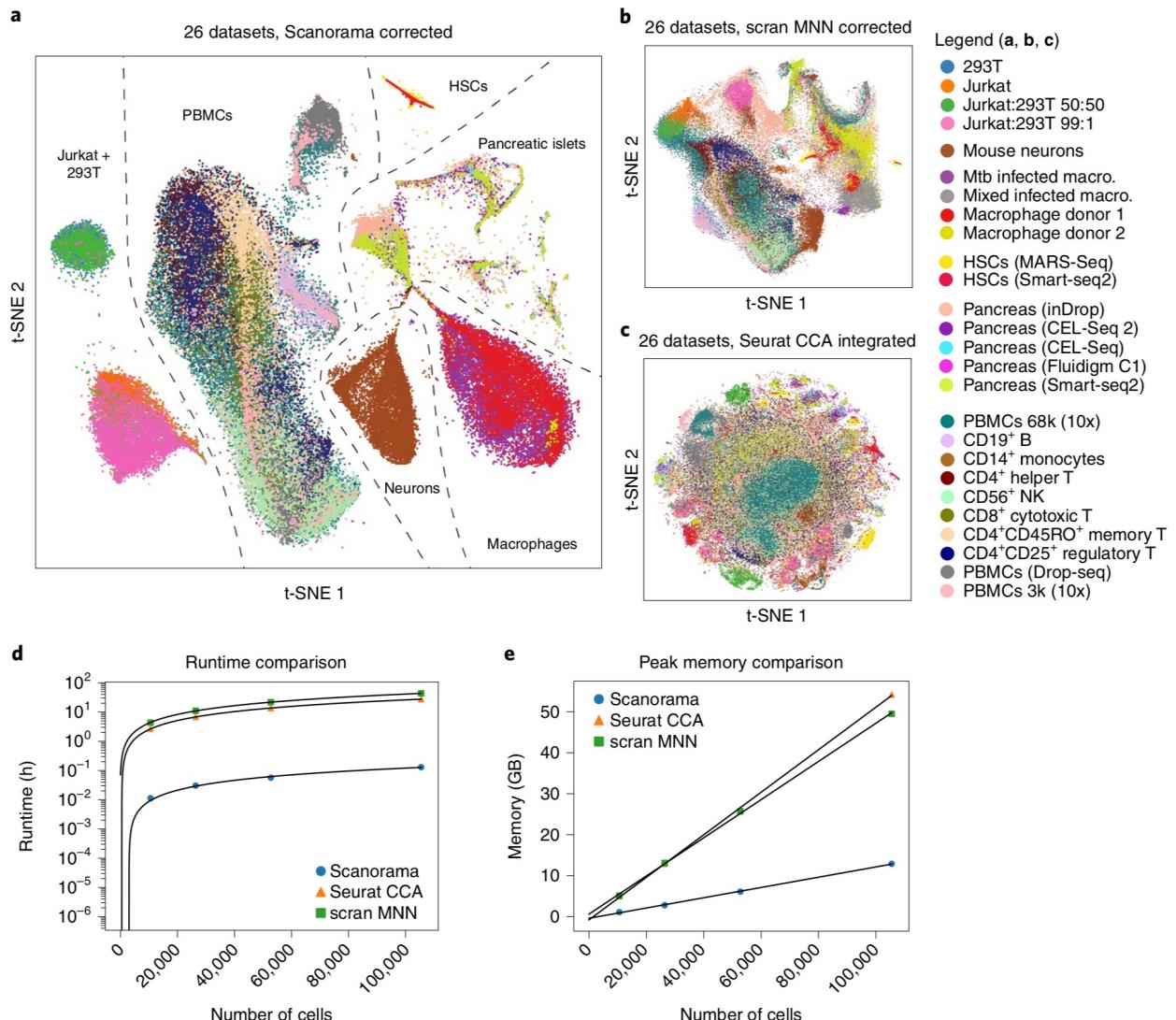
Scanorama

Scanorama

- Python implementation of fastMNN ?
 - Dimensionality reduction via SVD with all datasets
 - Find K-NN across datasets
 - Compute merging vectors
- **It scales well on large datasets**

