Generative AI for scRNA-seq data analysis

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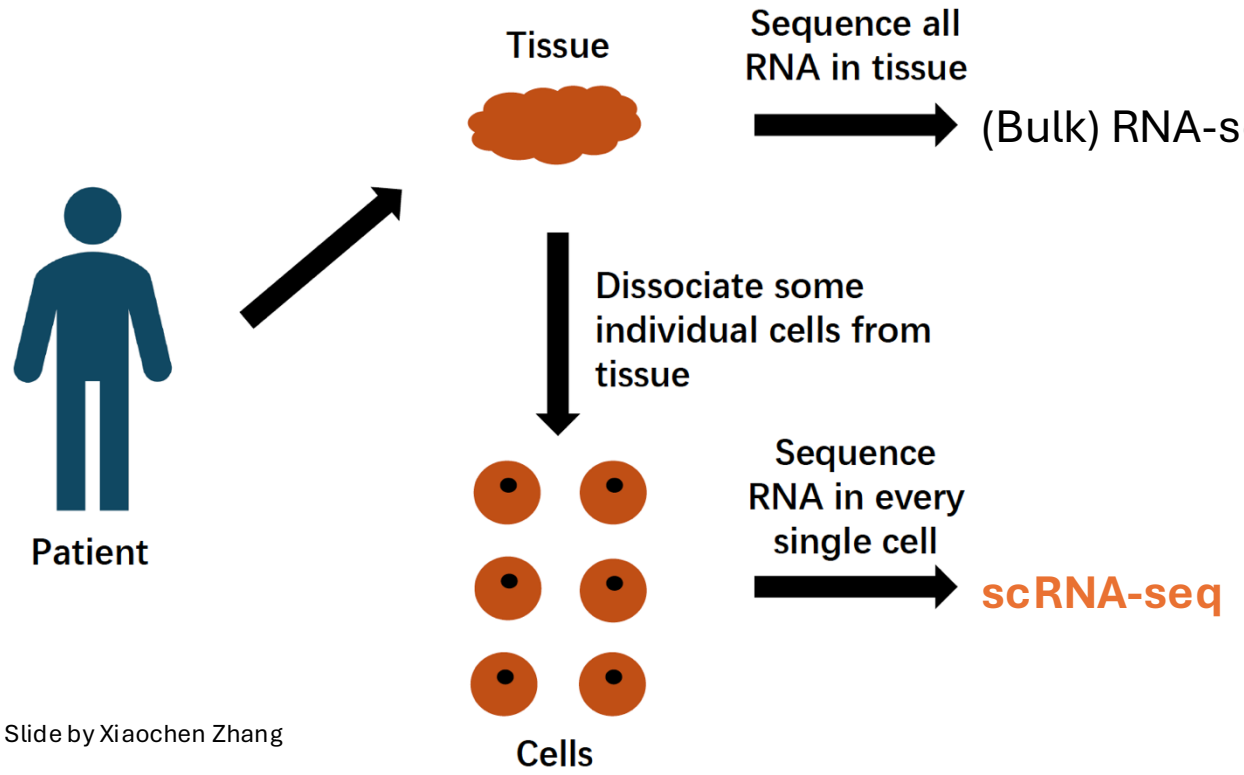
scRNA-seq: overview

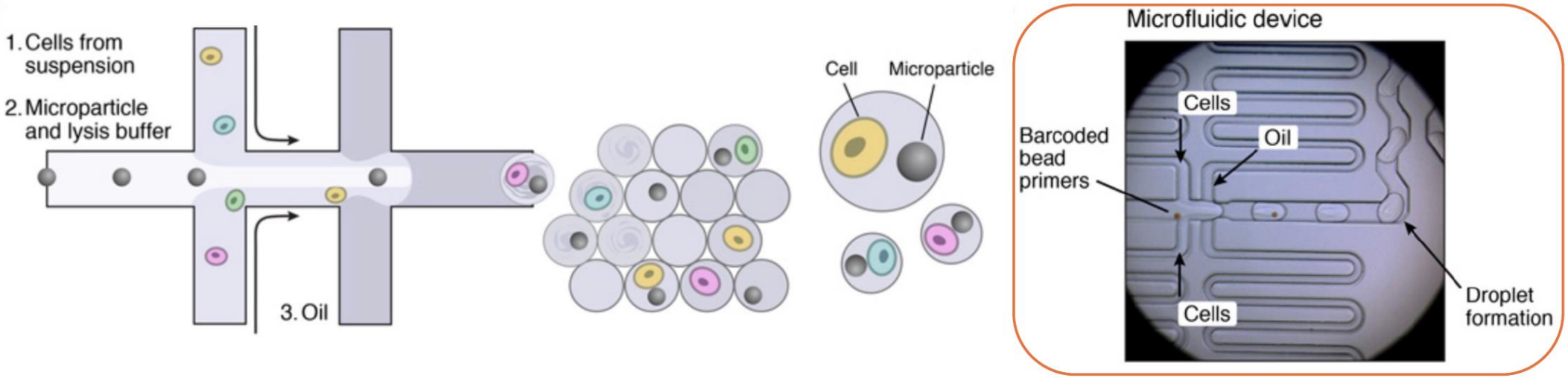


Slide by Xiaochen Zhang

- 'Omics' studies: biology at molecular level
- Bulk vs single cell
  - Bulk: marker discovery at population level
  - SC: marker discovery at **cell type** level
- 'Cell type'
  - Group of cells sharing similar gene expression profiles
  - More statistical power than single cells

What happened in the machine

Most scRNA-seq experiments use droplet-based platforms for dissociation.



Some scRNA-seq experiments use plate-based platforms for dissociation.

We assign each dissociated cell a barcode consisting of 4-20 bases to determine its identity.

Macosko EZ et al. (2015);
More details: Baran et al. (2018).

Slide by Xiaochen Zhang

Review of pipeline

Raw data processing

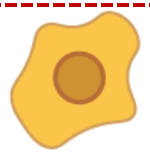
- Align reads to genes
- **Output**: count matrix (‘raw counts’)

Data pre-processing

- Quality control (QC)
- Batch correction
- Clustering + annotation (finding cell types)
- Normalisation
- **Output**: cleaned, normalised data with cluster labels or cell type annotation

Downstream analyses

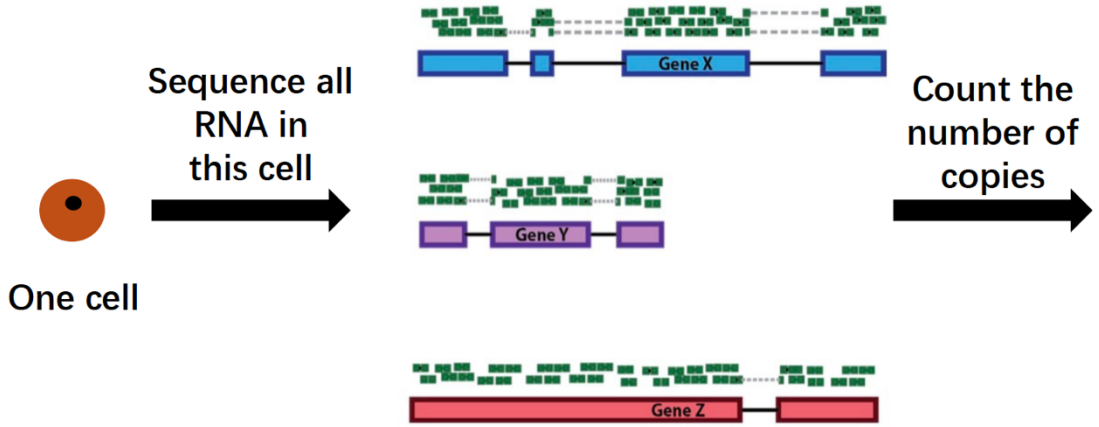
- Statistical modelling + visualisation
- Eg gene enrichment, cell type enrichment/depletion, cell-cell interaction, pseudo-time, …
- **Output**: biological interpretations of data



