Generative AI for scRNA-seq data analysis

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Generative AI for scRNA-seq data analysis

Why RNA?

Why not just DNA?

- The Human Genome Project (HGP) and the cure of cancer
  - Sequence 3 billion base pairs of A, G, C, T
  - ‘(HGP will) enable most individuals to live a … life without disease.’
  - ‘By 2010 individualised medicine would be a reality; physicians would routinely take check swabs from patients and send their DNA out for testing’
- This, alas, did not happen
  - Genome is a book too complicated to decipher
  - Eg E coli genome and gene regulation
- Multi-cellular organisms
  - ‘Cell types’

Stanford Encyclopedia of Philosophy

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Entry Contents

Bibliography

Academic Tools

The Human Genome Project

First published Wed Nov 26, 2008; substantive revision Thu Sep 14, 2023

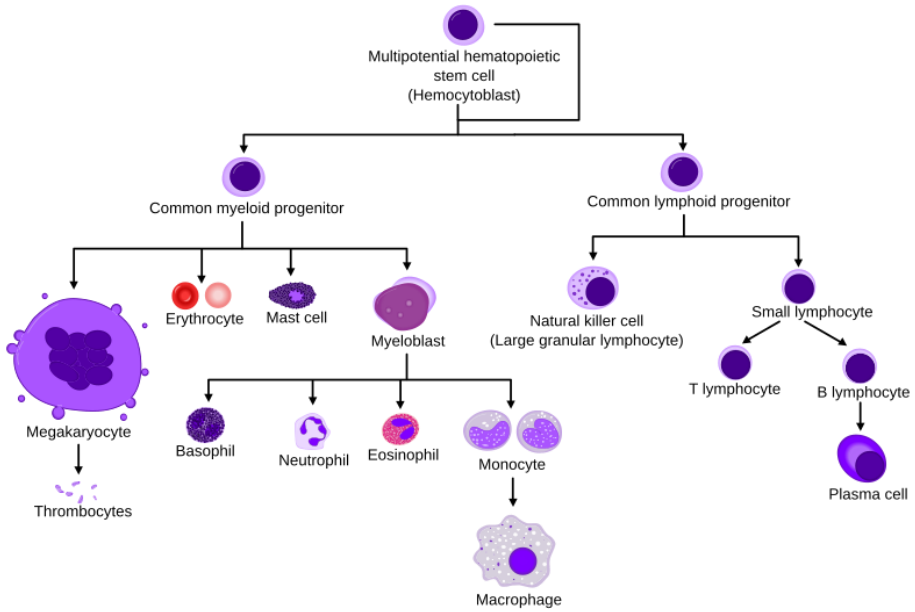
Cell

Leading Edge

Commentary

The cellular dogma

Stephen R. Quake[1,2,*]
[1]The Chan Zuckerberg Initiative, Redwood City, CA, USA
[2]Depts of Bioengineering and Applied Physics, Stanford University, Stanford, CA, USA

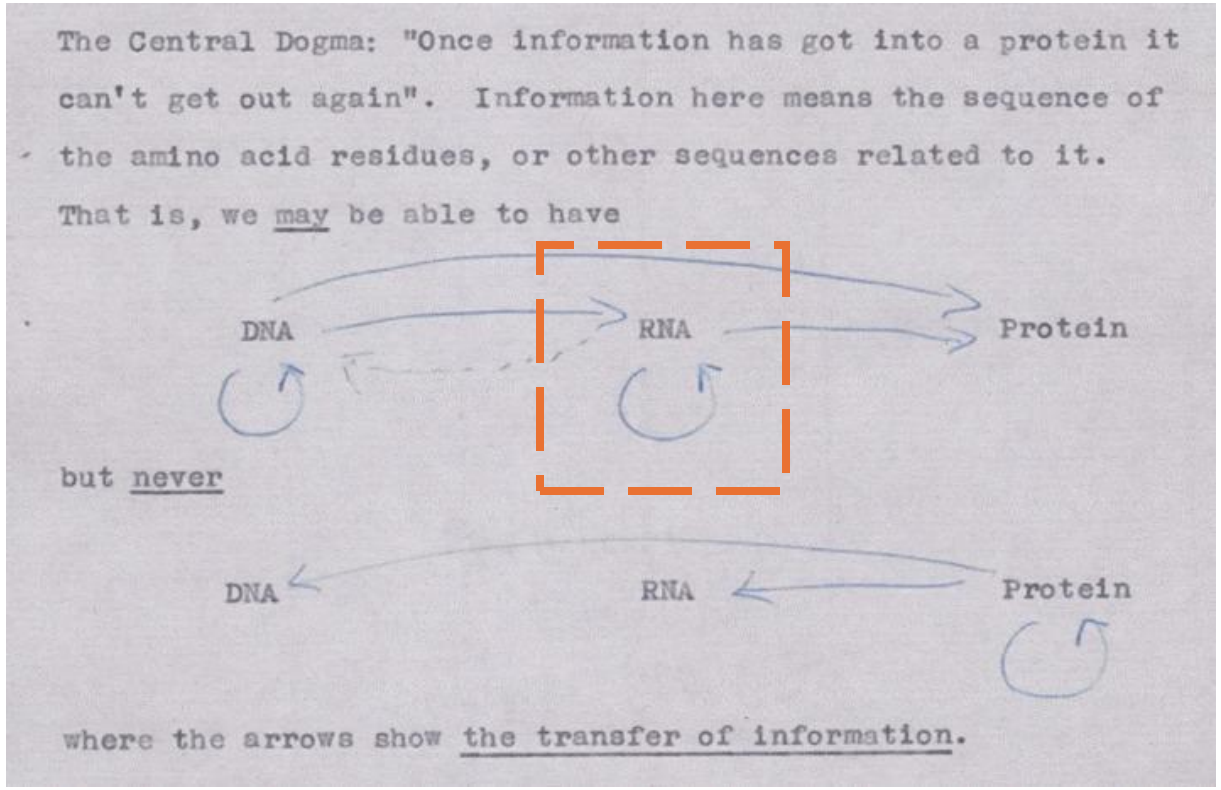


Haematopoiesis. Wikipedia

Genome: the complete set of genes or genetic materials present in a cell or organism.
Genomics: the branch of molecular biology concerned with the structure, function, evolution and mapping of genomes

From genotype to phenotype

- Crick's central dogma
  - Information flow at molecular level
  - Genomics, epigenomics, transcriptomics, proteomics, …
- mRNA and cell types
- From Human Genome Project to Human Cell Atlas
  - Single-cell omics