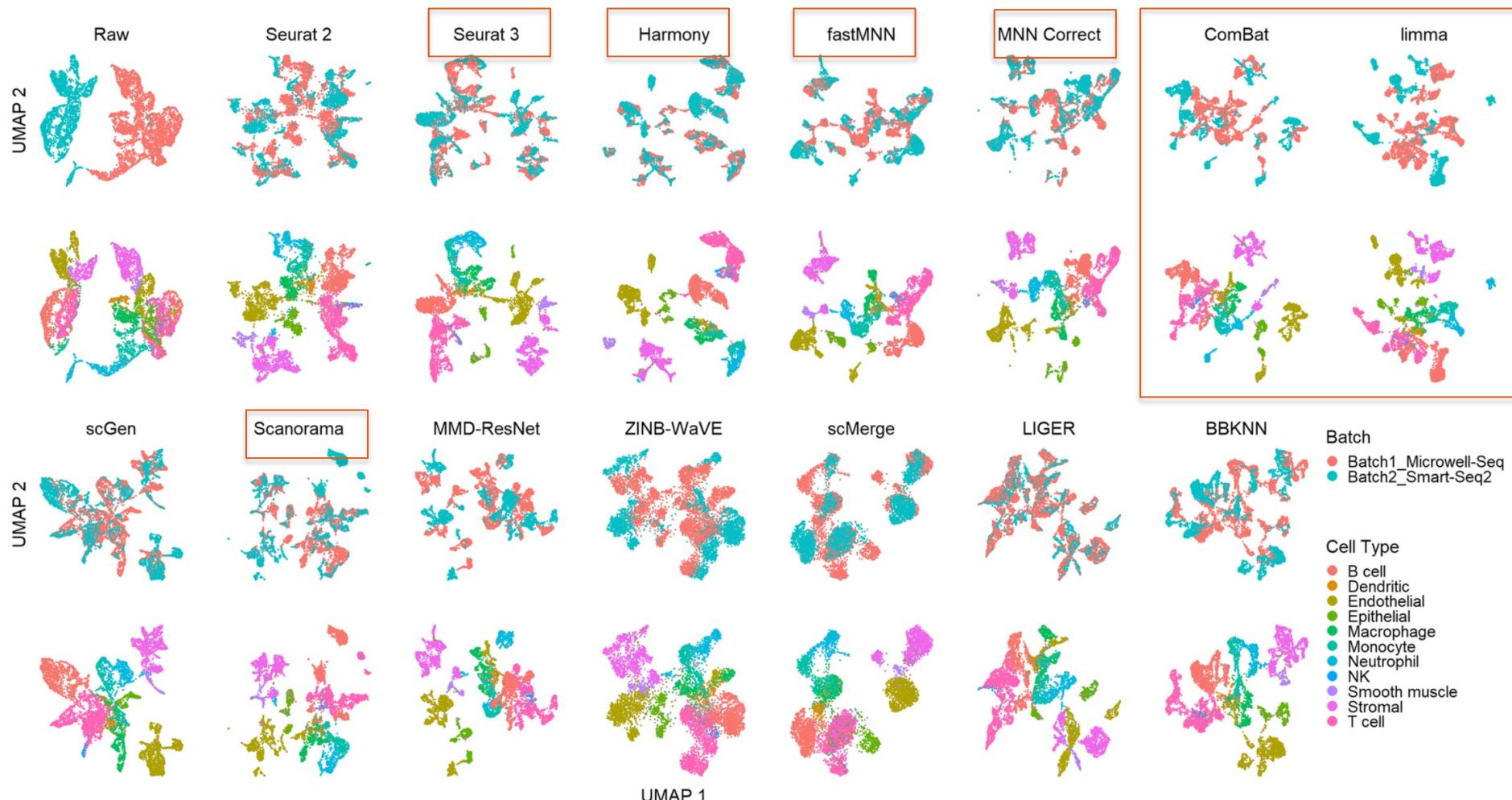


Scanorama

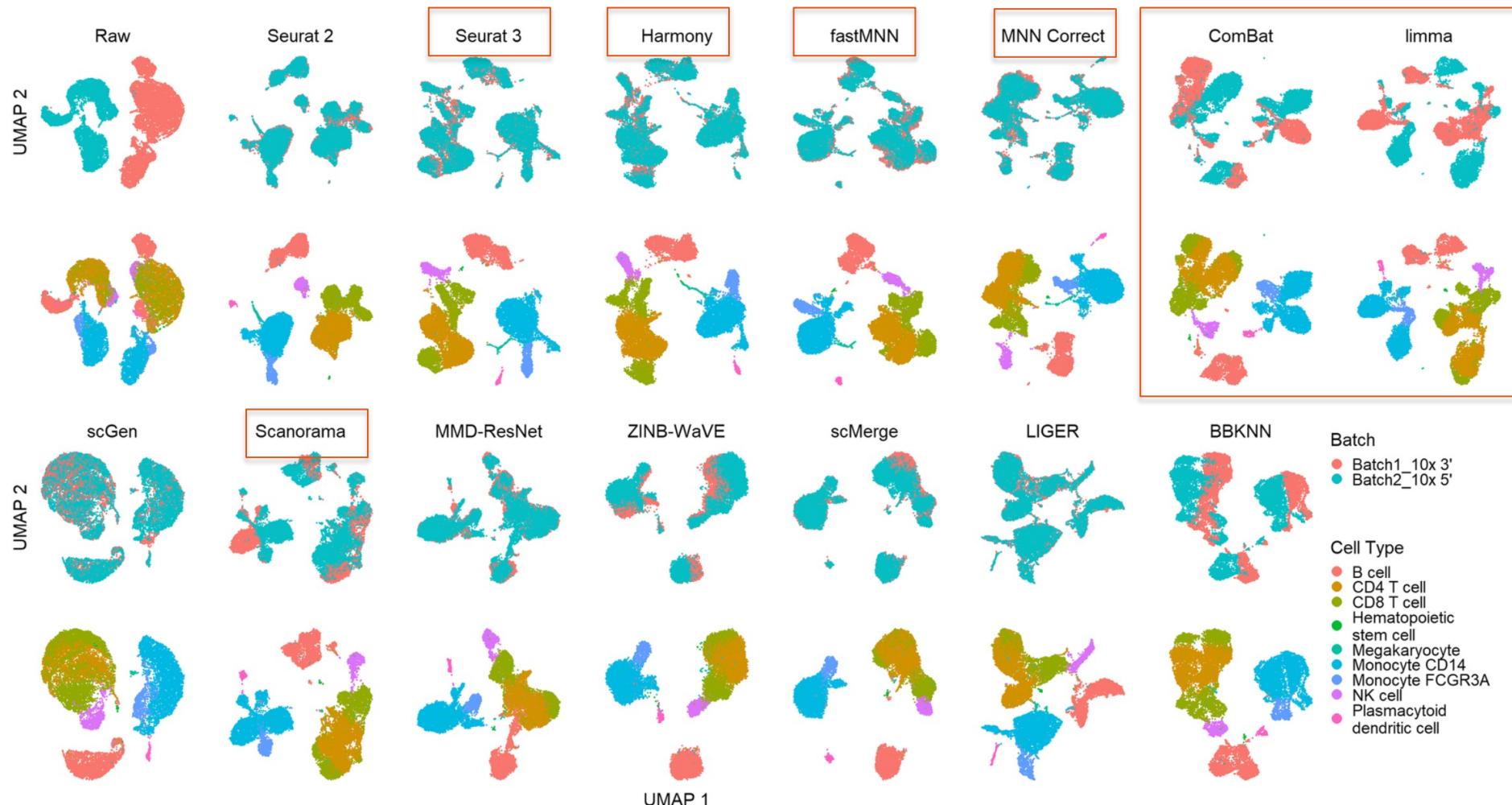


Evaluation of batch correction efficiency

Batch-correction performance assessment



Batch-correction performance assessment



Batch-correction performance assessment

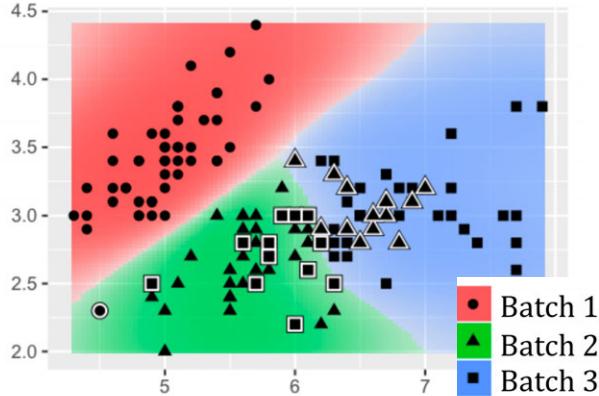
1. Evaluate mixing efficiency (Goal A)