scvi-tools

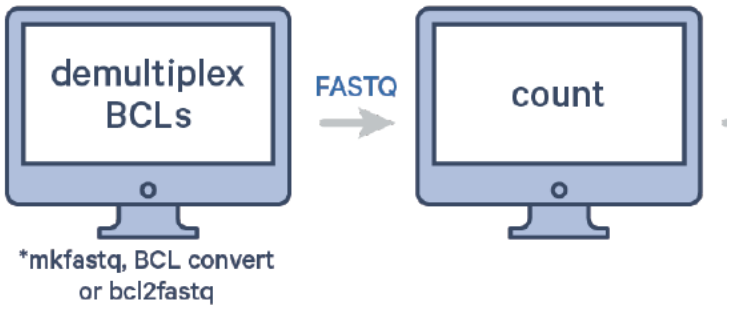
From sample to counts

Raw data processing → Data pre-processing → Downstream analyses



Gene	Count
Gene X	3
Gene Y	3
Gene Z	2

---------- Cell Ranger ----------



	pbmc2_10X_V2_AAACCTGAGATGGGTC	pbmc2_10X_V2_AAACCTGAGCGTAATA	pbmc2_10X_V2_AAACCTGAGCTAGGCA	pbmc2_10X_V2_AAACCTGAGGGTCTCC
TSPAN6	0	0	0	0
TNMD	0	0	0	0
DPM1	0	0	0	0
SCYL3	0	0	0	0
C1orf112	0	0	0	0
FGR	0	0	2	0
CFH	0	0	0	0
FUCA2	0	0	0	0
GCLC	0	0	0	0
NFYA	0	0	0	0
STPG1	0	0	0	0
NIPAL3	0	1	0	0
LAS1L	0	0	0	0
ENPP4	0	0	0	0

High dimension (typically > 10,000 cells * 20,000 genes)

Dimension reduction tools

Very Sparse (more than 90% of the matrix includes 0)

Feature selection, new computational methods

CellRanger: https://www.10xgenomics.com/support/software/cell-ranger/latest/getting-started/cr-what-is-cell-range

Quality control

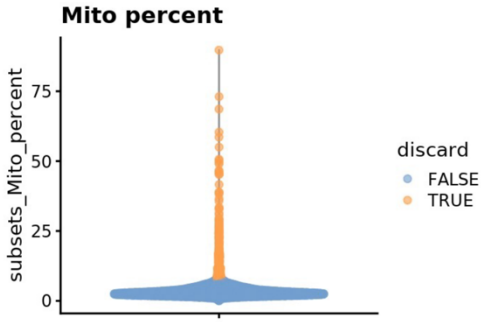
- Low-quality cells
  - Low gene counts (expressing too few genes)
  - High proportion of mitochondria genes
- Low-quality genes
  - Low cell counts (expressed in too few cells)
- Feature selection
  - Highly variable genes
- Not covered:
  - Normalisation, batch correction

```
sc.pp.filter_cells(adata, min_counts=3)
```

```
pbmc.var["mito"] = pbmc.var_names.str.startswith("MT-")
```

```
sc.pp.filter_genes(adata, min_counts=3)
```

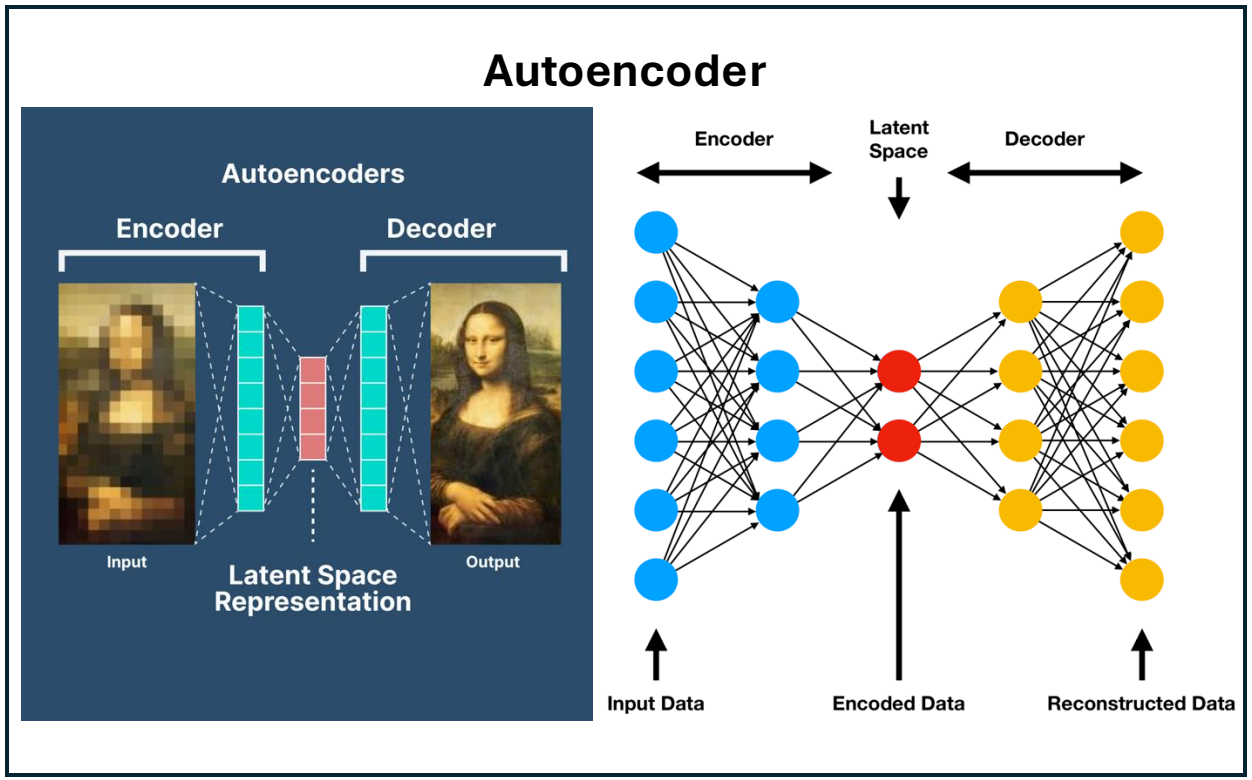
```
sc.pp.highly_variable_genes(
    adata,
    n_top_genes=1200,
    subset=True,
    layer="counts",
    flavor="seurat_v3",
    batch_key="cell_source",
)
```

