Single cell sample integration

Remi Montagne

Institut Curie

Initiation single cell - 10/20/2023

Introduction

Starting point: normalized, reduced **individual** matrices

Next step: start getting information

Introduction

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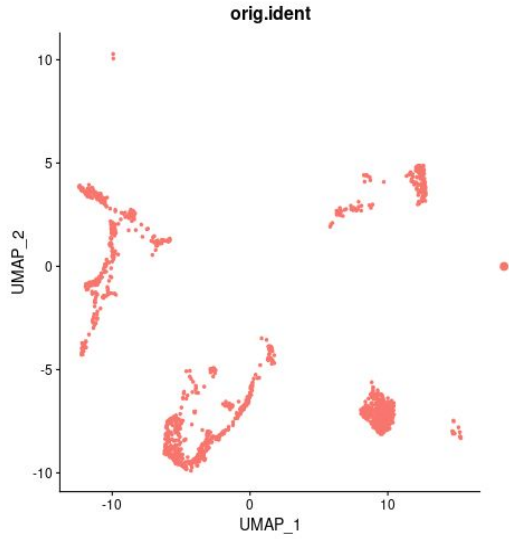
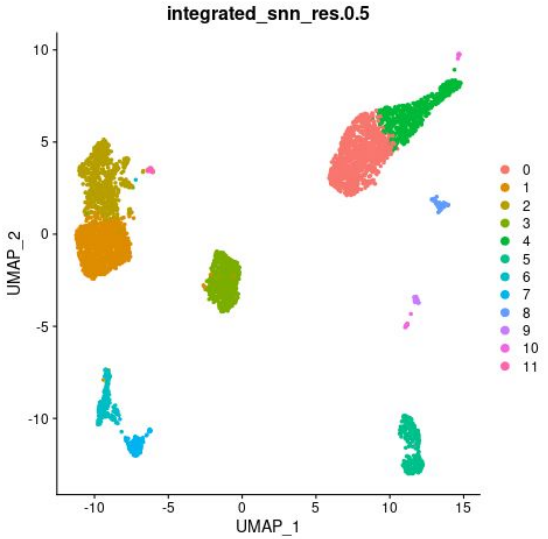
➔ Visualize the cells



➔ Understand what is in the samples (clustering)



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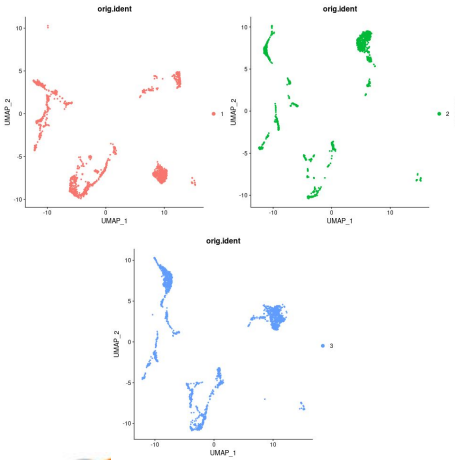
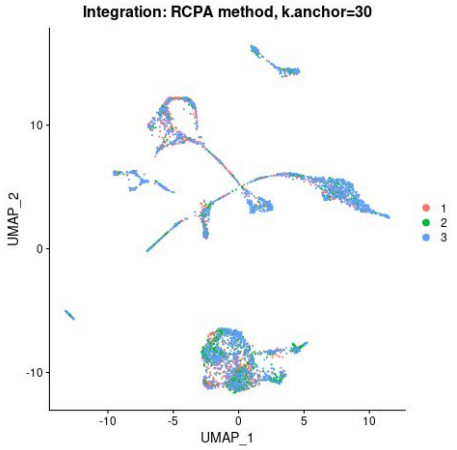
But should we do that

on individual samples?

On all samples together ?





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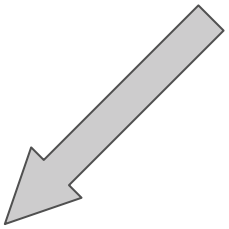

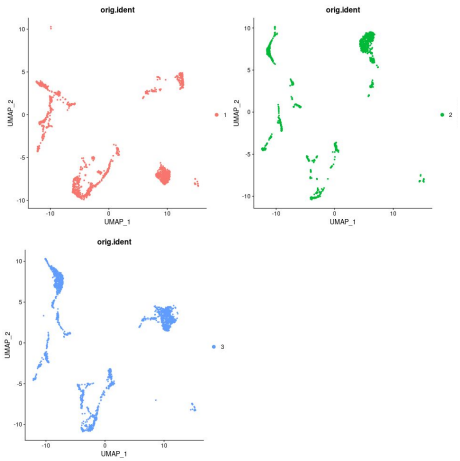

- Quick way to have a first look at data

- Repetitive
- Makes more sense to bring replicates together.
- Makes more sense to bring together similar samples (same experiment, organ…)

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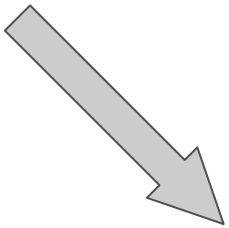
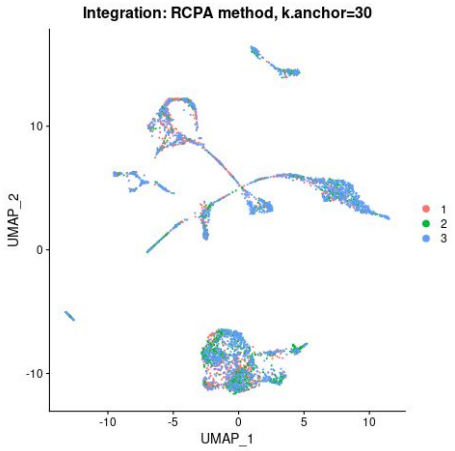
But should we do that

- Allows to work across multiple samples.
- Particularly important for cell populations visualization and identification
- Many cells : helps identifying rare populations

- Overcorrection ?



On all samples together ?



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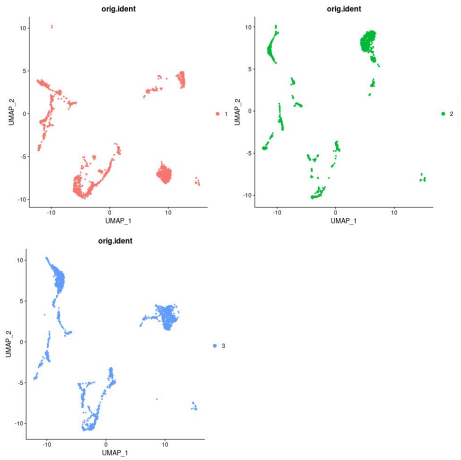

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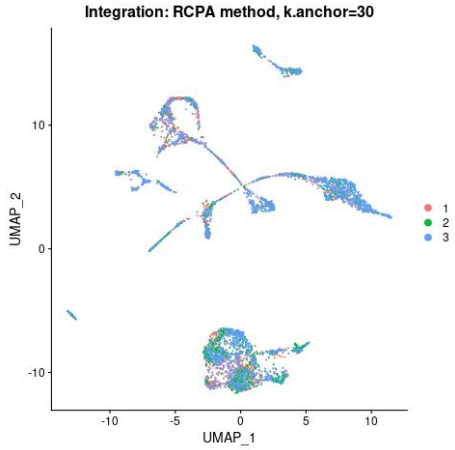

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We will do that on all samples together

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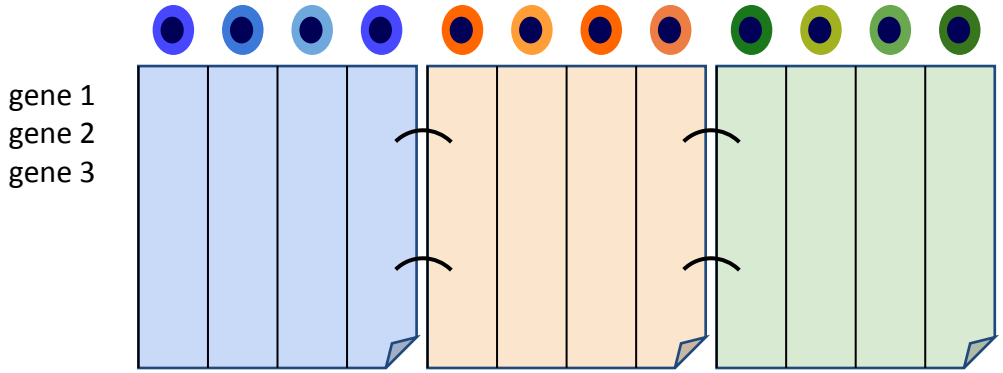
Problem: simple matrix concatenation does not always work



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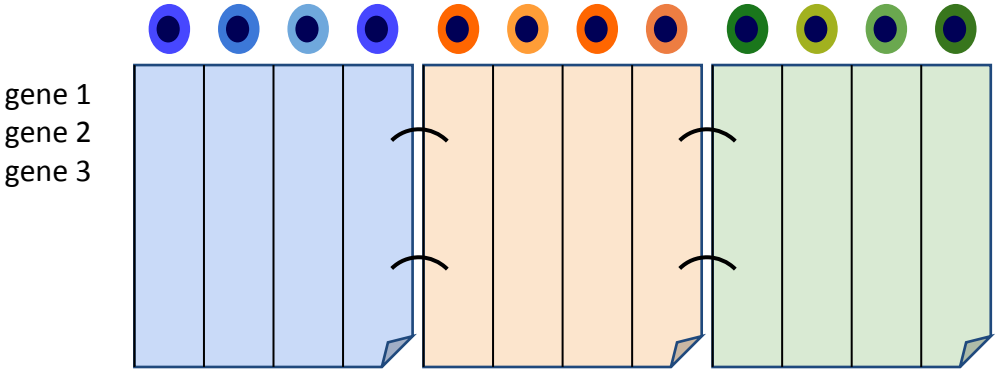