- How well mixed are the obtained clusters post-batch correction?
- How well does a classifier (i.e. SVM) perform pre/post-correction?

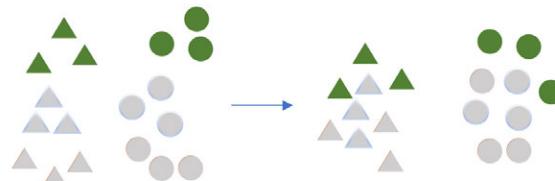
2. Evaluate preservation of remaining variance (Goals B, C)

- Evaluate proportion of removed variance, overlap of HVGs
- Evaluate preservation of within-batch cell topologies

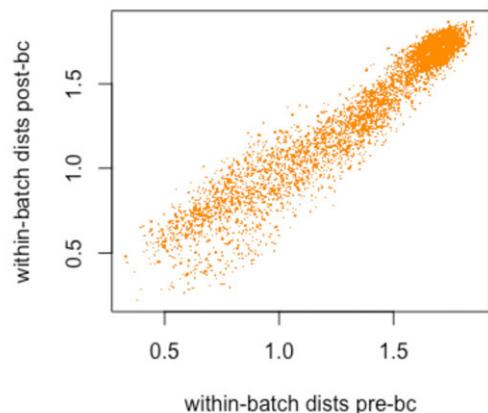
Classifiers must fail
to find batches



Local structure
preservation



Global structure
preservation



Batch-correction performance assessment

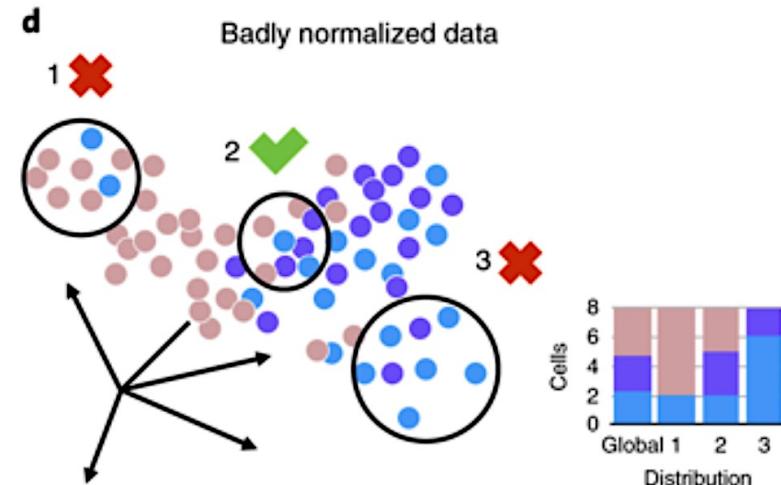
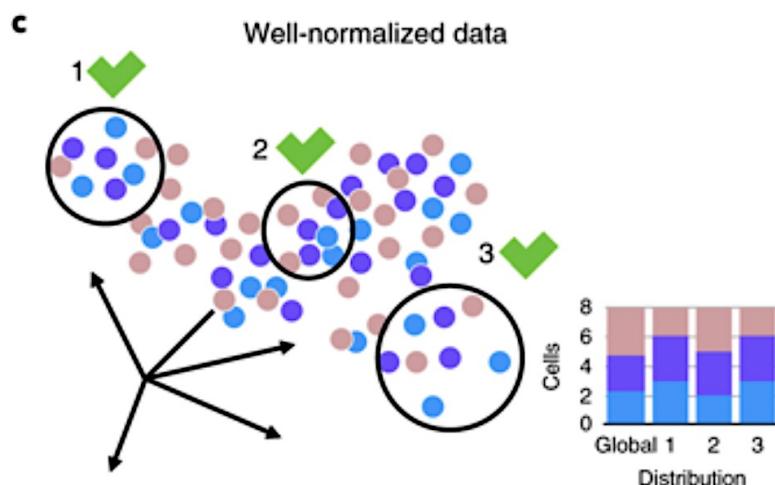
We wish to obtain corrected data where the following goals are met:

Goal:

1. The batch-originating variance is erased
2. Meaningful heterogeneity is preserved
3. No artefactual variance is introduced

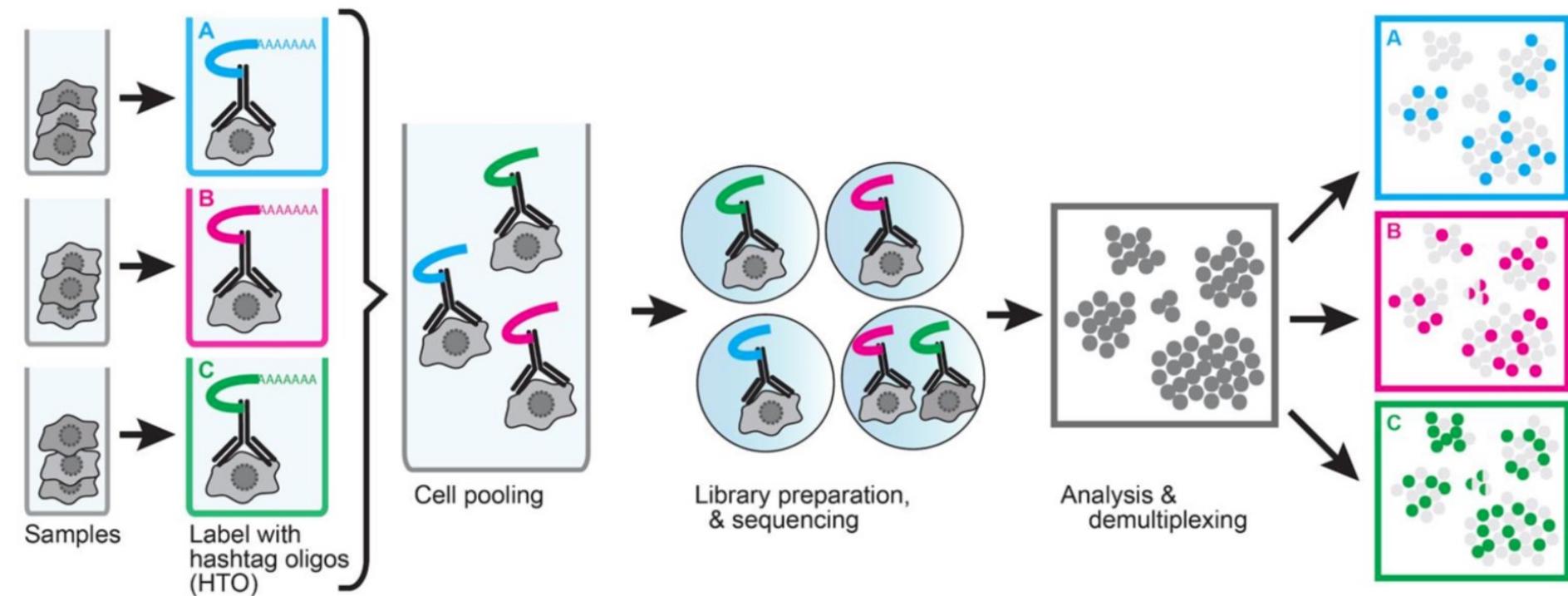
What it practically means:

- Similar cell types are intermixed across batches
- We are not mixing distinct cell types (across or within batches)
- We do not separate similar cells within batches

