Single Cell RNA-seq Data Integration

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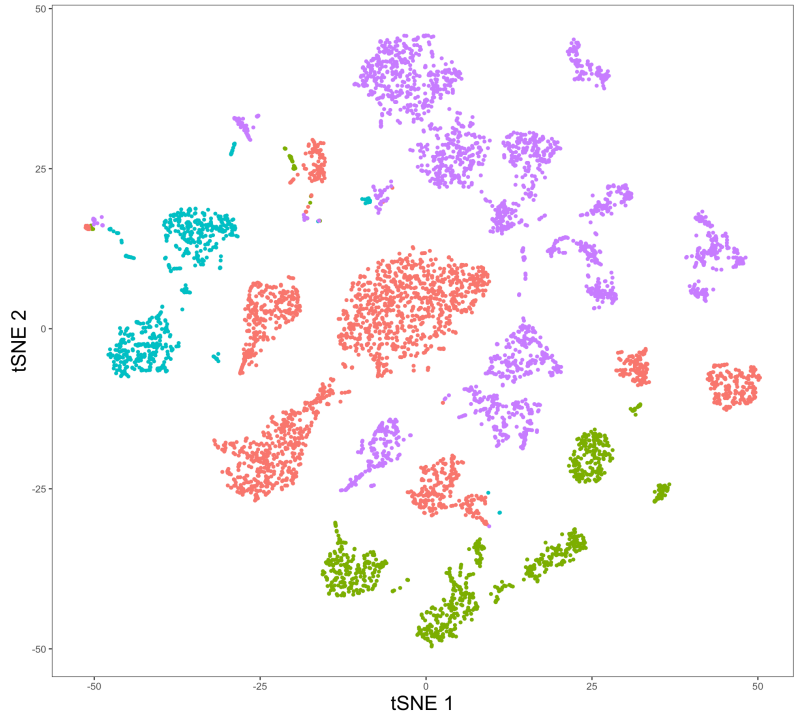
Leiden
Computational Biology Center

LUMC

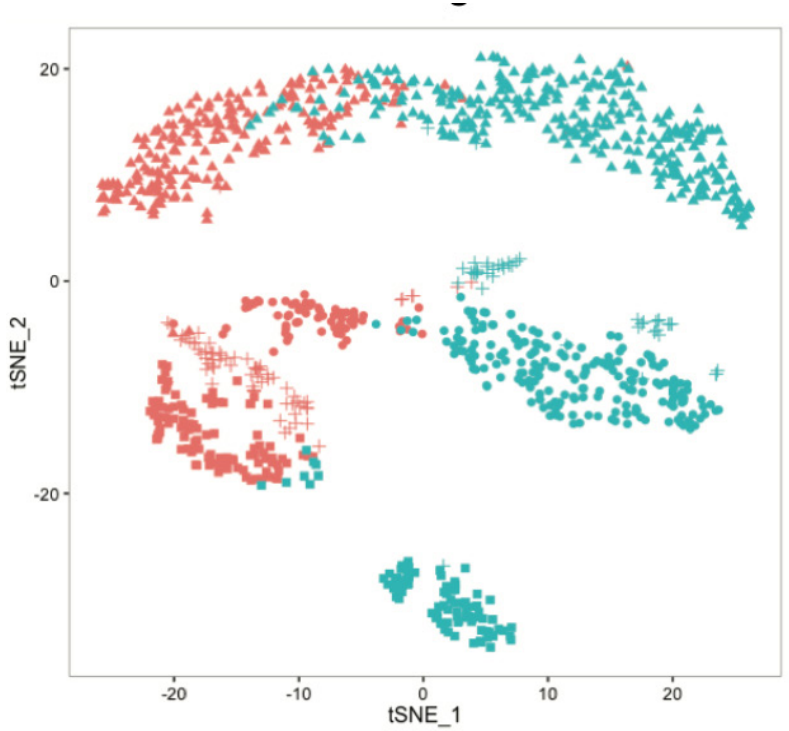
TU Delft

Why integrate?



Donor 1
Donor 2
Donor 3
Donor 4

Same tissue from different donors



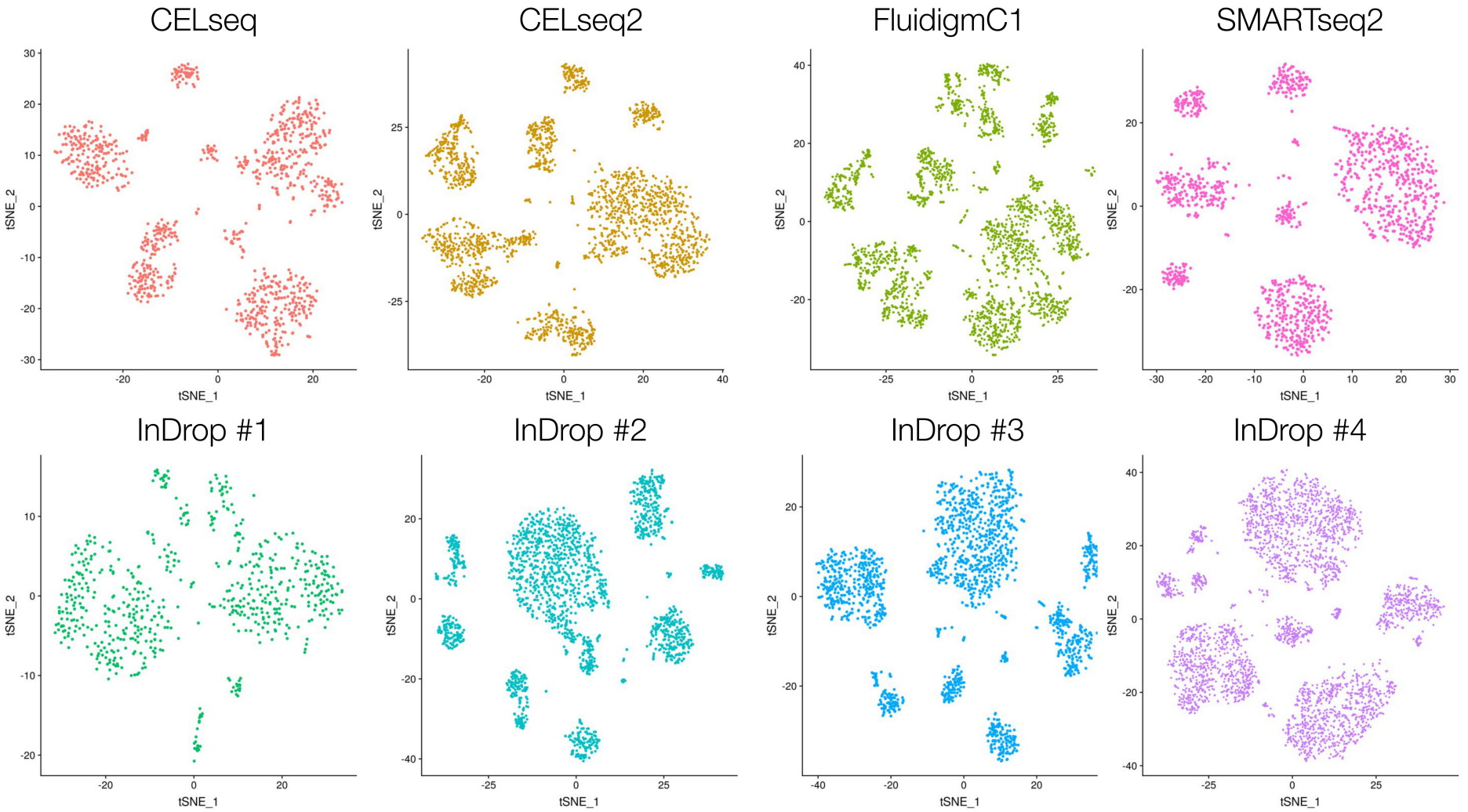
Sick
Healthy

▲ Cell Type 1
■ Cell Type 2
● Cell Type 3

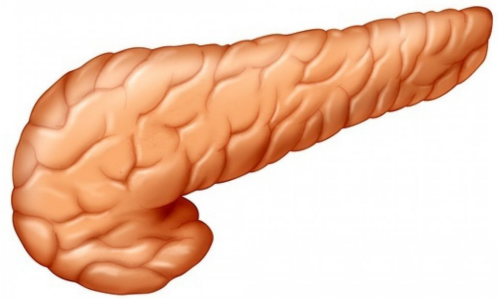
Cross condition comparisons

Building a cell atlas

8 maps of the human pancreas



Human pancreas



Baron et al. 2016, *Cell Syst.*

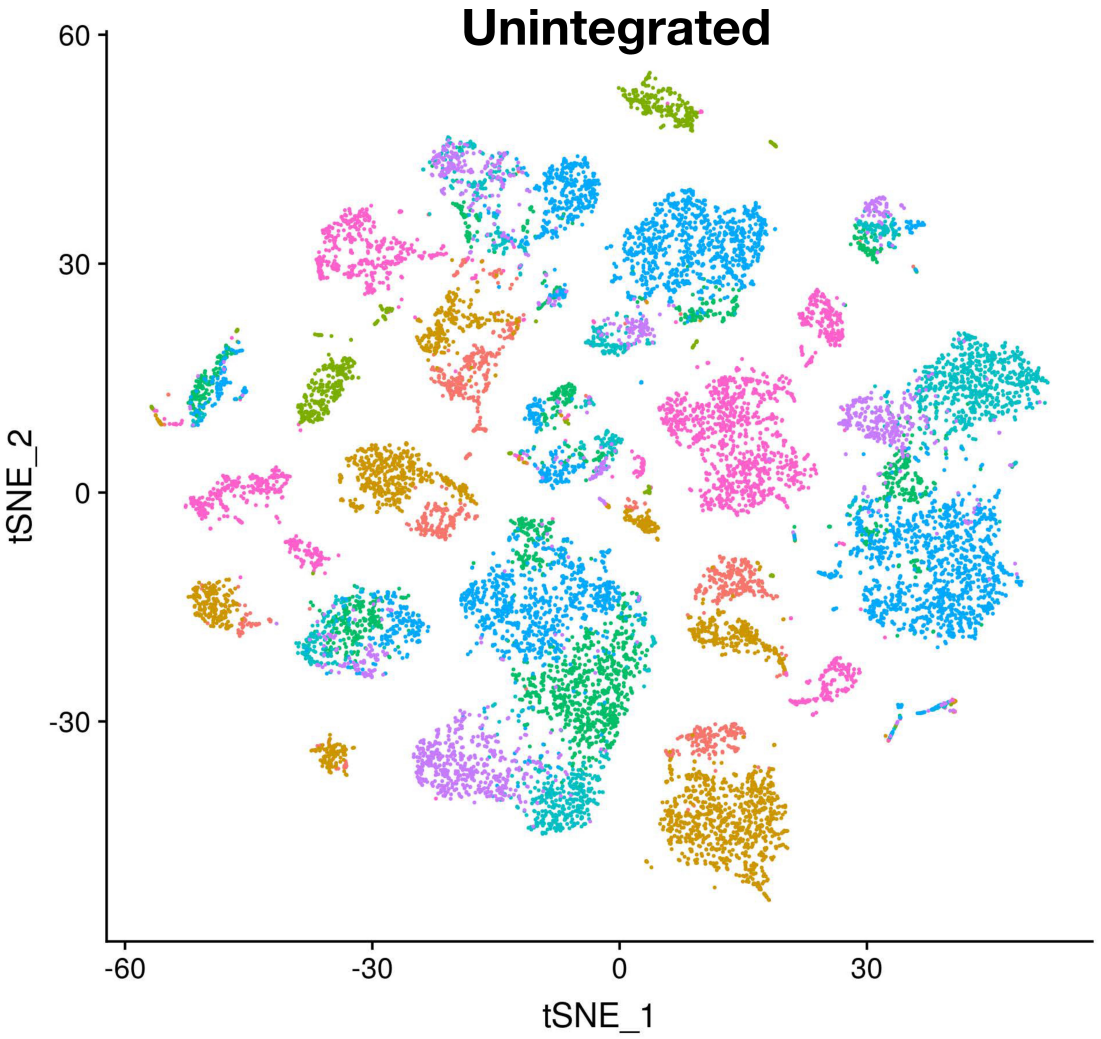
Lawlor et al. 2017, *Genome Res.*

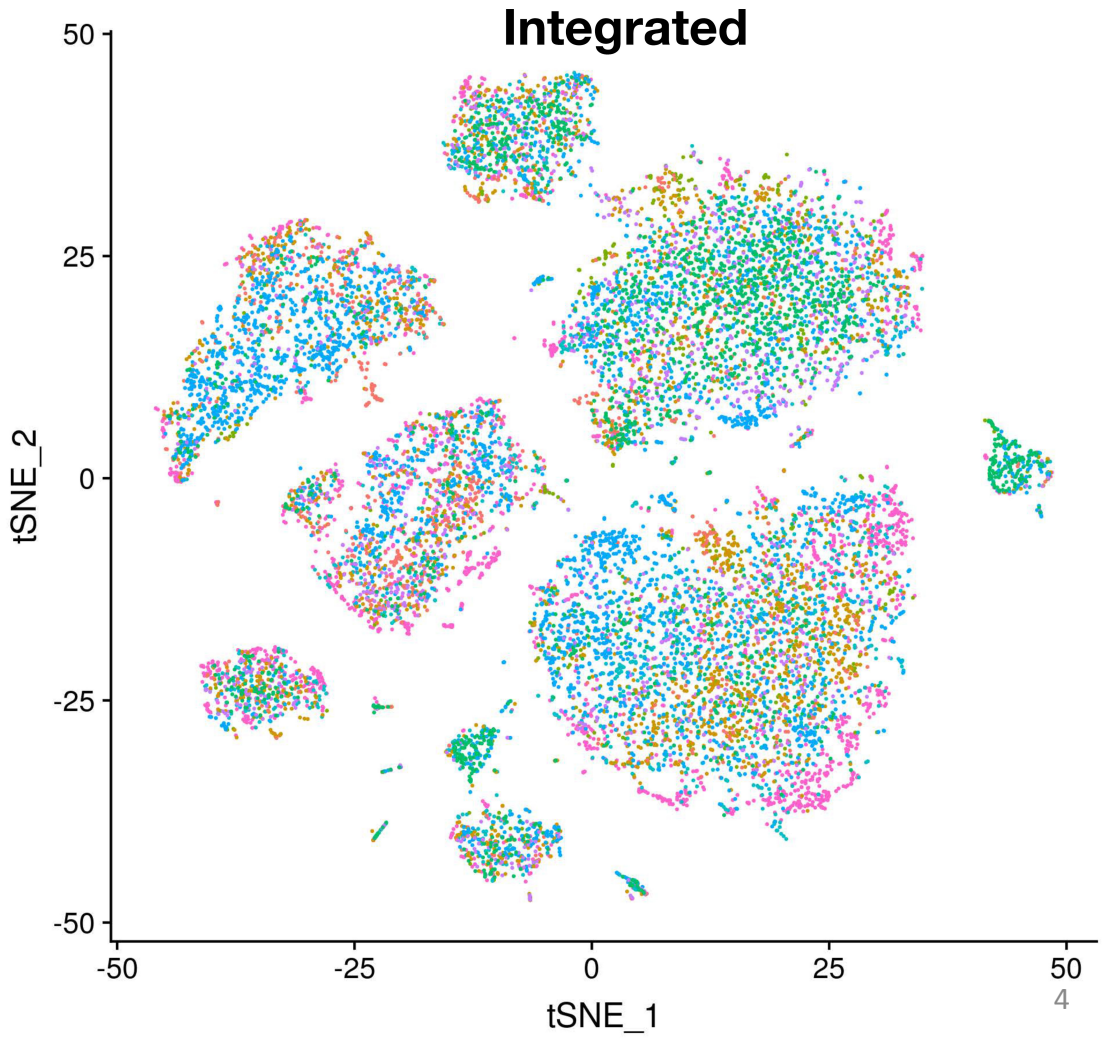
Grun et al. 2016, *Cell Stem Cell*

Muraro et al. 2016, *Cell Syst.*

Building a cell atlas

8 maps of the human pancreas

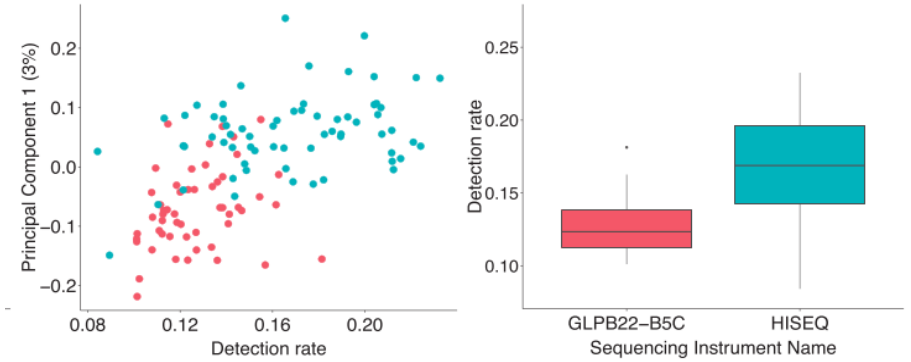




Confounders and batch effects

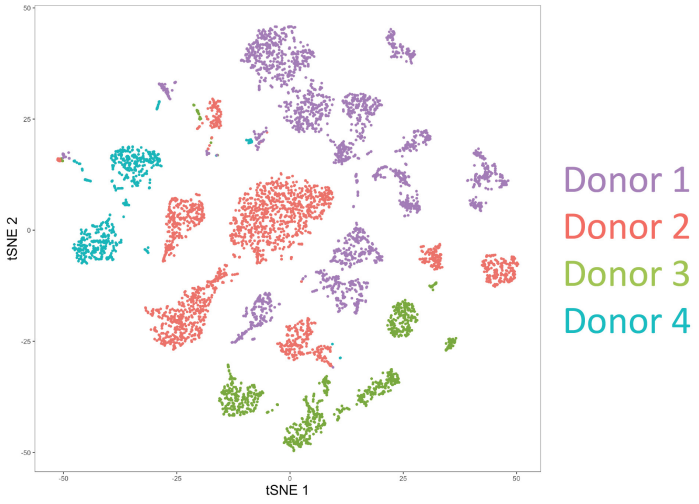
1. Technical variability
   - Changes in sample quality/processing
   - Library prep or sequencing technology
   - ‘Experimental reality’