HUMAN CELL ATLAS

PLOS | BIOLOGY

ESSAY

60 years ago, Francis Crick changed the logic of biology

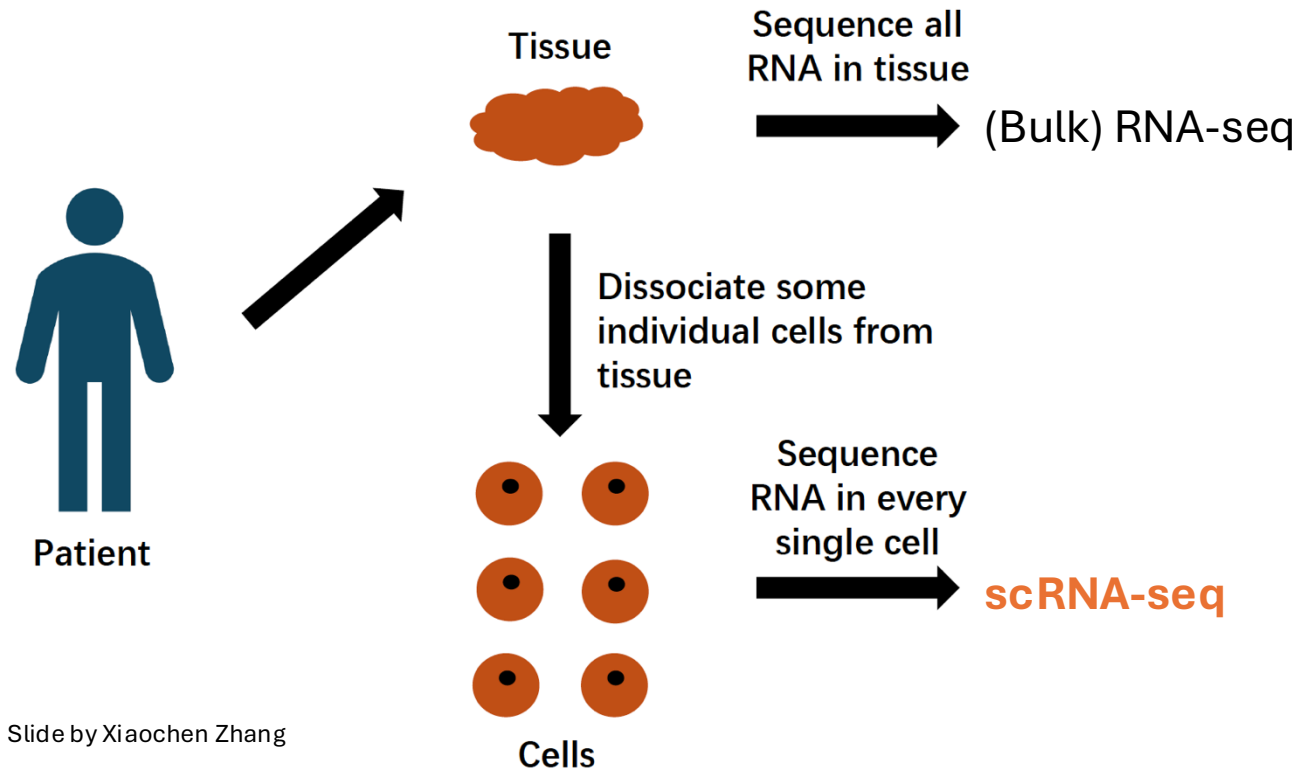
Matthew Cobb*
School of Biological Sciences, University of Manchester, Manchester, United Kingdom
* cobb@manchester.ac.uk

Genotype: the genetic constitution of an individual organism
Phenotype: the set of observable characteristics of an individual resulting from the interaction of its genotype with the environment.

Generative AI for scRNA-seq data analysis

How the data are generated?

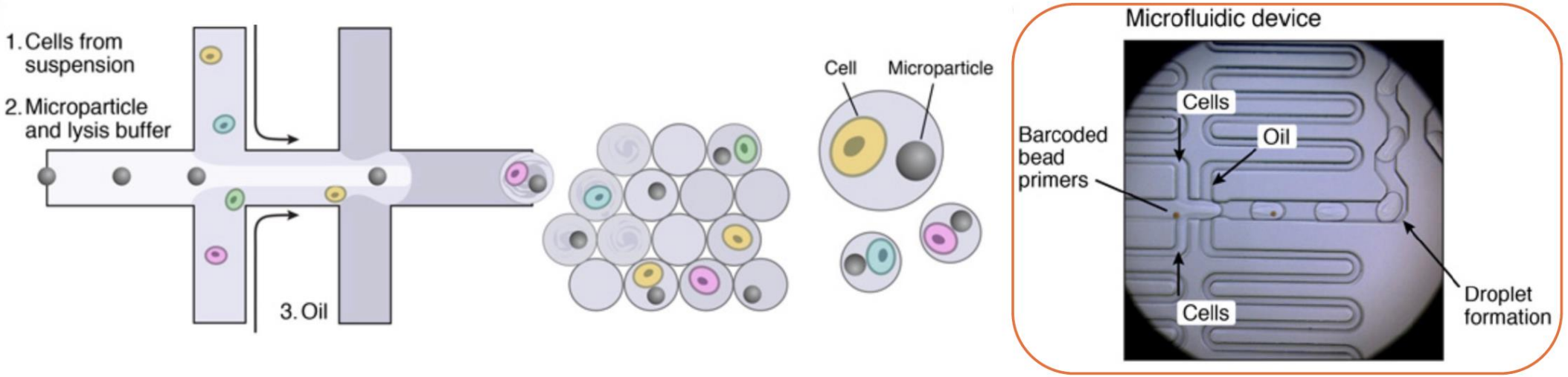
scRNA-seq: overview



Slide by Xiaochen Zhang

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

Generative AI for scRNA-seq data analysis

How the analysis is typically done?

Review of pipeline

Raw data processing

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

Data pre-processing

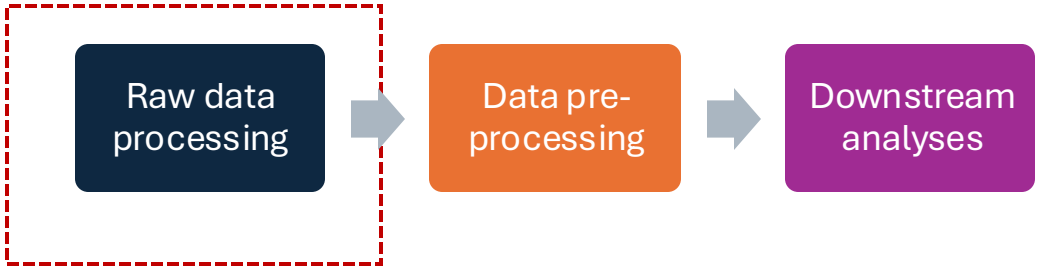
- Quality control (QC)
- Batch correction
- Clustering + annotation (finding cell types)
- Normalisation
- **Output**: cleaned, normalised data with cluster labels or cell type annotation