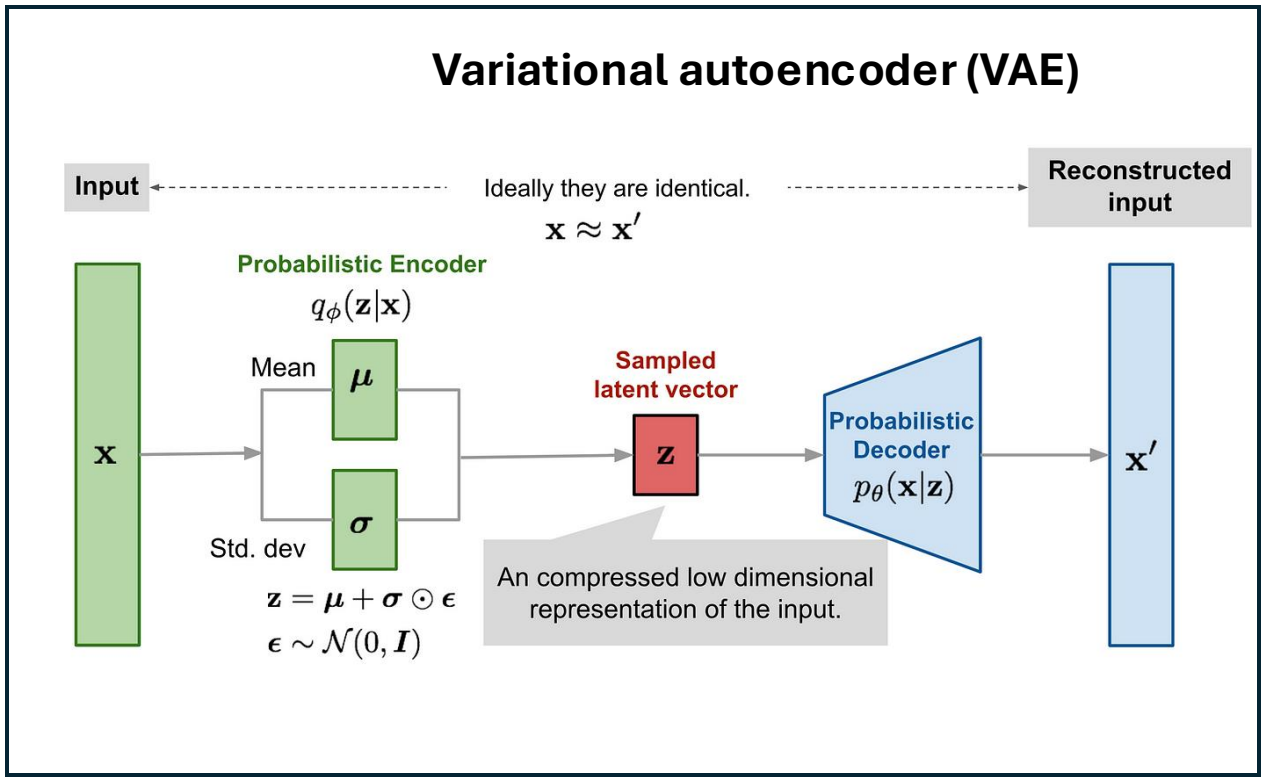


	Gene 1	Gene 2	Gene 3
	2	5	0
	1	1	0
	1	1	2
	1	0	4
Variance =	0.25	4.92	3.67

Scanpy pre-processing: https://scanpy.readthedocs.io/en/stable/api/preprocessing.html

Autoencoder





https://www.v7labs.com/blog/autoencoders-guide

https://www.compthree.com/blog/autoencoder/

scVI model

Article | Published: 30 November 2018

Deep generative modeling for single-cell transcriptomics

Romain Lopez, Jeffrey Regier, Michael B. Cole, Michael I. Jordan & Nir Yosef

Nature Methods **15**, 1053–1058 (2018) | Cite this article

scvi-tools

Six neural networks (NNs):

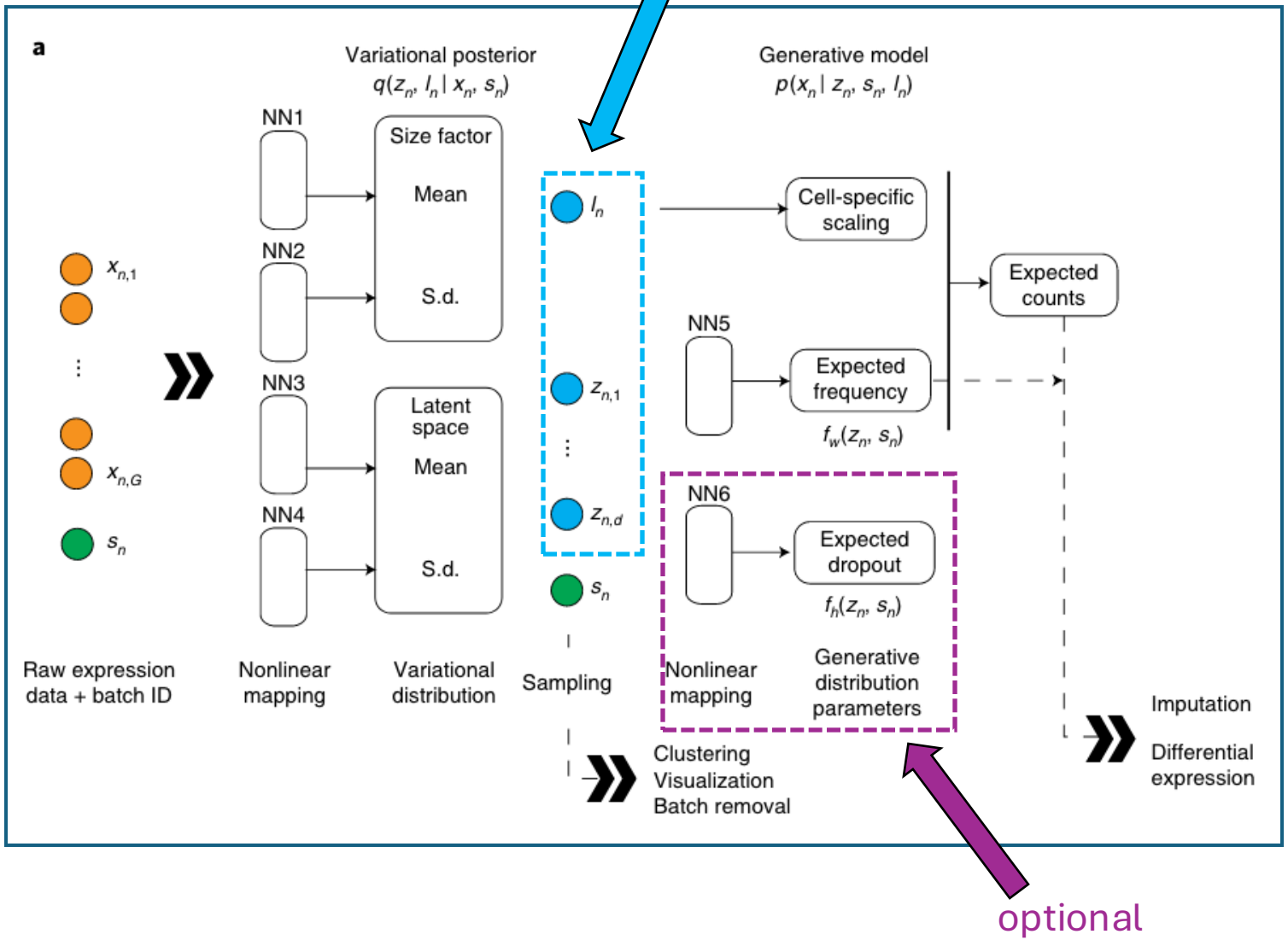
- NN1, NN2 **encode** mean & sd of l_n, size factor of cell n
- NN3, NN4 **encode** mean & sd latent variables (default $d = 10$)

Then, generate values from $z_{n,1}, \ldots, z_{n,d}$,

- NN5 **decodes** 'expected frequency', ie normalised counts
- NN6 regenerates dropout events (**optional**)