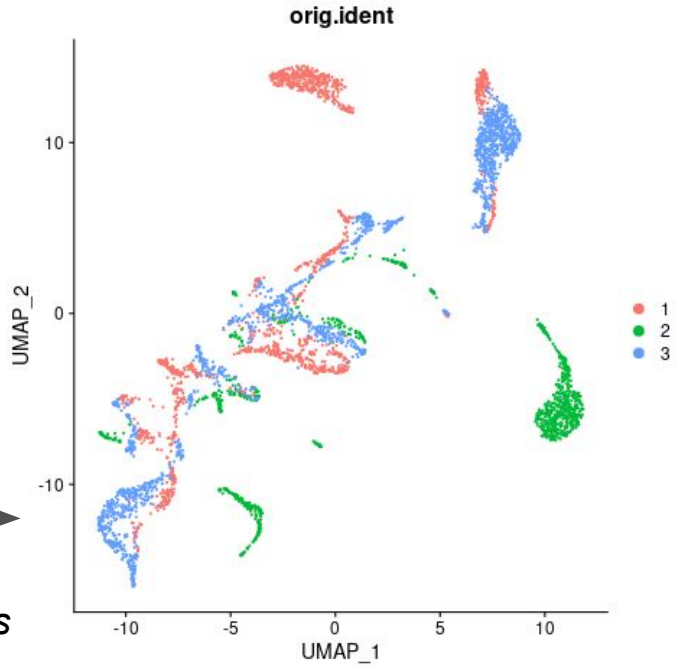

same model (PBMC), unaligned cells

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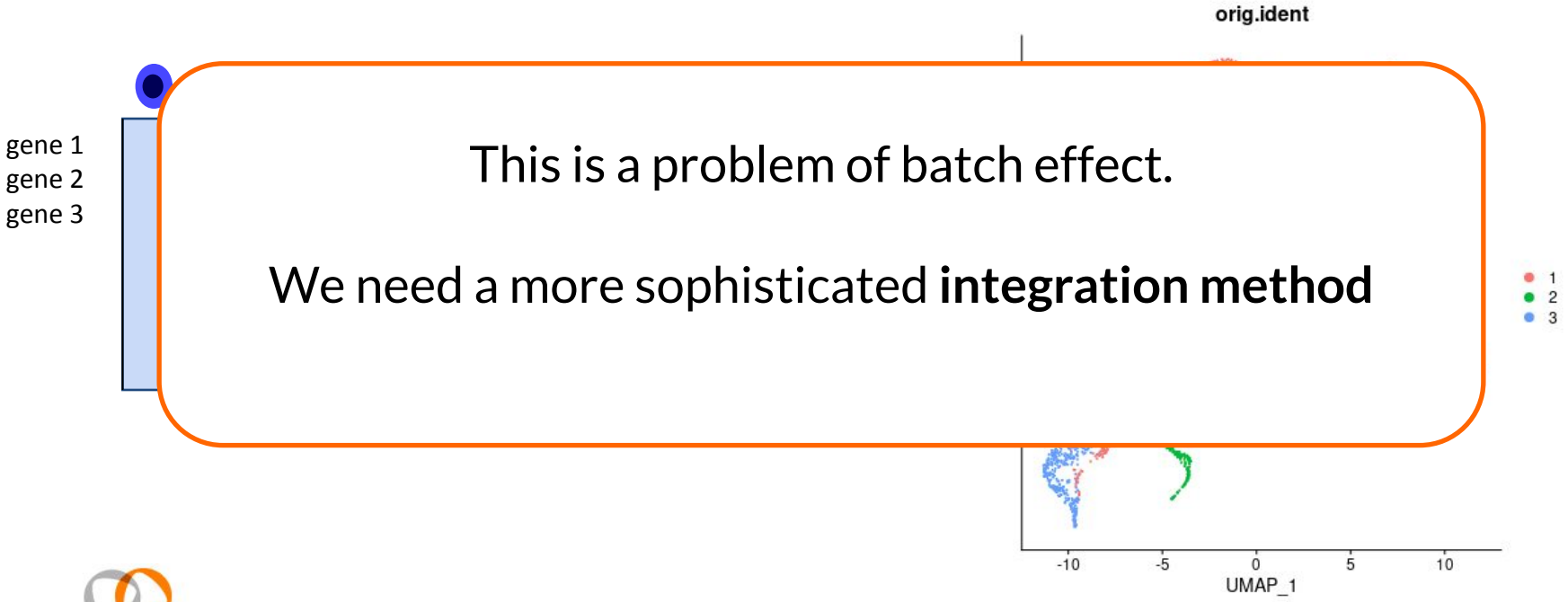


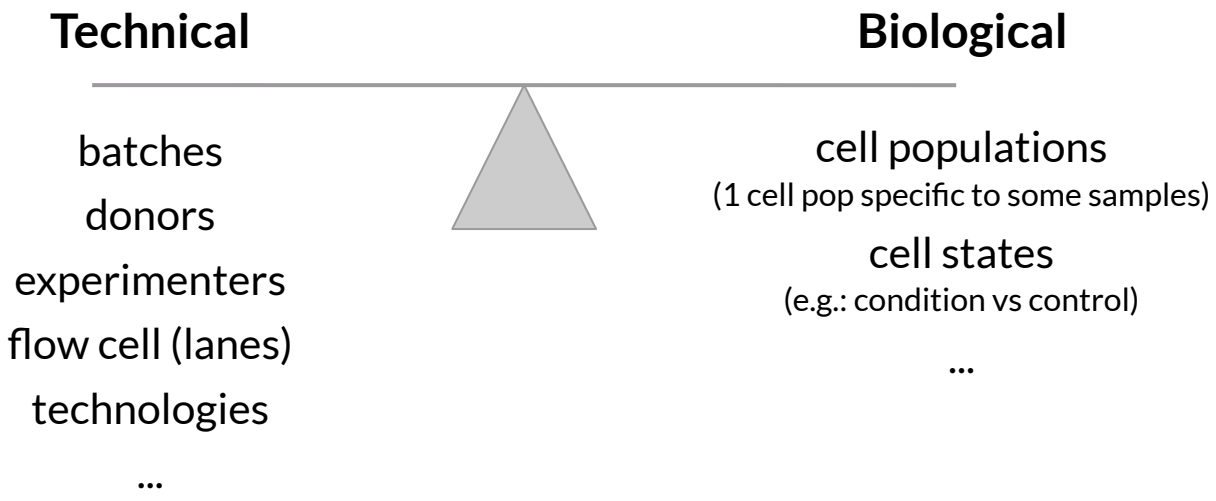
This is a problem of batch effect.

We need a more sophisticated **integration method**

Variability across samples

Variability across samples

2 sources of variability across samples



Variability across samples

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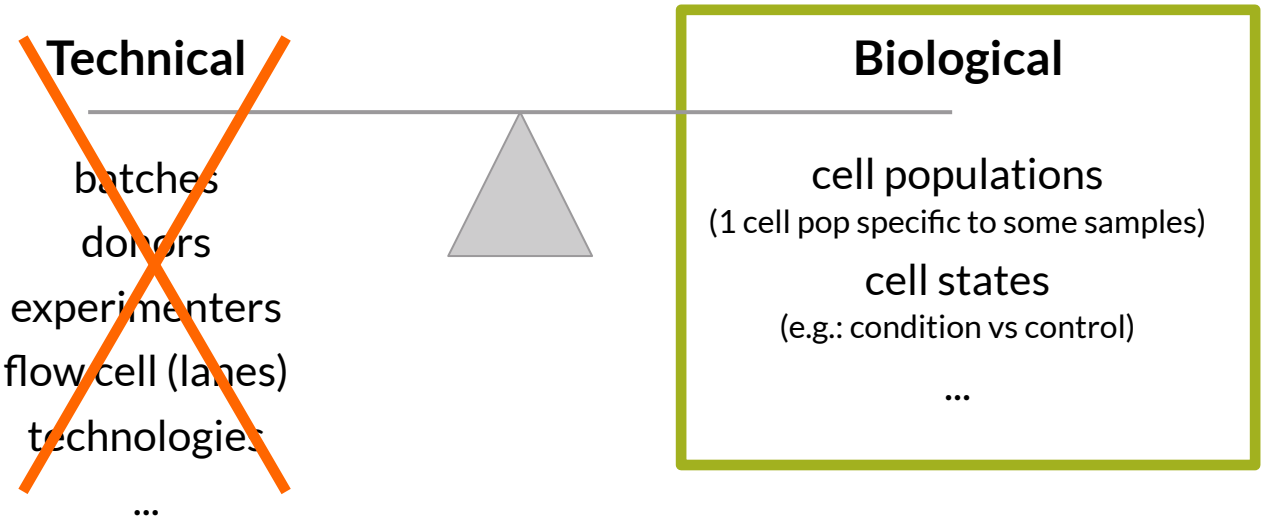