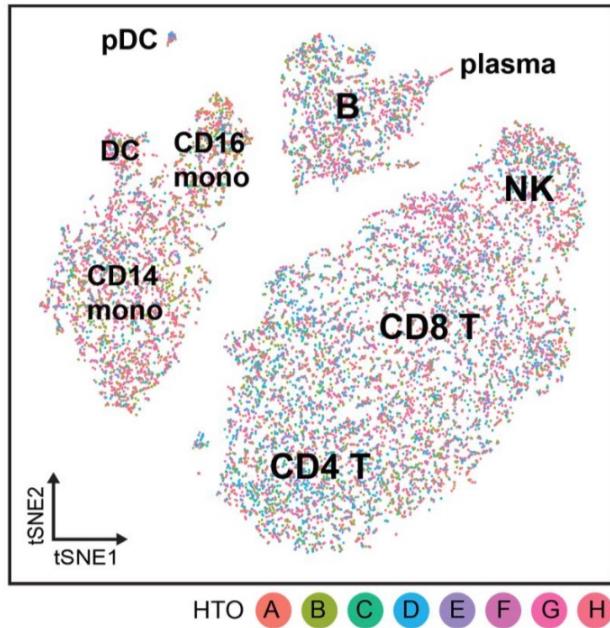
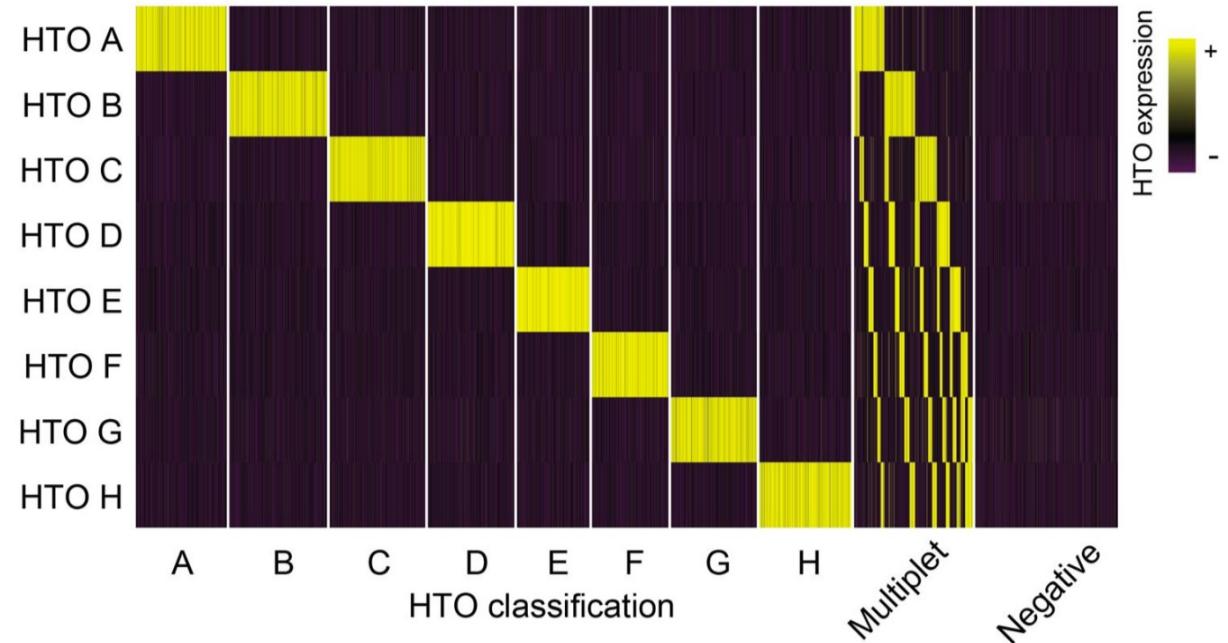


Avoiding batches

Cell hashing



Cell hashing



Summary

Summary

- Batch effects sometimes are not avoidable
- Many batch correction/integration methods available, mainly using joint dimension reduction, or joint clustering, or a combination of both
- Joint dimension reduction can yield interpretable factors and aid in the identification of equivalent states, but is computationally expensive
- Graph-based methods alone can be extremely fast, but may struggle when technical differences are on a similar scale to biological differences
- Performance assessment is challenging
- Sample multiplexing can help alleviate batch effects
- Simultaneous mRNA and protein profiling: REAP-seq and CITE-seq
- Several single cell multi-omics technologies can be integrated