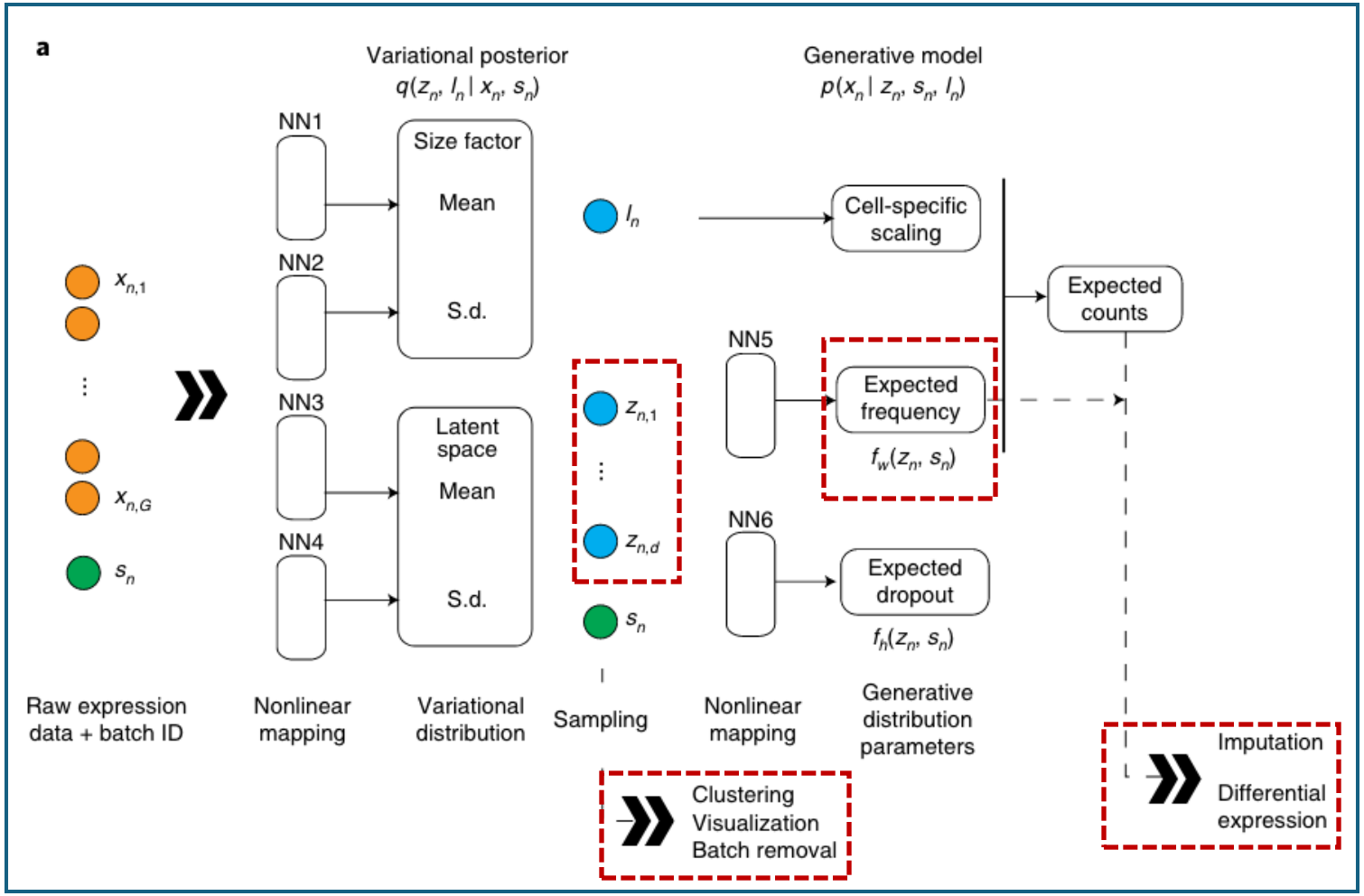
- Optional: generate l_n and recover counts

- Have to use raw counts as input



What has scVI done?

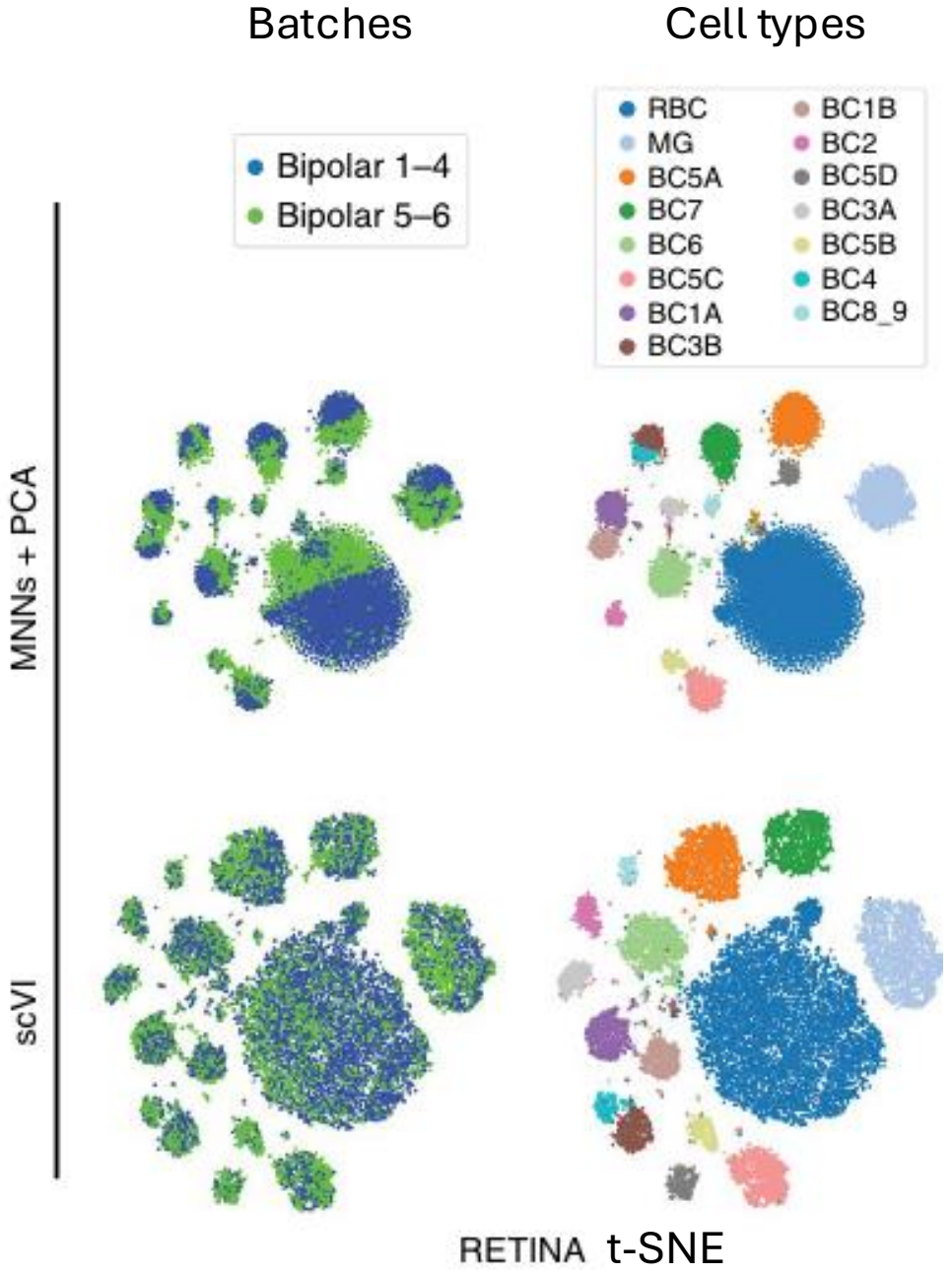
- Modelled latent variables
  - (Nonlinear) dimension reduction
  - Independent from size factor & batch
  - Suitable for visualisation & clustering
- Generated expected frequencies
  - Normalised (against size factor) & batch corrected counts
- Generated expected counts
  - Batch corrected (not normalised)
  - Useful for DE



Batch correction

Data
- 27,499 cells
- Cell type labels from original authors
- 2 batches (bipolar 1–4, bipolar 5–6)

