Integrating Different Samples in Single-cell RNA-seq

The Data Lab

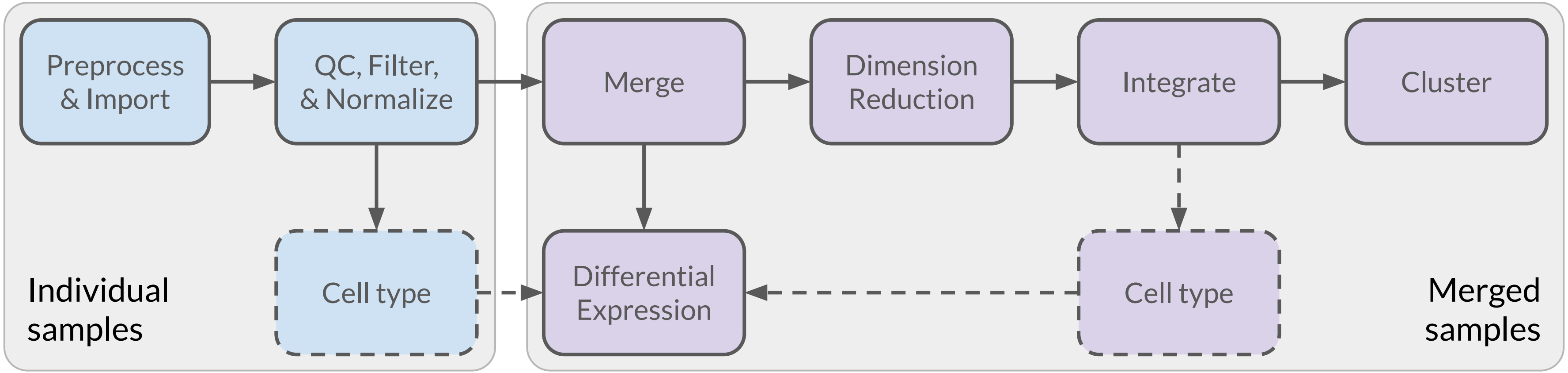
Working with multiple samples in scRNA-seq

Combine and harmonize samples for processing

Identify combined axes of variation across a set of samples

Correct for individual and batch effects among samples

Group cells across samples with similar (corrected) expression patterns



Preprocess & Import

QC, Filter, & Normalize

Cell type

Individual samples

Merge

Dimension Reduction

Integrate

Cluster

Differential Expression

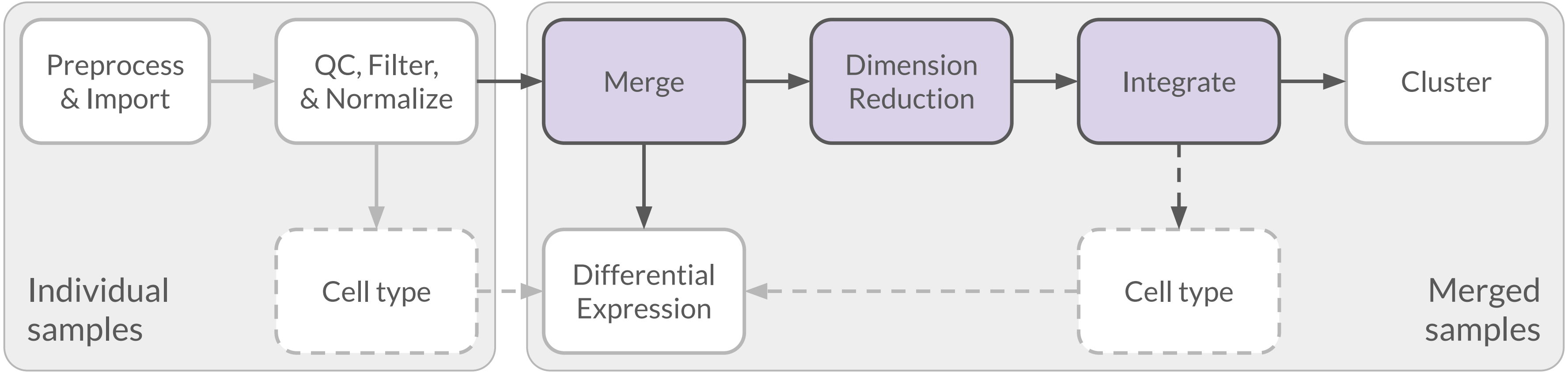
Cell type

Merged samples

Identify genes with expression differences among samples (within a cell type)

Infer cell labels across samples

Integration in scRNA-seq overview



Why integrate samples?

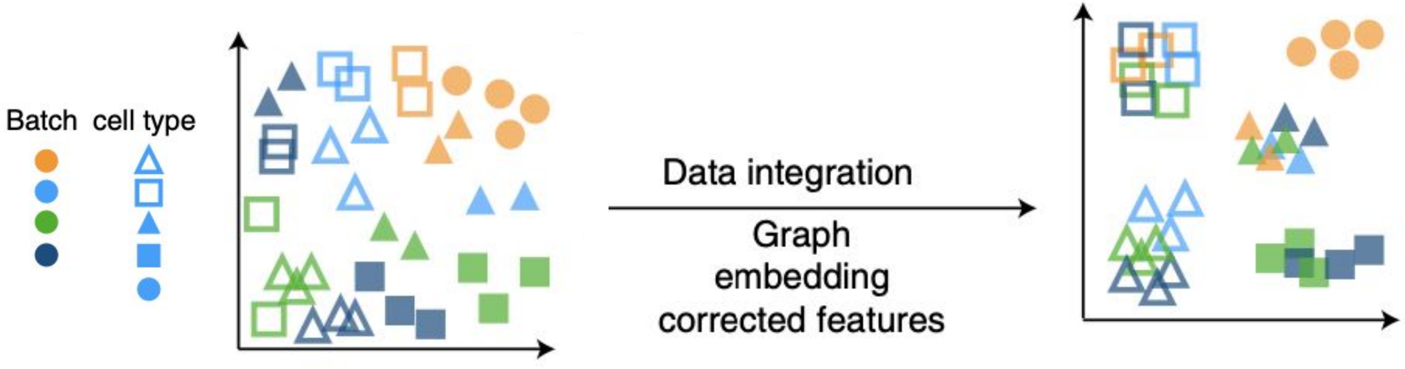
The goal of integration is to mitigate the *batch effects* caused by technical variation across samples, while still preserving biological information.

- Let's call each sample a "batch" of cells
- Cells in a given sample will share some technical variation
- This becomes a problem when we want to jointly consider several samples
  - Cells within a given sample appear more similar than they are, simply because they're from the same sample.
- To compare cells across samples, we need to remove this batch-level technical variation. Then, we can hopefully hone in on the more interesting biological variation 🕵️

What can('t) integration do for you?

- Integration is performed on reduced dimension representations (often principal components)
    - Integration also *returns* reduced dimension representations for downstream use
    - Some integration methods will "back-calculate" corrected gene expression values, but these aren't as important as you think!
    - For example, we do *not* use these for differential expression (stay tuned for more!)
    - Recommended reading on when to use, and not to use, corrected expression values: http://bioconductor.org/books/3.20/OSCA.multisample/using-corrected-values.html
- Integration allows us to...
    - Jointly visualize **cells** from multiple datasets
    - Jointly cluster **cells** from multiple datasets
    - Annotate or identify similar **cell types** across datasets

What does successful integration look like?



Before integration, the primary "clustering" is by batch

- Orange tends to group with orange, green with green, etc.