Technical ‘batch effects’ confound downstream analysis



2. Biological variability
   - Patient differences
   - Environmental/genetic perturbation
   - Evolution! (cross-species analysis)

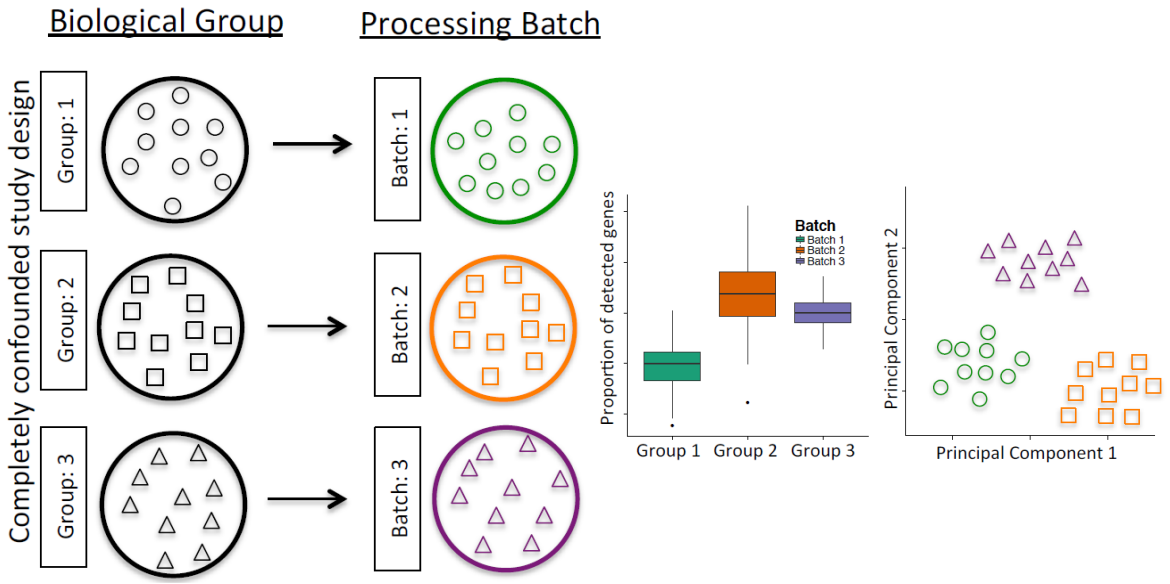
Biological ‘batch effects’ confound comparisons of scRNA-seq data



Shaham et al. (https://doi.org/10.1093/bioinformatics/btx196)

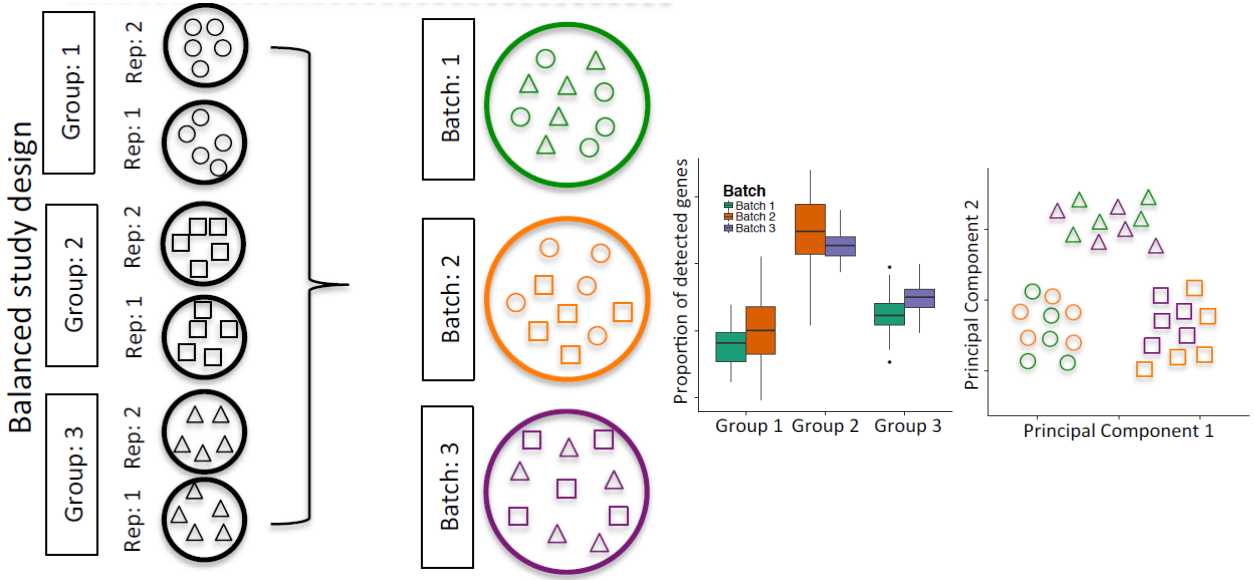
Confounders and batch effects

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.

Hicks et al. (https://doi.org/10.1093/biostatistics/kxx053)

Our agenda

- Single cell batch correction methods
- Performance assessment
- SpaGE – integration of scRNA-seq and spatial transcriptomics

Batch correction methods

- Many good options have been developed for bulk RNA-seq data:
  - RUVseq() or svaseq()
  - Linear models with e.g. removeBatchEffect() in limma or scater
  - ComBat() in sva
  - …

- But bulk RNA-seq methods make modelling assumptions that are likely to be violated in scRNAseq data
  - The composition of cell populations are either known or the same across batches
  - Batch effect is additive: batch-induced fold-change in expression is the same across different cell subpopulations for any given gene

Batch correction methods

- MNNcorrect (https://doi.org/10.1038/nbt.4091)
- CCA + anchors (Seurat v3) (https://doi.org/10.1101/460147)
- CCA + dynamic time warping (Seurat v2) (https://doi.org/10.1038/nbt.4096)
- LIGER (https://doi.org/10.1101/459891)
- Harmony (https://doi.org/10.1101/461954)
- Scanorama (https://doi.org/10.1101/371179)
- scMerge (https://doi.org/10.1073/pnas.1820006116)
- BBKNN (https://doi.org/10.1093/bioinformatics/btz625)
- scGen (https://doi.org/10.1038/s41592-019-0494-8)
- …

Two broad strategies:

- Joint dimension reduction
- Graph-based approaches

Batch correction methods

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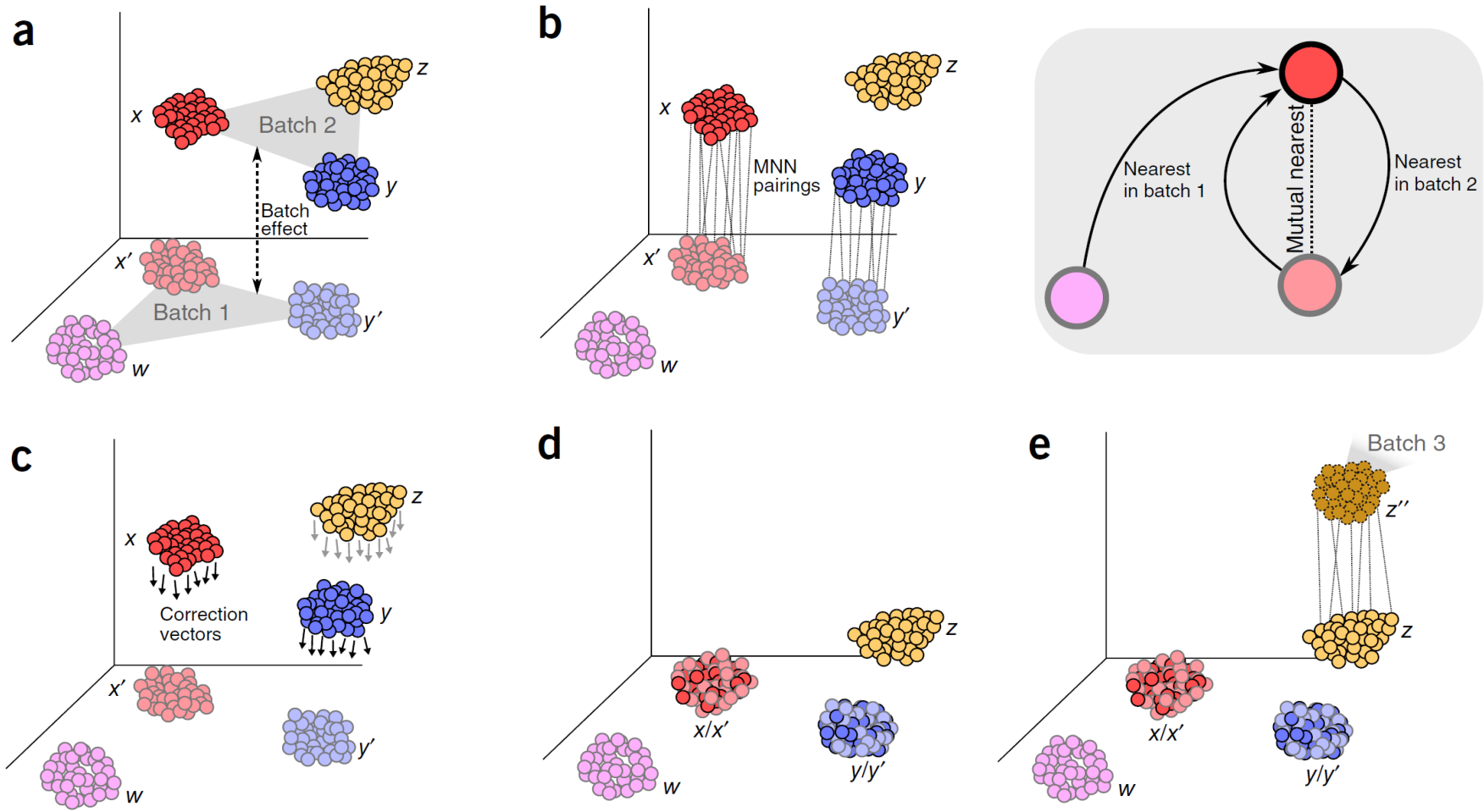
Mutual Nearest Neighbors (MNN)



Haghverdi et al. (https://doi.org/10.1038/nbt.4091)

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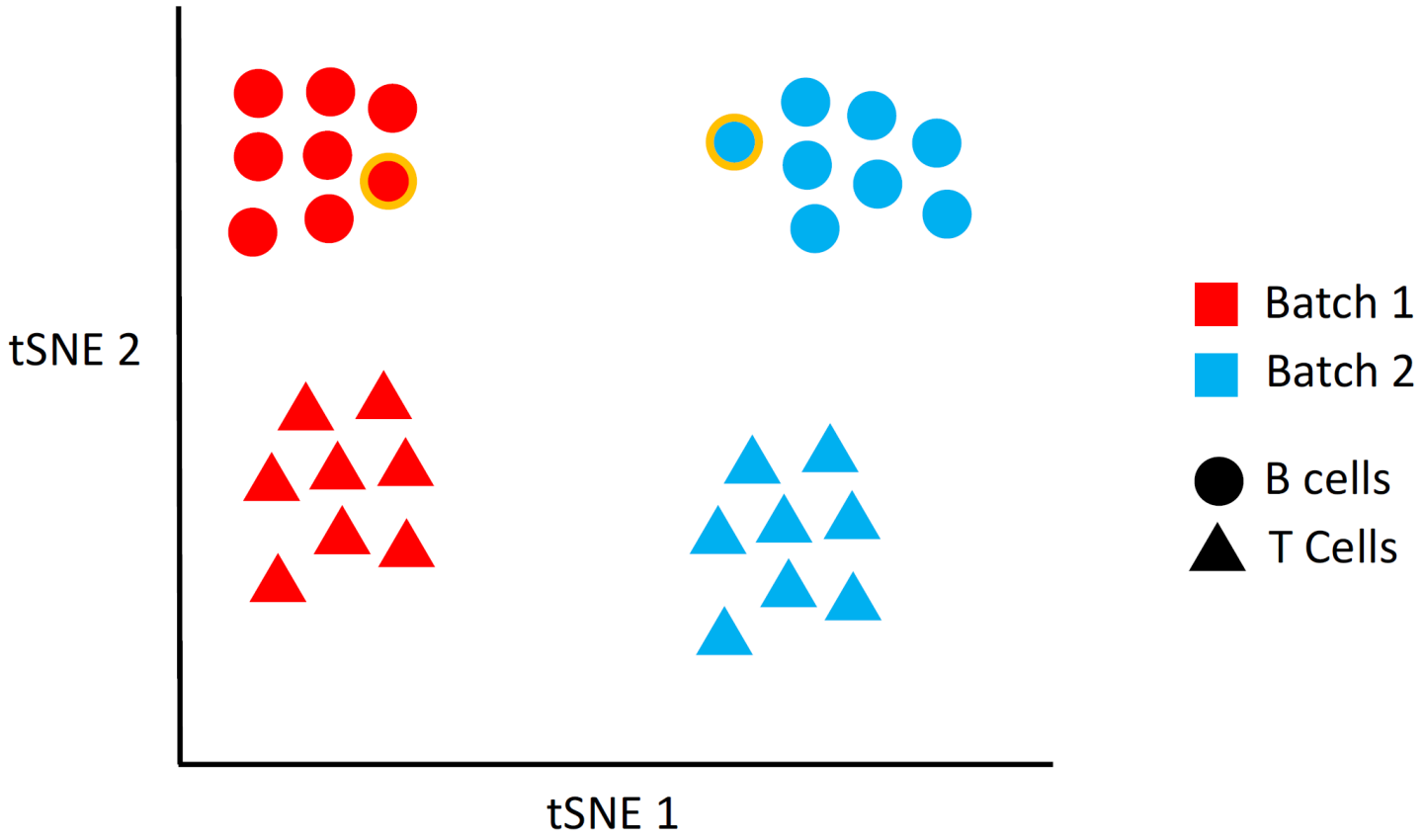

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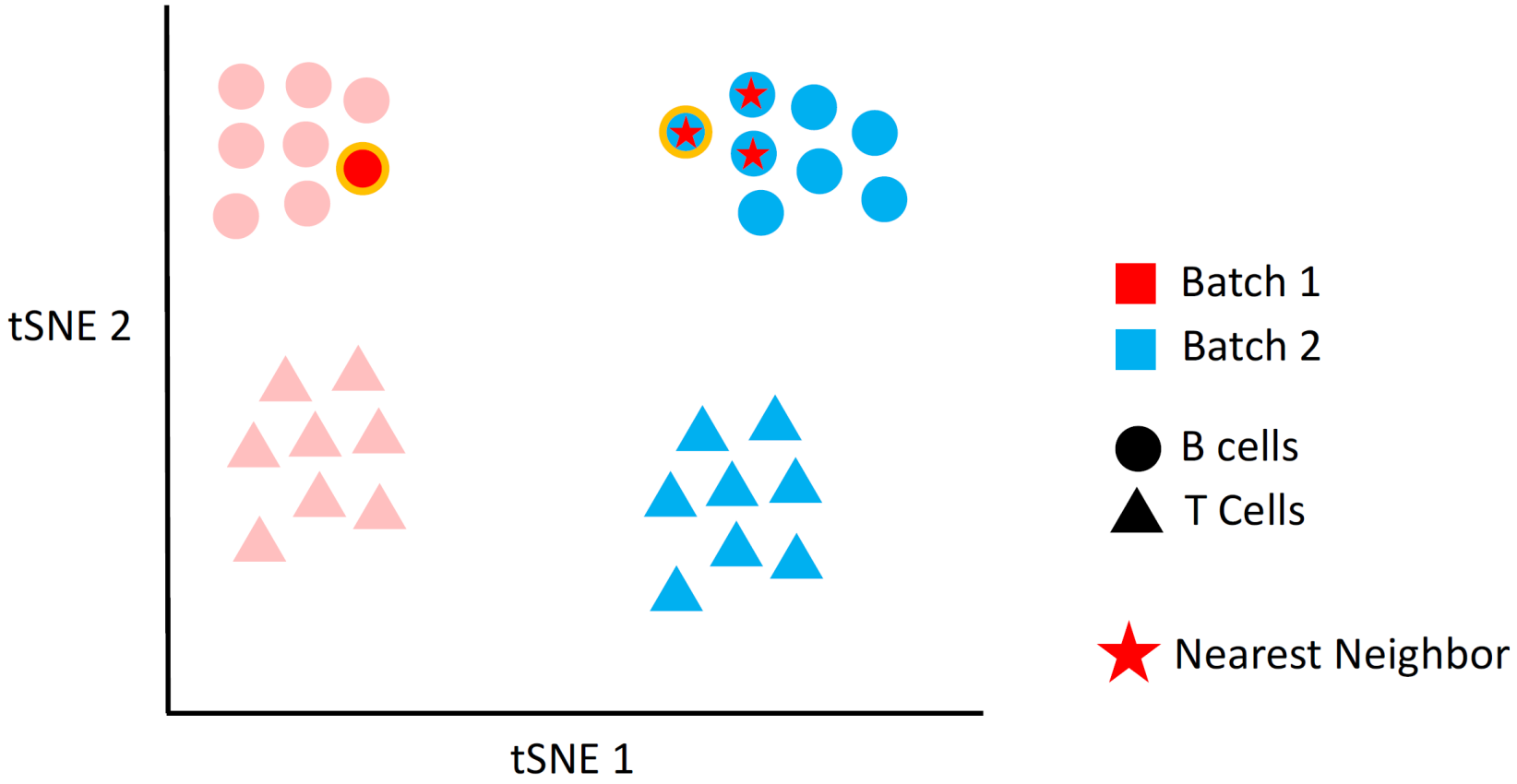