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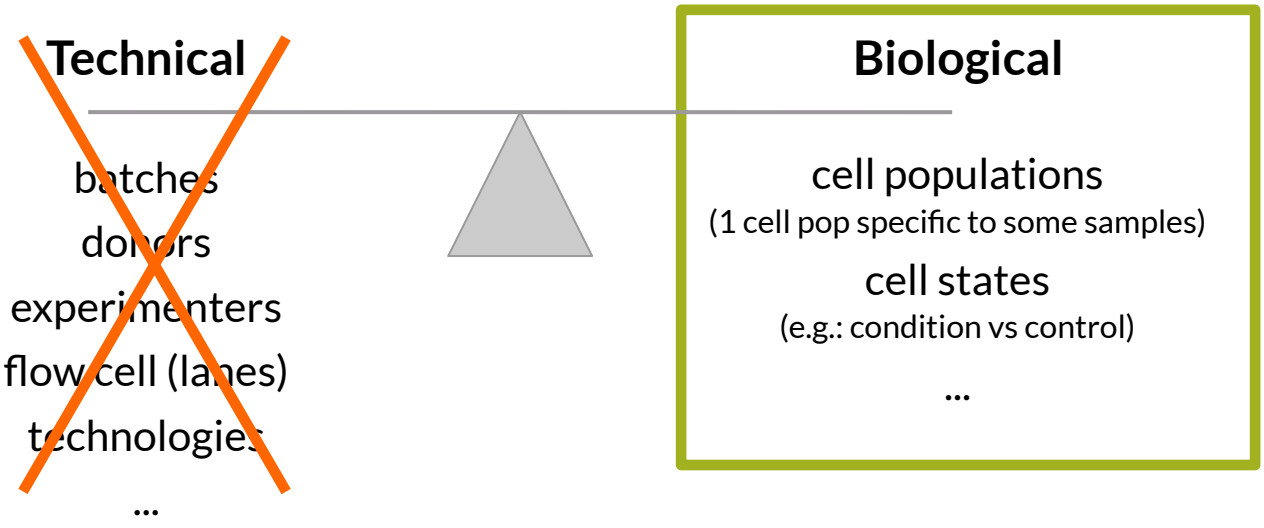


→ Solutions:

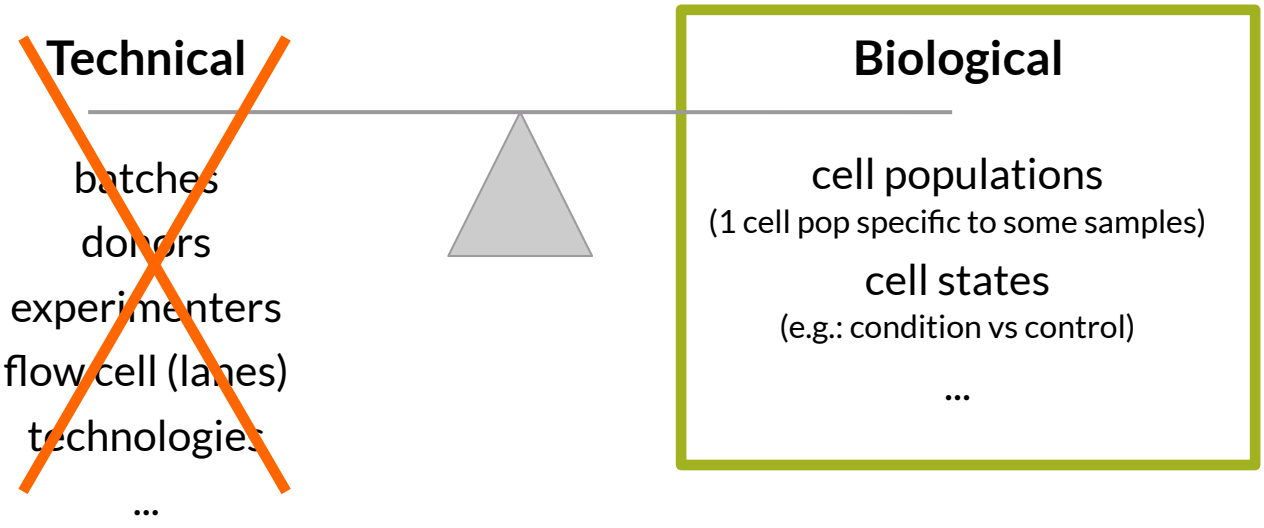
Strategies to avoid factors causing batch effect in the lab

Solution: Technical factors that potentially lead to batch effects may be avoided with mitigation strategies in the lab and during sequencing. Examples of lab strategies include: sampling cells on the same day, using the same handling personnel, reagent lots, protocols, reducing PCR amplification bias, and generally using the same equipment. Sequencing strategies can include multiplexing libraries across flow cells. For example, if samples came from two patients, pooling libraries together and spreading them across flow cells can potentially spread out the flow cell-specific variation across samples.

https://www.10xgenomics.com/resources/analysis-guides/introduction-batch-effect-correction

Variability across samples

2 sources of variability across samples



→ Solutions:

Strategies to avoid factors causing batch effect in the lab

Computational data integration

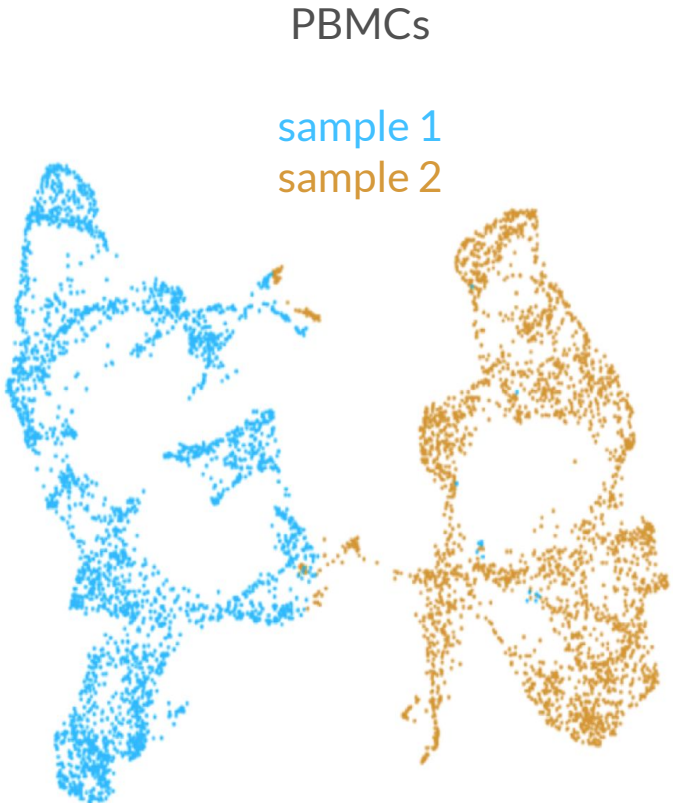
When to integrate

When to integrate

- **Integrate** when obvious batch effect between samples, typically seen on low dimension visualization

In this example, the sample of origin would be a huge bias for clustering

The samples need integration to align cell types/clusters and then identify them correctly



https://www.10xgenomics.com

When to integrate

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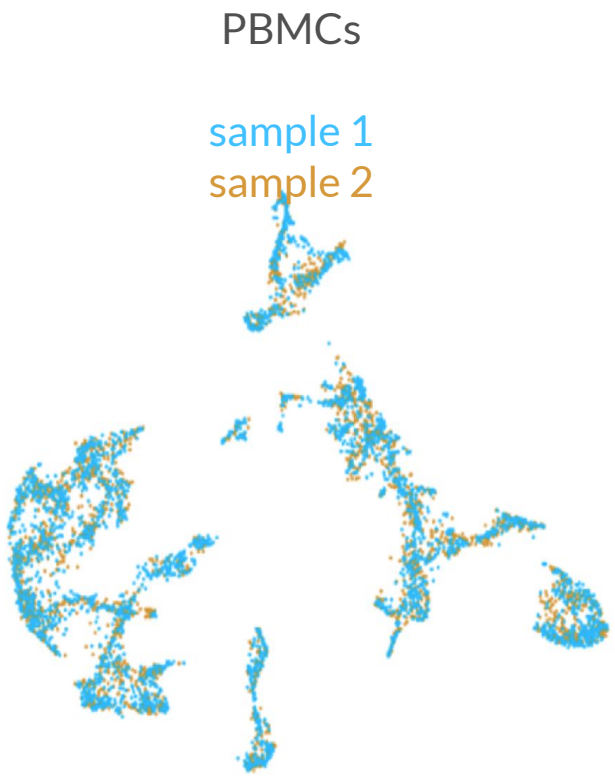
- Do not integrate otherwise:
  e.g.: replicates generated in the same time and exactly in the same manner may not need integration



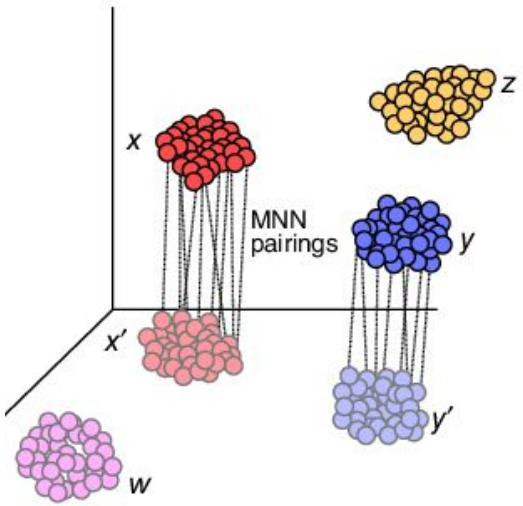
https://www.10xgenomics.com

Integration with Seurat

Integration with Seurat

Many methods

- Over 49 methods (Luecken et al., Nat Methods 2022)
- Seurat integration: group of **similarity-based** methods (most methods)

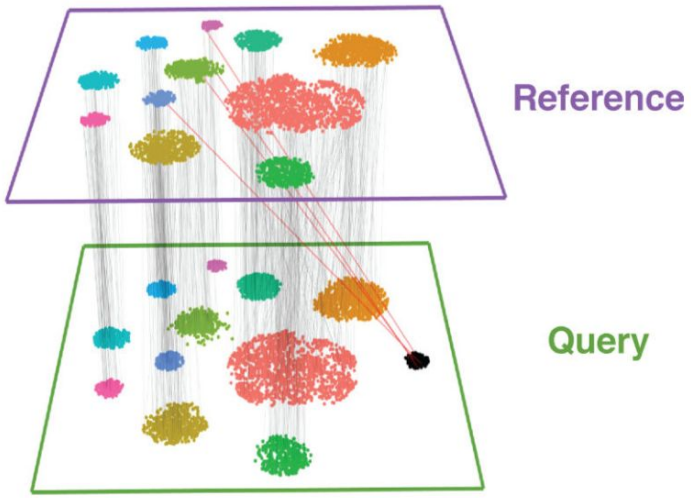


Stuart *et al.*, Cell 2019

Integration with Seurat

Principle

- Integration is always **pairwise:** correct a sample, **the query** to match the expression data of another sample, **the reference**.