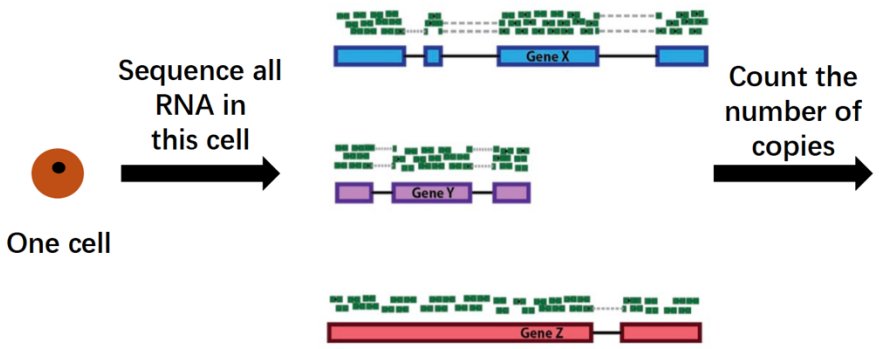
Downstream analyses

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

scvi-tools

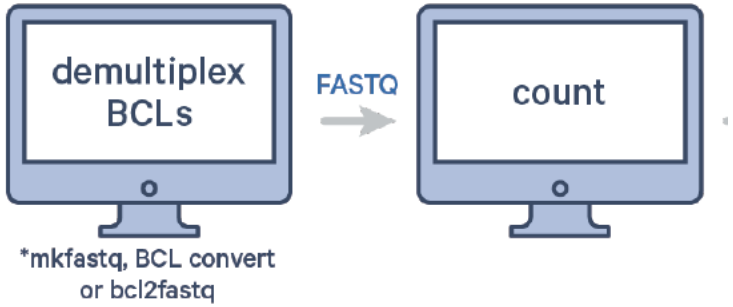
From sample to counts





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-ranger

Quality control

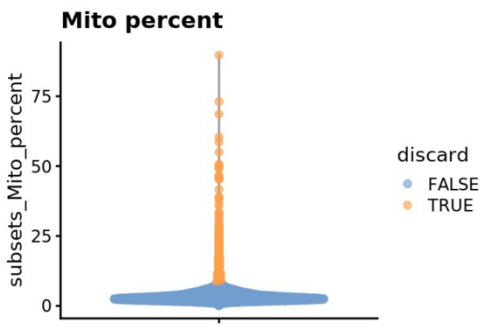
- Low-quality cells
  - Low gene counts (expressing too few genes)
  - High proportion of mitochondria genes
- Low-quality genes
  - Low cell counts (expressed in too few cells)
- Feature selection
  - Highly variable genes
- Not covered:
  - Normalisation, batch correction

```
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",
)
```



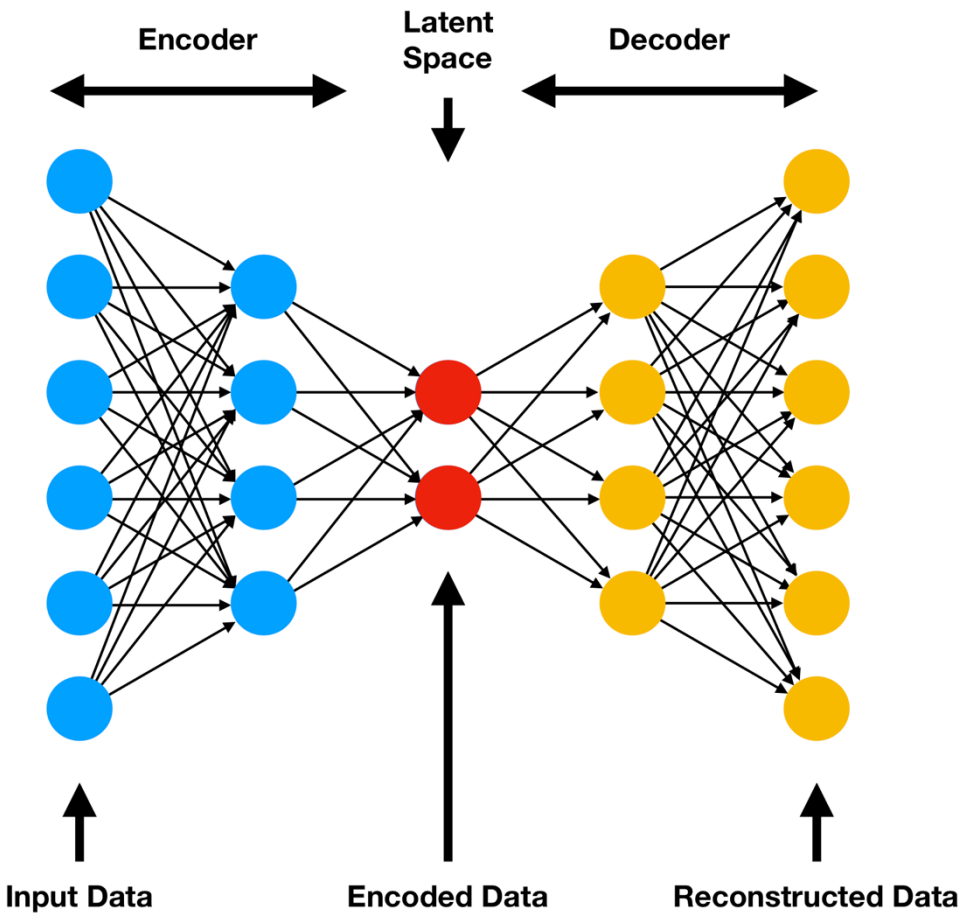
	Gene 1	Gene 2	Gene 3
	2	5	0
	1	1	0
	1	1	2
	1	0	4
Variance =	0.25	4.92	3.67

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

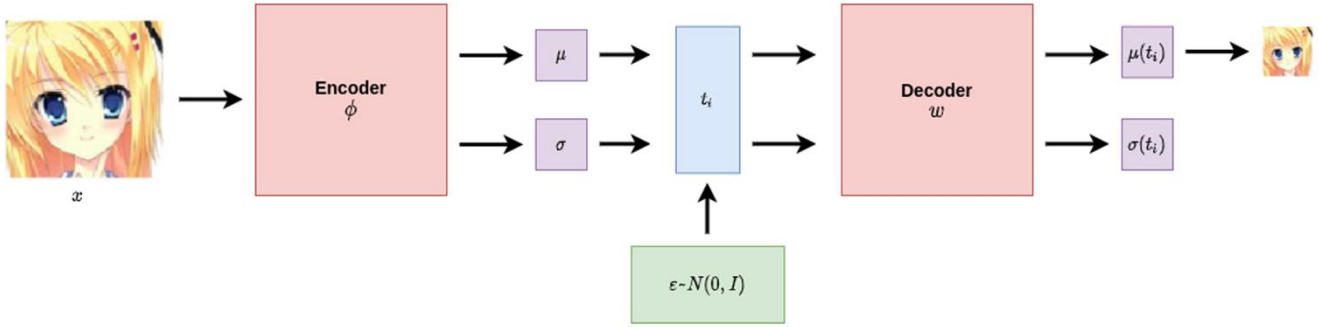
Generative AI for scRNA-seq data analysis

What is generative AI?

Autoencoders



Is PCA an autoencoder?



Variational autoencoder (VAE)

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

Generative modelling of images

A Comprehensive Study of Autoencoders' Applications Related to Images

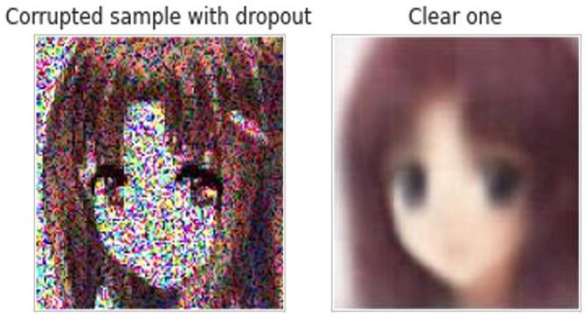
Volodymyr Kovenko, Ilona Bogach[a]

Vinnytsia National Technical University, Khmelnytsky highway 95, Vinnytsia, 21021 Ukraine

Training

training data

Applications



denoising by reconstruction

reconstruction
(not a goal in itself)