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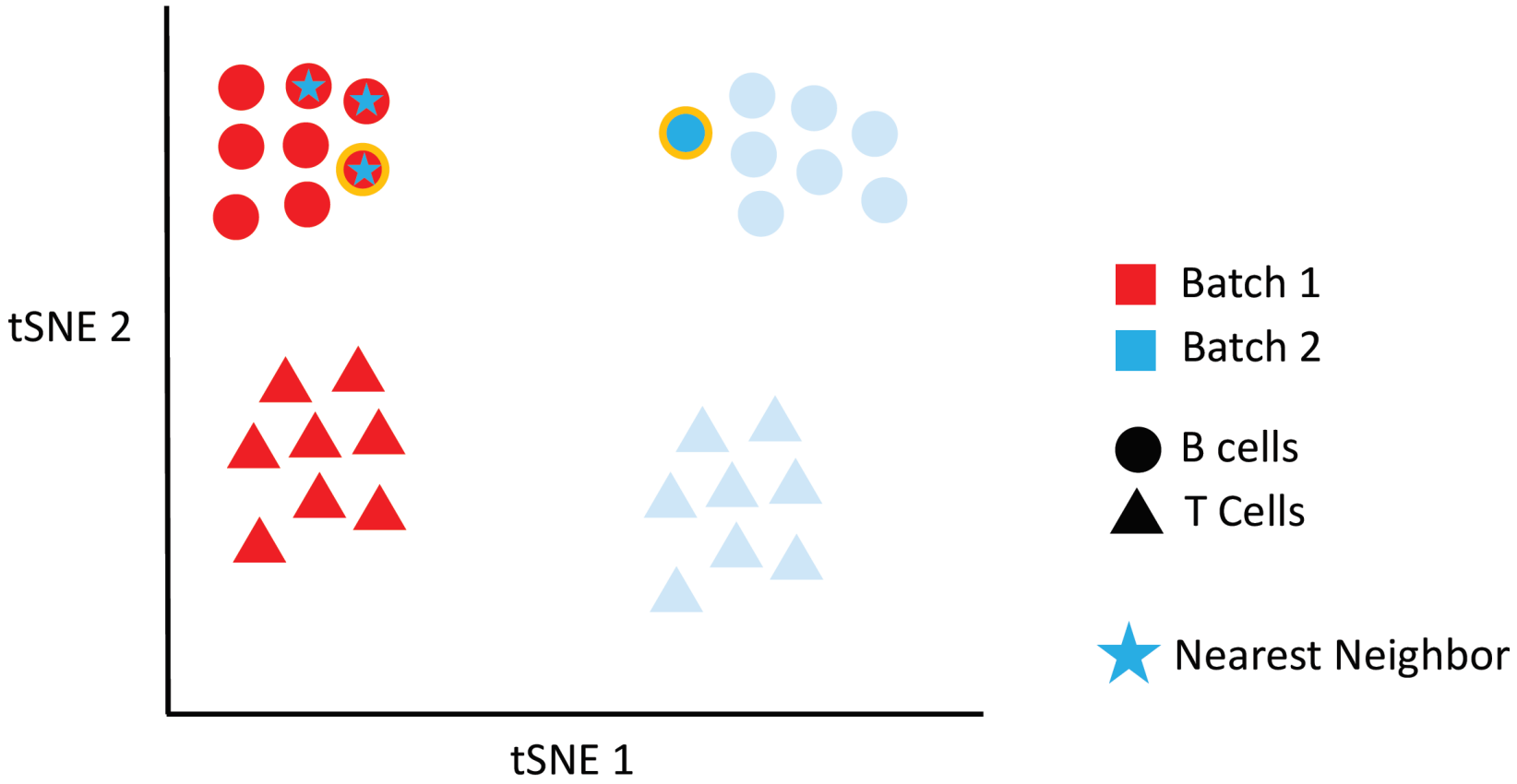

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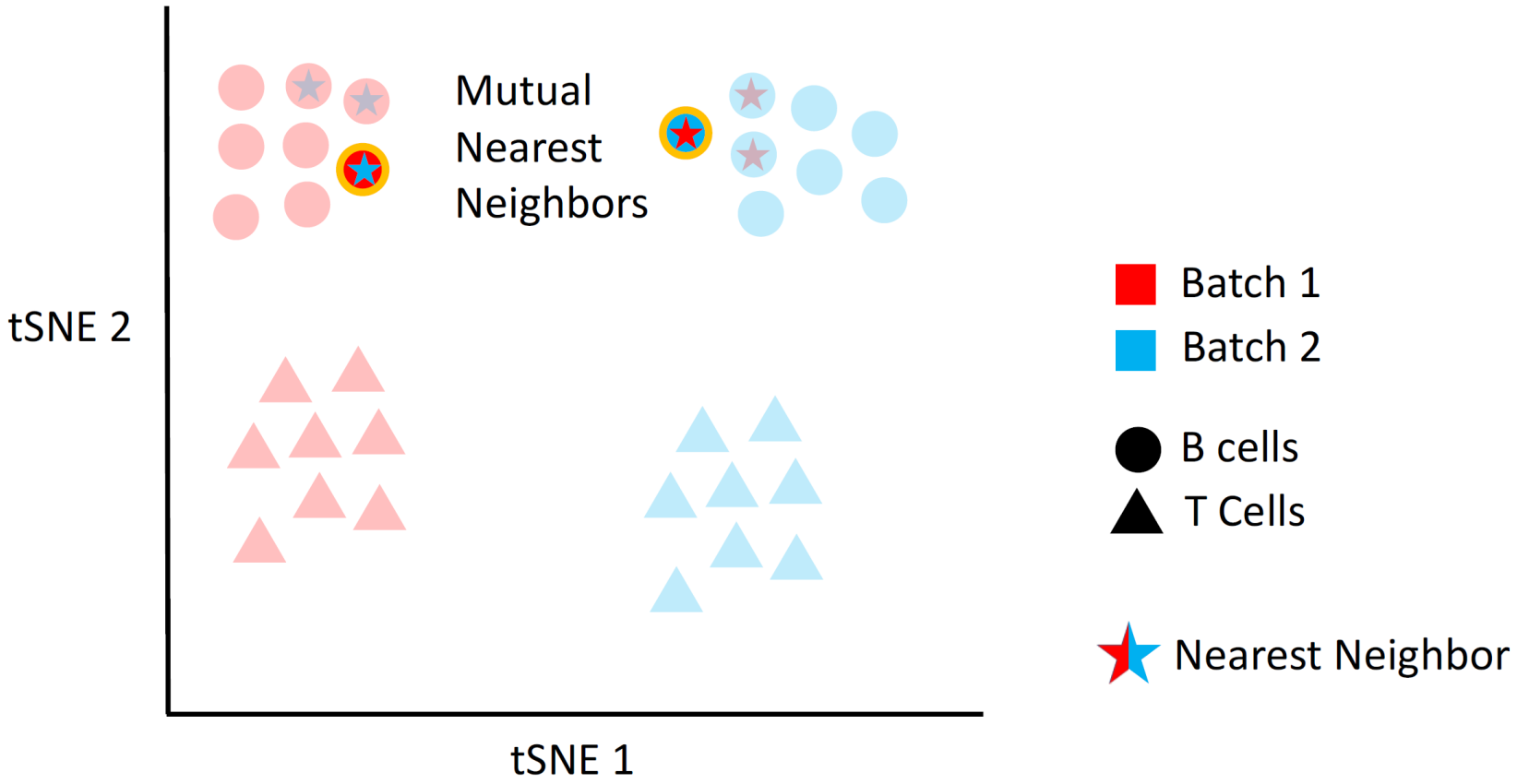

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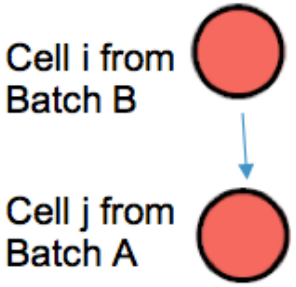
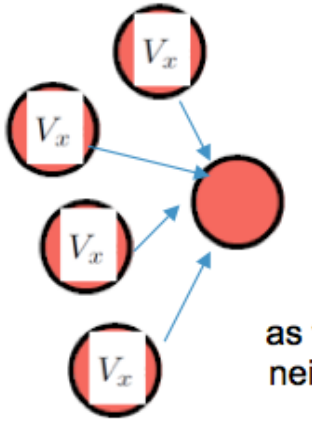


1) For each MNN pair, a pair-specific batch-correction vector is computed as the vector difference between the expression profiles of the paired cells.

$$V_x = \begin{pmatrix} gene1_a - gene1_b \\ gene2_a - gene2_b \\ gene3_a - gene3_b \\ \dots \\ geneN_a - geneN_b \end{pmatrix}$$

2) A cell-specific batch-correction vector is then calculated as a weighted average of these pair-specific vectors, as computed with a Gaussian kernel.

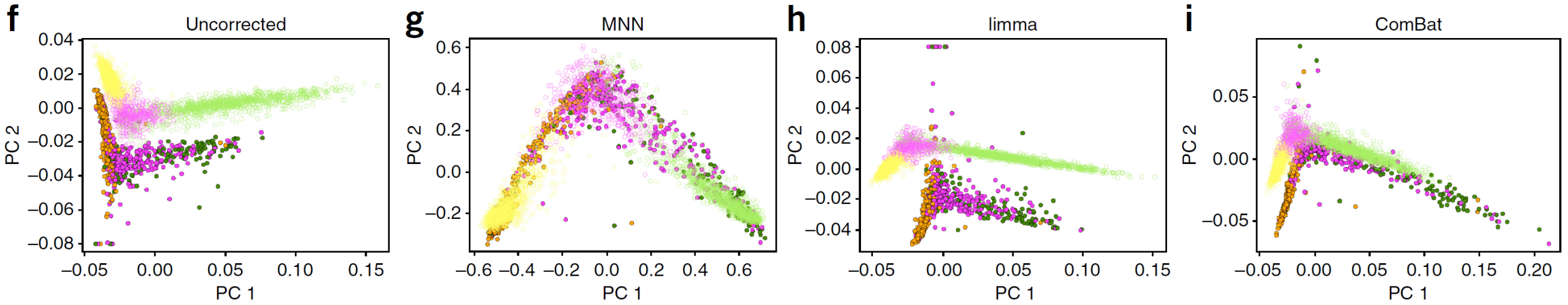


Gaussian Kernel Smoothing

Real valued function
$f : R^p \rightarrow R$
as the weighted average of neighboring observed data

Batch Correction vector for each cell

Mutual Nearest Neighbors (MNN)



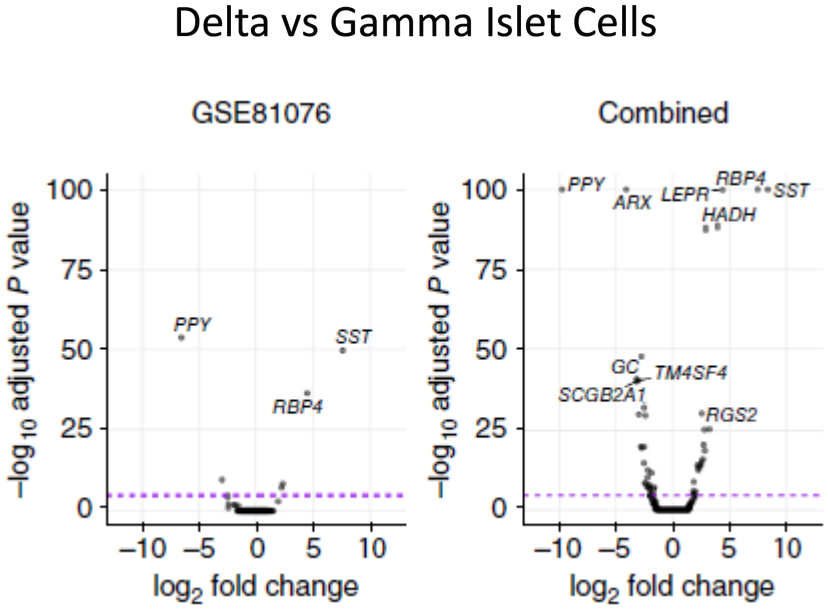
SMART-seq2
- MEP
- GMP
- CMP

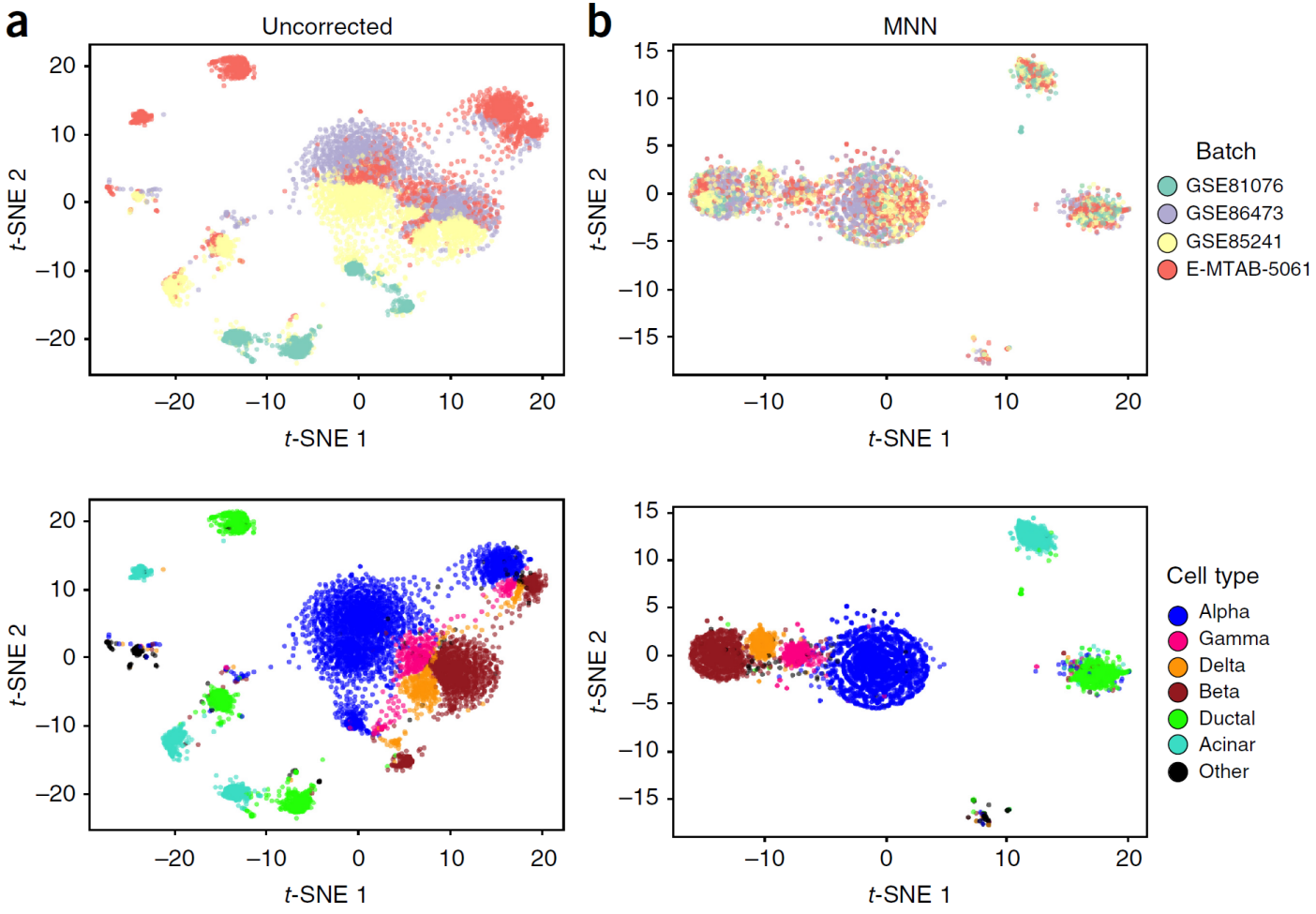
MARS-seq
- MEP
- GMP
- CMP

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

Mutual Nearest Neighbors (MNN)

