# Benchmarking Sample Representations from Single-Cell Data: Metrics for Biologically Meaningful Embeddings

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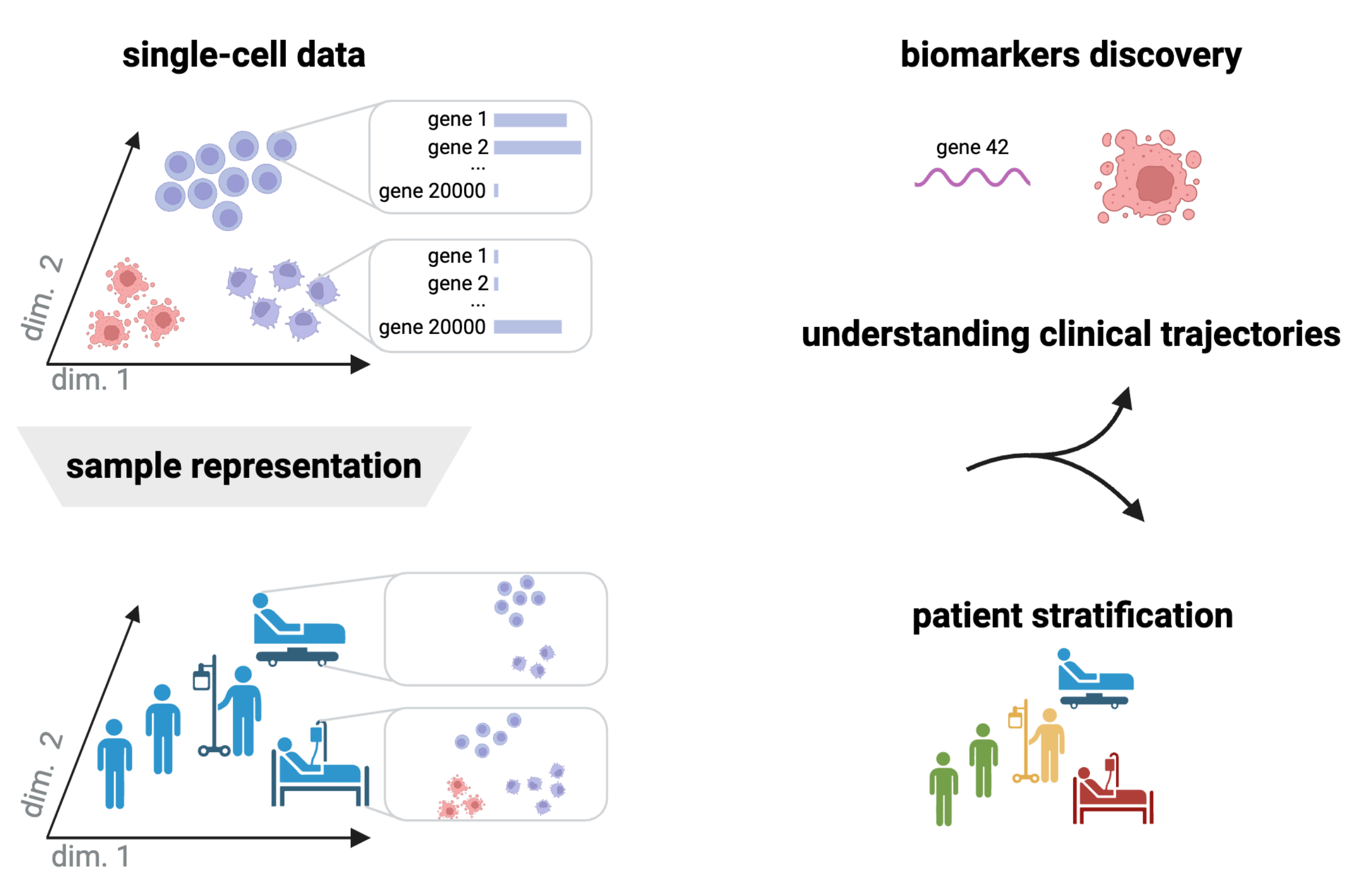
## From cells to humans with sample representation