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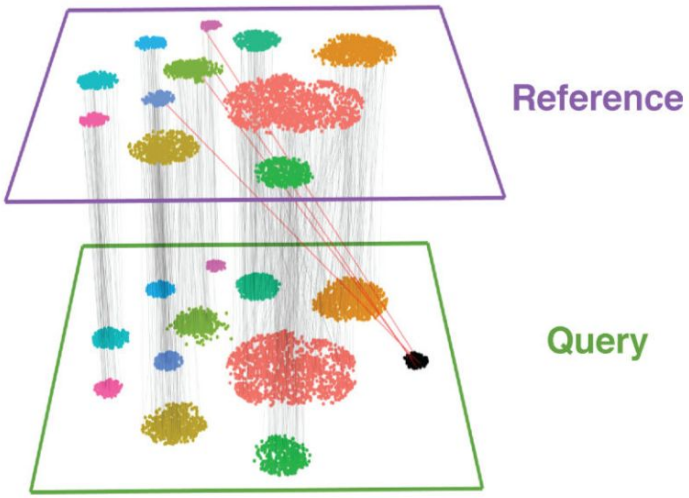
- Seurat identifies pairs of close cells across both datasets, the **anchors** (Mutual Nearest Neighbors).



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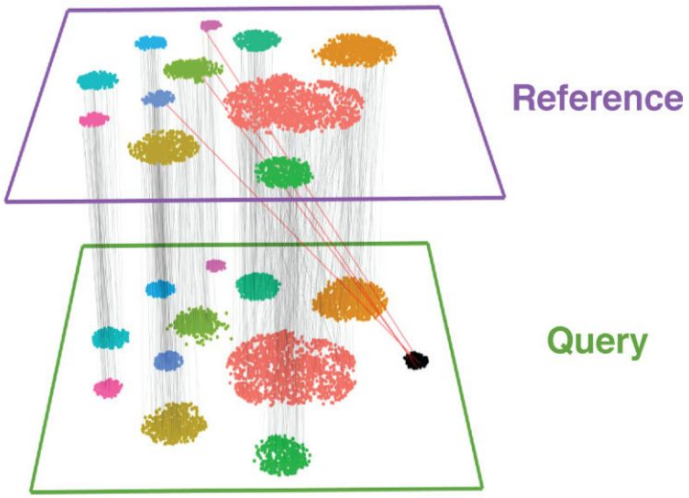
- The difference between them is used to compute a **correction**.



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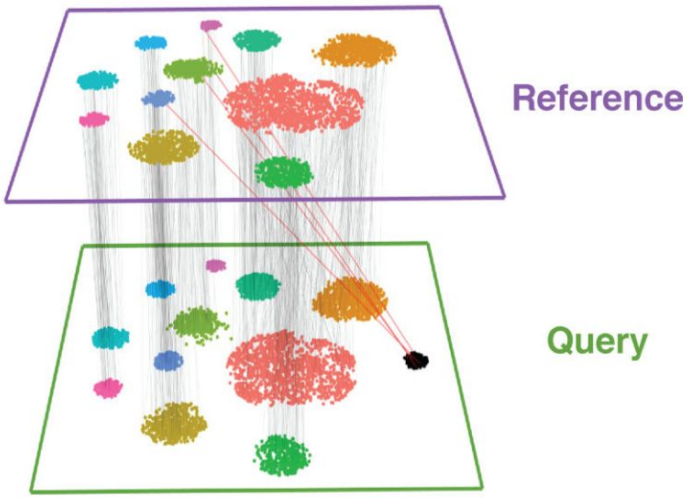
- Seurat identifies pairs of close cells across both datasets, the **anchors** (Mutual Nearest Neighbors).
- The difference between them is used to compute a **correction**.
- The correction is used to **align** all the **query** cells on the **reference** cells.



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Integration with Seurat

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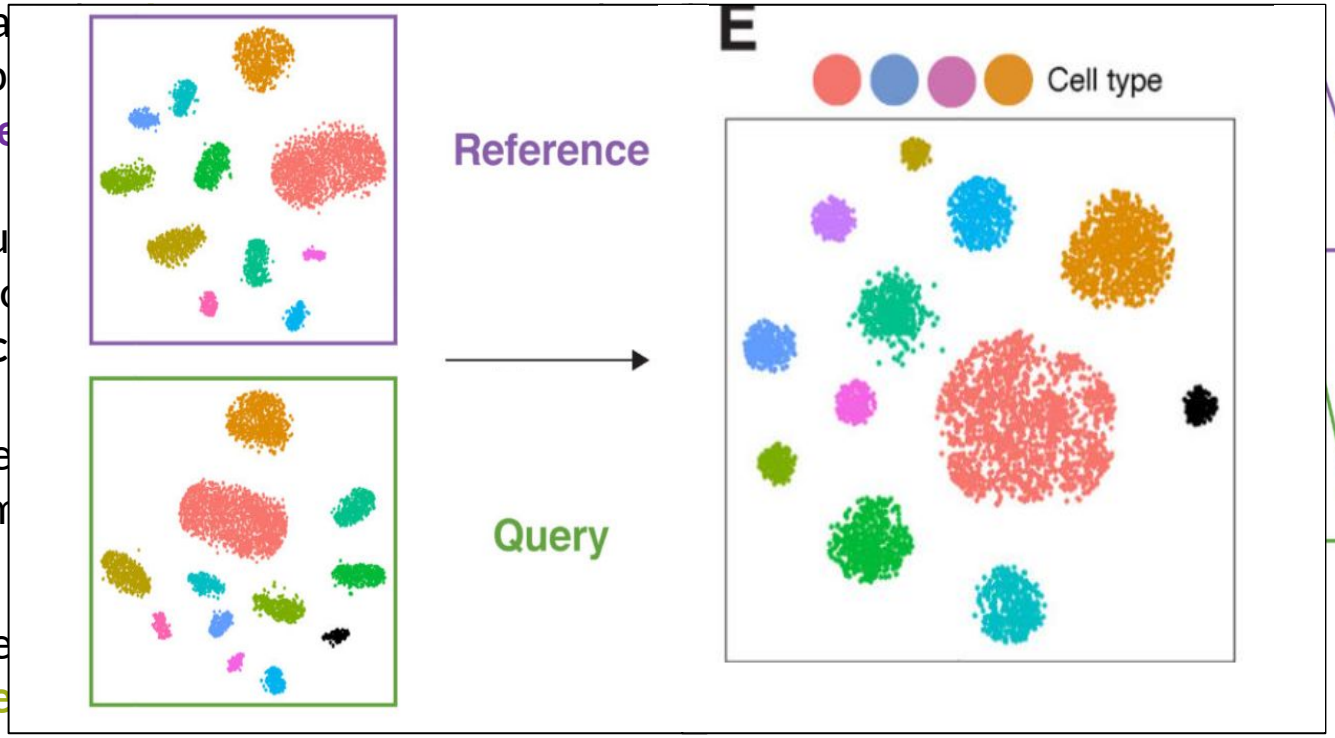