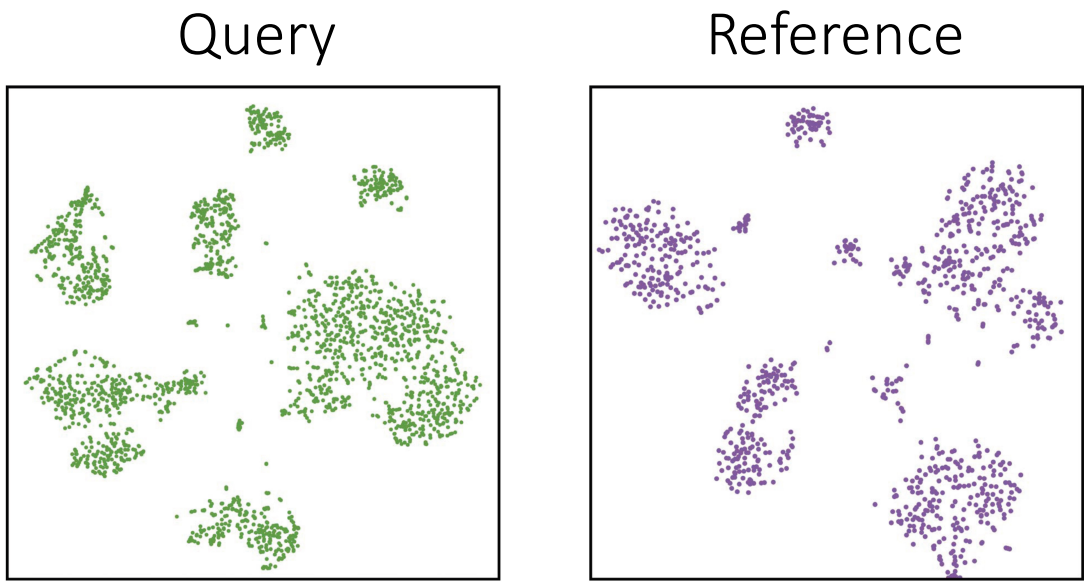
- Pooling experiments -> increased statistical power





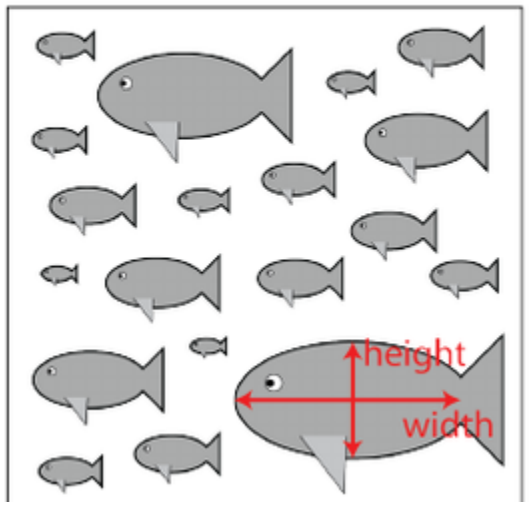
CCA + anchors (Seurat v3)

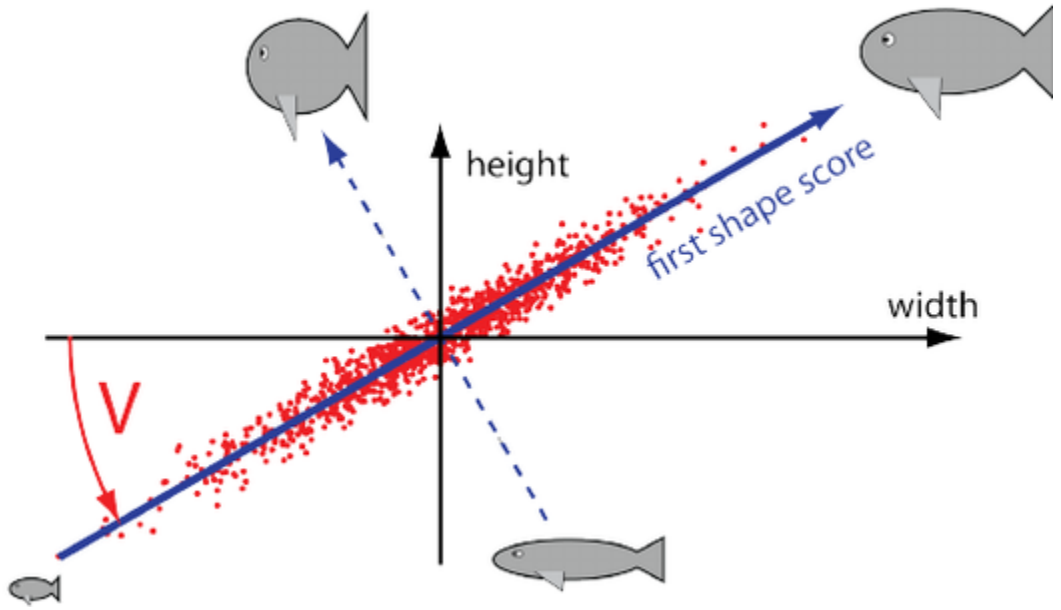
1. Find corresponding cells across datasets
2. Compute a data adjustment based on correspondences between cells
3. Apply the adjustment



Stuart et al. (https://doi.org/10.1101/460147)

Principle component analysis

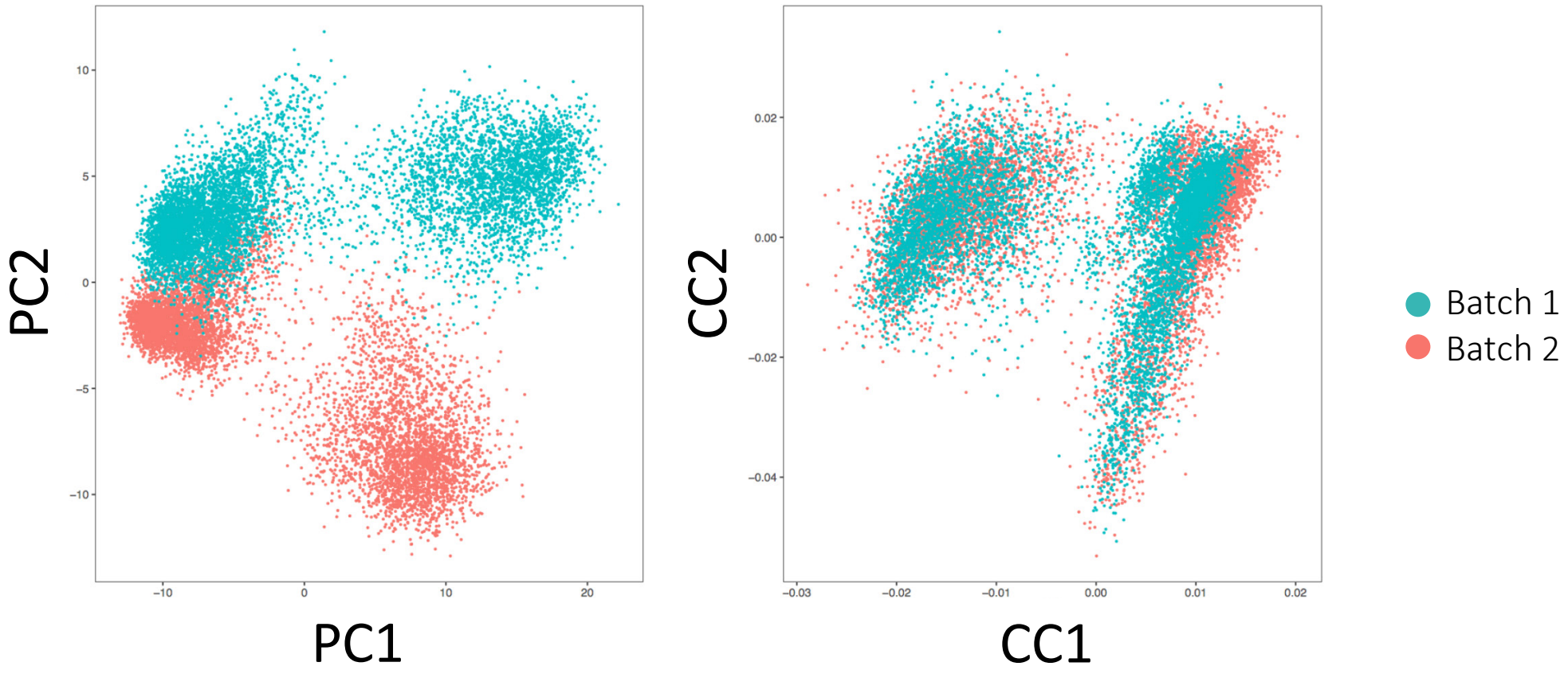




Werner et al. (https://doi.org/10.1371/journal.pone.0113083)

Finding corresponding cells

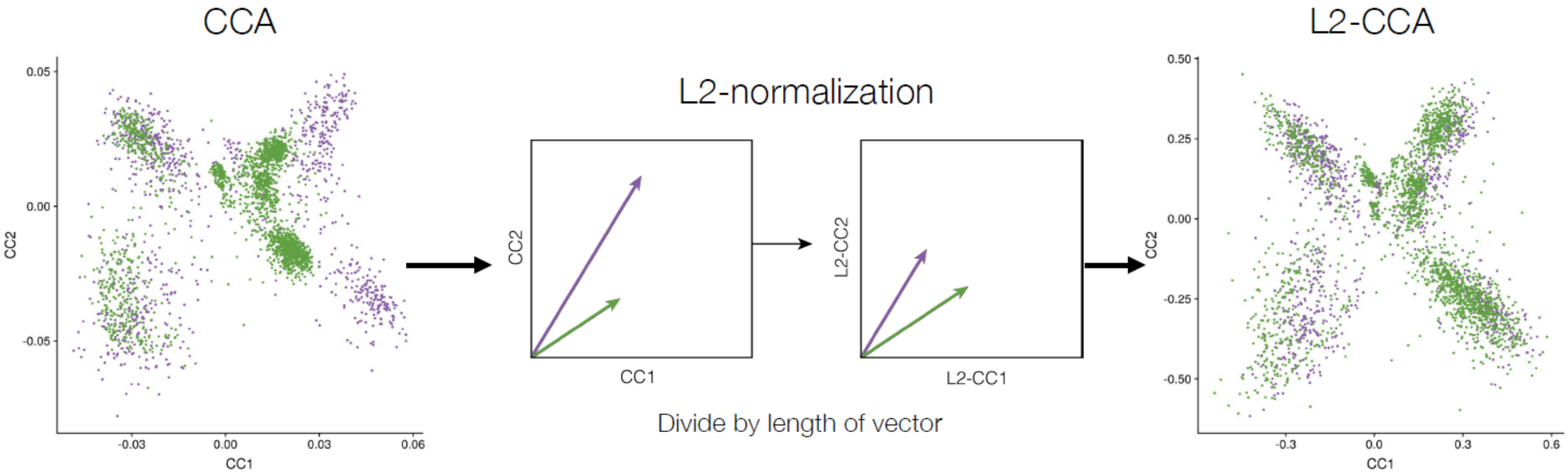
Canonical correlation analysis and normalization



CCA captures correlated sources of variation between two datasets

Finding corresponding cells

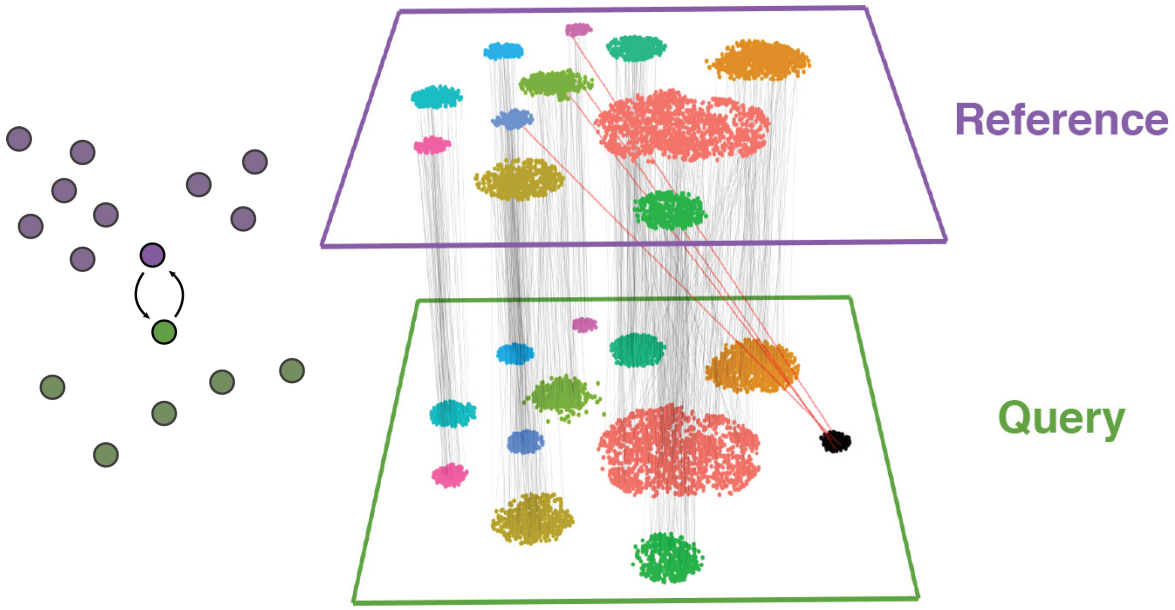
Canonical correlation analysis and normalization

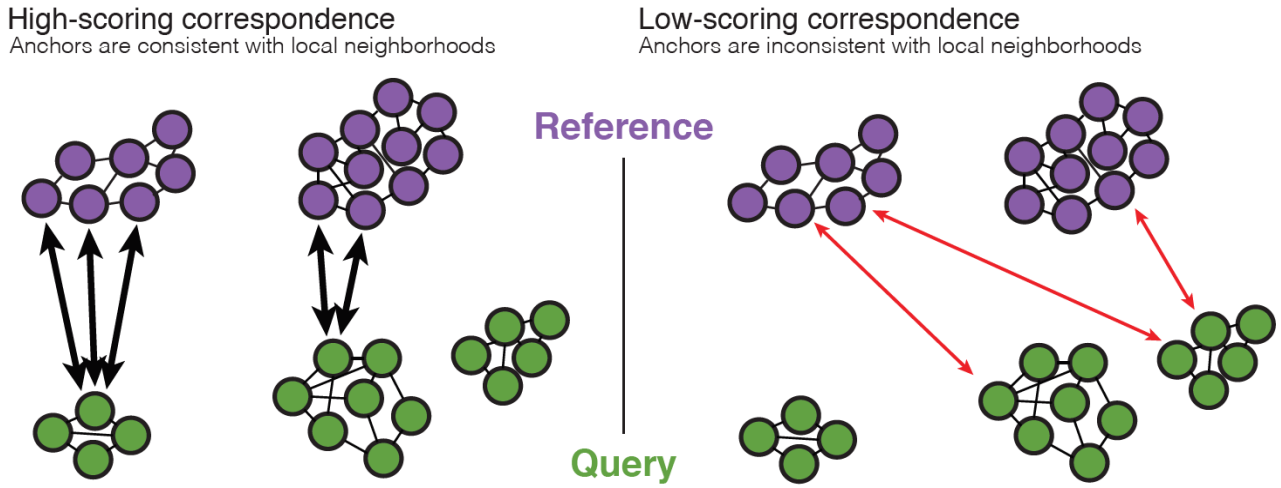


L2-normalization corrects for differences in scale

Finding corresponding cells

Anchors: mutual nearest neighbors





Finding corresponding cells

Data integration

1. Calculate the matrix B, where each column represents the difference between the two expression vectors for every pair of anchor cells a