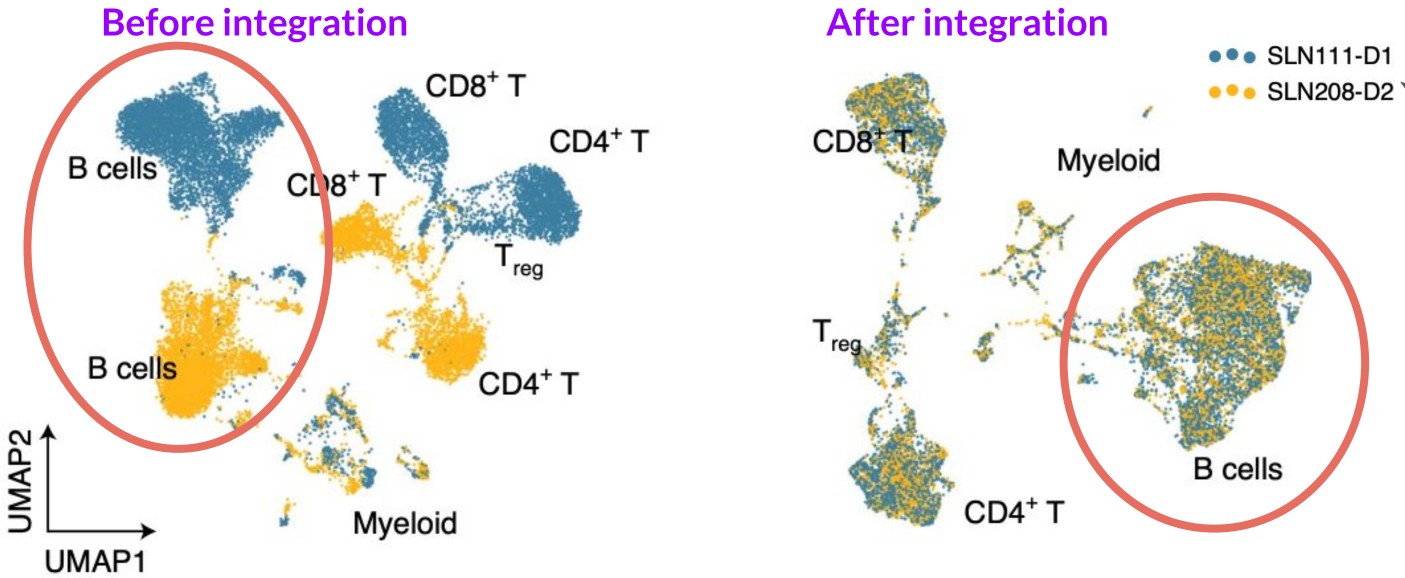
After successful integration:

- Batches show lots of mixing
- Cell types ("biology") cluster together, and do *not* show lots of mixing

Successful integration depends on *shared information* across batches.

Figure adapted from Lueken *et al*. (2022) https://doi.org/10.1038/s41592-021-01336-8

Example of (what looks like!) successful integration


Before integration

After integration

spleen/lymph node samples
from two different mice

Gayoso *et al.* (2021) https://doi.org/10.1038/s41592-020-01050-x

How to evaluate integration

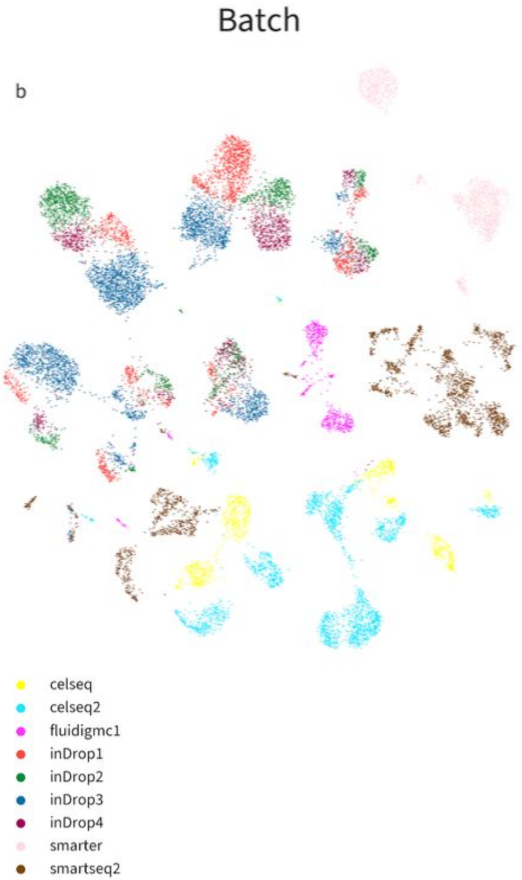
- Compare before and after UMAP vibes

  - Before integration, batches (datasets) will mostly cluster together
  - After integration...
    - Batches should not group together but should be highly mixed across the UMAP
    - Biologically similar cells (tissue, cell type, disease vs healthy) should group together
  - Usually, when it fails, *it fails.*

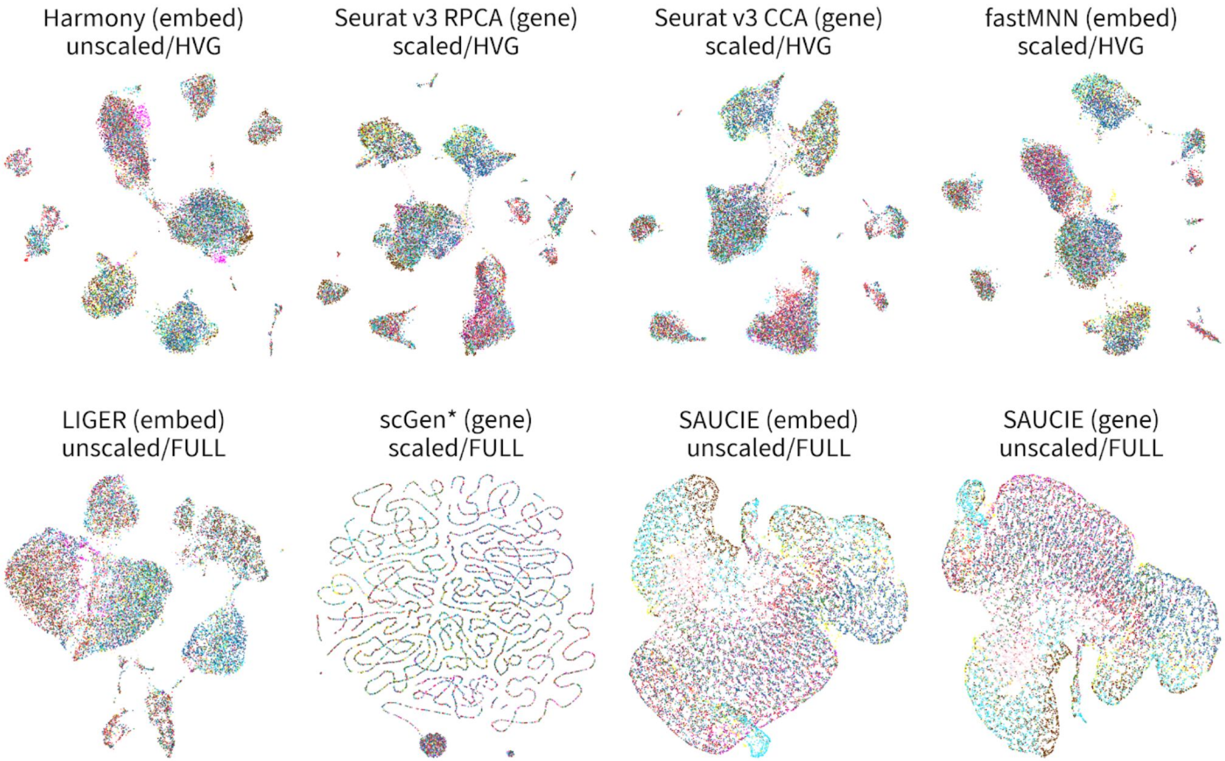
- There are several metrics for evaluating batch correction
  - Luecken *et al.* (2022) is an excellent reference https://doi.org/10.1038/s41592-019-0619-0
  - Caution: Metrics do not measure "was integration successful," but other proxies which sometimes can help us tell if integration was successful (or at least not unsuccessful)

How I stopped worrying and learned to love (the) UMAPs

- Some examples from Luecken et al. (2022)
    - Luecken, M.D., Büttner, M., Chaichoompu, K. et al. Benchmarking atlas-level data integration in single-cell genomics. (2022). https://doi.org/10.1038/s41592-021-01336-8
    - Panels from Figure S13 are shown on the next two slides
        - https://static-content.springer.com/esm/art%3A10.1038%2Fs41592-021-01336-8/MediaObjects/41592_2021_1336_MOESM1_ESM.pdf