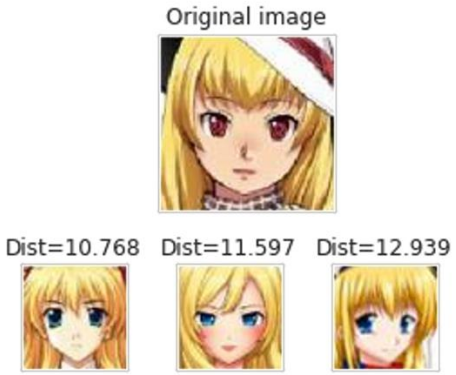


image embedding

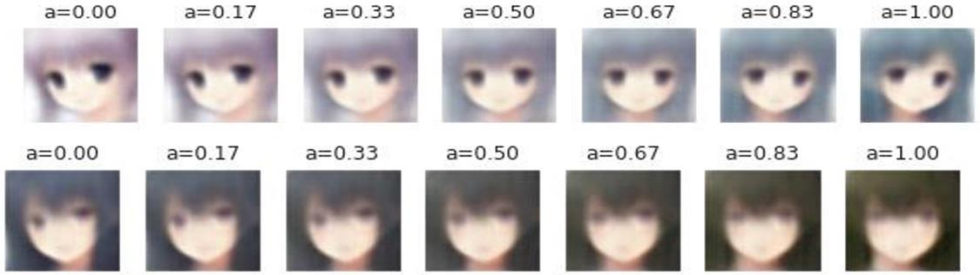


image interpolation (in latent space)

Key: dimension reduction (encoding) to a space (manifold) that is easier to manipulate

Generative AI for scRNA-seq data analysis

www.recipetineats.com

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 $d = 10$)

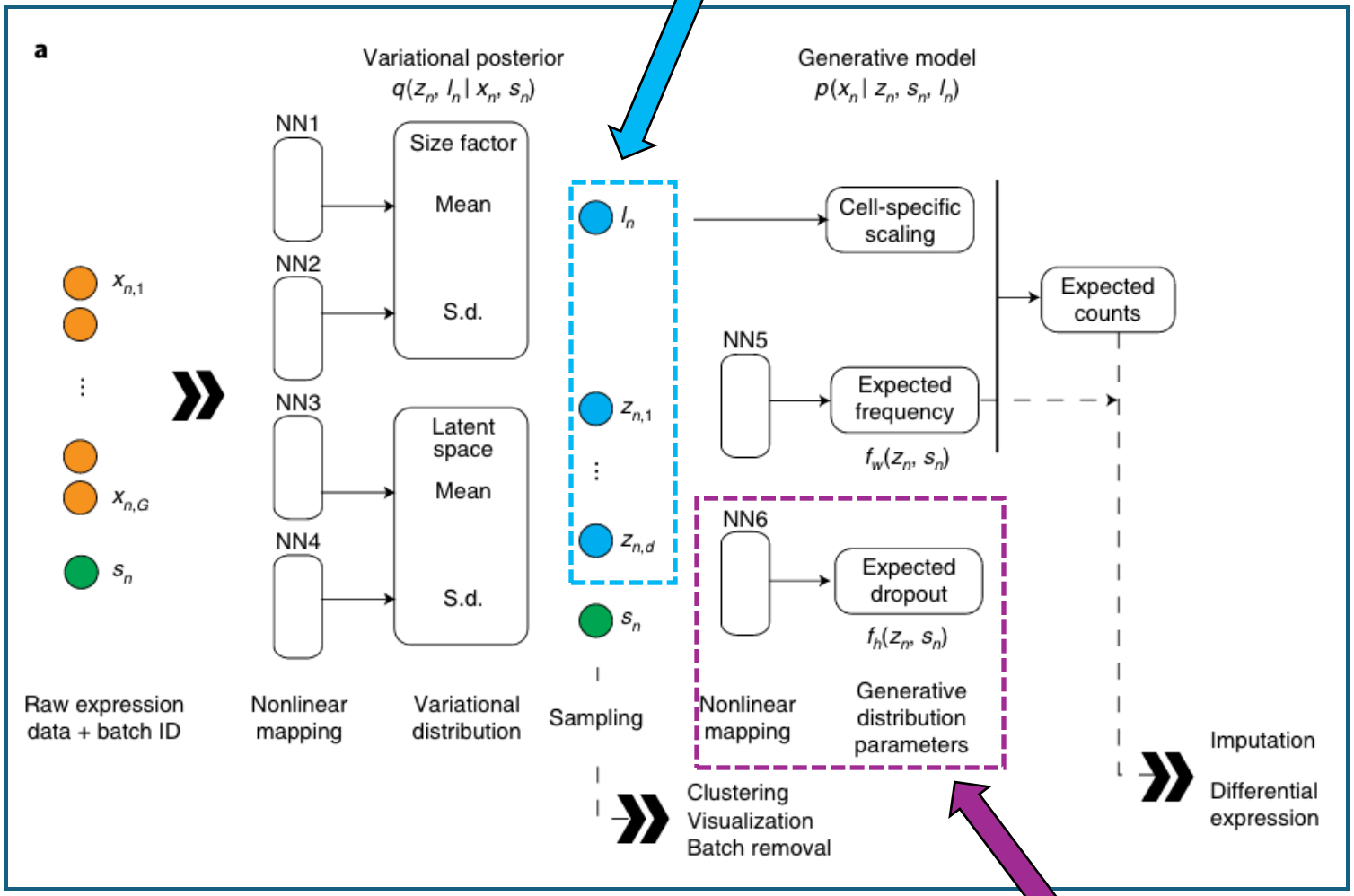
Then, generate values from $z_{n,1}, \ldots, z_{n,d}$,