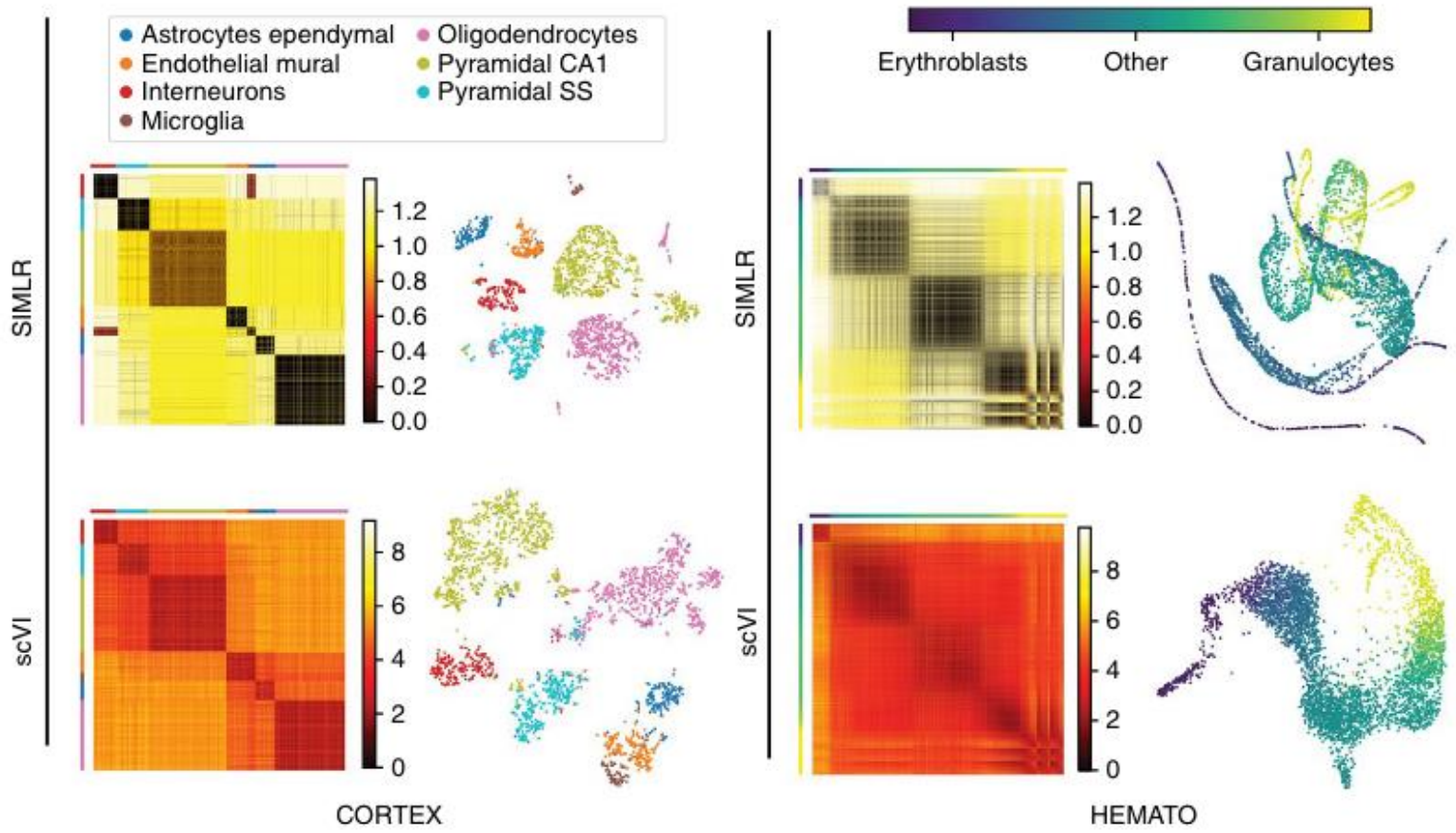
Goal
Comparing scVI and MNNs on batch correction



Biologically meaningful clustering

CORTEX
- 3,005 mouse cortex cells
- Reliable highly differentiated cell types

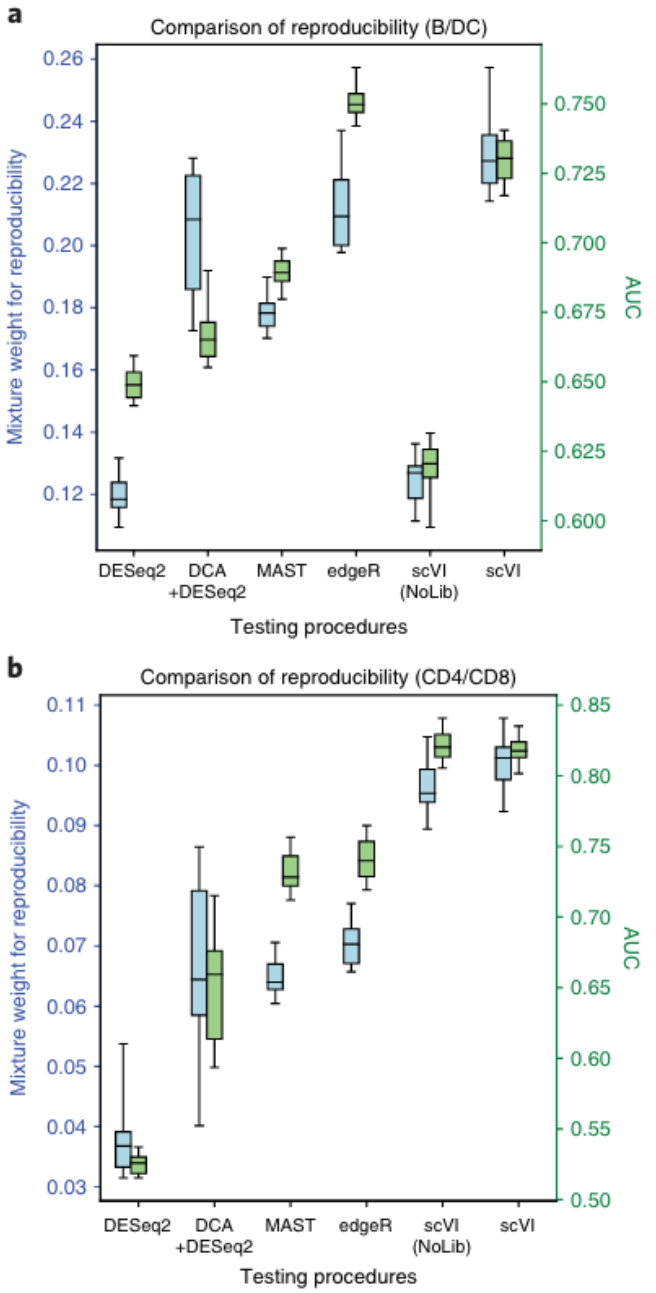
HEMATO
- 4,016 hematopoietic progenitor cells
- Cells along a developmental trajectory



Differential expression

- After scVI processing, data are denoised
- Can use any tools for DE
- But scVI also does its own DE (probabilistic modelling)

- Importance of library size in DE (compared to eg clustering)



scANVI: semi-supervised VAE

Article

TRANSPARENT PROCESS OPEN ACCESS

molecular systems biology

Probabilistic harmonization and annotation of single-cell transcriptomics data with deep generative models

Chenling Xu[1,†], Romain Lopez[2,†], Edouard Mehlman[2,3,†], Jeffrey Regier[4], Michael I Jordan[2,5] & Nir Yosef[1,2,6,7,*]

Motivation

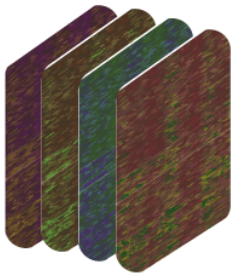
- Unsupervised batch correction is not always appropriate

- Eg when batch cofounds biology
  - Disease samples in batch 1, control in batch 2
  - This happens a lot, due to …