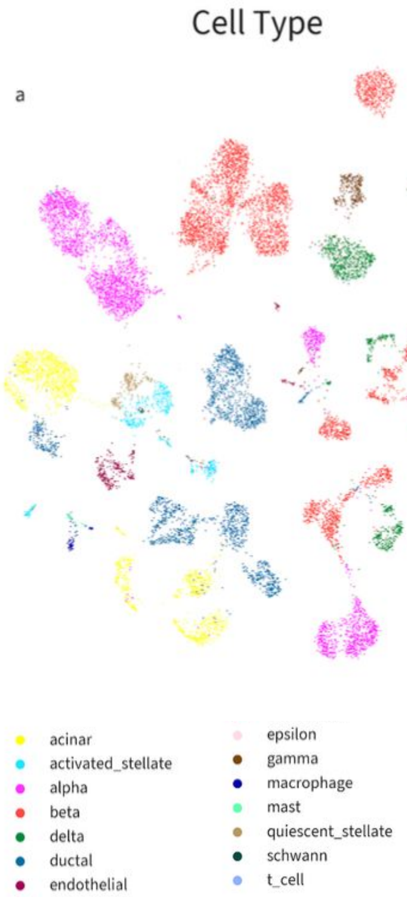


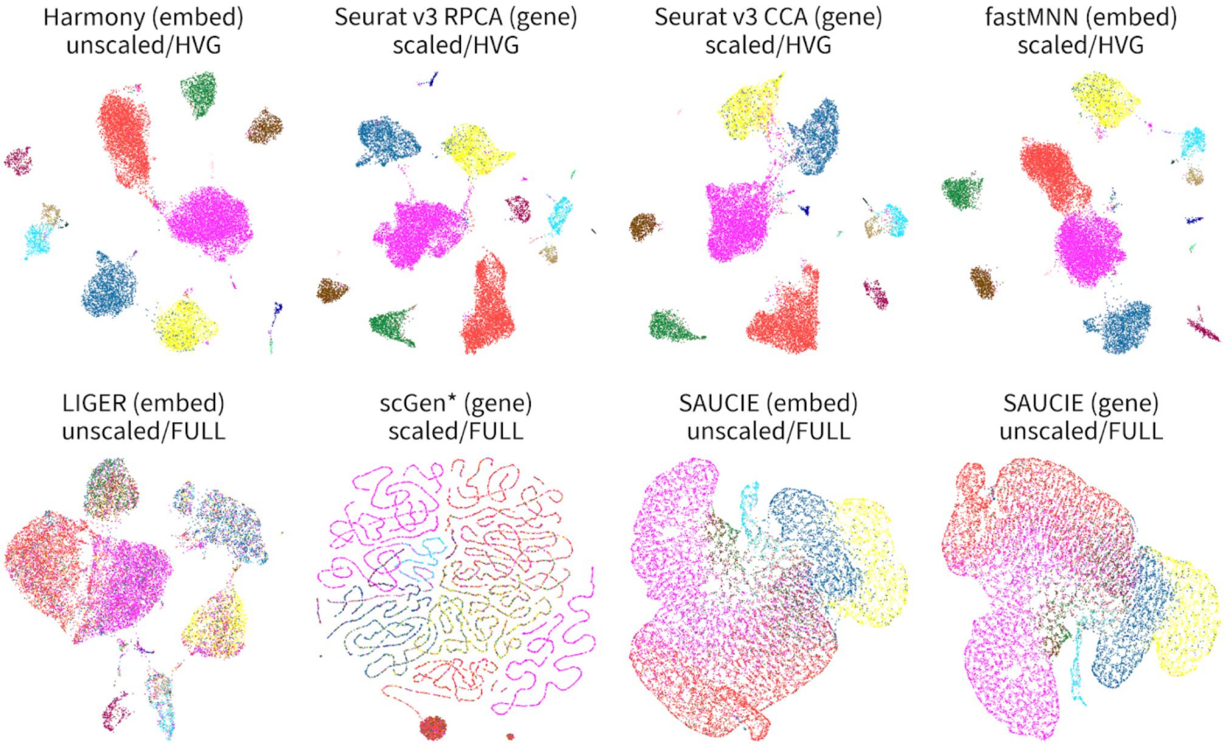
Top 4 "best" integration methods



Bottom 4 "worst" integration methods

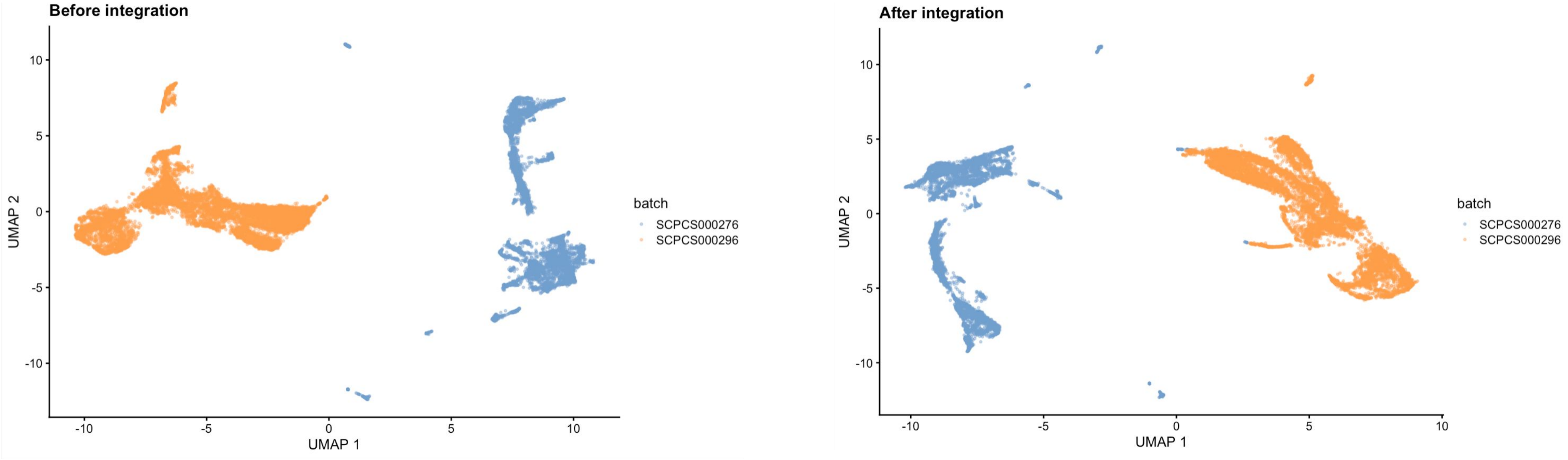
Top 4 "best" integration methods





Bottom 4 "worst" integration methods

An example of failed integration

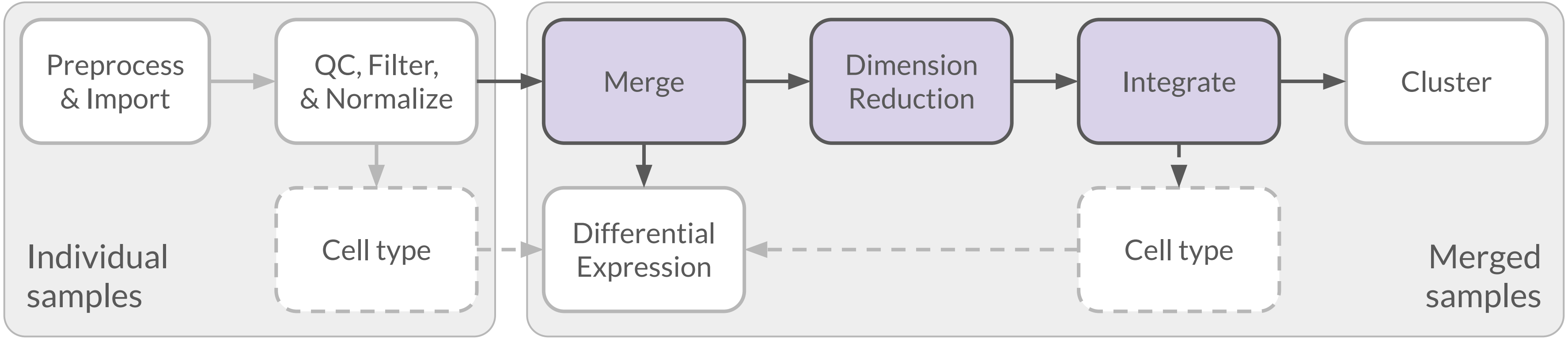


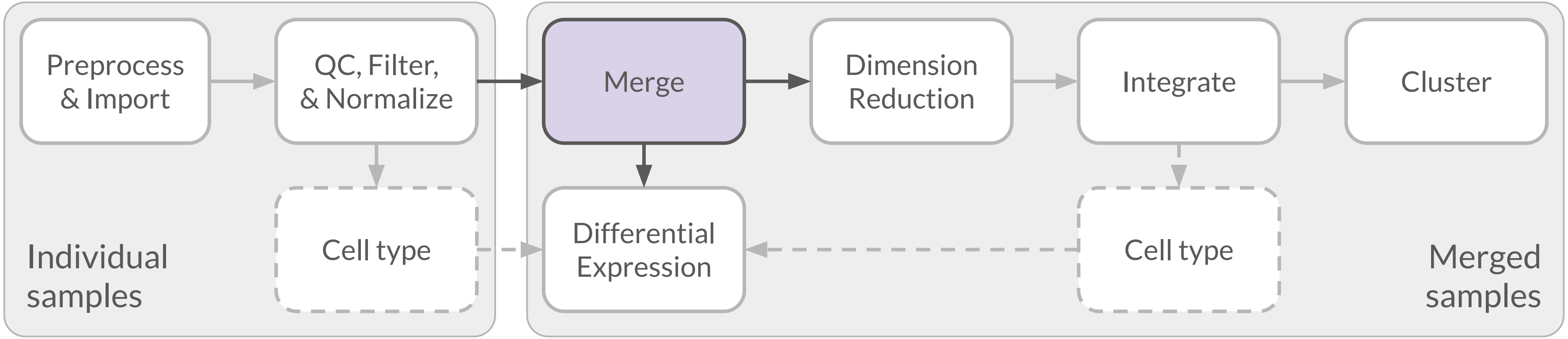
Will it integrate?