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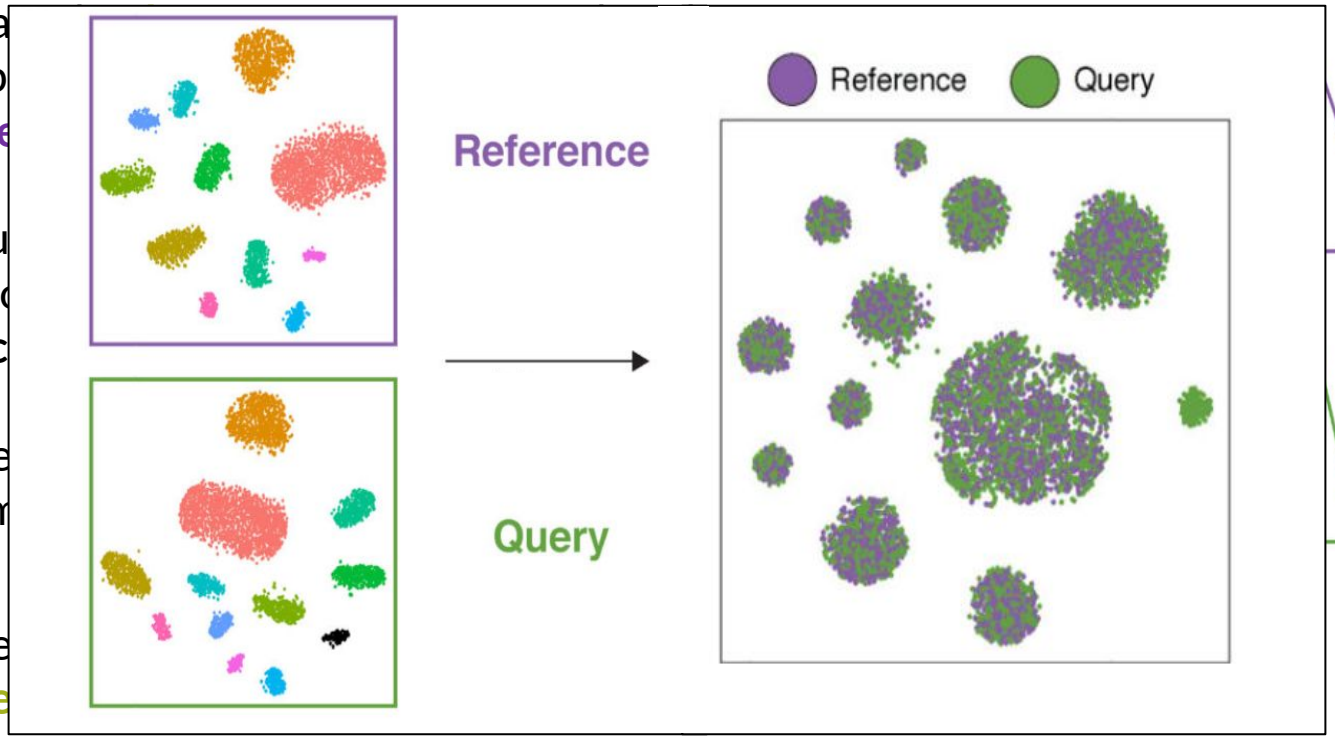
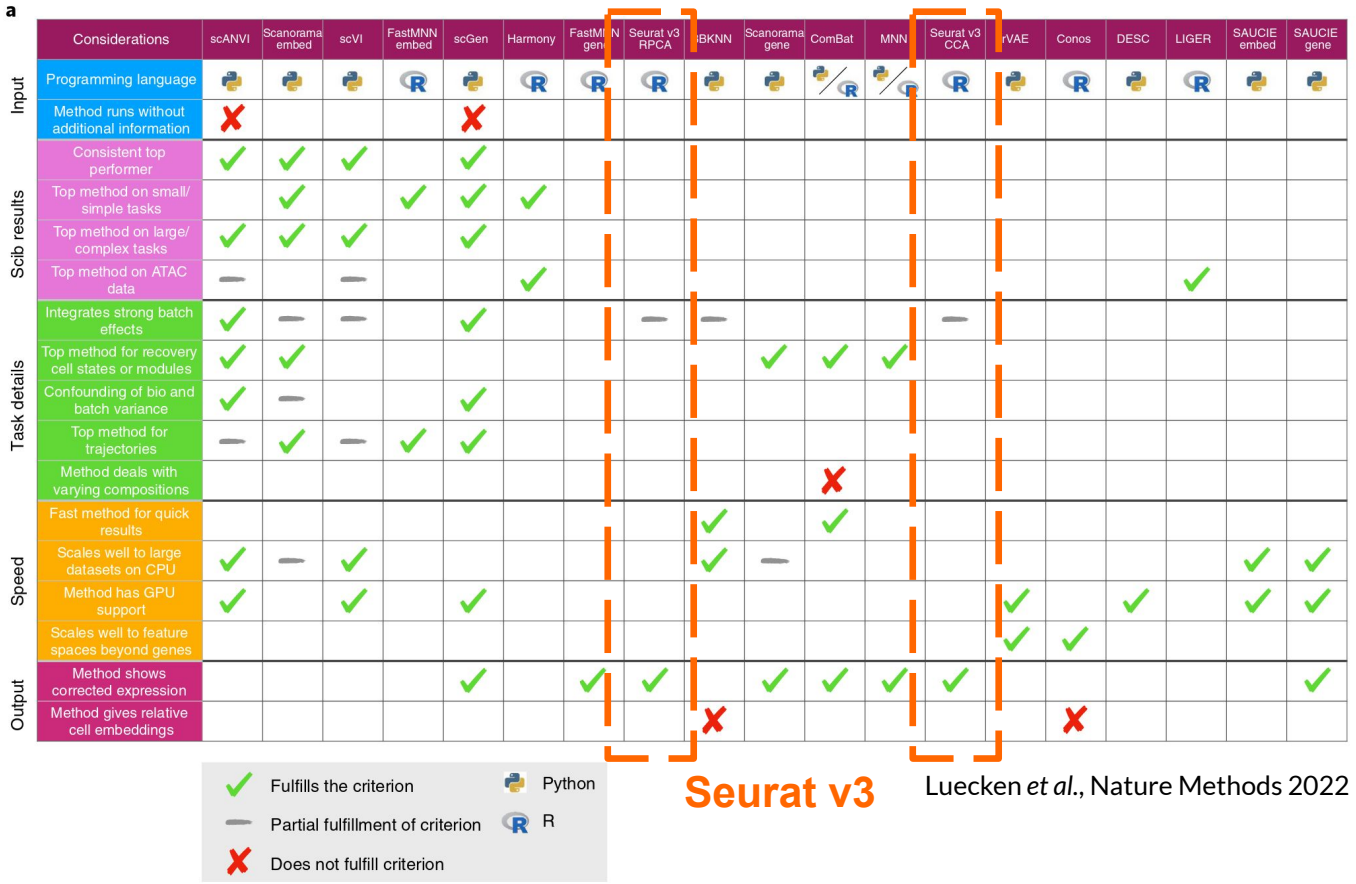


Stuart *et al.*, Cell 2019

Benchmarking methods

Benchmarking methods



Luecken *et al.*, Nature Methods 2022

A few benchmarks, that do not agree with each other

Büttner *et al.*, Nat. Methods. 2019
Chen *et al.*, Nat. Biotechnol 2020
Tran *et al.*, Genome Biol. 2020

Benchmarking methods

Do not hesitate to test several methods

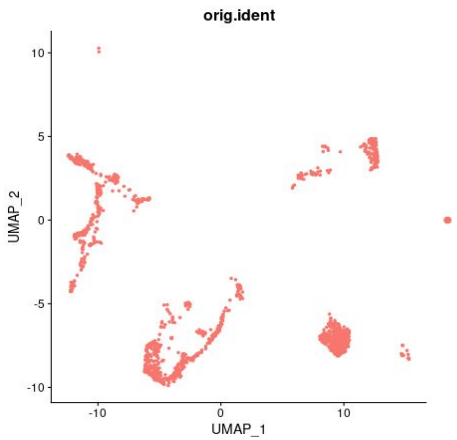
Luecken *et al.*, Nature Methods 2022

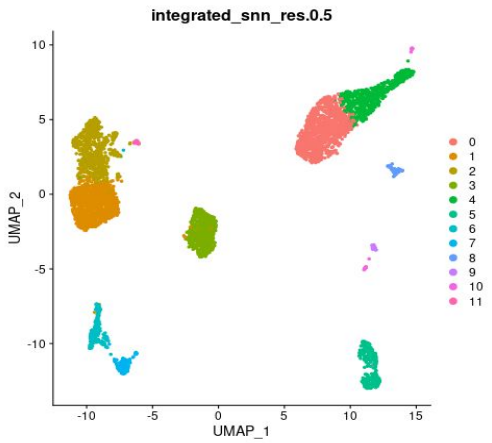
What is integration for

What is integration for

- For computational efficiency, integration is only performed on the most variable genes, not all the genes.

- It is intended for **visualization** and **clustering**



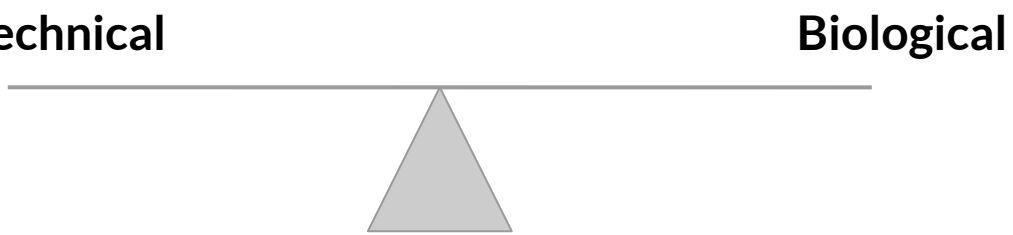


- For differential expression analysis, we go back to raw data

Stuart *et al.*, Cell 2019

Conclusion

A good integration method

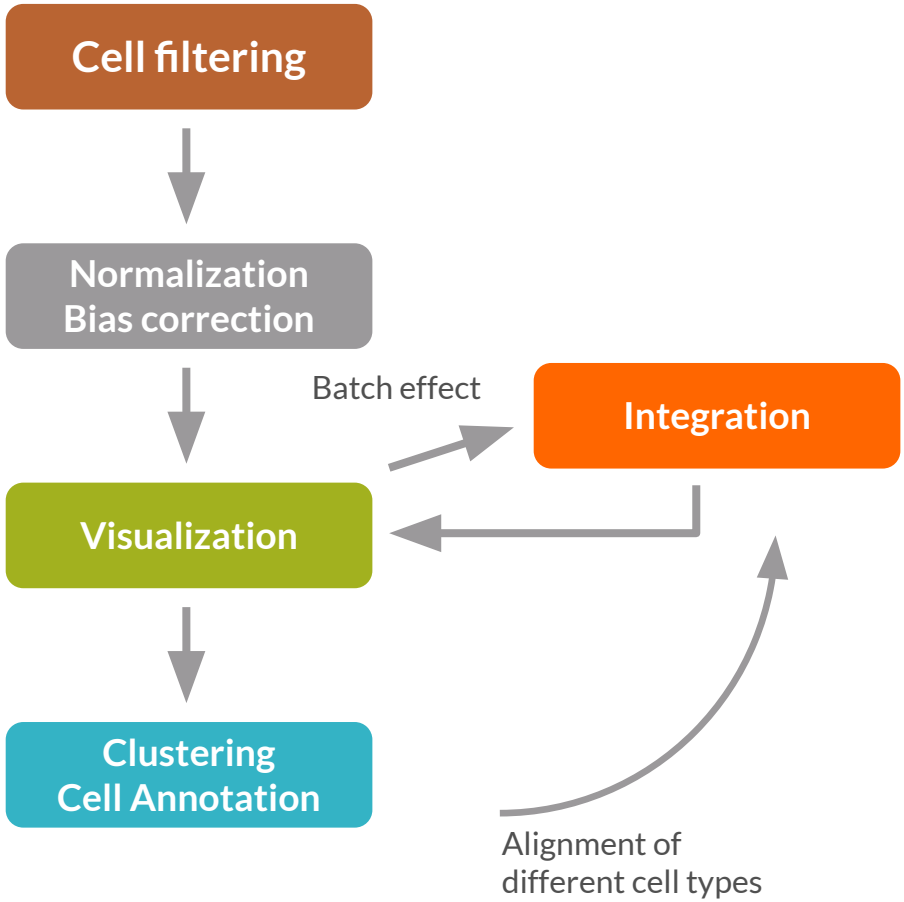


- Corrects for technical variability:
  - samples
  - donors
  - experimenter
  - technologies