**Single-cell data** enables understanding cell biology with unprecedented resolution

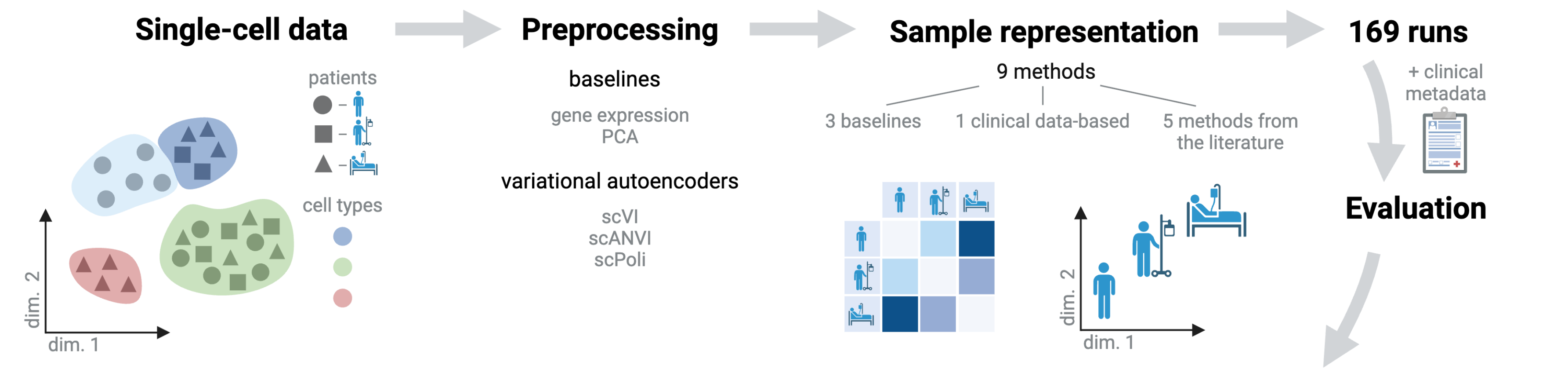


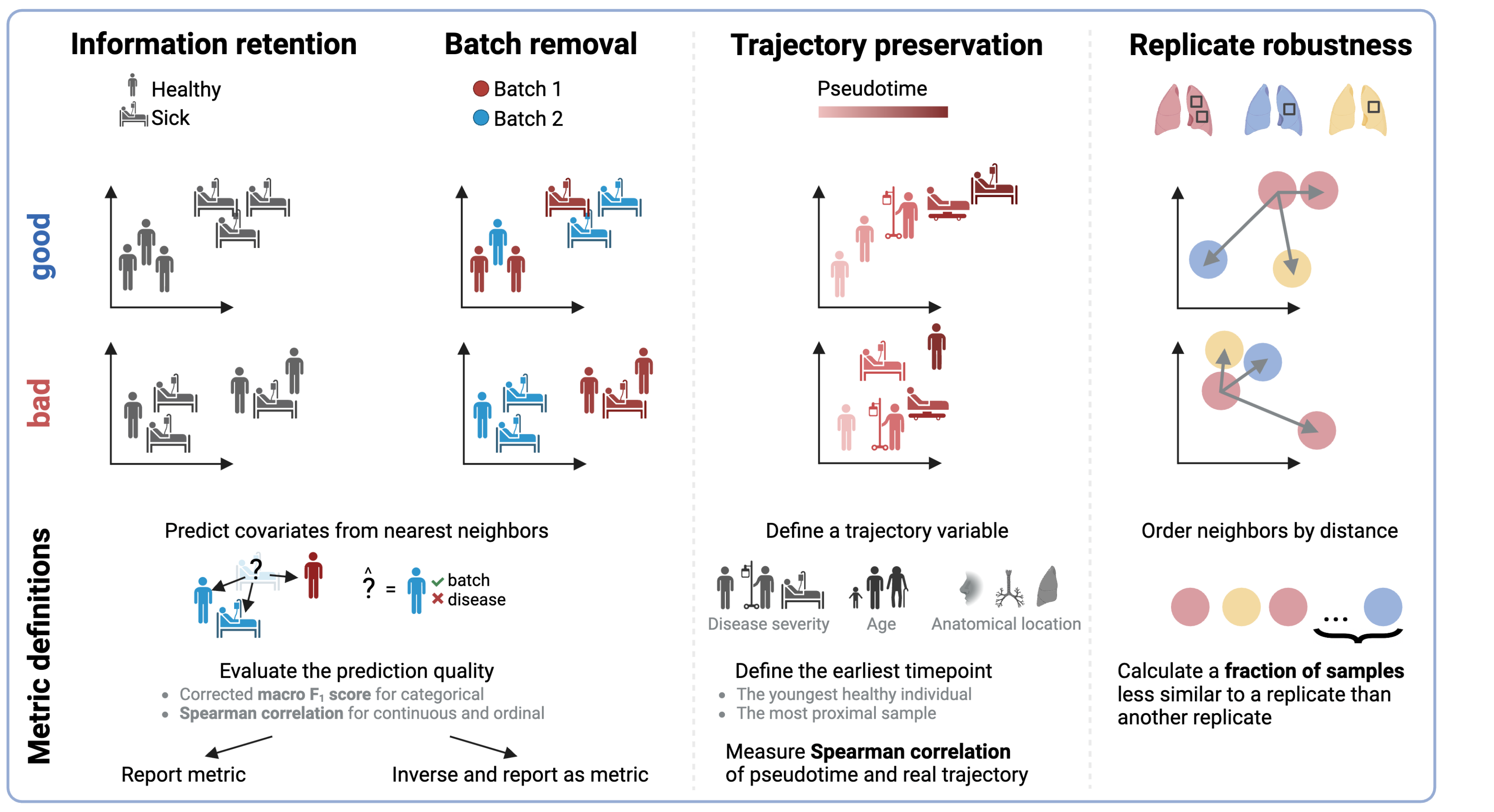
With datasets growing in size[1], it is now possible to analyze **sample-level variation**.