- Datasets that don't have shared cell types or states will be hard to integrate
  - Patient and xenograft
  - Healthy and normal
  - Data from different tissue types
  - Data from different organisms

- The extent of "overlap" among datasets may also influence which integration method you should use, along with the results themselves

Integration in scRNA-seq overview





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Differential Expression

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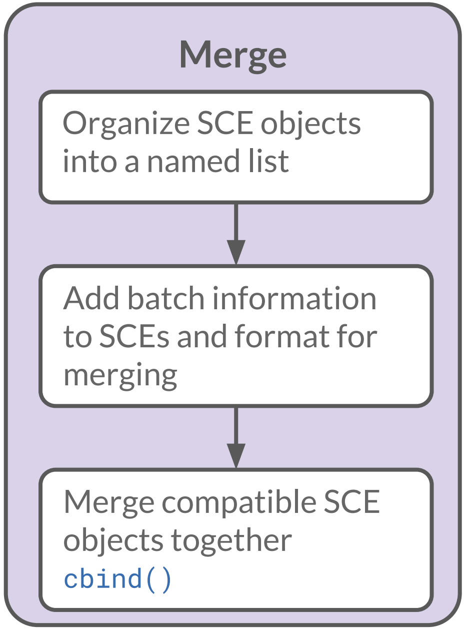
Merged samples



Merge

Organize SCE objects into a named list

Add batch information to SCEs and format for merging

Merge compatible SCE objects together `cbind()`

- It's useful to *merge SCEs together* for many downstream analyses, but this merging requires some bookkeeping:
  - After merging, how can we still tell which batch (sample) each cell came from?
    - We need to add this information into SCEs
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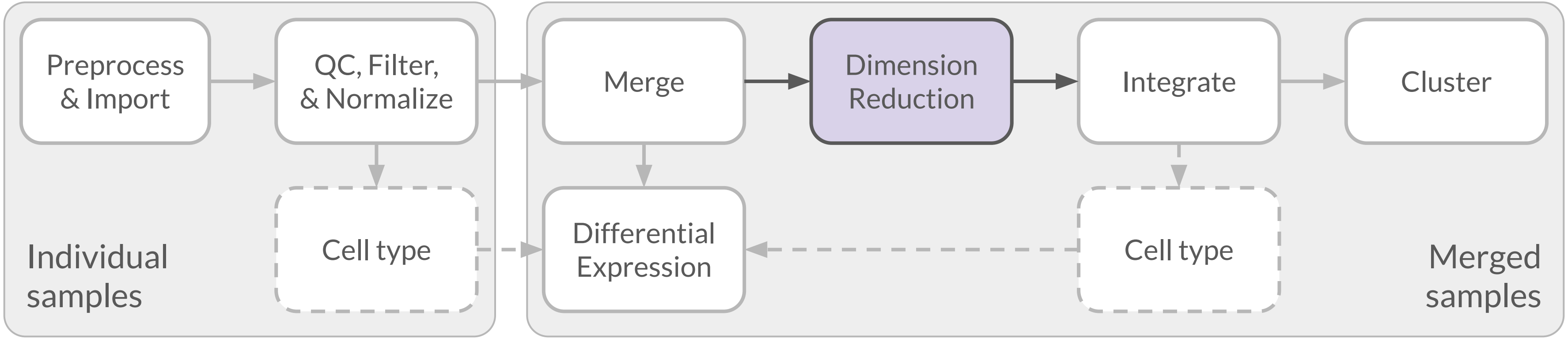

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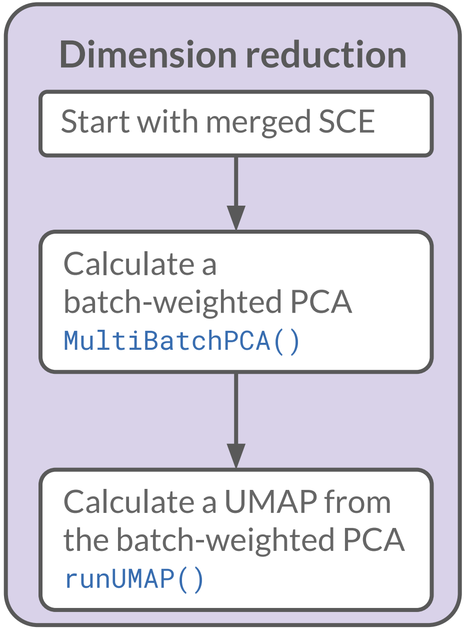


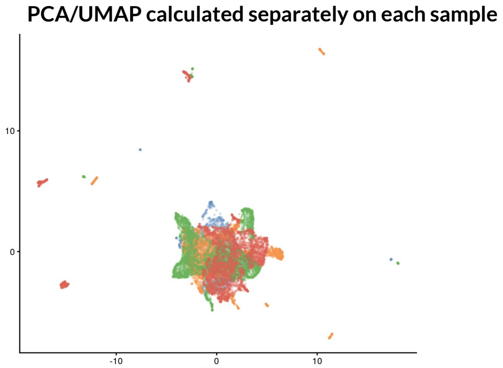
Dimension reduction

Start with merged SCE

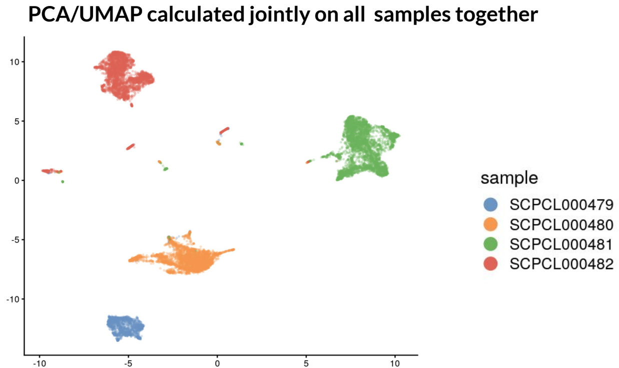
Calculate a batch-weighted PCA `MultiBatchPCA()`

Calculate a UMAP from the batch-weighted PCA `runUMAP()`

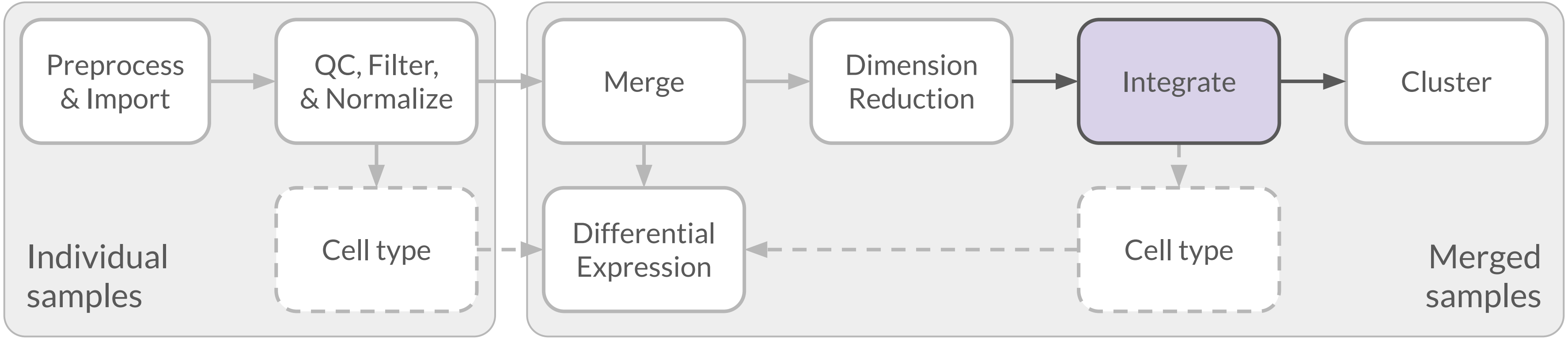
- Dimension reduction techniques like PCA and UMAP start by scaling data to be centered at 0.
- To use PCA/UMAP across samples, we need to calculate the variation jointly