- 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

random variables



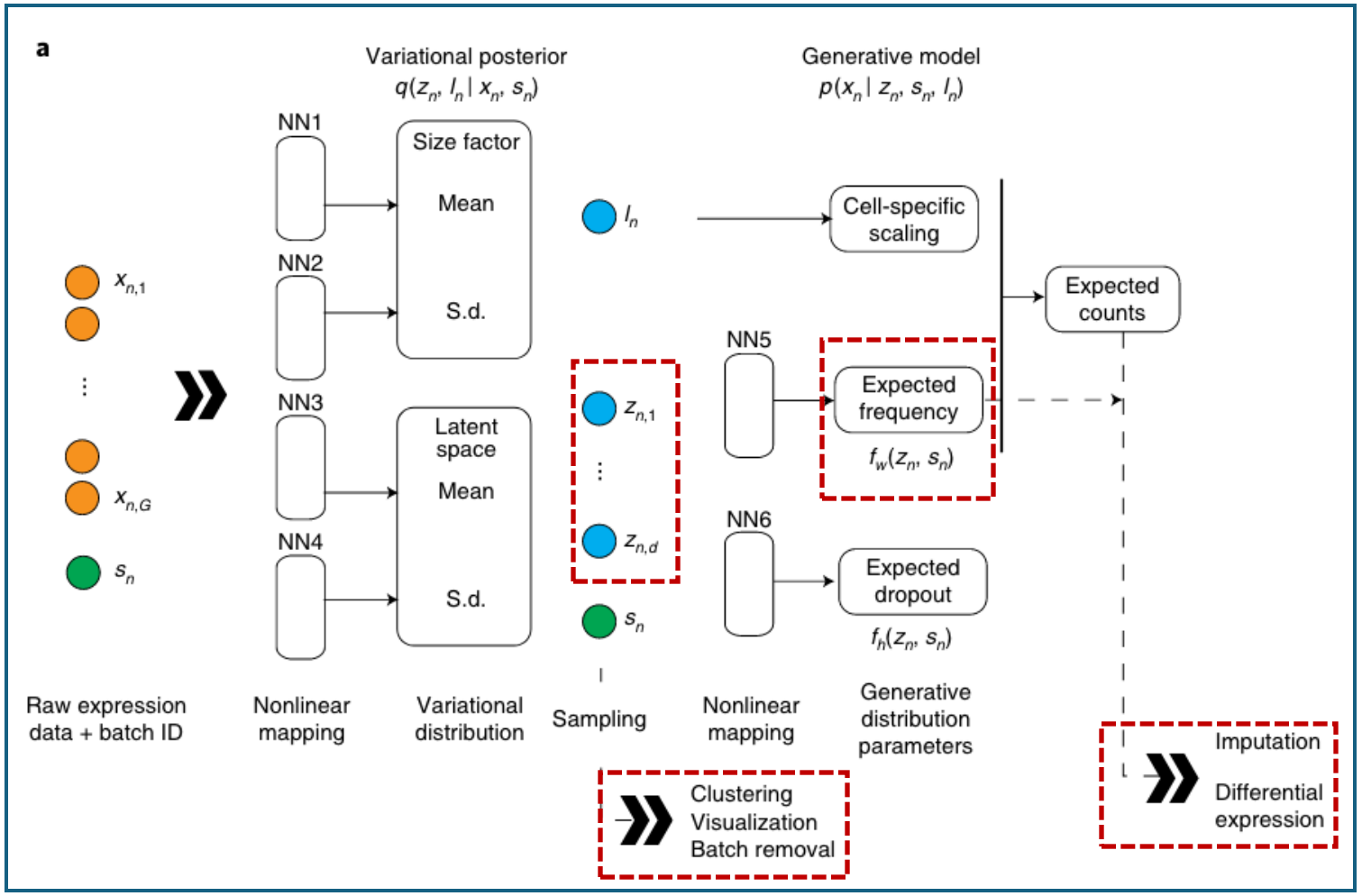
optional

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



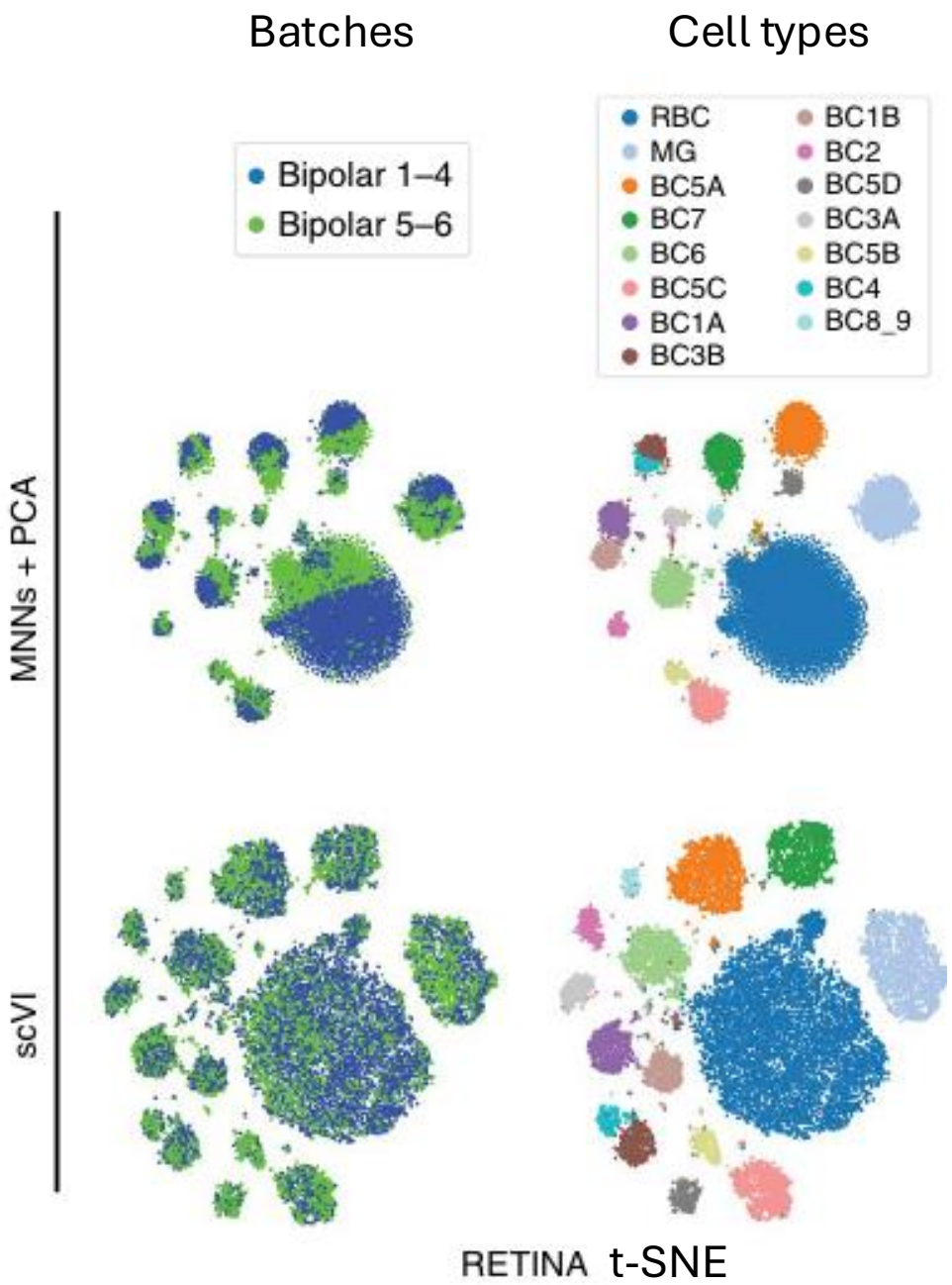
Batch correction

Data

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

Goal

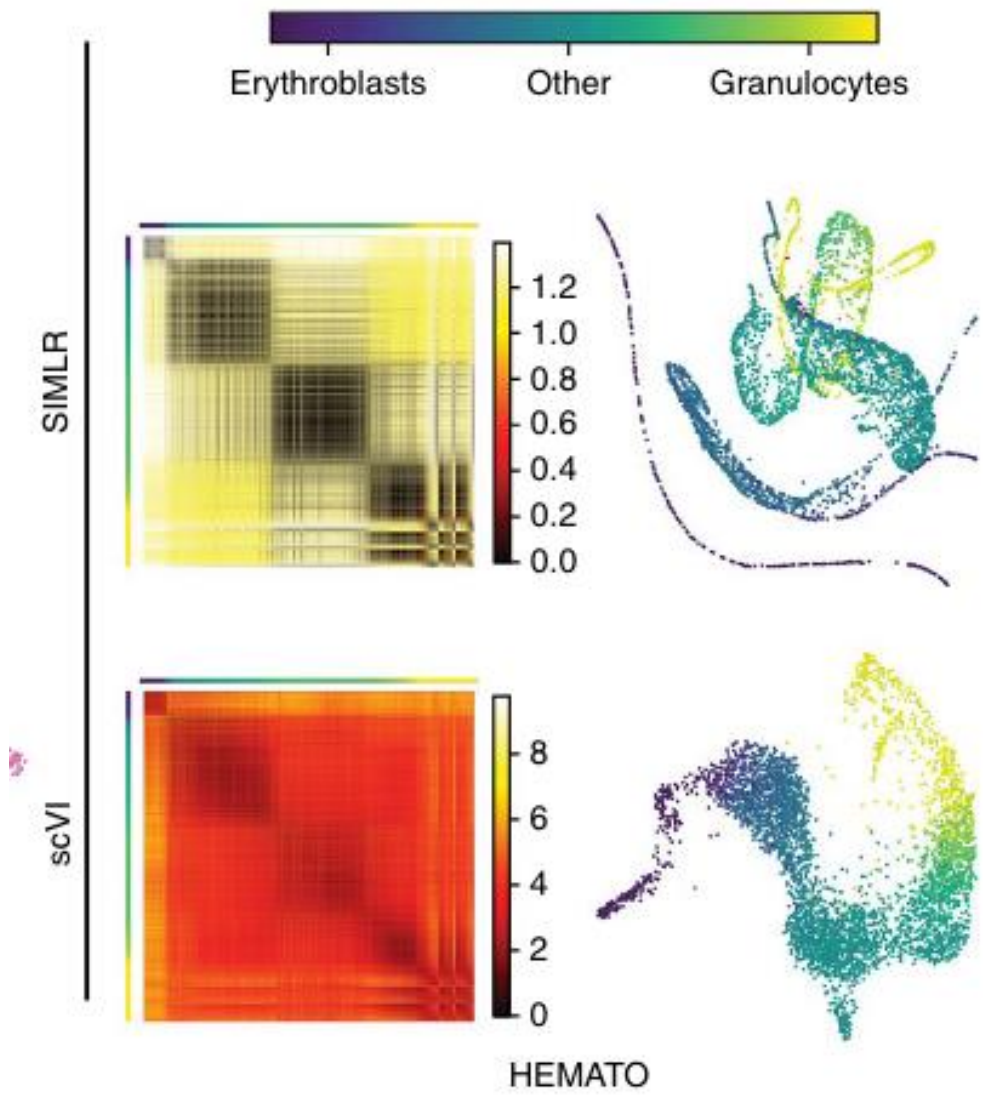