Several methods for sample representation are published[2-8], but a **consistent comparison** and **biologically relevant metrics** are still lacking.

We present a Single-cell-based Patient Representation Evaluation (SPARE) benchmark. We defined **4 application-inspired metrics**, and used these to compare **9 sample representation methods** on **5 datasets** from **2 tissues** comprising **4.5 million cells** and **1668 samples**.

## Establishing best practices for sample representation



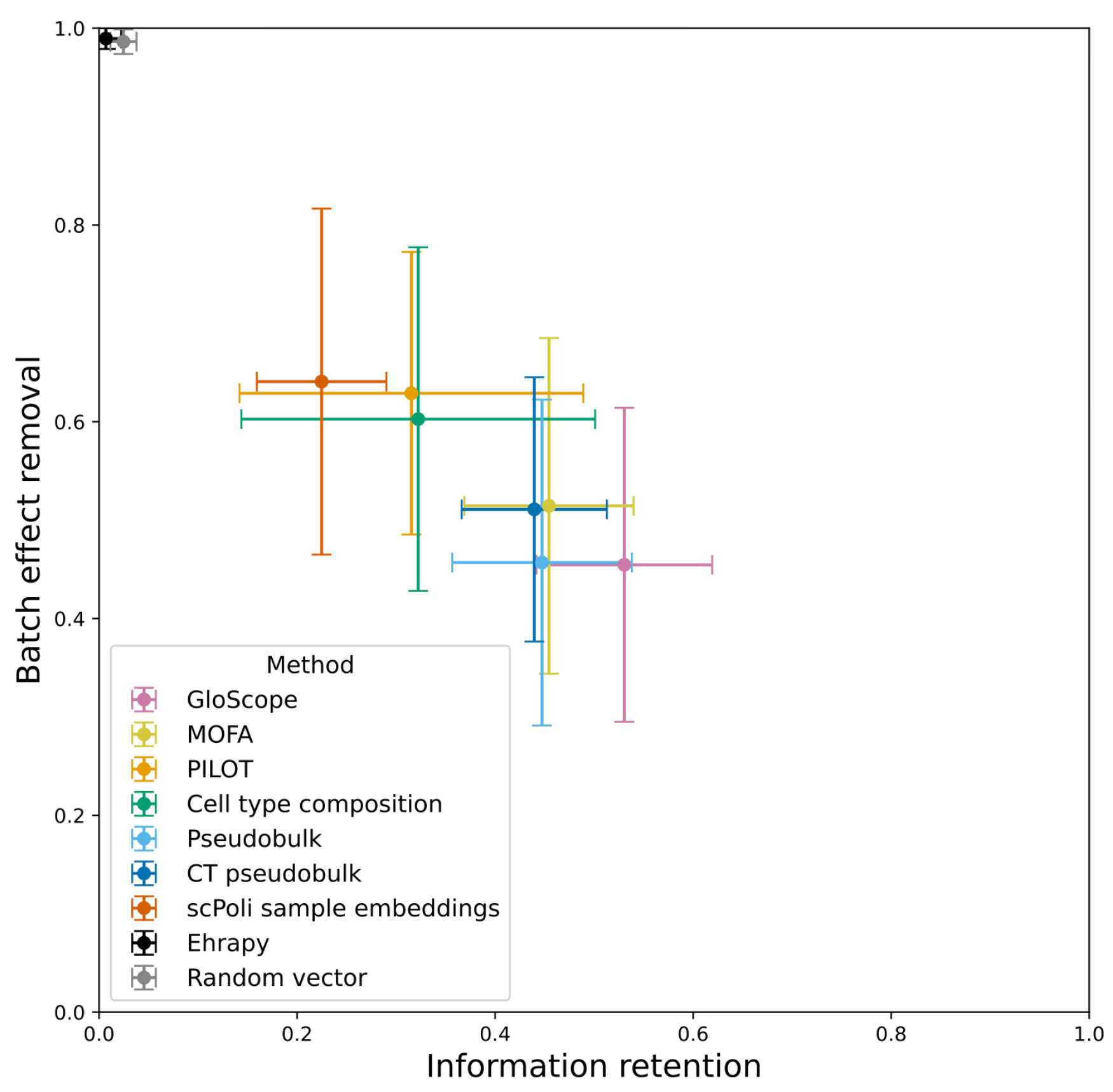

Information retention
Healthy
Sick
Batch removal
Batch 1
Batch 2
Trajectory preservation
Pseudotime
Replicate robustness
good
bad
Metric definitions
Predict covariates from nearest neighbors
?
$\hat{?}$ =
✓ batch
✗ disease
Evaluate the prediction quality
Corrected macro $F_1$ score for categorical
Spearman correlation for continuous and ordinal
Report metric
Inverse and report as metric
Define a trajectory variable
Disease severity
Age
Anatomical location
Define the earliest timepoint
The youngest healthy individual
The most proximal sample
Measure Spearman correlation of pseudotime and real trajectory
Order neighbors by distance
...
Calculate a fraction of samples less similar to a replicate than another replicate

## Density-based method, GloScope, often performs the best

| Dataset | Representation | Information retention | Batch removal | Replicate robustness | Trajectory preservation | Total |