PCA/UMAP calculated separately on each sample



PCA/UMAP calculated jointly on all samples together



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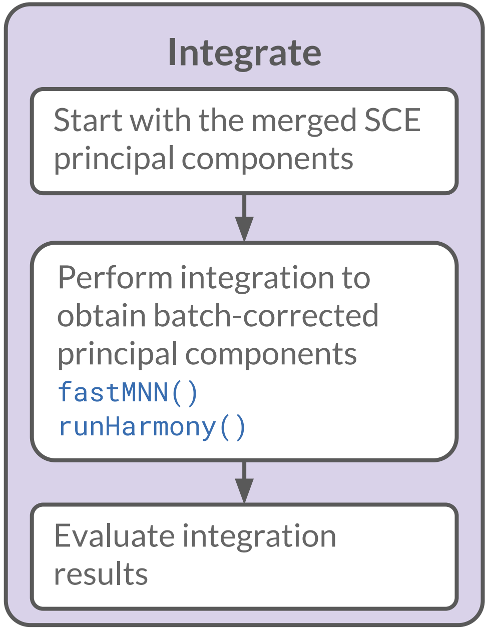


Integrate

Start with the merged SCE principal components

Perform integration to obtain batch-corrected principal components

```
fastMNN()
runHarmony()
```

Evaluate integration results

- We can evaluate results by…
  - Comparing before/after UMAPs
  - Calculating metrics that tell us how well cells and batches mix
- If integration is successful, we should see…
  - Batches are well-mixed across the UMAP
  - Cell types (or similar biological grouping, if known) group together separately
  - Remember: Success depends on overlap among batches

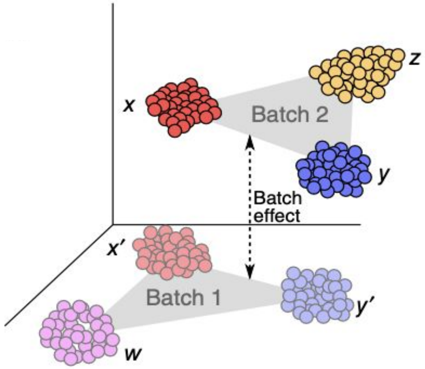
Let's have a closer look at methods we'll be using

- **MNN**: Mutual nearest neighbors
  - Specifically, we'll use FastMNN 🚀
  - Haghverdi, L, Lun, A, Morgan, M, *et al. Batch effects in single-cell RNA-sequencing data are corrected by matching mutual nearest neighbors.* (2018) https://doi.org/10.1038/nbt.4091

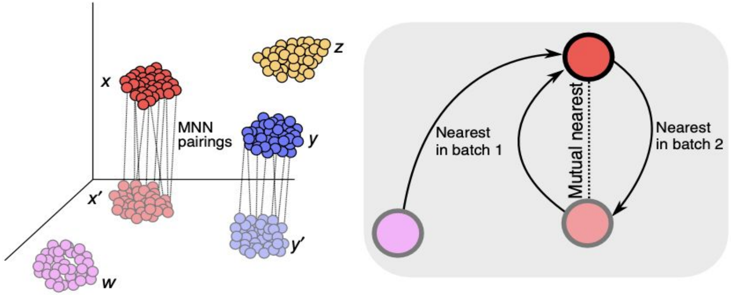
- **Harmony**
  - Korsunsky, I, Millard, N, Fan, J, *et al. Fast, sensitive and accurate integration of single-cell data with Harmony*. (2019) https://doi.org/10.1038/s41592-019-0619-0

Mutual nearest neighbors batch correction

- Imagine we have 2 batches, each with 3 cell types
  - Red (x) and blue (y) are shared but pink (w) and yellow (z) are not!
  - Before beginning integration, cosine distances are first calculated among pairs of cells *within each sample*
  - This enables expression profile comparisons and sets up the data for integration

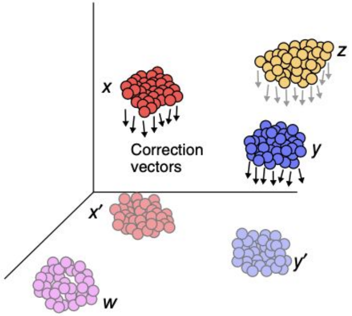


- First, we identify pairs of cells with mutually similar expression profiles
  - These are our "mutual nearest neighbors"

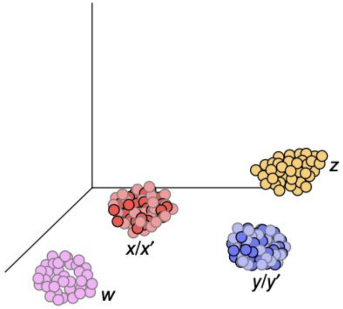


Mutual nearest neighbors batch correction

- Next, compute a batch correction vector for each MNN pair



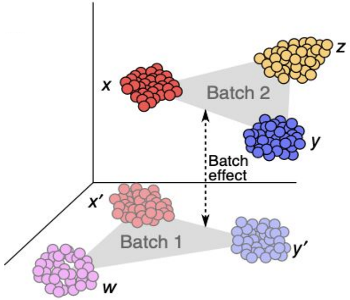
- Finally, calculate the weighted average of these vectors to get cell-specific batch corrections to perform the final integration
  - Note that **w** and **z** don't "look" as "integrated"! Why?



Some assumptions that MNN makes

- At least one cell population is present in both batches

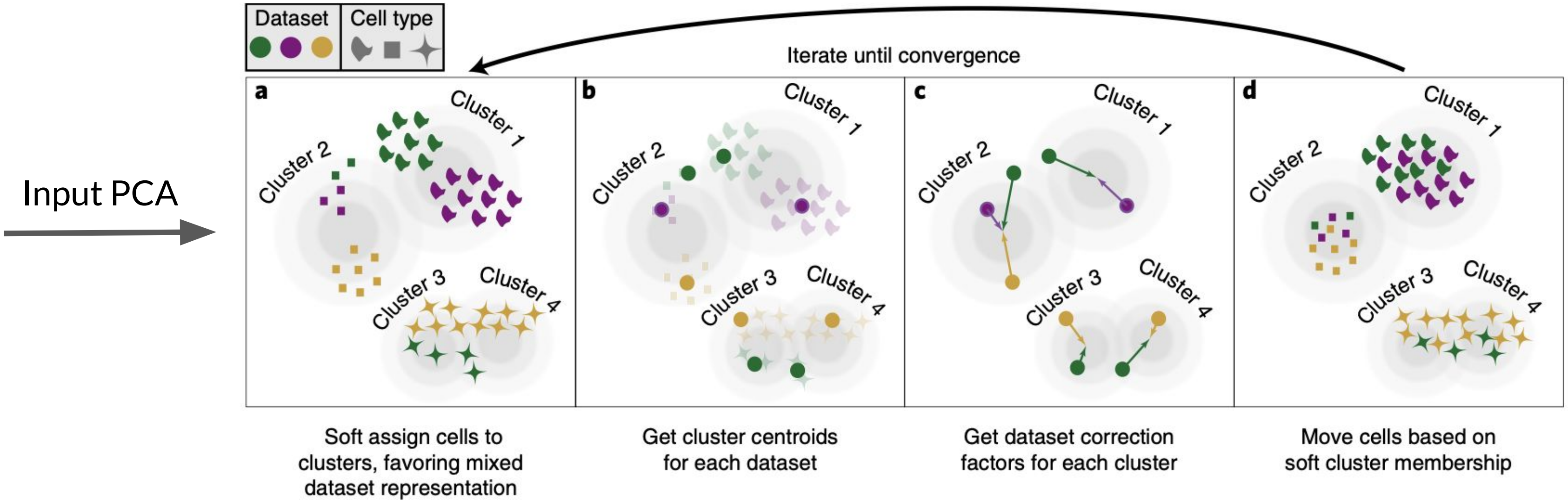
- The batch effect is almost orthogonal to the biological effects
    - Roughly means, batches and biology are expected to have *separate variation*



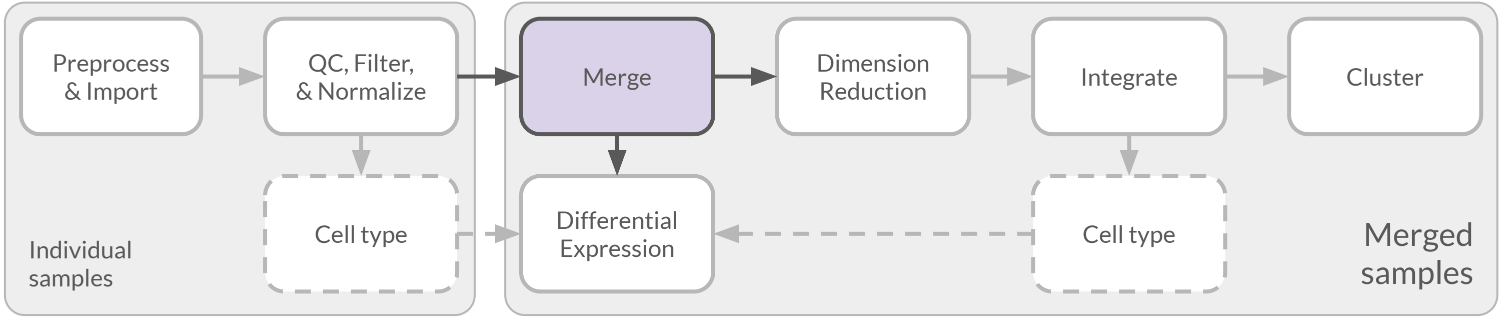
- The batch-effect variation is much smaller than the biological-effect variation across cell types