Comparing scVI and MNNs on batch correction



Preservation of continuous biology

HEMATO

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



Good. But not quite enough.

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

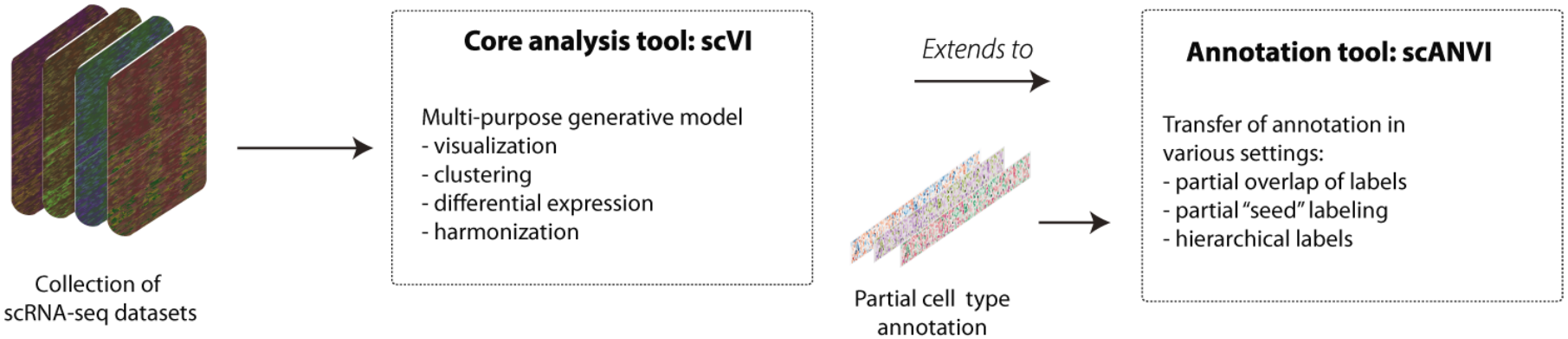
- Unsupervised batch correction is not always appropriate