- Preserves biological signal
  - cell types across different samples, tissues
  - cell trajectories
  - differences (cell subtypes, cell states) between condition and control
  - population (cell subtypes, cell states) unique to a condition...

Conclusion

Preparation of the data is not always a linear process

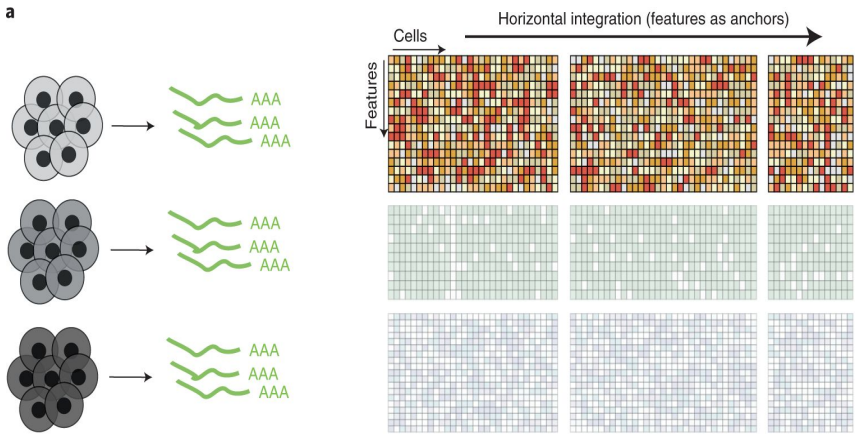


Conclusion

Different types of integrations

- Horizontal: different samples same modality

We saw horizontal integration



Luecken *et al.*, Nat Met 2021

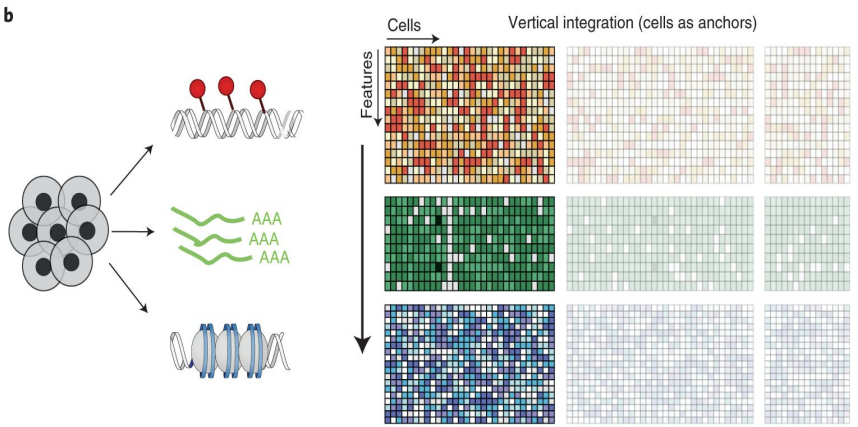
Conclusion

Different types of integrations

- Horizontal: different samples
  same modality

We saw horizontal integration

- Vertical: same sample
  different modalities
  (multiomics)



Luecken *et al.*, Nat Met 2021

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Different types of integrations

- Horizontal: different samples
  same modality

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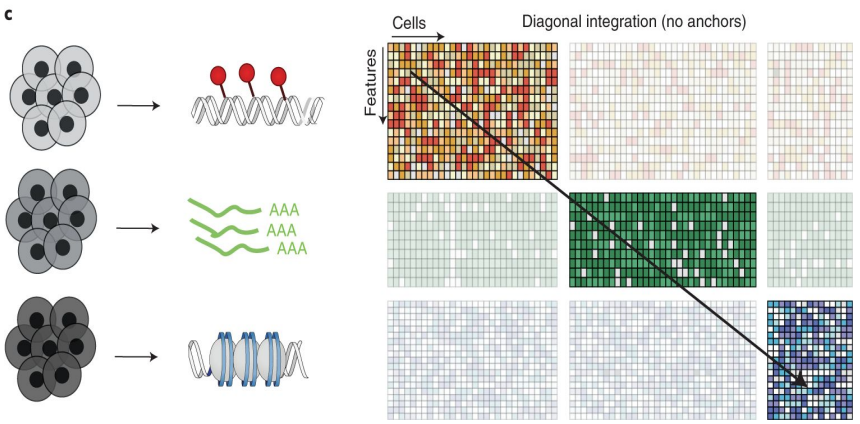
- Vertical: same sample
  different modalities
  (multiomics)

- Diagonal: different samples
  different modalities



Luecken *et al.*, Nat Met 2021

Acknowledgements

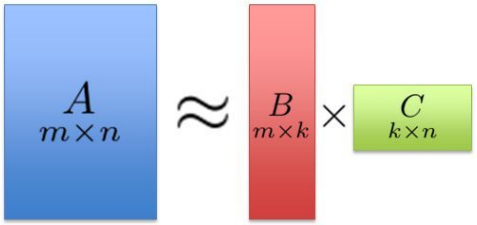
Parts of this course are inspired by

The *Swiss Institute of Bioinformatics* course Single Cell Transcriptomics

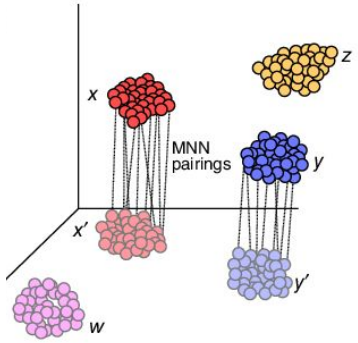
Integration with Seurat

Many methods

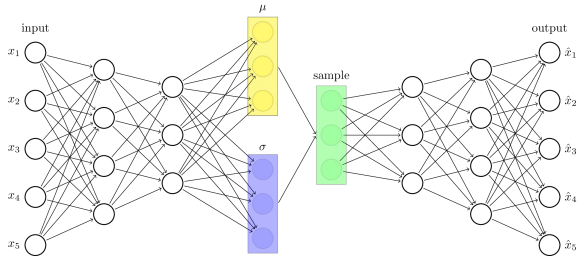
1. Linear decomposition methods



2. similarity-based
(in reduced dimension space)



3. Deep learning



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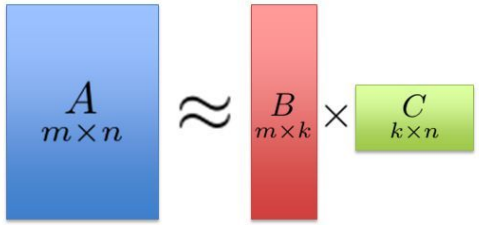
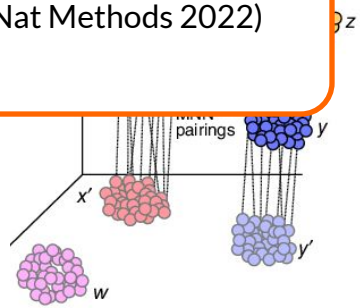
Many methods

1. Linear decomposition methods

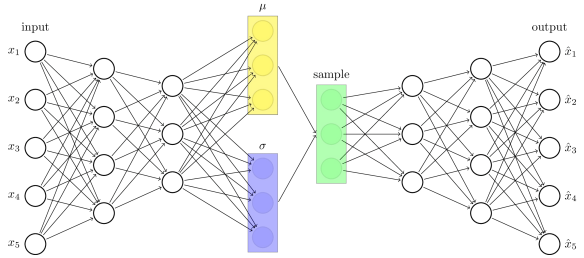


Over 49 methods (Luecken et al., Nat Methods 2022)

2. similarity-based
(in reduced dimension space)



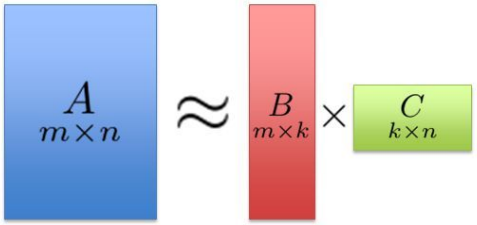
3. Deep learning



Integration with Seurat

Many methods

1. Linear decomposition methods



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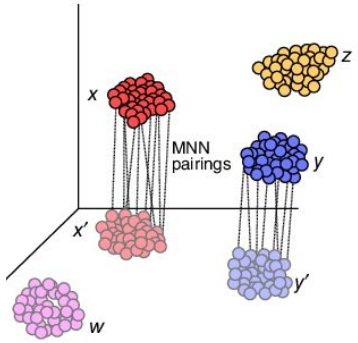
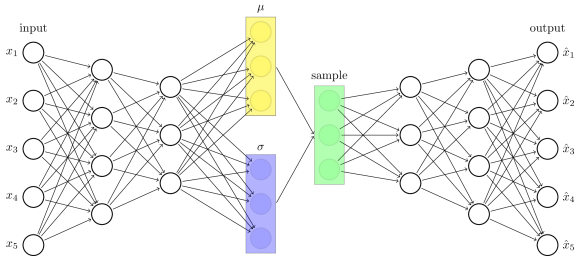