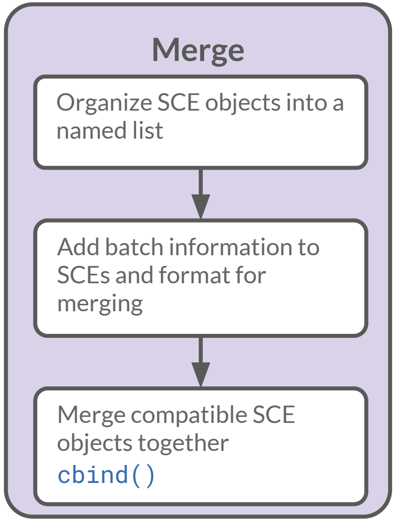
Harmony batch correction

- "Soft k-means clustering algorithm"


Input PCA
Dataset
Cell type
Iterate until convergence
a
b
c
d
Cluster 1
Cluster 2
Cluster 3
Cluster 4
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

Before we dive in, let's see how some of this sausage gets made





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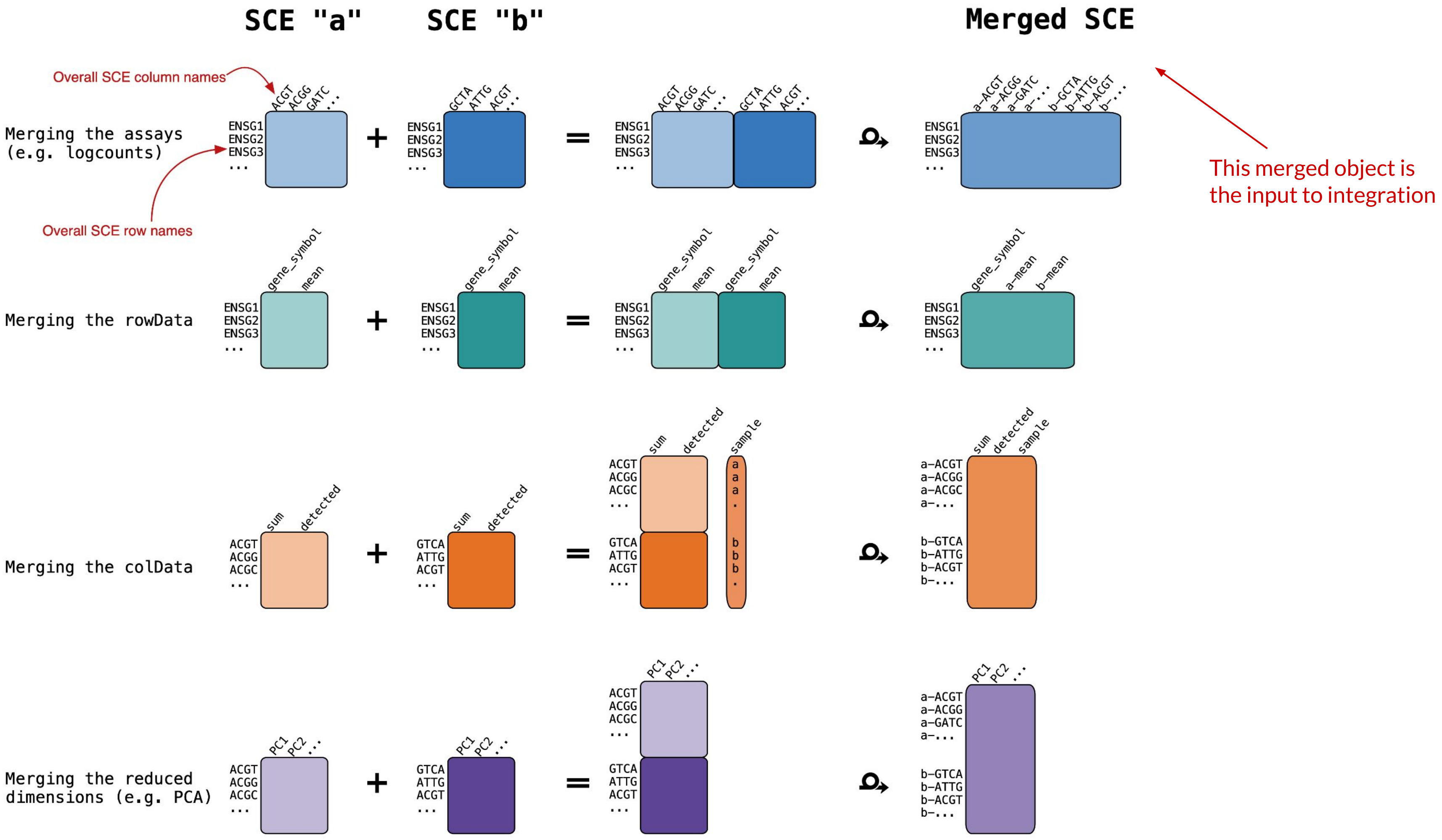


SCE "a"

SCE "b"

Merged SCE

Overall SCE column names

Overall SCE row names

This merged object is the input to integration

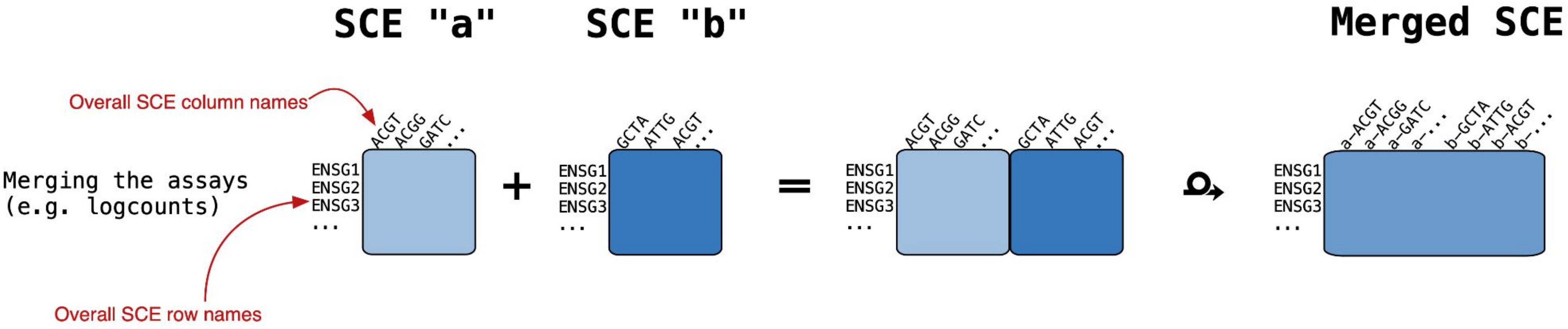
Merging the assays (e.g. logcounts)

Merging the rowData

Merging the colData

Merging the reduced dimensions (e.g. PCA)

Merging SCE assays

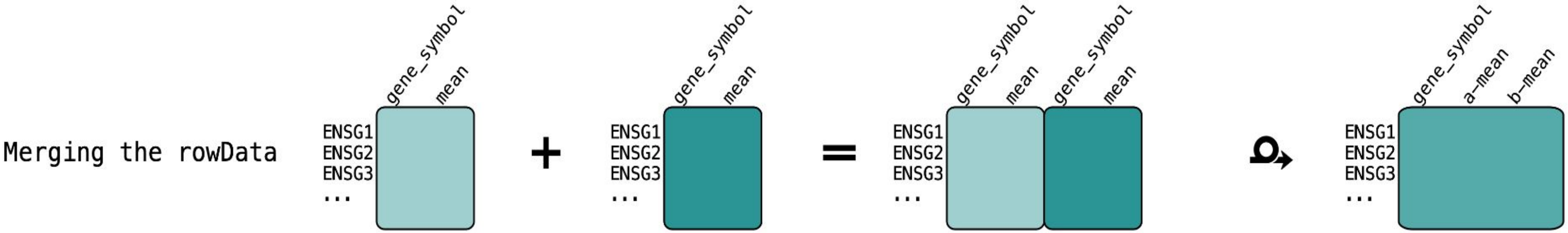


Merging the assays (e.g. logcounts)