- Batch effects removed, part of biological difference also removed

- A bit of guidance (supervision) helps
  - Eg control and control should be similar across batches
  - Eg CD4 T cells should stay together on UMAP/t-SNE

- Guidance comes in the form of (partial) labels

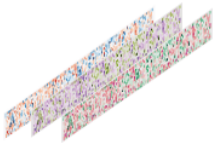


Collection of scRNA-seq datasets

Core analysis tool: scVI

Multi-purpose generative model
- visualization
- clustering
- differential expression
- harmonization

Extends to



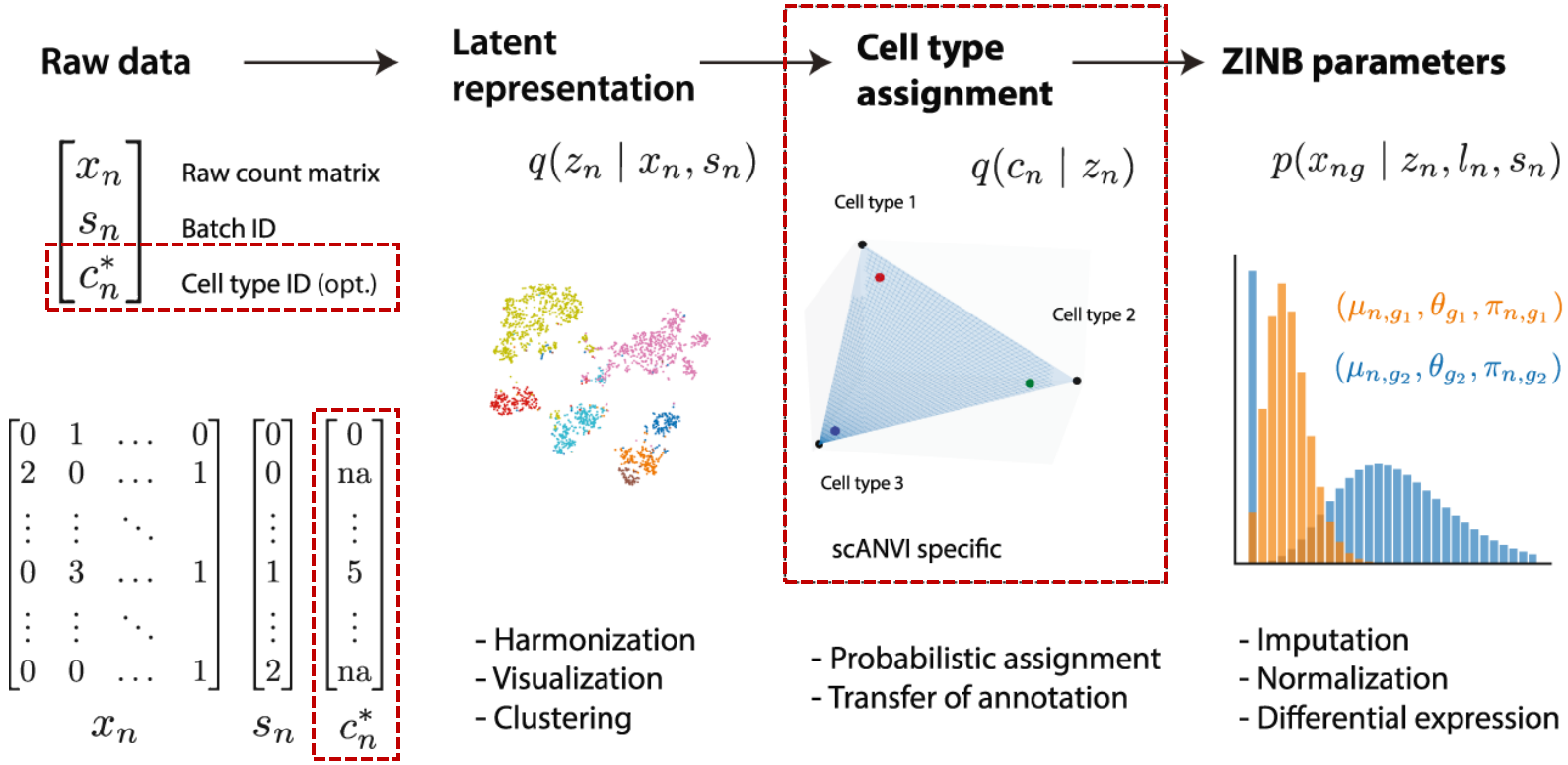
Partial cell type annotation

Annotation tool: scANVI

Transfer of annotation in various settings:
- partial overlap of labels
- partial "seed" labeling
- hierarchical labels

scANVI model

- Added cell type labels
  - Could be simply cluster labels
  - Can be partial
- Restriction: cells of same label have similar latent representations
- Result: batch correction by aligning cells from same type



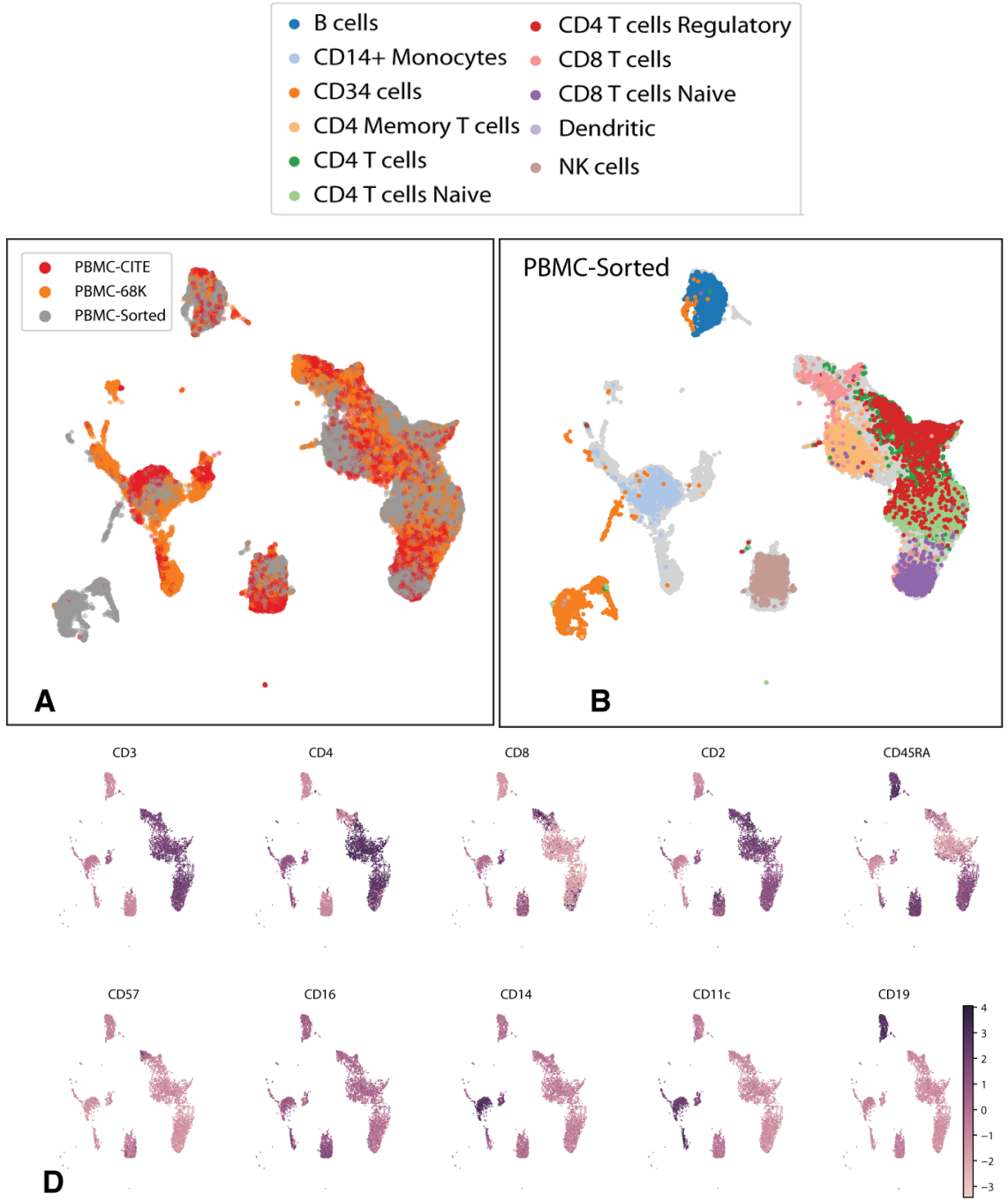
Case study

Data

- Three PBMC (peripheral blood mononuclear cells) datasets
  - 169,850 cells in total
- PBMC-Sorted: annotated
- PBMC-65k: unannotated
- PMBC-CITE: unannotated, also contains 10 surface protein counts
- Protein markers serve as validation