Seurat

3. Deep learning

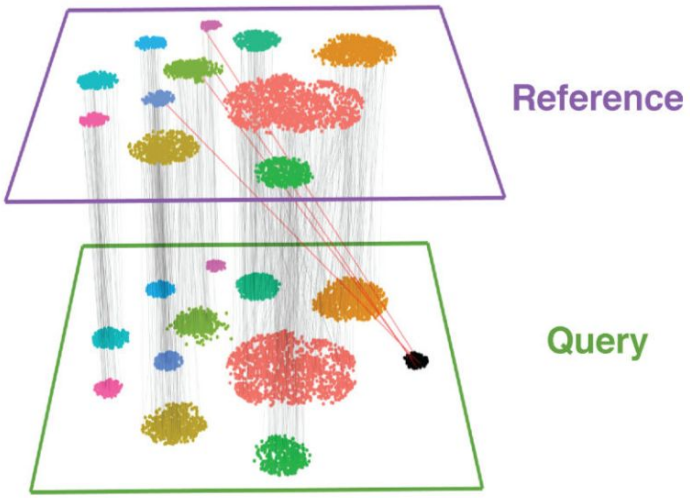


Integration with Seurat

Dimension reduction

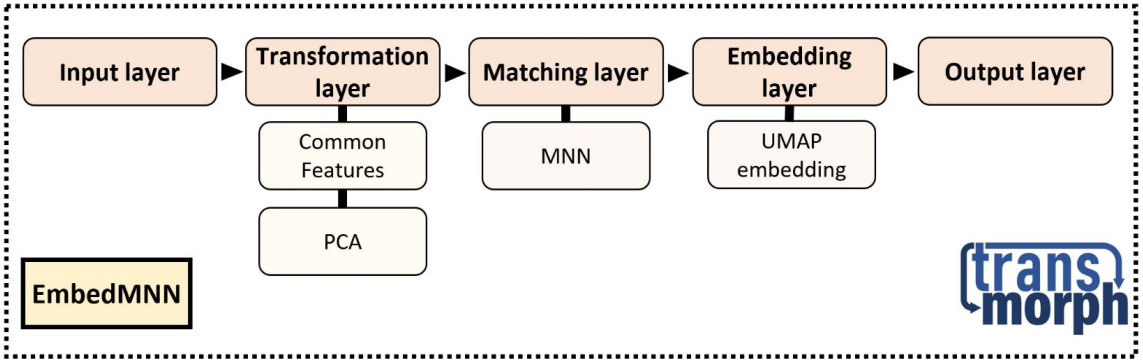
- Integration is performed in low dimension space.
- The reduction method is an important parameter

	focus on	when	limits
CCA	Finding highly variable genes between samples	Dataset with many differences	Can overcorrect biological signal
RPCA	Telling signal and noise appart from each other	Less different dataset, huge datasets	Can fail to align populations perfectly
LSI	Identify latent structure of texts (here DNA sequences)	scATAC-Seq	



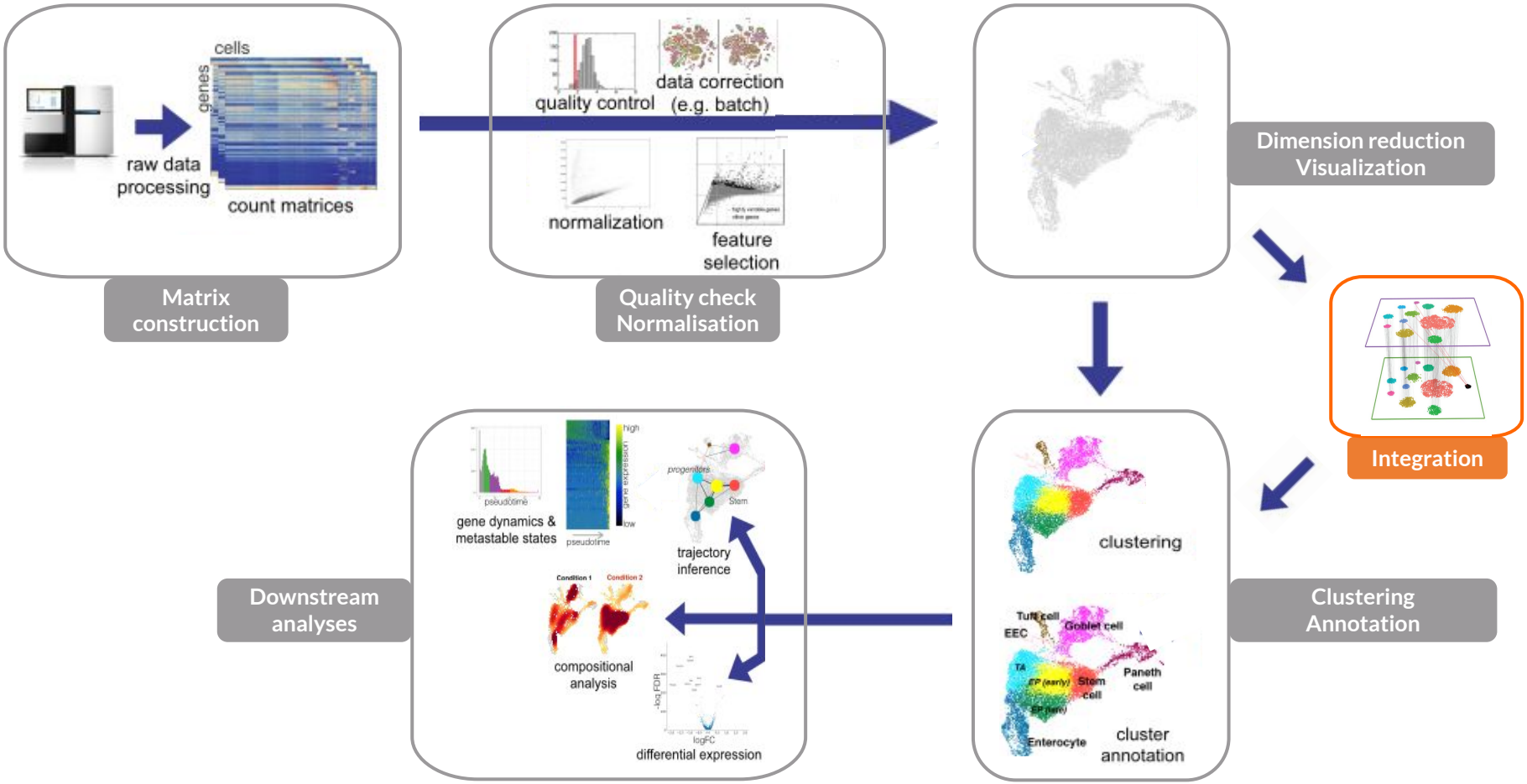
Stuart *et al.*, Cell 2019

Benchmarking methods



- Developped by A. Fouché, L. Chadoutaud, A. Zinovyev in U900
- Framework breaking down integration algorithms into building blocks
- Allow to combine the building blocks into personalized integration workflow
- Databank for benchmarking

Introduction



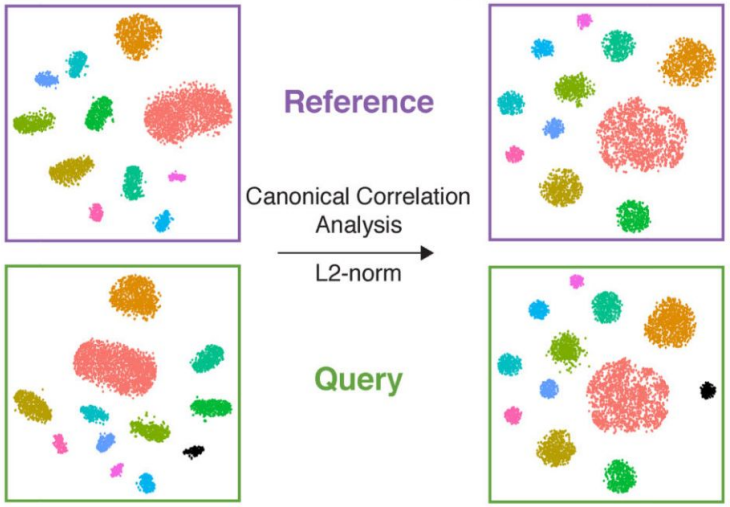
Integration with Seurat

Algorithm

1) Dimension reduction

- Like PCA or UMAP, it projects the cells into a lower space

- The dimension reduction methods used here roughly align similar cells.