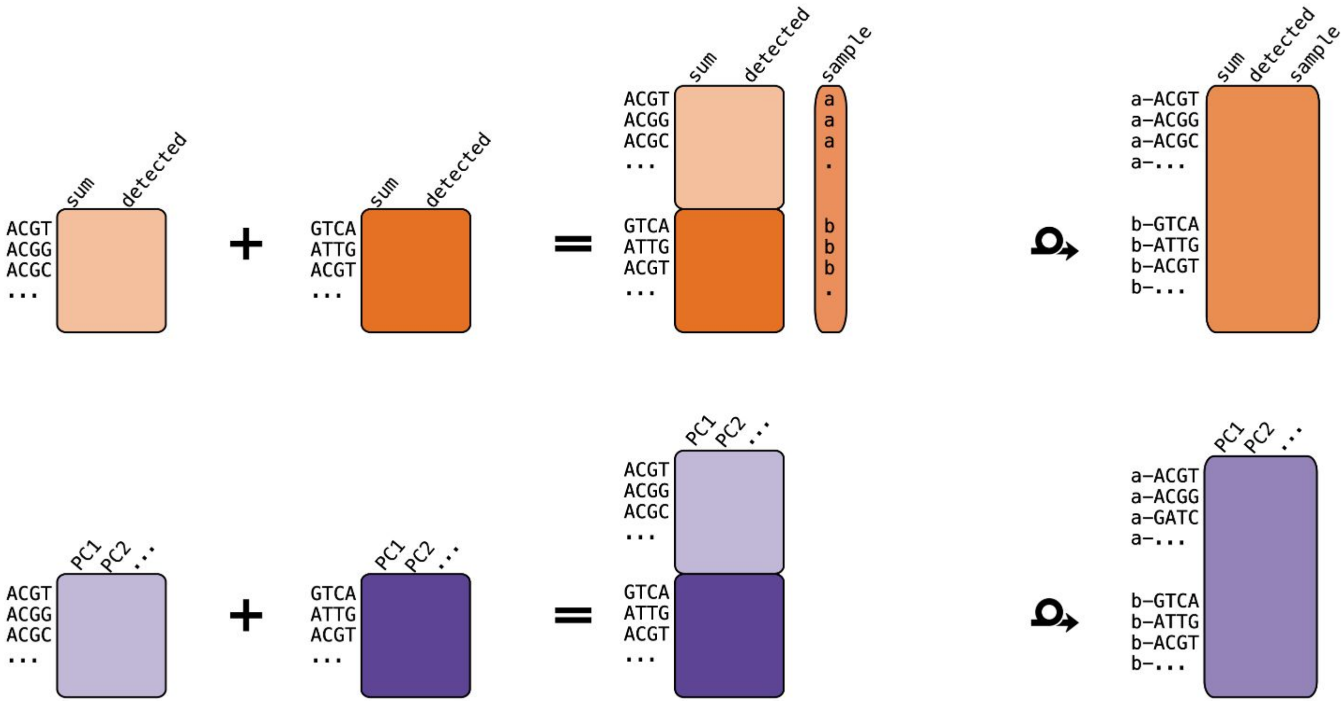
Overall SCE column names

Overall SCE row names

Per-gene (feature) data: Each row is a gene



Per-cell data: Each row is a cell


Merging the colData

Merging the reduced dimensions (e.g. PCA)

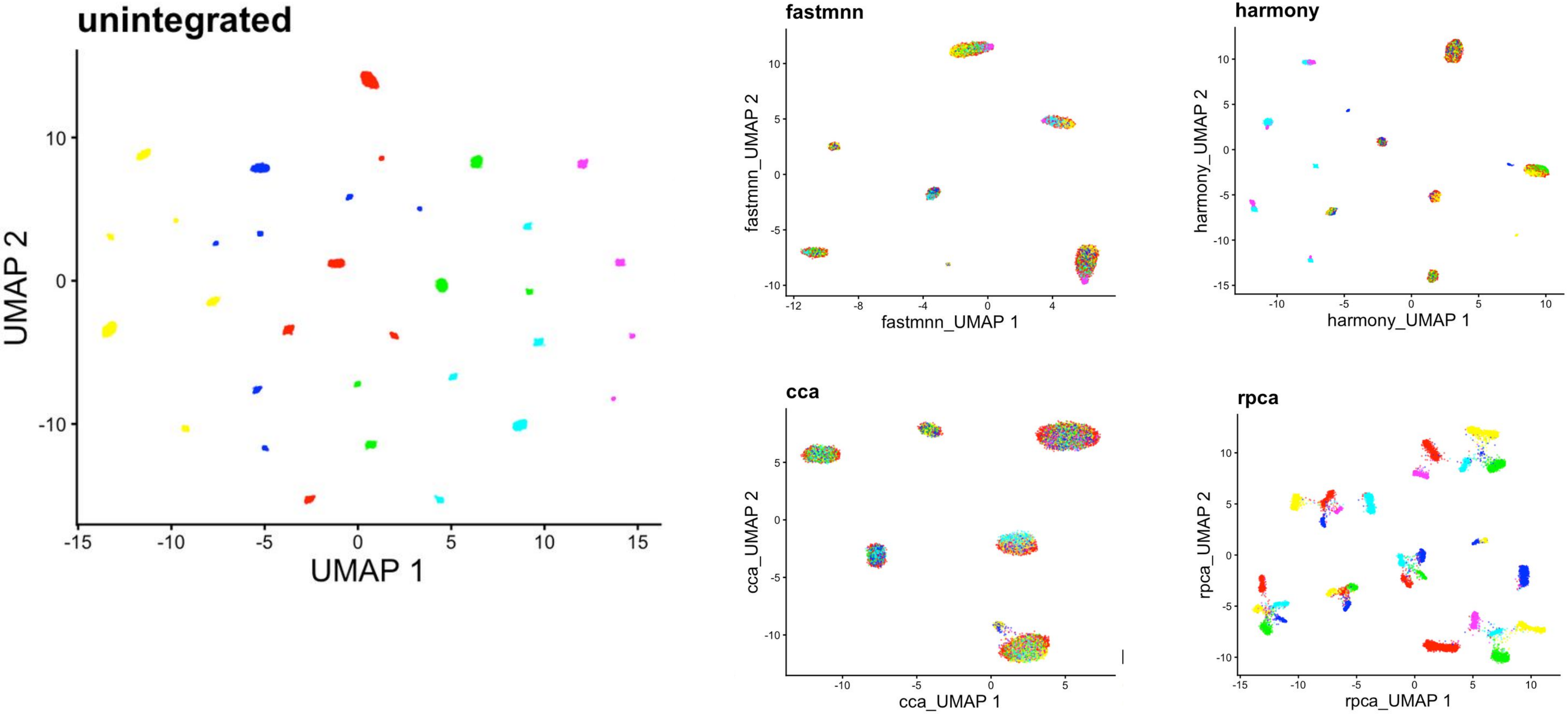
The following slides show some results we obtained benchmarking integration algorithms.

We performed some benchmarking on simulated data from Luecken *et al.*

- We evaluated several methods, four of which we'll show here:
  - **FastMNN**
  - **Harmony**
  - **Seurat using CCA** (canonical correlation analysis)
  - **Seurat using RPCA** (reciprocal PCA)
  - (We'll note that we also looked at scVI, which seemed to work well but is slow on CPU and it's in Python which is beyond the scope of our workshop!)
- We chose these methods based on performance in Luecken *et al.* and their usability
- See https://github.com/AlexsLemonade/sc-data-integration for our benchmarking code