$$B = X[,a] - Y[,a]$$

2. Construct a weight matrix W that defines the strength of association between each query cell c, and each anchor i

$$W_{c,i} = \frac{\tilde{D}_{c,i}}{\sum_{1}^{j=k.weight} \tilde{D}_{c,j}}$$

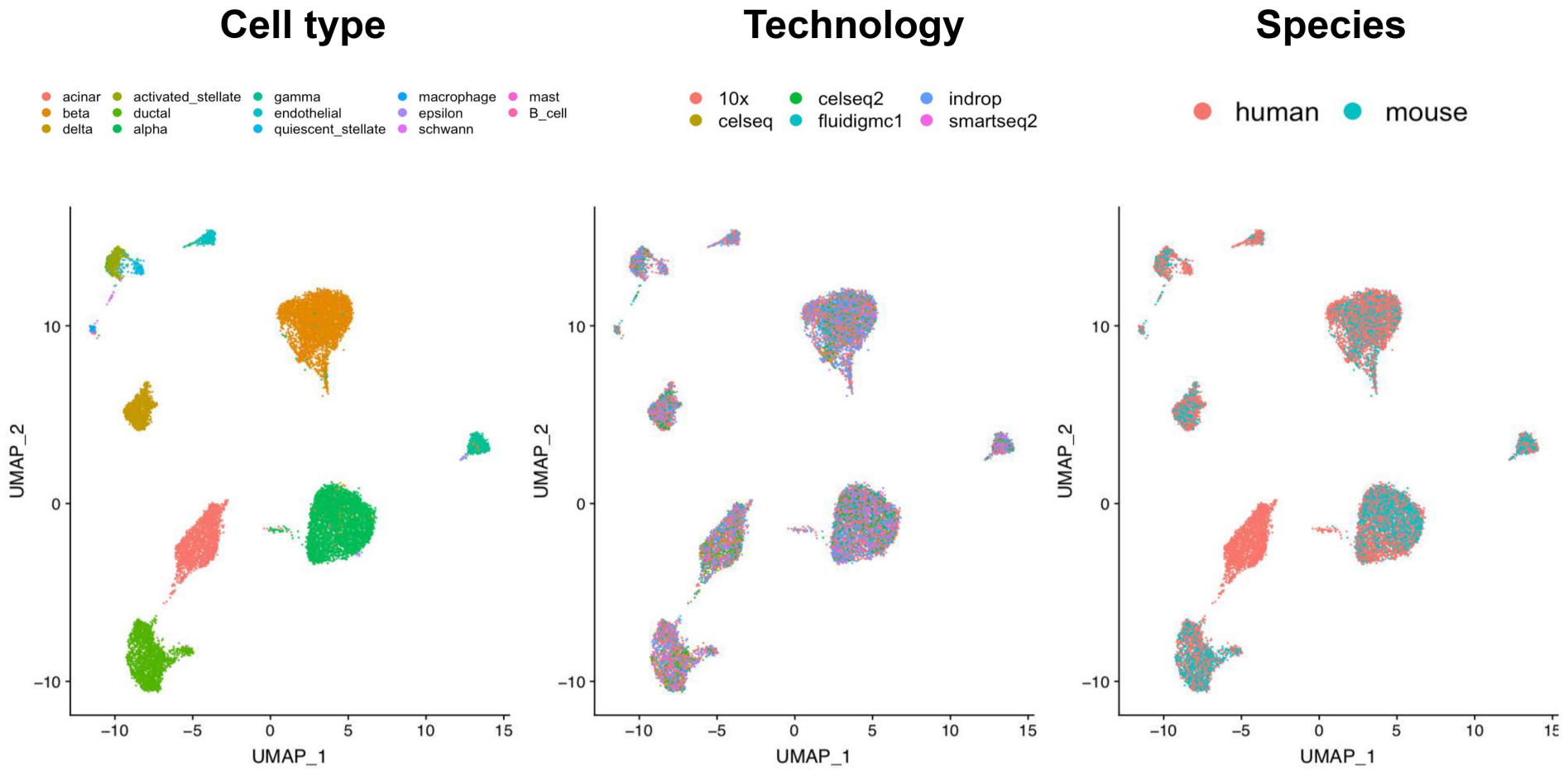
3. Calculate a transformation matrix C using the previously computed weights matrix and the integration matrix as

$$C = BW^T$$

4. Subtract the transformation matrix C from the original expression matrix Y to produce the integrated expression matrix $\hat{Y}$

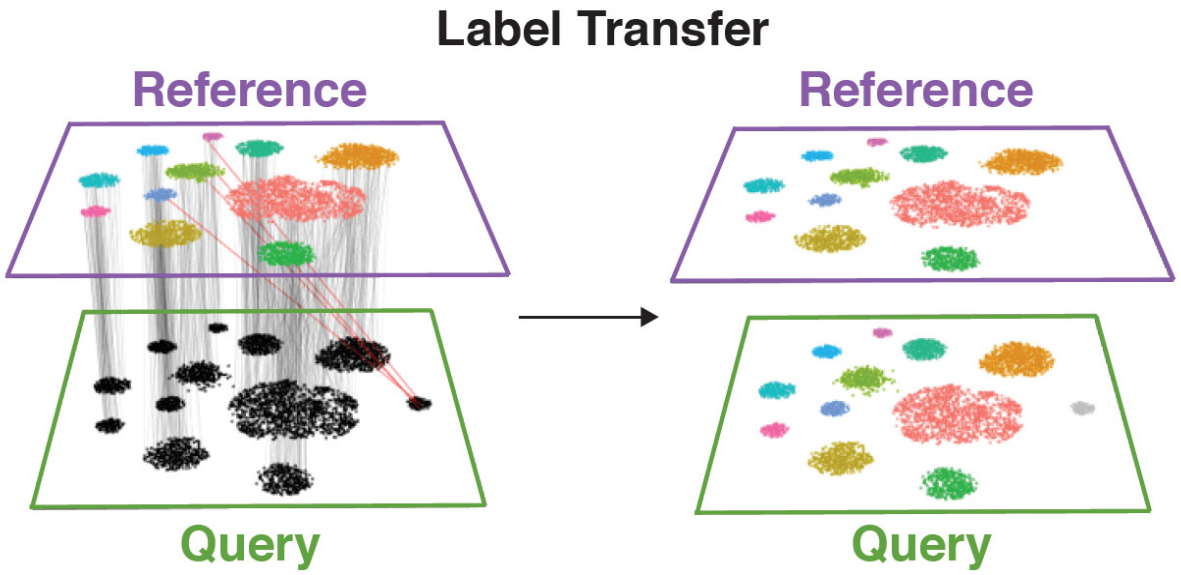
$$\hat{Y} = Y - C$$

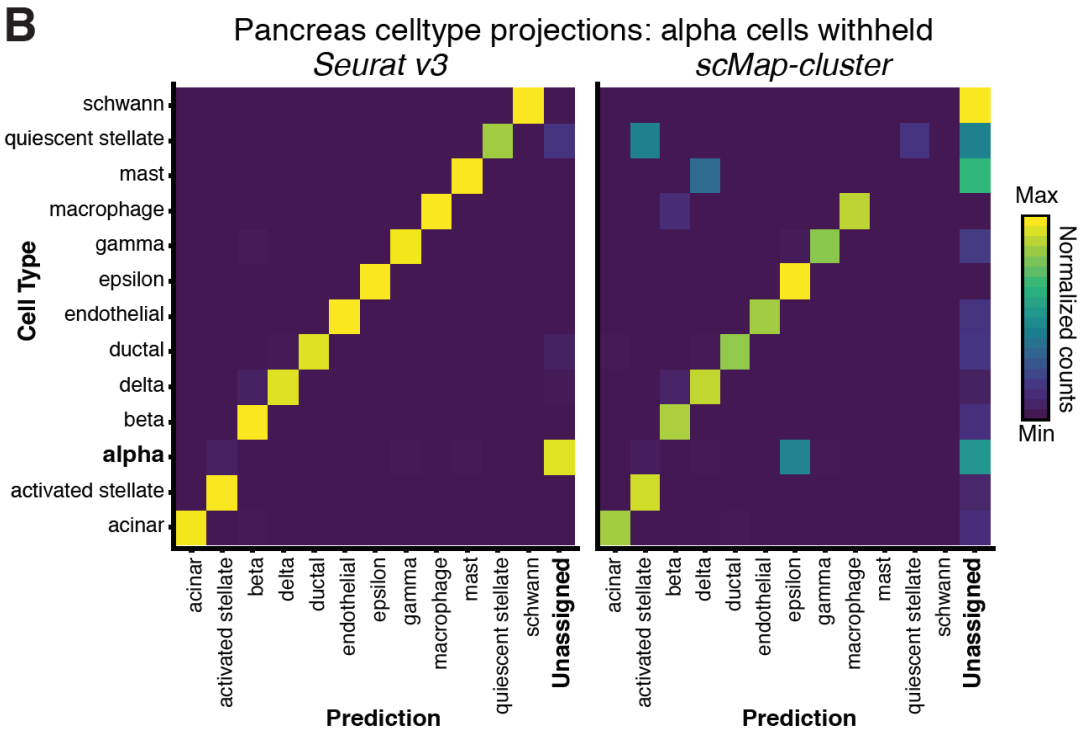
CCA + anchors (Seurat v3)



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

Label transfer (classification)

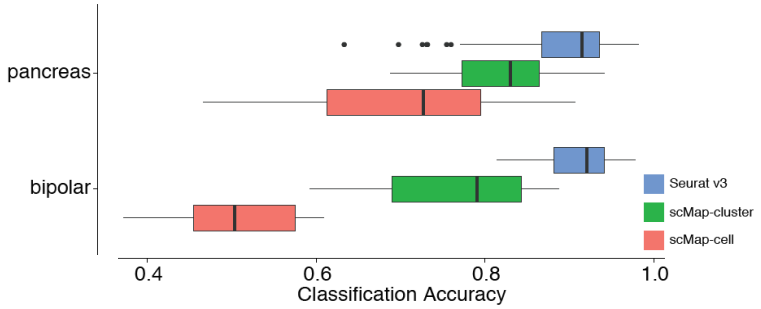




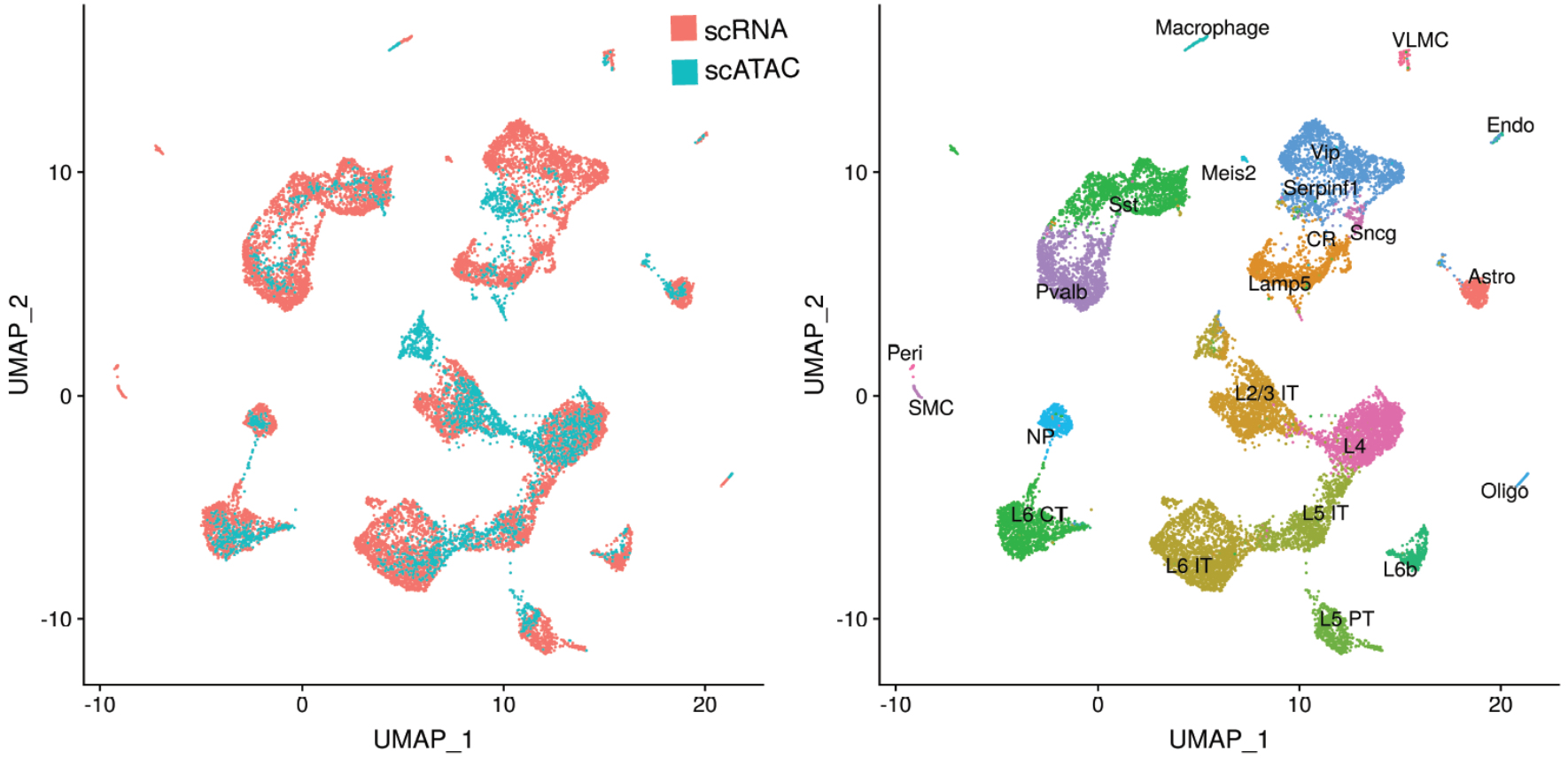
Weighted vote classifier

What is the classification of each cells nearest anchors?

$$P_l = LW^T$$



Integration across modalities



Using the corrected values

- Batch correction facilitates cell-based analysis of population heterogeneity in a consistent manner across batches.
  - No need to identify mappings between separate clusterings
  - Increased number of cells allows for greater resolution of population structure

- BUT…
- It is not recommended to use the corrected expression values for gene-based analyses (e.g. differential expression)
- Arbitrary correction algorithms are not obliged to preserve the magnitude (or even direction) of differences in per-gene expression when attempting to align multiple batches

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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*

Tran et al. (https://doi.org/10.1186/s13059-019-1850-9)

Performance assessment

- Qualitative (visualization)

- Quantitative:
  - Silhouette score
  - kBET: k-nearest-neighbor batch-effect test
  - …

Silhouette score

A score for each cell that assesses the separation of cell types, with a high score suggesting that cells of the same cell type are close together and far from other cells of a different type.

$$s(i) = \frac{b(i) - a(i)}{\max(a(i), b(i))}$$

$a(i)$ is the average distance of cell i to all other cells within i's cluster.

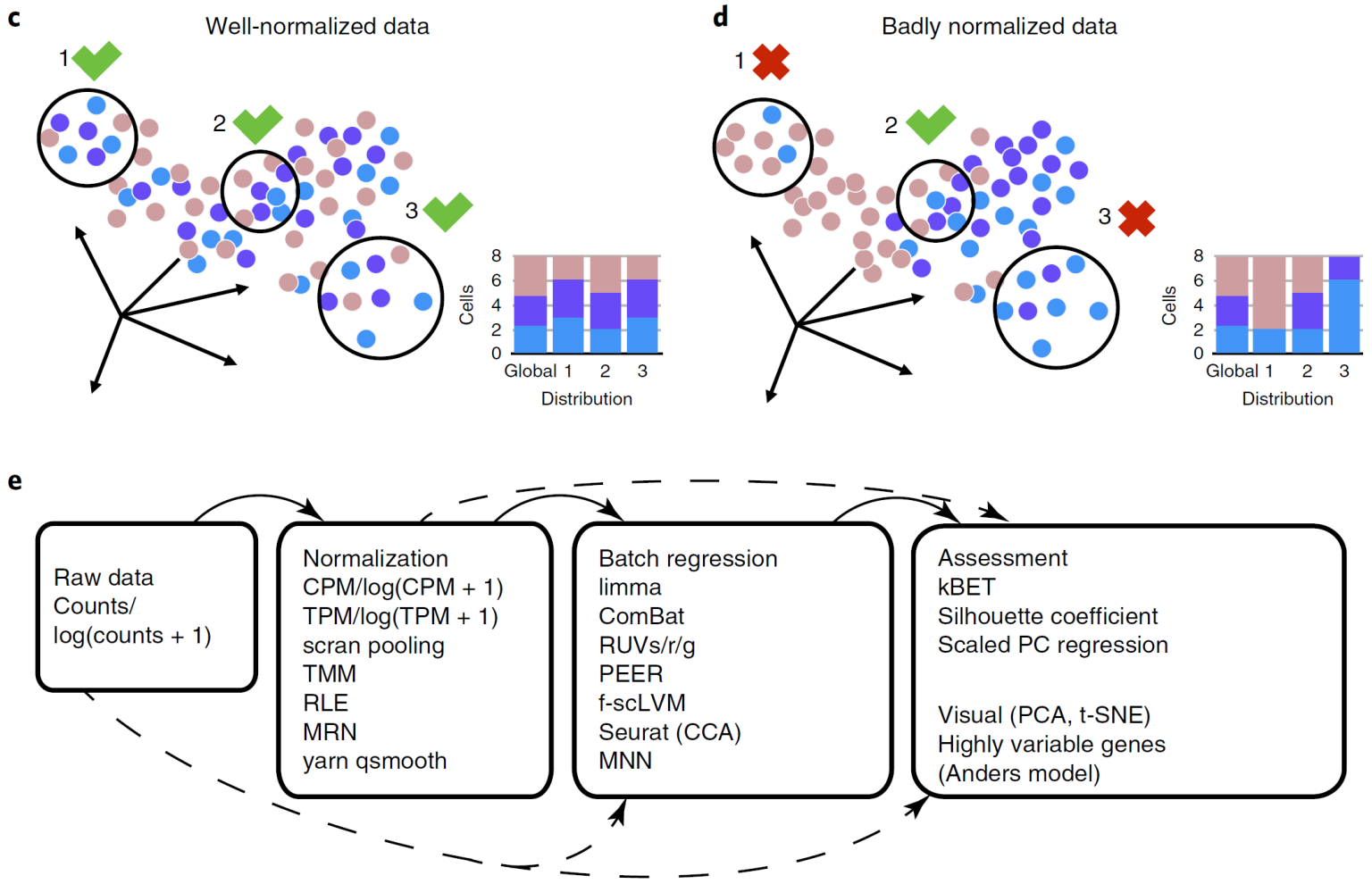
$$a(i) = \frac{1}{|C_i|} \sum_{\forall j} d(x_i, x_j)$$