- 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

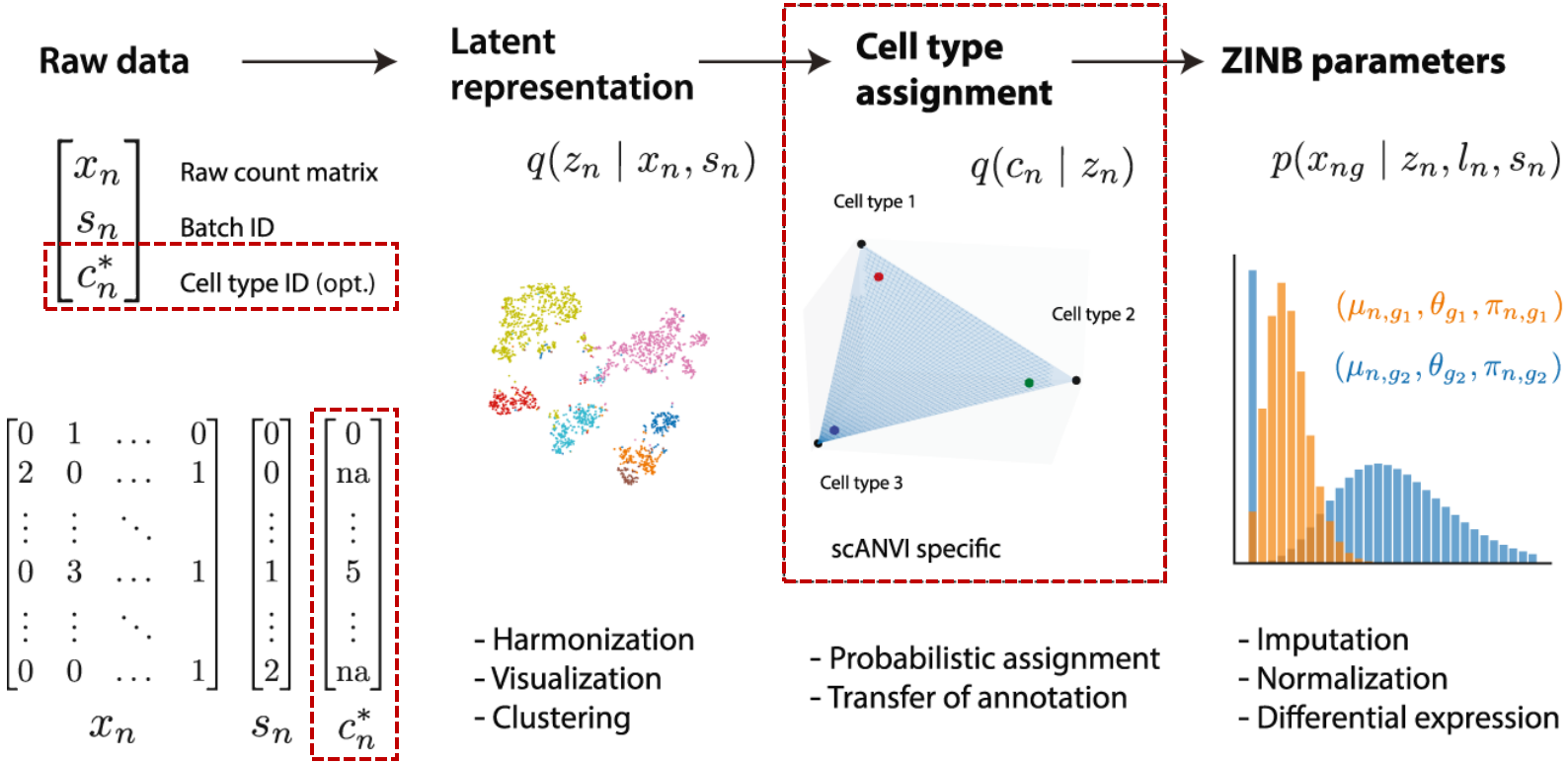


scANVI model

- 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



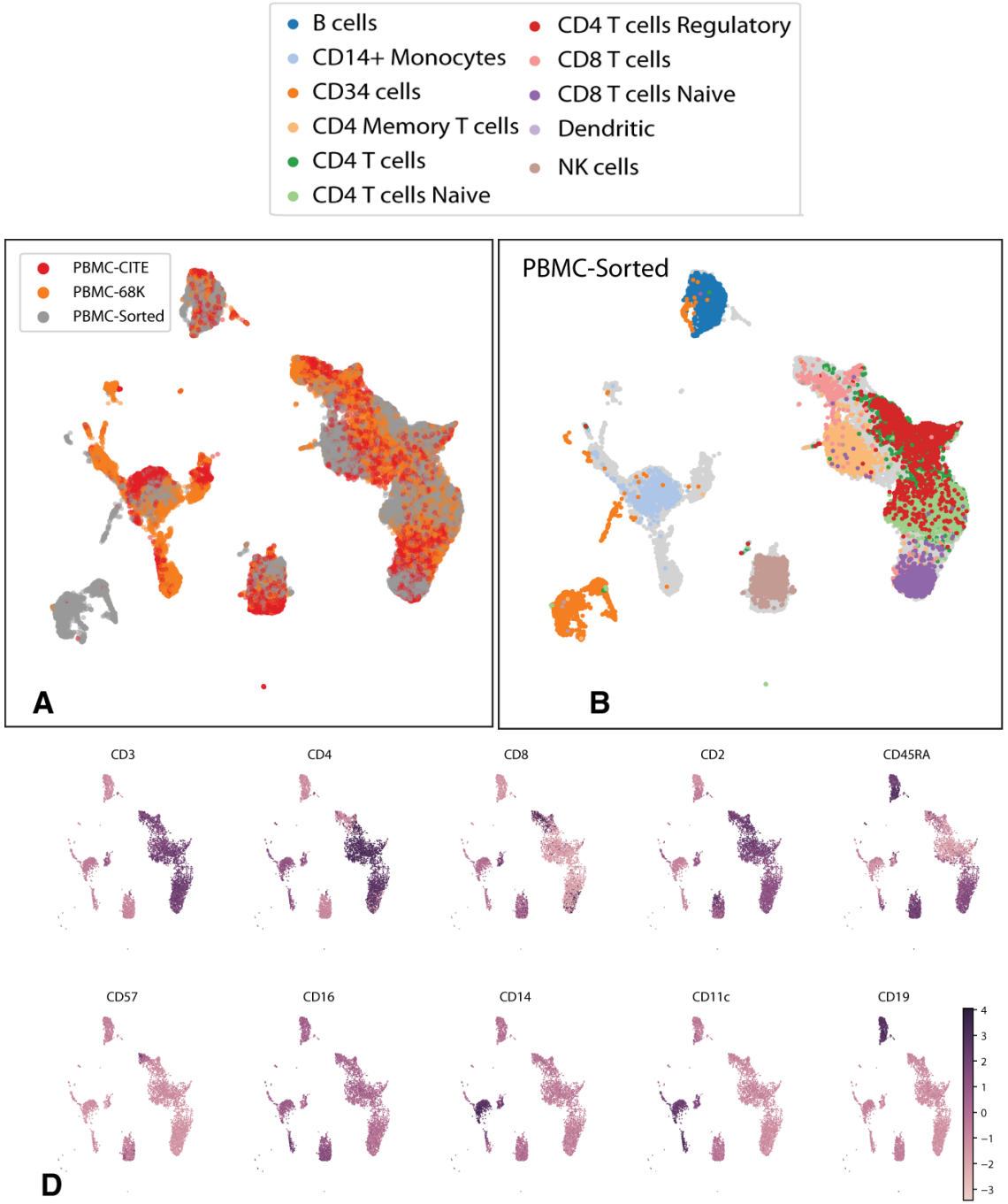
Case study

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)



Put them into use

Building large-scale cell atlas

Motivation

- Integrate large number of studies from hundreds of patients

Challenges

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

Why scANVI

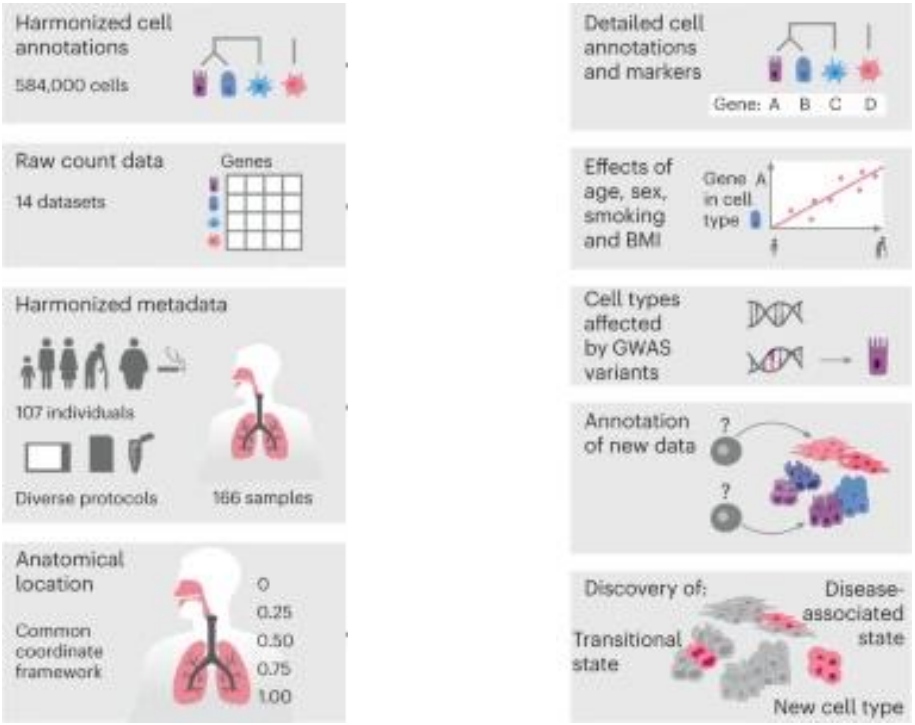
- Flexible and biology-preserving batch correction
- Computationally scalable
    - Takes longer to train compared to other methods, on smaller scale data
    - Computational time increases not fast when sample size increases
    - Reason: back propagation, small batch learning (no need to handle huge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