Scenario 1: All cell types are present in all batches

UMAPs colored by Batch



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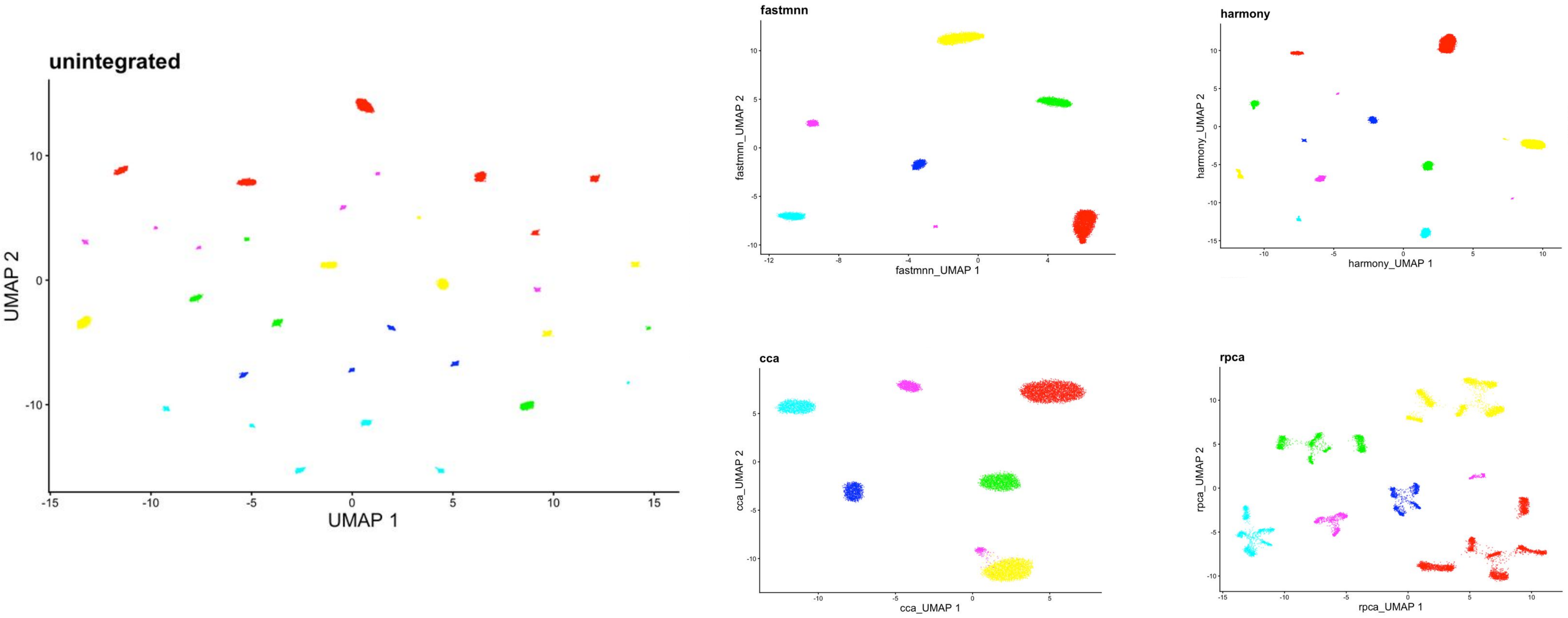
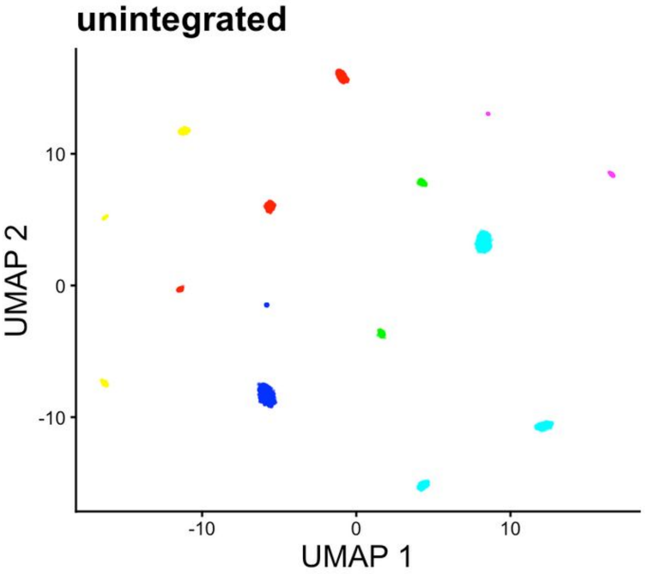
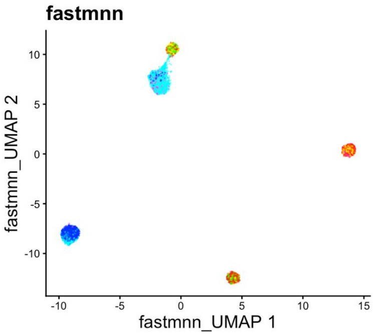
UMAPs colored by Cell Type

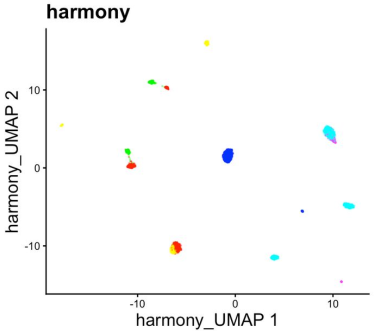


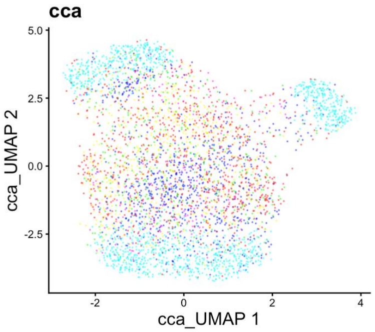
Scenario 2: Cell types are not present in all batches, and not all batches have cells in common

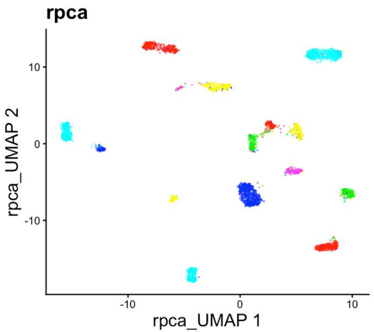
UMAPs colored by Batch











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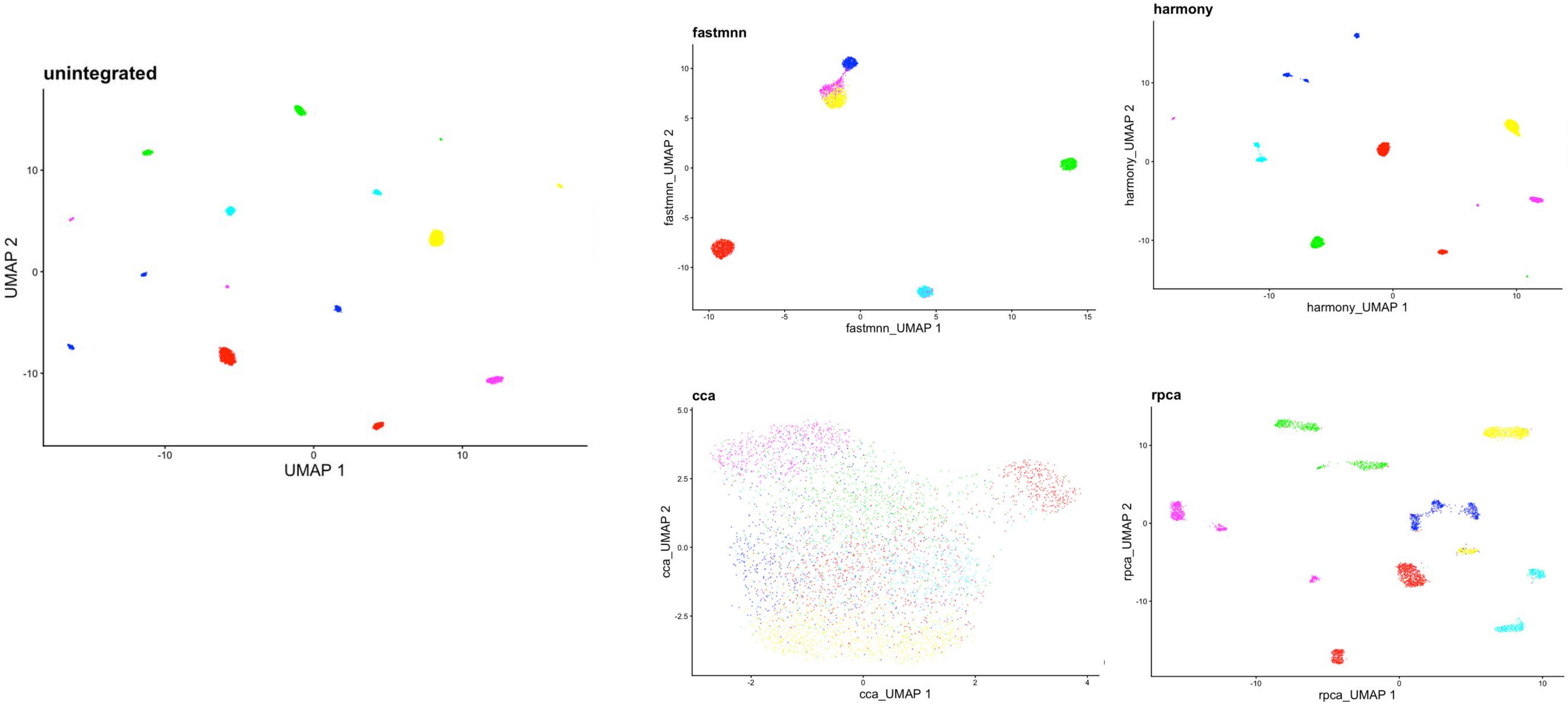
UMAPs colored by Cell Type


unintegrated
UMAP 1
UMAP 2
fastmnn
fastmnn_UMAP 1
fastmnn_UMAP 2
harmony
harmony_UMAP 1
harmony_UMAP 2
cca
cca_UMAP 1
cca_UMAP 2
rpca
rpca_UMAP 1
rpca_UMAP 2