$b(i)$ is the average distance of i to all cells in the nearest cluster to which i does not belong.

$$b(i) = \min_{\forall j, j \notin C_i} d(x_i, x_j)$$

Silhouette score: $$S = \frac{1}{N} \sum s(i)$$

kBET: *k*-nearest-neighbor batch-effect test

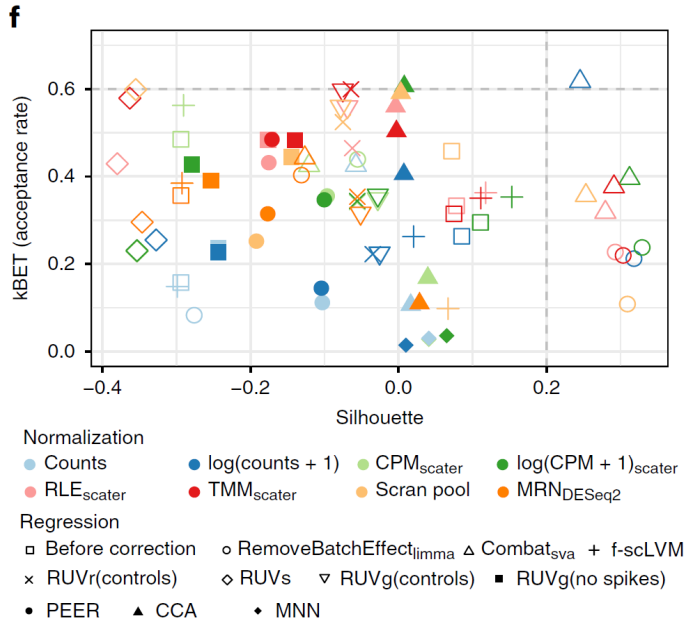


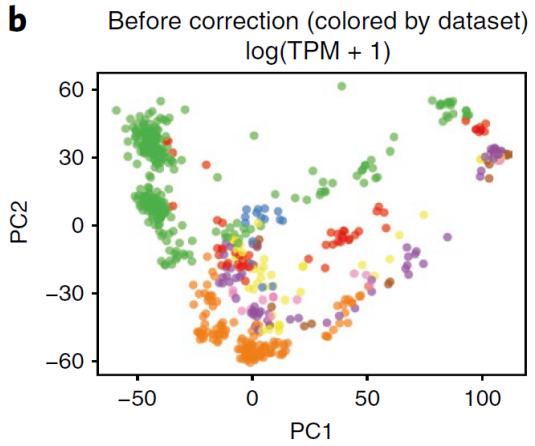
Büttner et al. (https://doi.org/10.1038/s41592-018-0254-1)

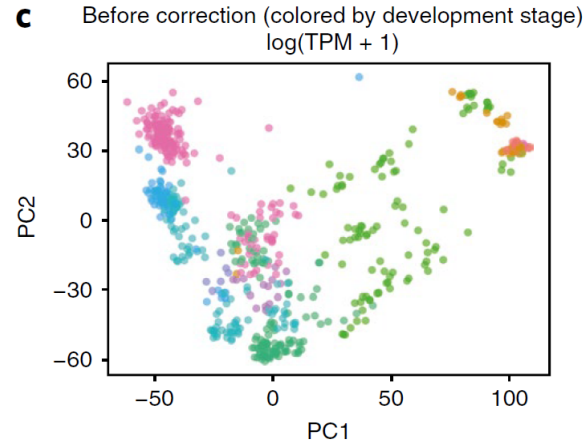
kBET assesses data-integration quality

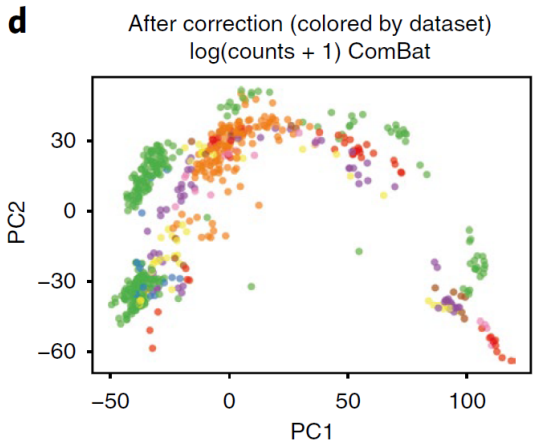
Development stage

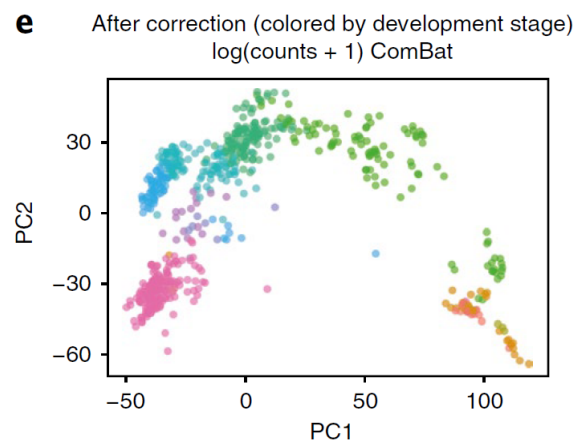
Dataset	Oocyte	Zygote	Pronucleus	2-cell	4-cell	8-cell	16-cell	32-cell	Morula	Blastocyst
Biase et al. 2014[16]	9			20	20					7
Boroviak et al. 2015[20]						3				12
Deng et al. 2014[21]	4			38	14	47	58			133
Fan et al. 2015[19]	9	9		13	7	7			7	13
Goolam et al. 2016[18]				16	64	32	6	6		
Liu et al. 2016[17]	6	5		5	6	6			6	15
Wu et al. 2016[23]	2	2		4	2	2				3
Xue et al. 2013[22]	2		3	3	3	3			3	











Summary (so far)

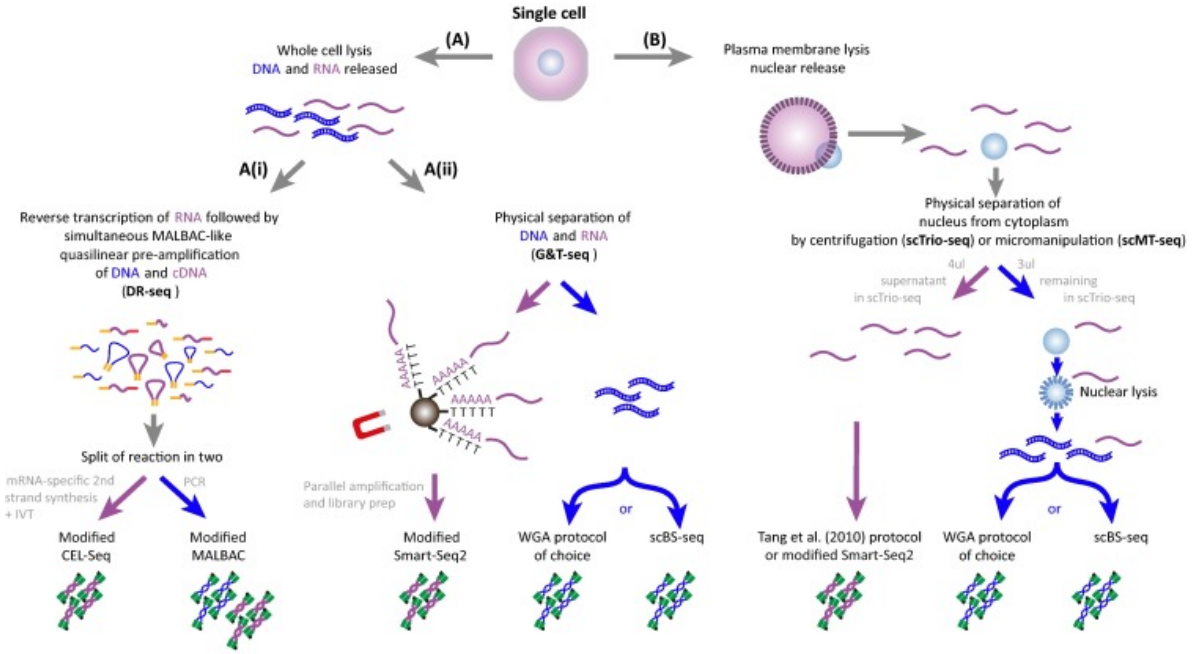
- Integration can allow us to **improve the interpretation** of single-cell data, and build a **multi-modal view** of the tissue

- Numerous methods now available for integration, mainly using **joint dimension reduction**, or **joint clustering**, or a combination of both

- Methods yielding **corrected expression matrix** can be used for further downstream analysis.

Single cell Multi-omics

Same cell



(C)	Loss of nucleic acids	Nature of RNA-seq	Nature of gDNA-seq	Shown amenable to bisulphite-sequencing
DR-seq	Minimal risk of loss	3'end tag transcript seq	MALBAC-like amplified gDNA, contaminated with co-amplified cDNA	no
G&T-seq (like)	Potential loss of mRNA and DNA molecules	Full-length transcript seq	In line with chosen WGA	yes
scTrio-seq	Loss of nearly half of cytoplasmic and all nuclear mRNA-molecules	Full-length transcript seq	reduced representation bisulphite-seq	yes
scMT-seq	Loss of some cytoplasmic and all nuclear mRNA-molecules during micromanipulation	Full-length transcript seq	reduced representation bisulphite-seq	yes

Trends in Genetics

Macaulay et al. (https://doi.org/10.1016/j.tig.2016.12.003)

nature biotechnology
LETTERS
https://doi.org/10.1038/s41587-019-0290-0

High-throughput sequencing of the transcriptome and chromatin accessibility in the same cell

Song Chen, Blue B. Lake and Kun Zhang*

Simultaneous epitope and transcriptome measurement in single cells

Marlon Stoeckius, Christoph Hafemeister, William Stephenson, Brian Houck-Loomis, Pratip K Chattopadhyay, Harold Swerdlow, Rahul Satija & Peter Smibert

Nature Methods **14**, 865–868(2017) | Cite this article

nature methods
ARTICLES
https://doi.org/10.1038/s41592-019-0547-z

Simultaneous profiling of 3D genome structure and DNA methylation in single human cells

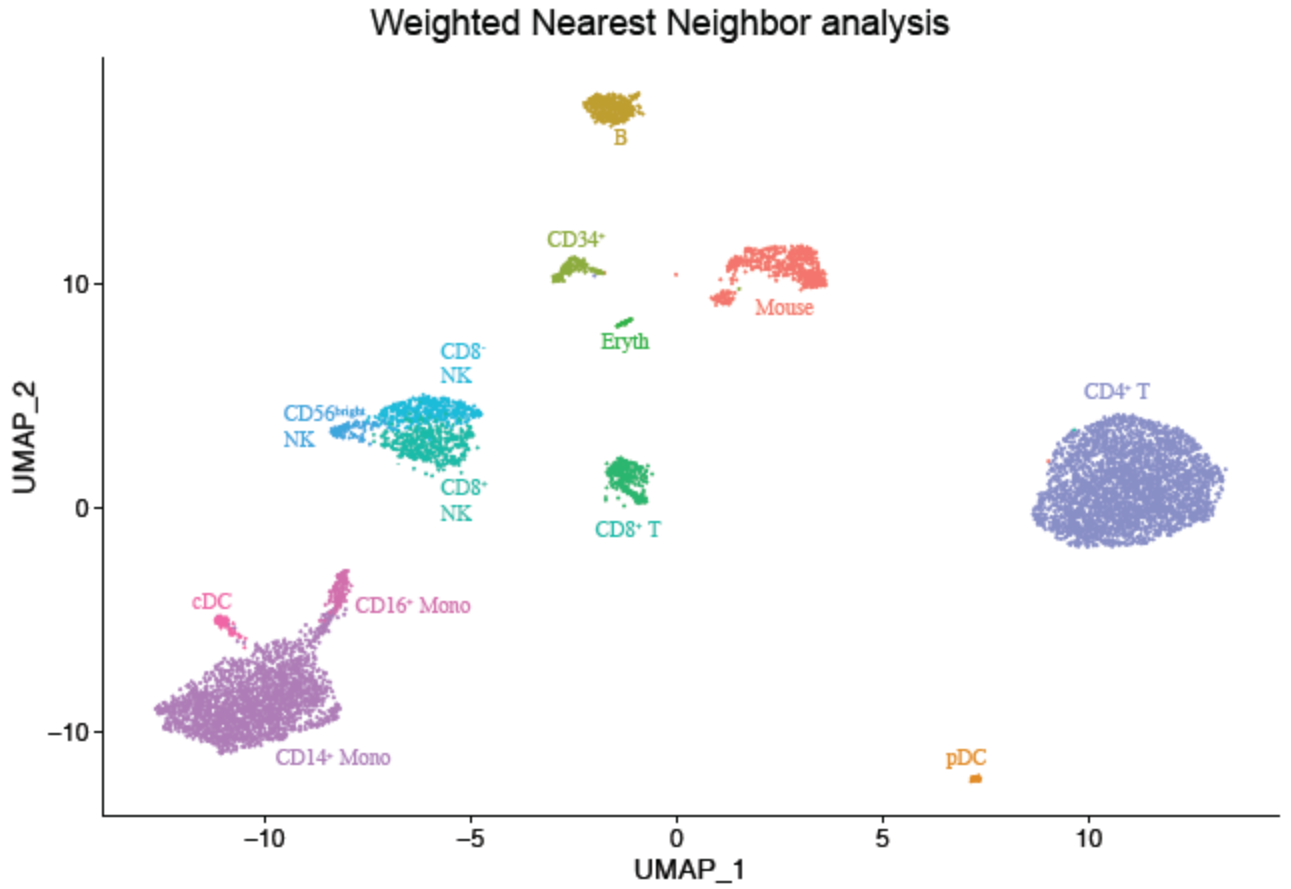
Dong-Sung Lee[1,5], Chongyuan Luo[2,3,5], Jingtian Zhou[2,5], Sahaana Chandran[1], Angeline Rivkin[2], Anna Bartlett[2], Joseph R. Nery[2], Conor Fitzpatrick[4], Carolyn O'Connor[4], Jesse R. Dixon[1*] and Joseph R. Ecker[2,3*]

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

Stephen J. Clark, Ricard Argelaguet, ChantriolInt-Andreas Kapourani, Thomas M. Stubbs, Heather J. Lee, Celia Alda-Catalinas, Felix Krueger, Guido Sanguinetti, Gavin Kelsey, John C. Marioni, Oliver Stegle & Wolf Reik

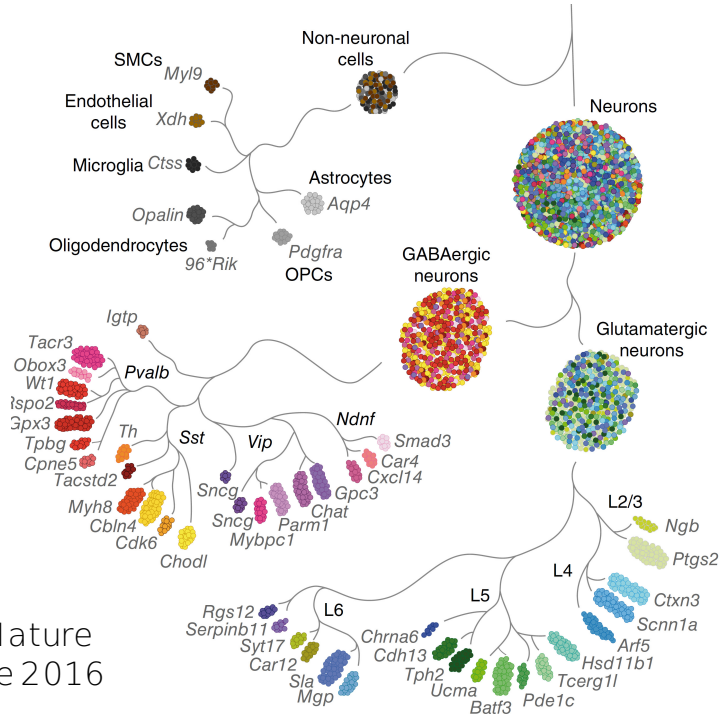
Nature Communications **9**, Article number: 781 (2018) | Cite this article