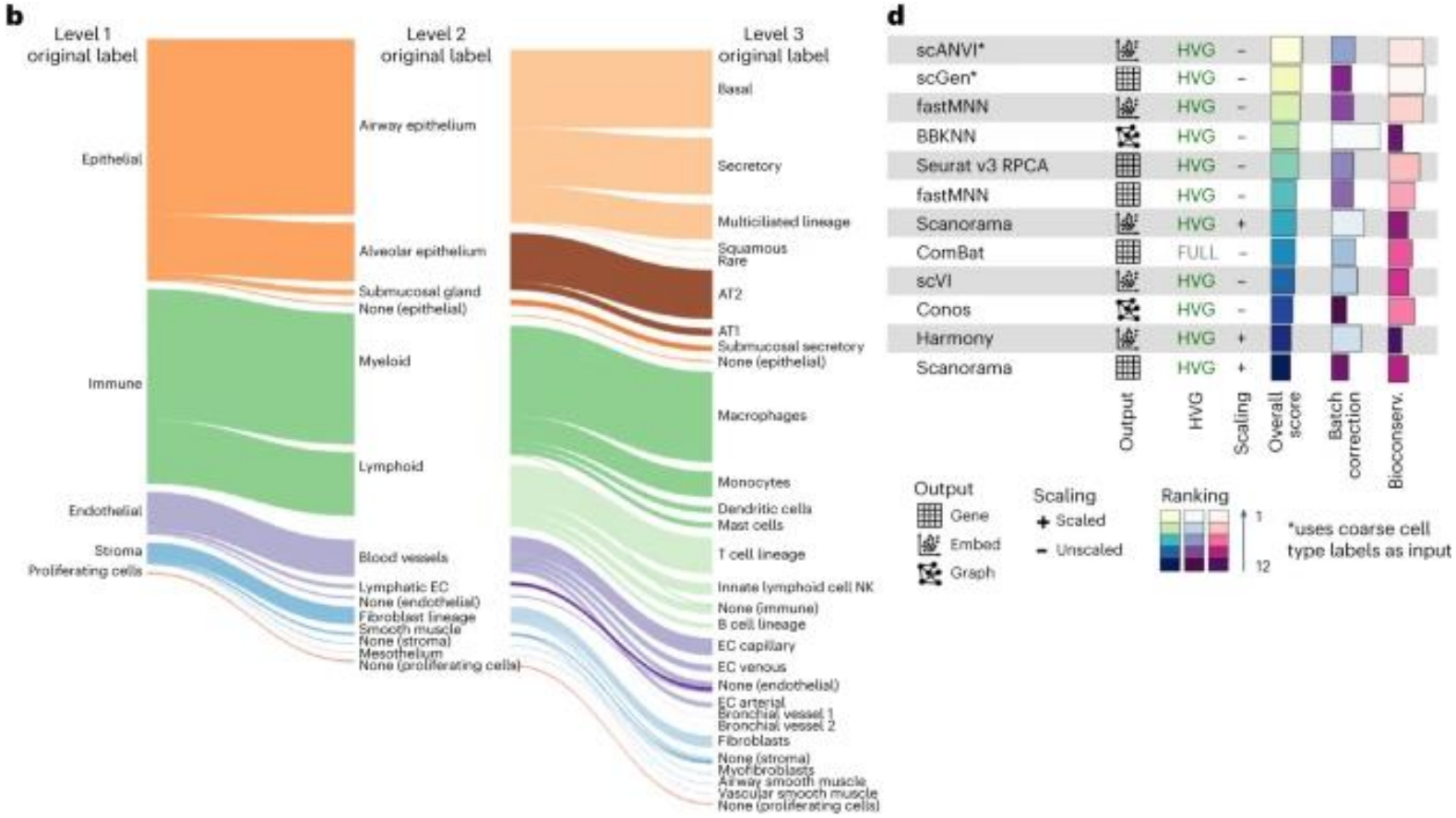
An integrated cell atlas of the lung in health and disease

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

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

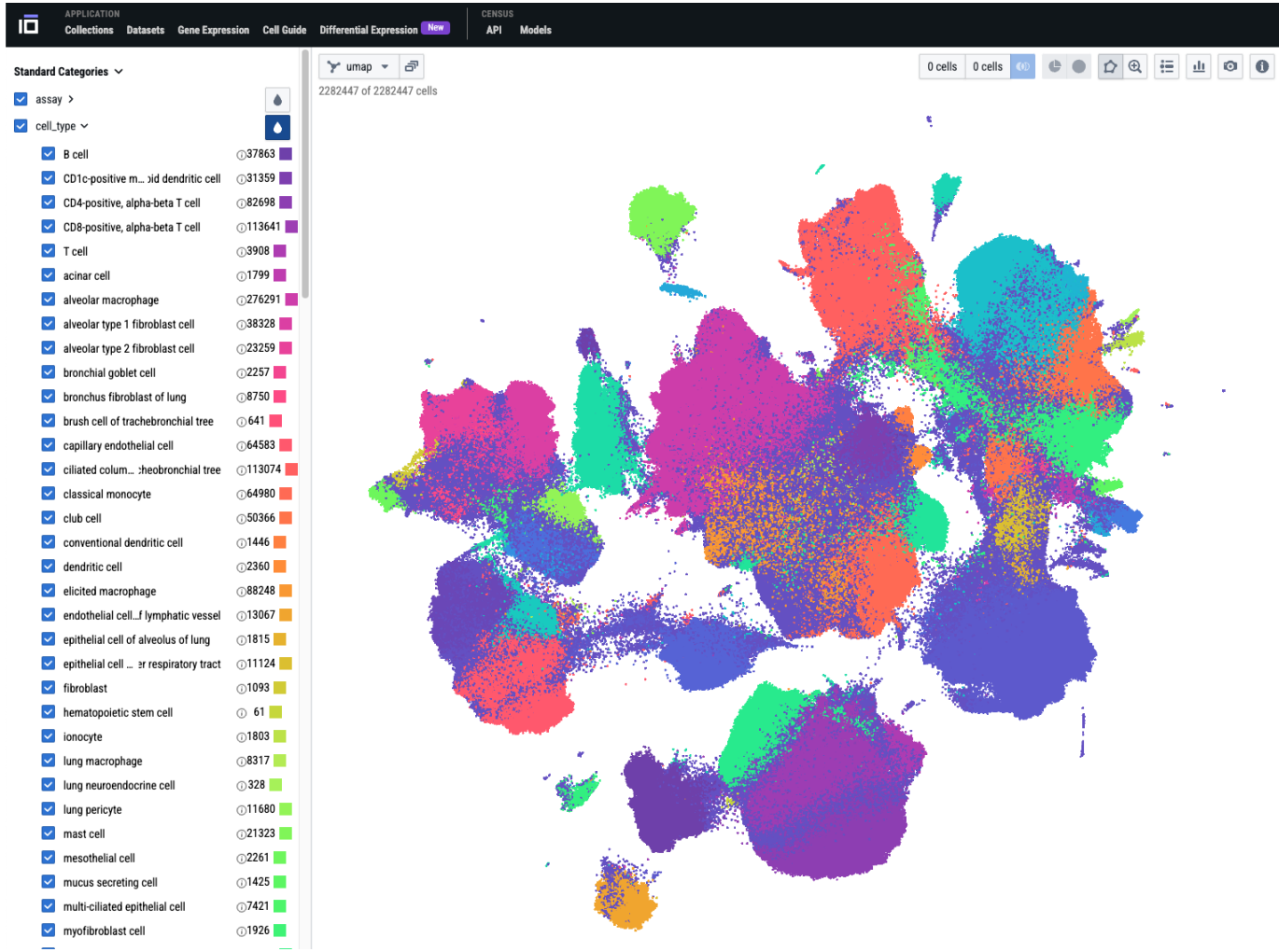
CZI Single-Cell Biology, Human Cell Atlas (HCA)

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

Mr Atlas on Collins Street