Stuart *et al.*, Cell 2019

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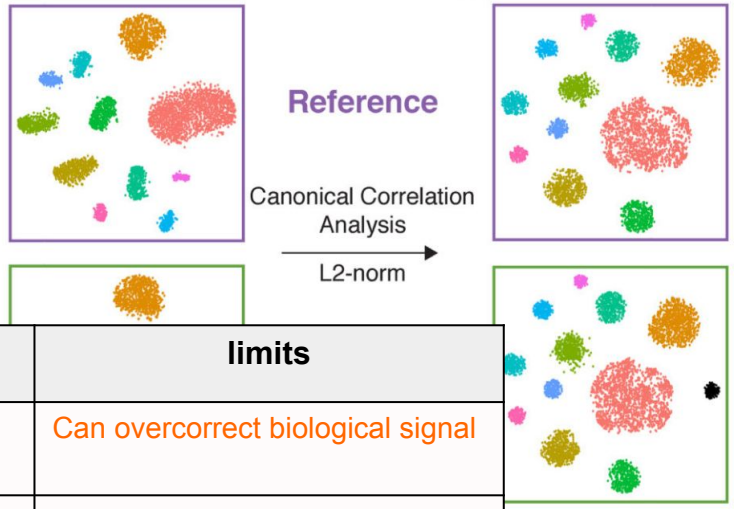

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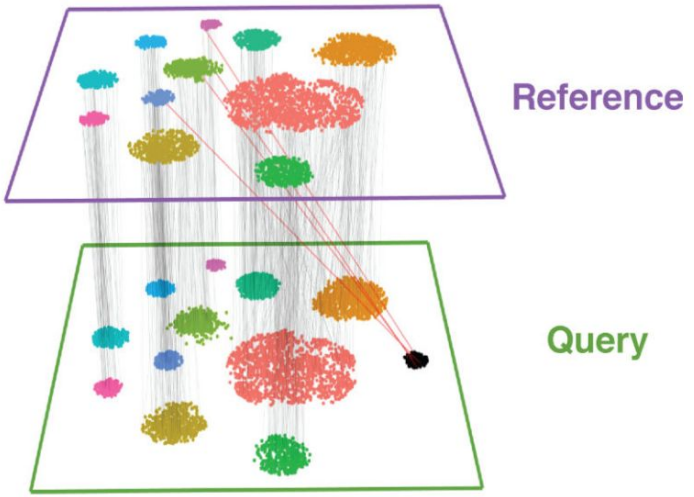
Integration with Seurat

Algorithm

1) Dimension reduction → 2) Identify anchors (MNN)

- MNN: Mutual Nearest Neighbors
- In reference and query, identify 2 cells that are close (neighbors) in terms of euclidean distance: **anchors**
- Identify many anchors



Stuart *et al.*, Cell 2019

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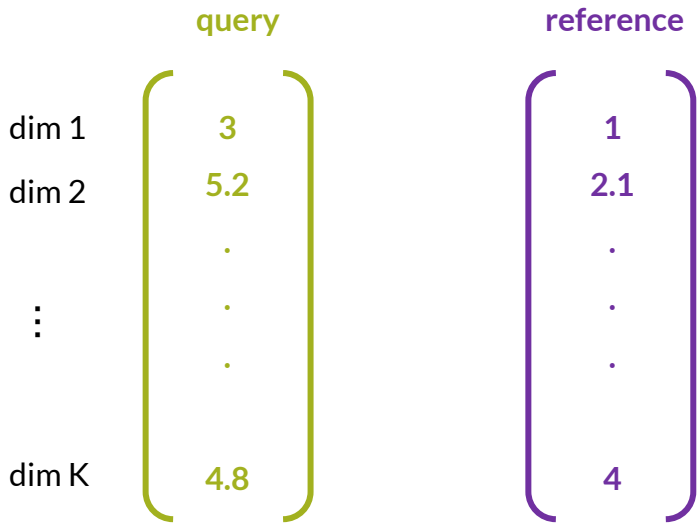
- Note: a cell is represented as a vector

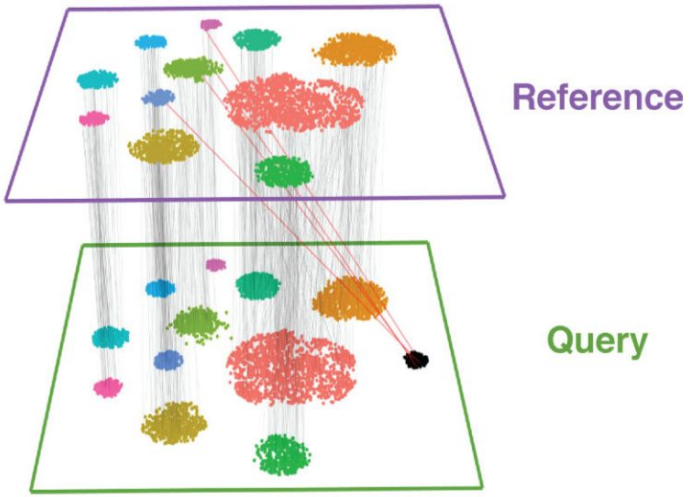


Stuart *et al.*, Cell 2019

Integration with Seurat

Algorithm

1) Dimension reduction → 2) Identify anchors (MNN) → 3) Filter and score anchors



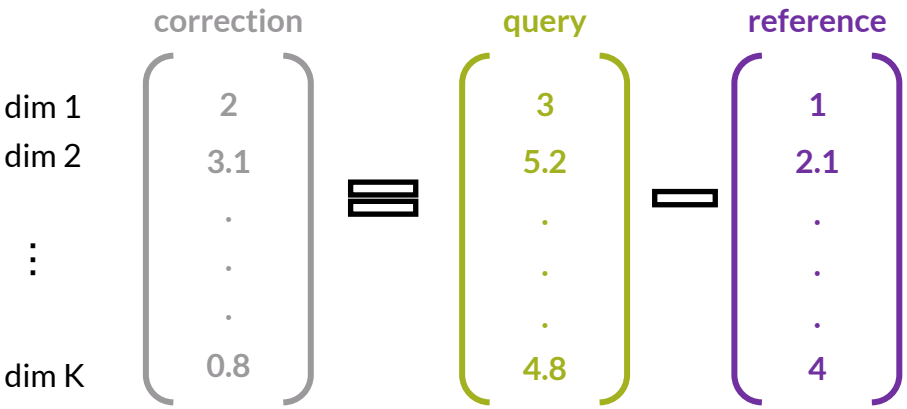
Stuart *et al.*, Cell 2019

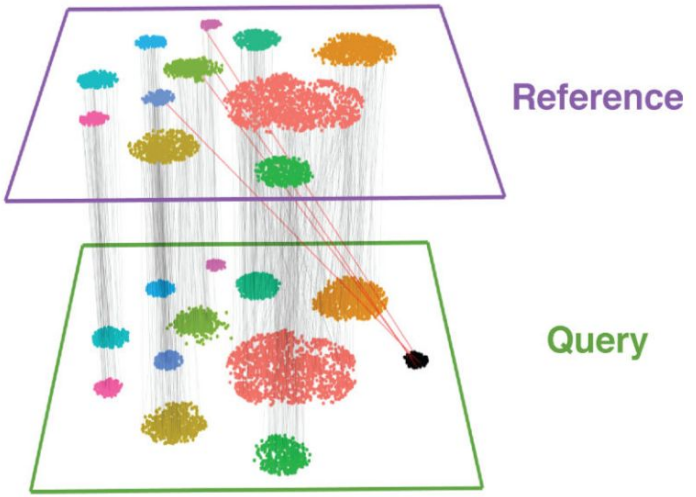
Integration with Seurat

Algorithm

1) Dimension reduction → 2) Identify anchors (MNN) → 3) Filter and score anchors → 4) Integrate

- Deduce correction from anchors





Stuart *et al.*, Cell 2019

Integration with Seurat

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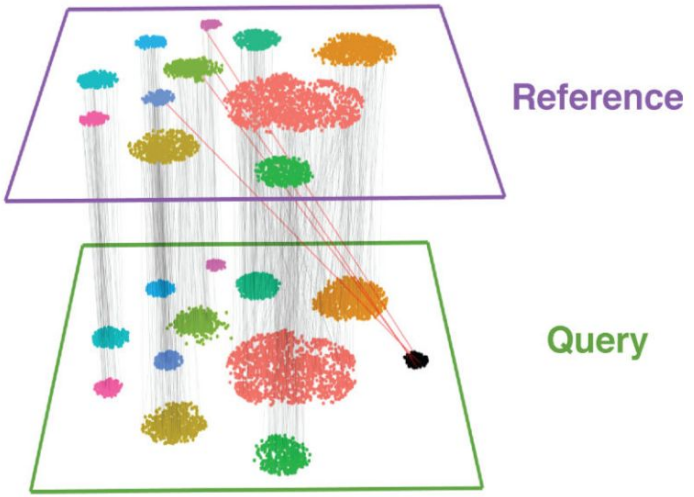
- Deduce correction from anchors
- Apply correction vector to all query cells.



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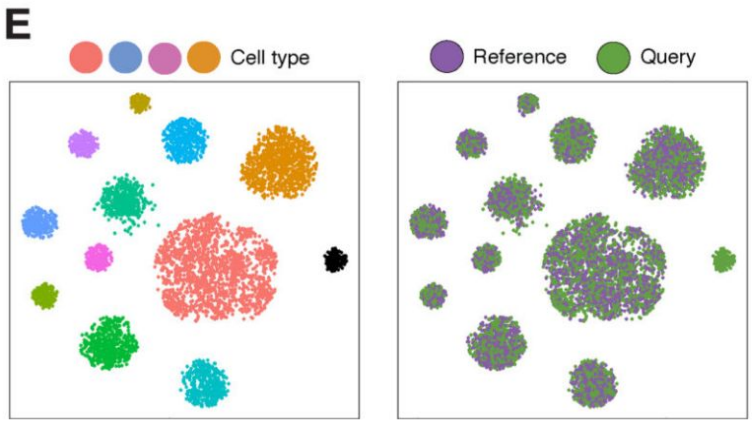

Stuart *et al.*, Cell 2019