Seurat v4



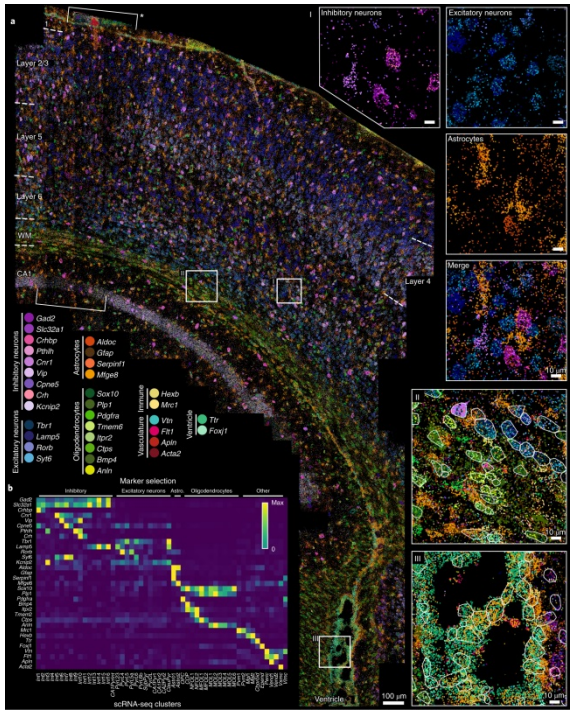
Yuhan Hao et al. (https://www.biorxiv.org/content/10.1101/2020.10.12.335331v1)

SpaGE: Spatial Gene Enhancement using scRNA-seq



Tasic et al, Nature Neuroscience 2016

Codeluppi et al, Nature methods 2018



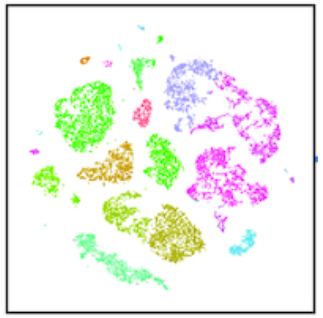
scRNA-seq
Whole transcriptome
Loss of spatial information

Spatial transcriptomics
Retain spatial information
Limited in the number of genes

Abdelaal et al., *NAR* (https://doi.org/10.1093/nar/gkaa740)

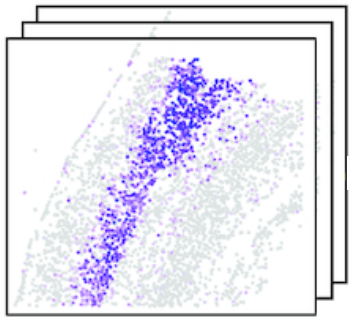
SpaGE overview

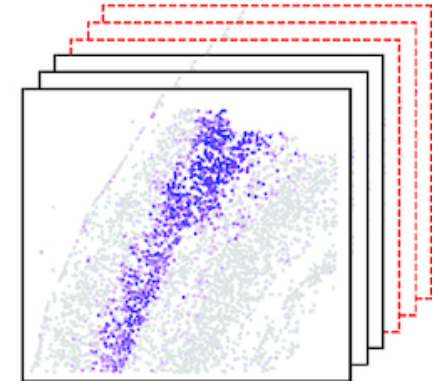
scRNA-seq (reference)
(~25,000 genes)



+

Spatial transcriptomics (query)
(~ 10-1,000 genes)





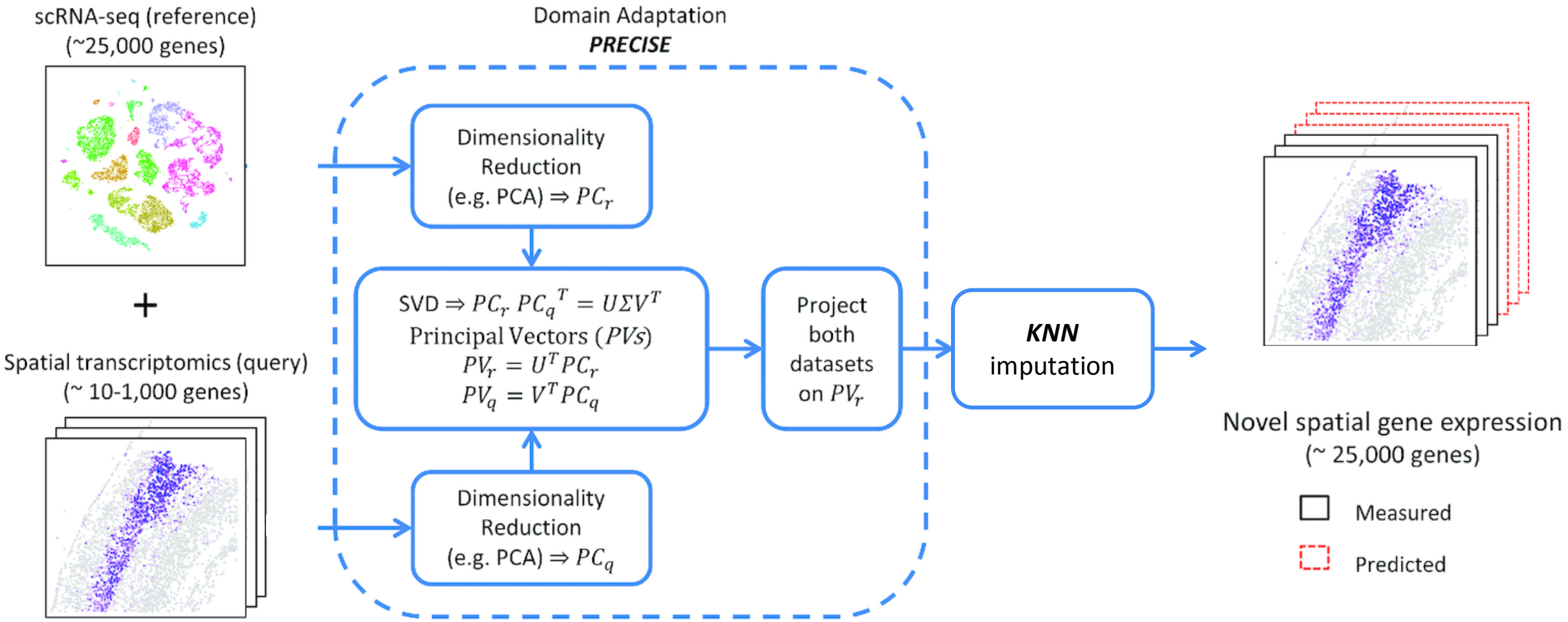
Novel spatial gene expression
(~ 25,000 genes)

Measured

Predicted

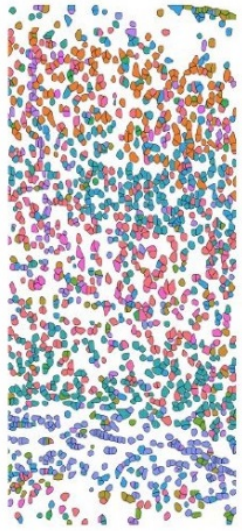
PRECISE: Mourragui et al, Bioinformatics. 2019

SpaGE overview

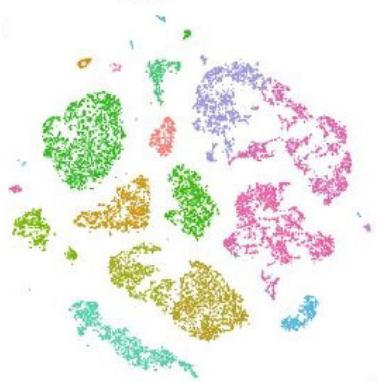


PRECISE: Mourragui et al, Bioinformatics. 2019

SpaGE on primary visual cortex (VISp)

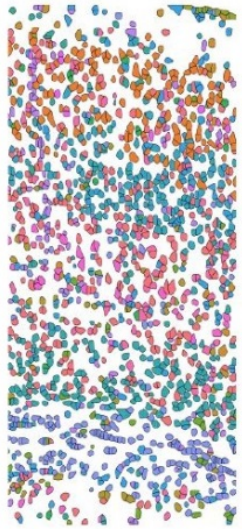


STARmap
1,549 cells
1,020 genes
Wang et al. Science 2018



scRNA-seq
14,249 cells
34,617 transcripts
Tasic et al. Nature 2018

SpaGE on primary visual cortex (VISp)



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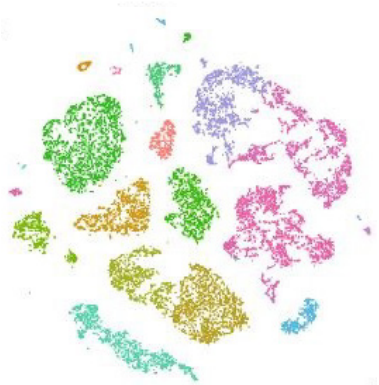

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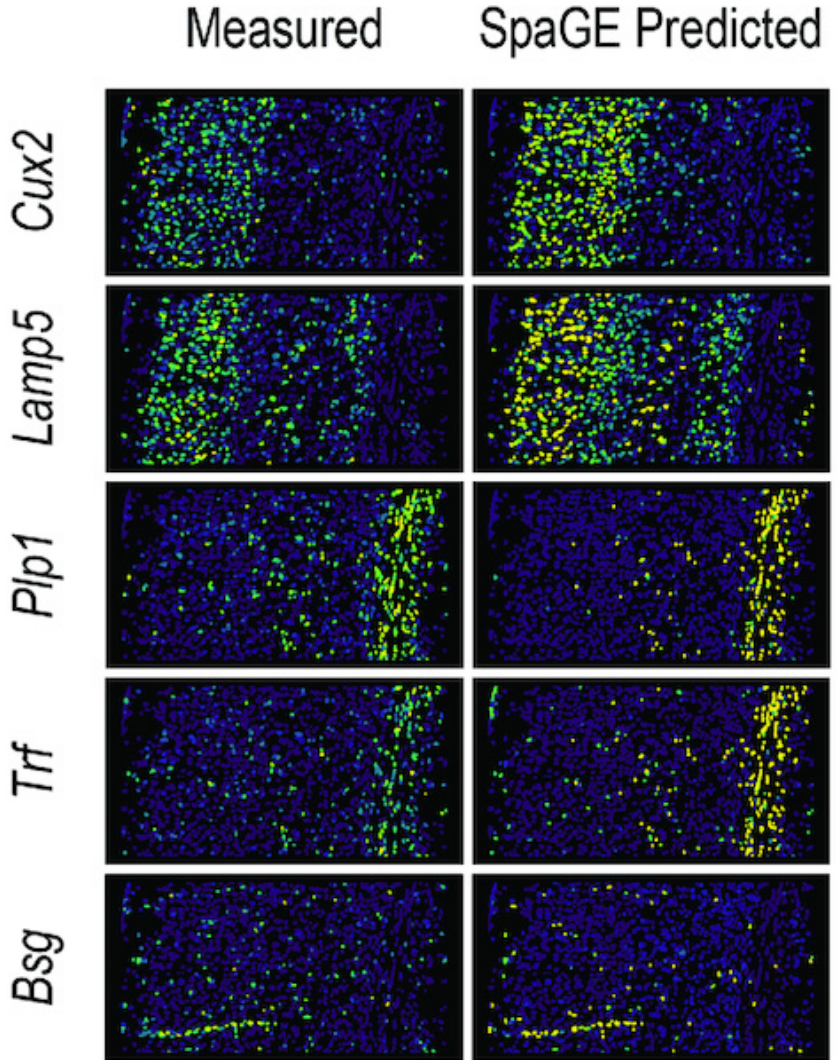
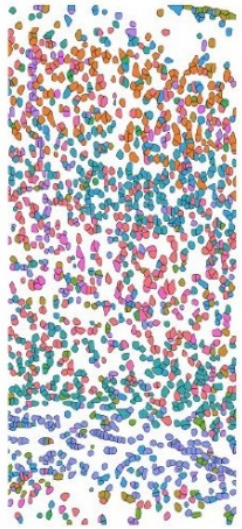


SpaGE outperforms Seurat, Liger and gimVI



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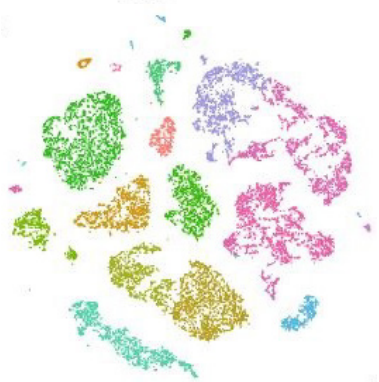
Wang et al. Science 2018



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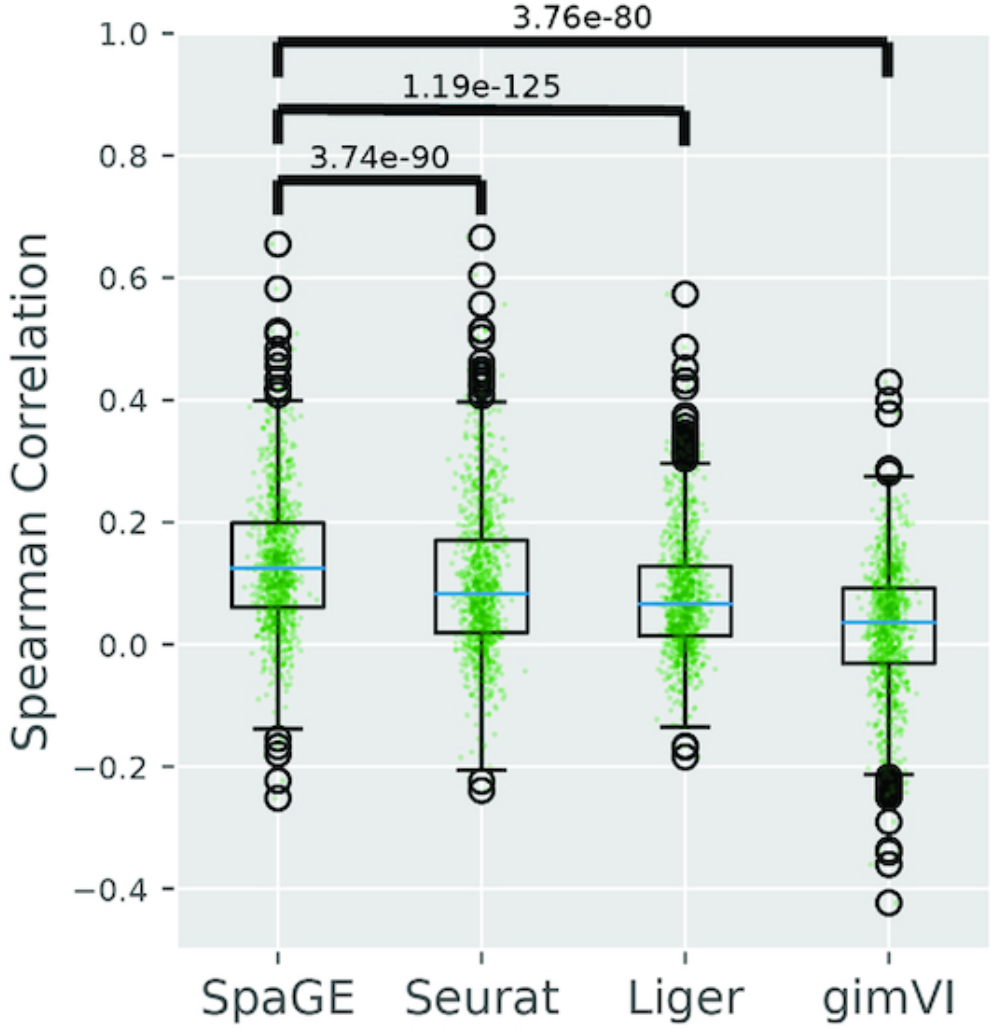
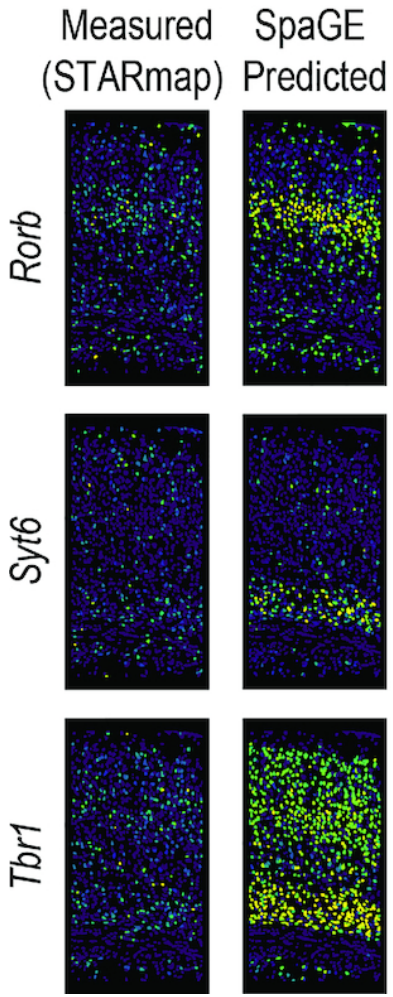