Challenges

- Partially labelled
- Not all cell types available across three datasets (imbalanced design)



Building large-scale cell atlas

Motivation

- scRNA-seq still more costly than bulk
- Each study contains limited donors (budget)
- Cell atlas: integrate large number of studies from hundreds of patients

Challenges

- Serious batch effects
- Large number of cells (millions), huge computational burden

Why scANVI

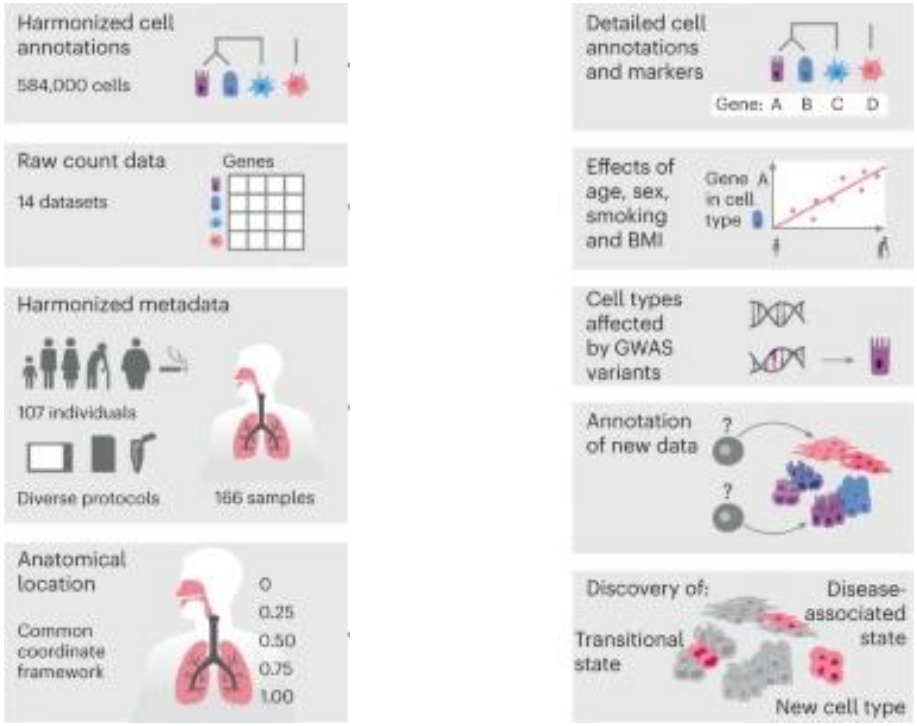
- Flexible and biology-preserving batch correction
- Computationally scalable
  - Takes longer to train compared to other methods, on smaller scale data
  - Computational time increases not fast when sample size increases
  - Reason: back propagation, small batch learning (no need to handle huge matrices)

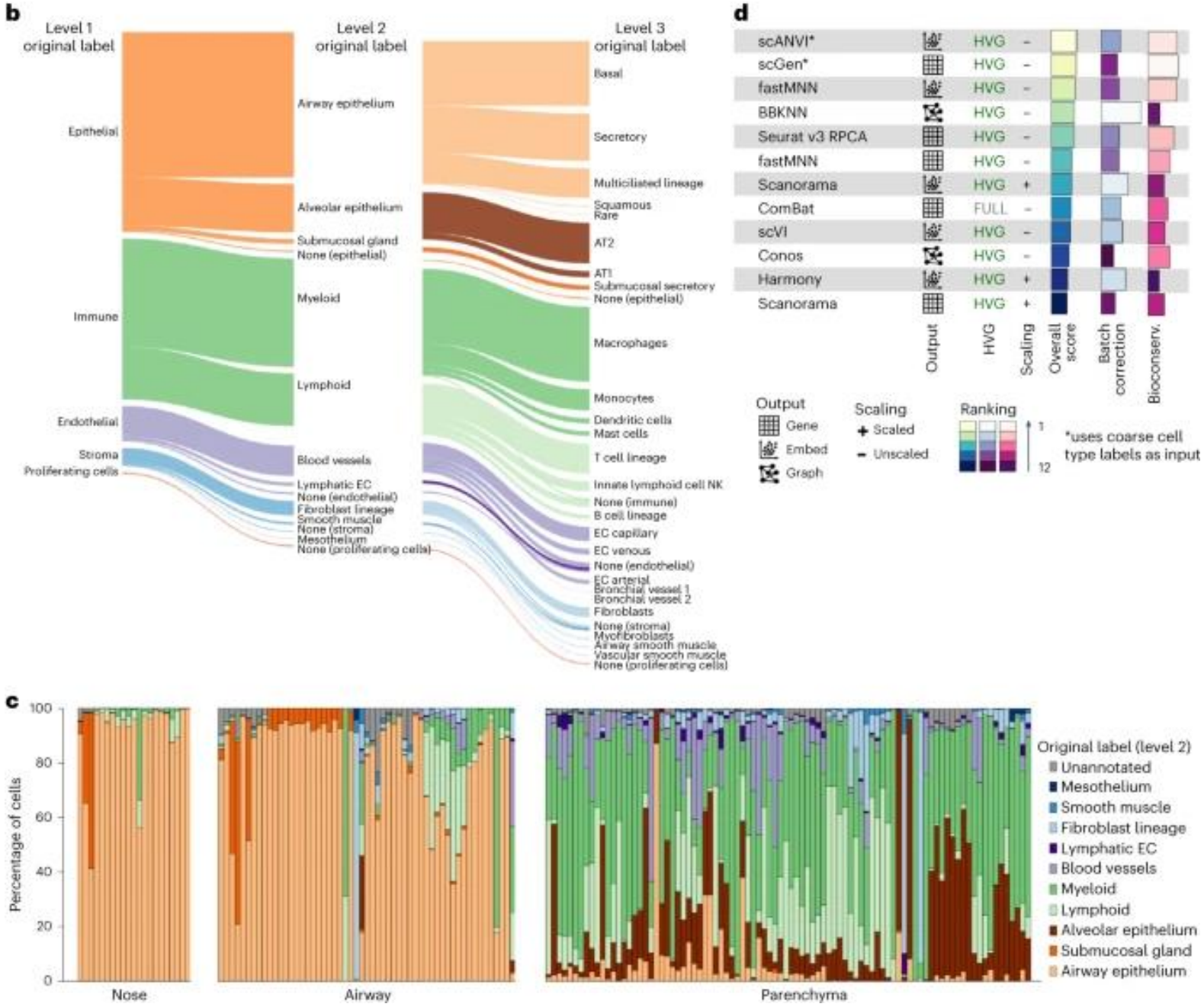
An integrated cell atlas of the lung in health and disease