Wrongly measured, correctly predicted



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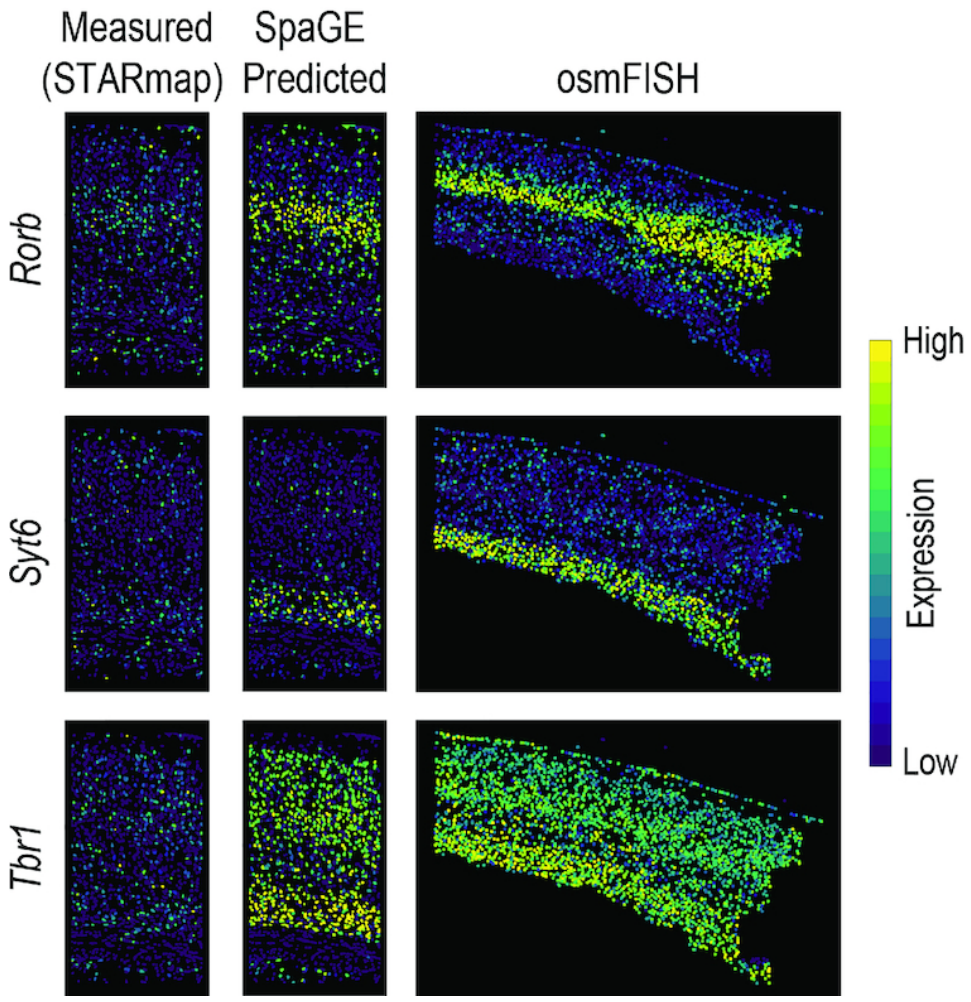
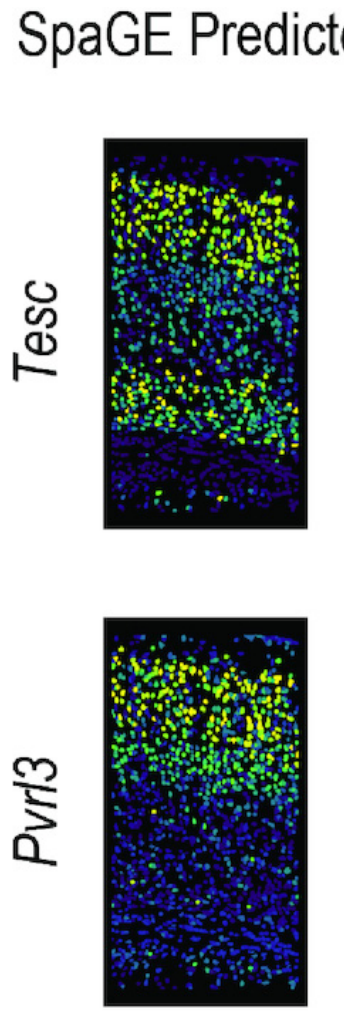
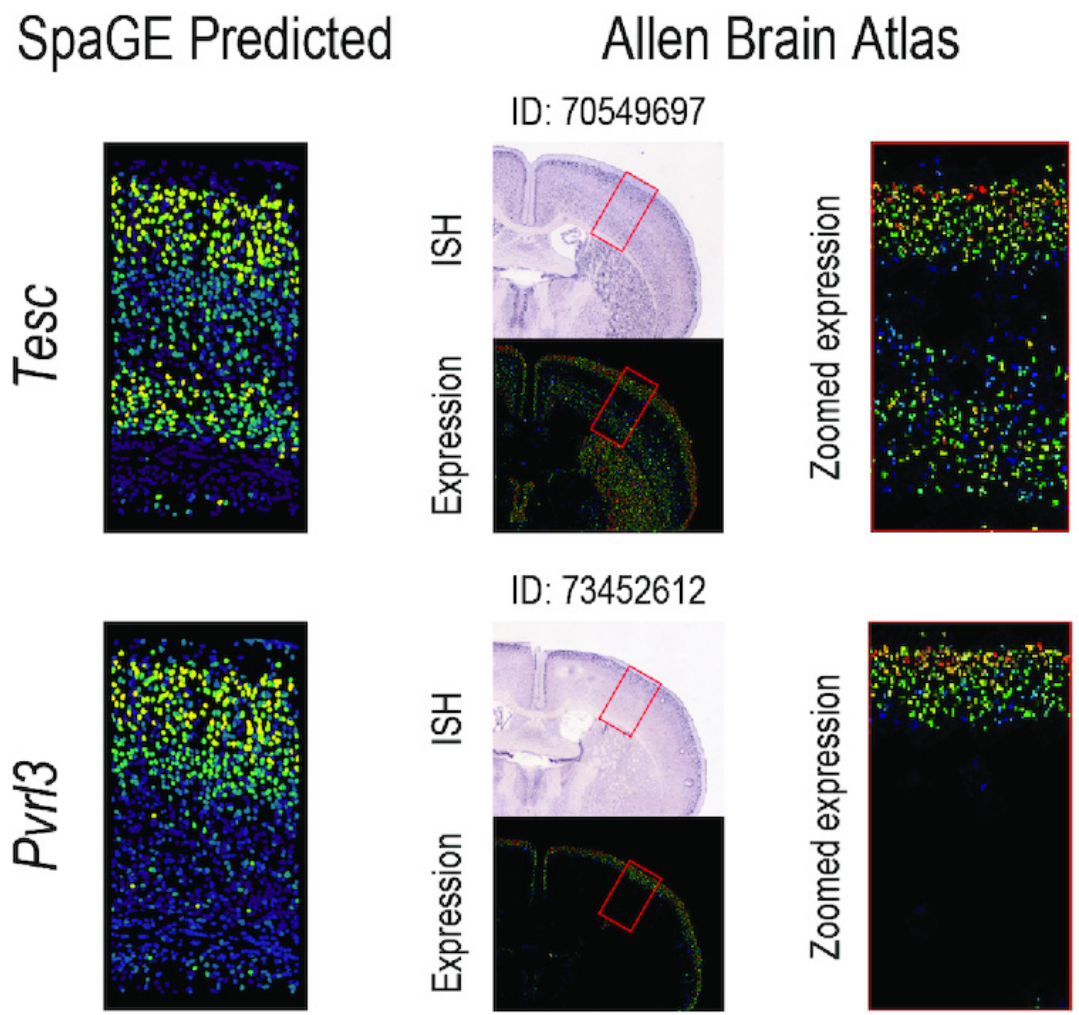


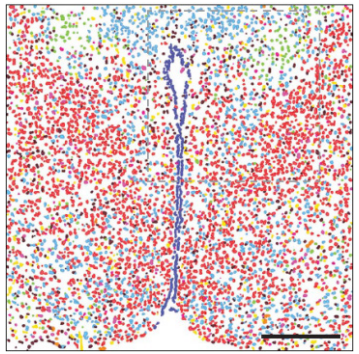
Predicting unmeasured genes



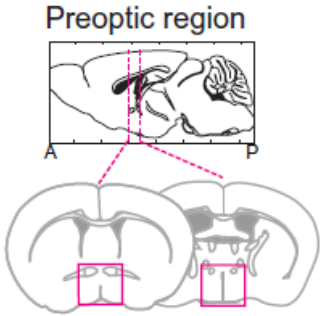
Predicting unmeasured genes



SpaGE scales to large spatial datasets

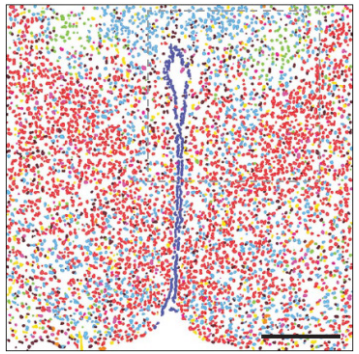


MERFISH
64,373 cells
155 genes
Moffit et al. Science 2018



scRNA-seq
31,299 cells
18,646 transcripts
Moffit et al. Nature 2018

SpaGE scales to large spatial datasets



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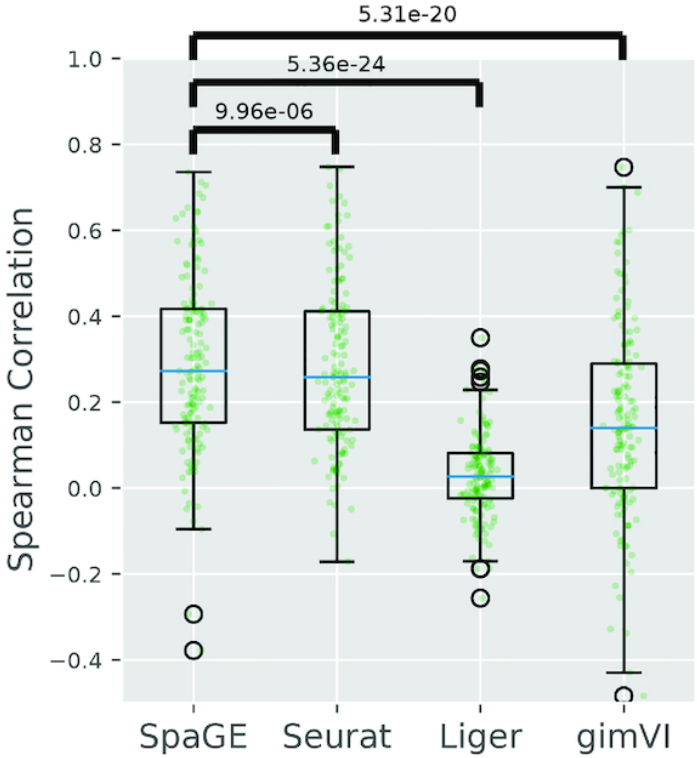

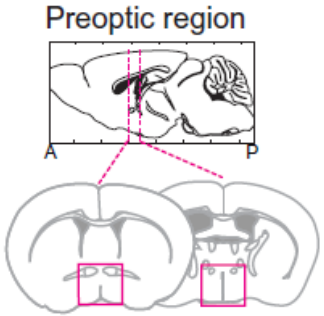

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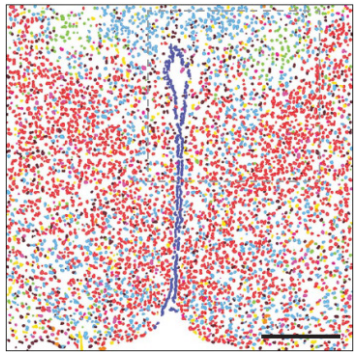

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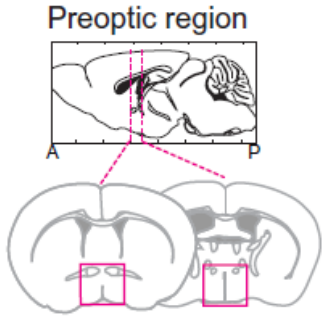

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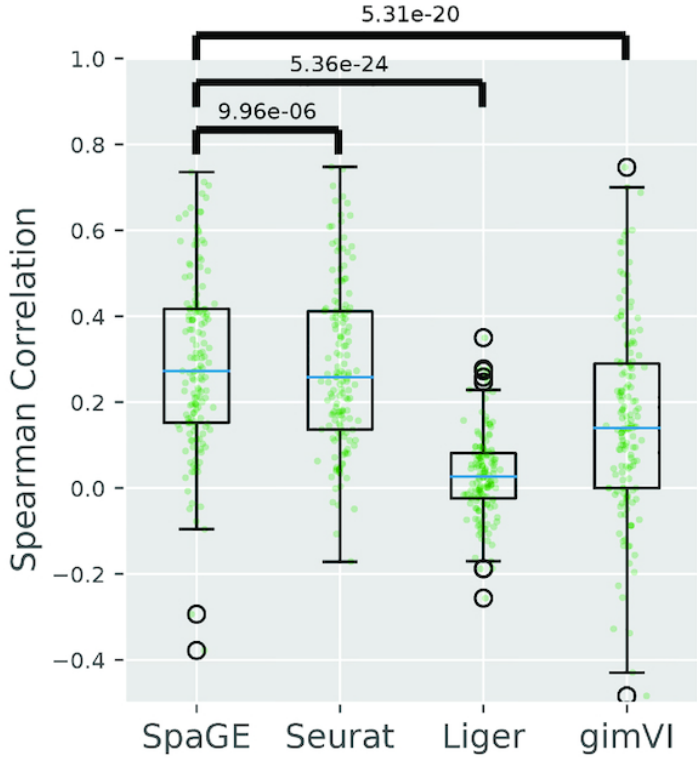

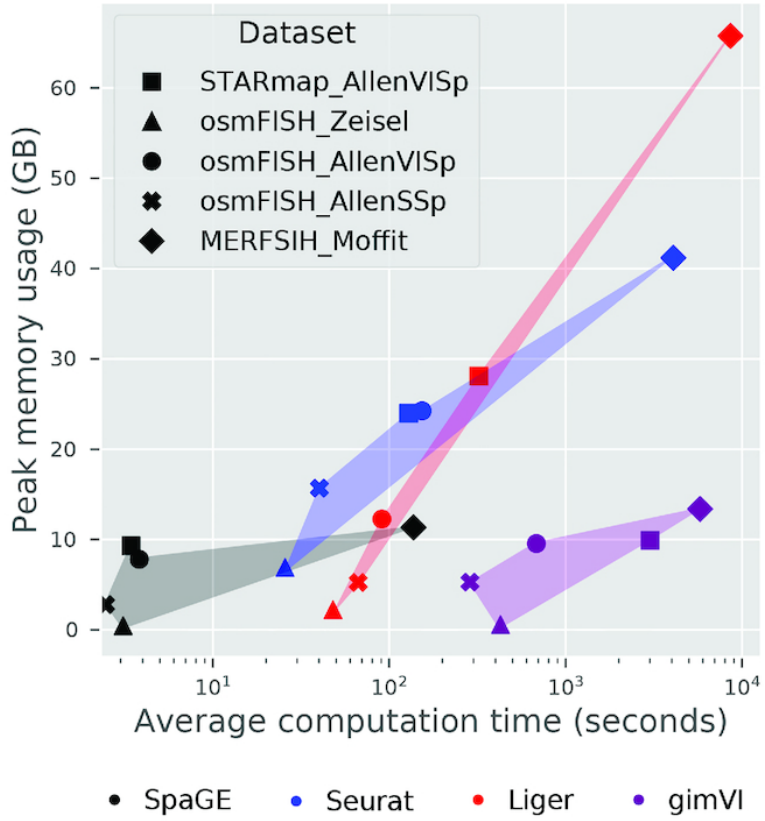

Summary

- SpaGE predicts spatial expression of unmeasured genes at the single cell level

- SpaGE outperforms state-of-the-art methods using is simpler, scalable and more flexible approach.

Data integration practical

- MNN correction
- Seurat v3
- Four pancreatic datasets

Resources

- Stuart et al. “Comprehensive integration of single cell data”

https://www.biorxiv.org/content/10.1101/460147v1

- Haghverdi et al. “Batch effects in single-cell RNA-sequencing data are corrected by matching mutual nearest neighbors”

https://doi.org/10.1038/nbt.4091

- Tim Stuart “Integration and harmonization of single-cell data” (Satija Lab single cell genomics day 2019)

https://satijalab.org/scgd/

- Andrew Butler “Batch Correction and Data Integration for Single Cell Transcriptomics” (Satija Lab single cell genomics day 2018)

https://satijalab.org/scgd18/

- Orchestrating Single-Cell Analysis with Bioconductor

https://osca.bioconductor.org/

- Hemberg’s group course: Analysis of single cell RNA-seq data

https://scrnaseq-course.cog.sanger.ac.uk/website/index.html

- Seurat Integration and Label Transfer tutorial

https://satijalab.org/seurat/v3.0/pancreas_integration_label_transfer.html

Thank You!

t.r.m.abdelaal-1@tudelft.nl