Lisa Sikkema, Ciro Ramírez-Suástegui, Daniel C. Strobl, Tessa E. Gillett, Luke Zappia, Elo Madissoon, Nikolay S. Markov, Laure-Emmanuelle Zaragosi, Yuge Ji, Meshal Ansari, Marie-Jeanne Arguel, Leonie Apperloo, Martin Banchero, Christophe Bécavin, Marijn Berg, Evgeny Chichelnitskiy, Mei-i Chung, Antoine Collin, Aurore C. A. Gay, Janine Gote-Schniering, Baharak Hooshiar Kashani, Kemal Inecik, Manu Jain, Theodore S. Kapellos, Lung Biological Network Consortium, ... Fabian J. Theis

+ Show authors

Nature Medicine **29**, 1563–1577 (2023) | Cite this article

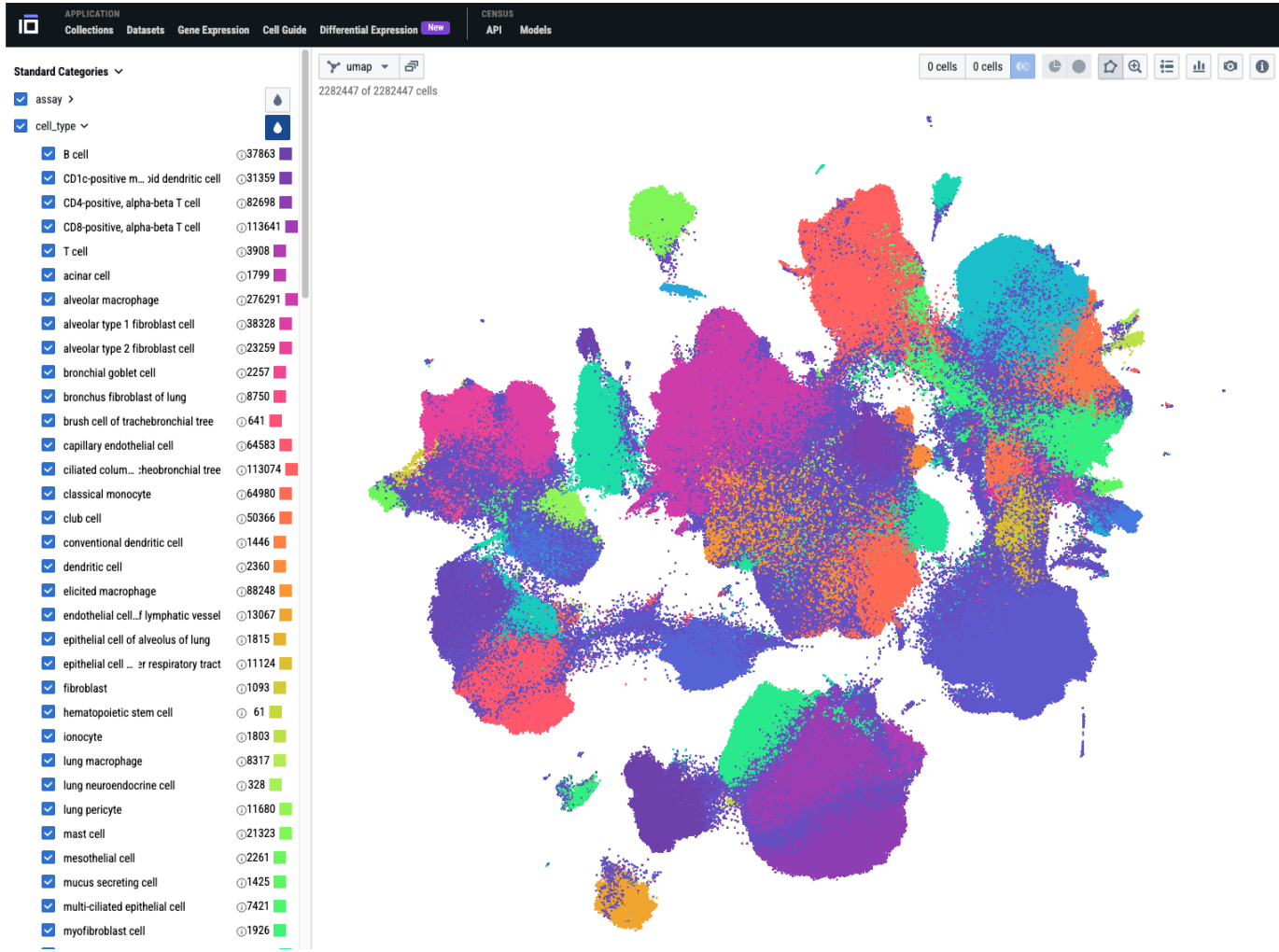



b
Level 1 original label
Level 2 original label
Level 3 original label
Epithelial
Immune
Endothelial
Stroma
Proliferating cells
Airway epithelium
Alveolar epithelium
Submucosal gland
None (epithelial)
Myeloid
Lymphoid
Blood vessels
Lymphatic EC
None (endothelial)
Fibroblast lineage
Smooth muscle
None (stroma)
Mesothelium
None (proliferating cells)
Basal
Secretory
Multiciliated lineage
Squamous
Rare
AT2
AT1
Submucosal secretory
None (epithelial)
Macrophages
Monocytes
Dendritic cells
Mast cells
T cell lineage
Innate lymphoid cell NK
None (immune)
B cell lineage
EC capillary
EC venous
None (endothelial)
EC arterial
Bronchial vessel 1
Bronchial vessel 2
Fibroblasts
None (stroma)
Myofibroblasts
Airway smooth muscle
Vascular smooth muscle
None (proliferating cells)
d
scANVI*
scGen*
fastMNN
BBKNN
Seurat v3 RPCA
fastMNN
Scanorama
ComBat
scVI
Conos
Harmony
Scanorama
HVG
FULL
Output
HVG
Scaling
Overall score
Batch correction
Bioconserv.
Output
Gene
Embed
Graph
Scaling
+ Scaled
− Unscaled
Ranking
1
12
*uses coarse cell type labels as input
c
Percentage of cells
100
80
60
40
20
0
Nose
Airway
Parenchyma
Original label (level 2)
Unannotated
Mesothelium
Smooth muscle
Fibroblast lineage
Lymphatic EC
Blood vessels
Myeloid
Lymphoid
Alveolar epithelium
Submucosal gland
Airway epithelium

Query your own lung samples

Use of atlas: ‘reference mapping’

- Get cell type annotations
- Compare with your own annotations
- Marker genes
- Levering more samples
  - Eg I don’t have enough control samples in my study



The integrated Human Lung Cell Atlas

CZI Single-Cell Biology, Human Cell Atlas (HCA)

The integrated Human Lung Cell Atlas (HLCA) represents the first large-scale, integrated single-cell reference atlas of the human lung. It consists of over 2 million cells from the respiratory tract of 486 individuals, and includes 49 different datasets. It is split into the HLCA core, and the extended or full HLCA. The HLCA core includes data of healthy lung tissue from 107 individuals, and includes manual cell type...

Show More

Publication	Sikkema et al. (2023) Nat Med
Contact	Malte D. Luecken
Other	HLCA landing page

Dataset	Tissue	Disease	Assay	Organism	Cells		
An integrated cell atlas of the human lung in health and disease (full)	4 tissues	normal 15 diseases	9 assays	Homo sapiens	2,282,447	Download	Explore
An integrated cell atlas of the human lung in health and disease (core)	3 tissues	normal	5 assays	Homo sapiens	584,944	Download	Explore

Discussions

- scVI & scANVI serve as data pre-processing tools
- Decoded data are ready for downstream analysis
- Scalable and flexible
- But lack interpretability compared to linear methods
  - There is no ‘loading’ for latent variables
- Suitable for building atlases and transfer learning
  - Train on large scale data
  - Download trained model and fine tune on own data

Ἄτλας on Collins Street