|---|---|---|---|---|---|---|
| COMBAT | scPoli – GloScope | **0.37** | 0.57 | – | **0.79** | **0.58** |
| | scANVI$_s$ – GloScope | 0.31 | 0.47 | – | 0.71 | 0.50 |
| | scANVI$_b$ – CT pseudobulk | 0.24 | **0.70** | – | 0.62 | 0.48 |
| | counts – MOFA | 0.23 | 0.53 | – | 0.03 | 0.21 |
| Stephenson | scVI$_s$ – MOFA | **0.48** | 0.47 | – | **0.45** | **0.47** |
| | scVI$_b$ – MOFA | 0.47 | 0.44 | – | 0.45 | 0.45 |
| | scANVI$_b$ – MOFA | 0.41 | 0.42 | – | 0.45 | 0.43 |
| | scPoli – Pseudobulk | 0.06 | **0.58** | – | 0.07 | 0.17 |
| Onek1k | scPoli – GloScope | 0.60 | 0.55 | – | 0.41 | **0.52** |
| | scANVI$_s$ – GloScope | **0.63** | 0.47 | – | **0.42** | 0.51 |
| | Cell type composition | 0.54 | 0.65 | – | 0.40 | 0.50 |
| | scPoli – MOFA | 0.00 | **0.97** | – | 0.00 | 0.20 |
| HLCA | scVI$_b$ – Pseudobulk | **0.54** | 0.36 | – | **0.81** | **0.61** |
| | scANVI$_b$ – Pseudobulk | 0.48 | 0.46 | – | 0.81 | 0.61 |
| | scVI$_s$ – Pseudobulk | 0.54 | 0.37 | – | 0.80 | 0.61 |
| | Ehrapy | 0.00 | **0.98** | – | 0.07 | 0.23 |
| COPD | PCA – GloScope | **0.54** | 0.69 | **0.98** | 0.26 | **0.61** |
| | scANVI$_b$ – CT pseudobulk | 0.48 | 0.61 | 0.96 | **0.33** | 0.59 |
| | scANVI$_b$ – GloScope | 0.47 | 0.61 | 0.99 | 0.32 | 0.59 |
| | Random vector$_{10}$ | 0.01 | **0.97** | 0.37 | 0.02 | 0.25 |

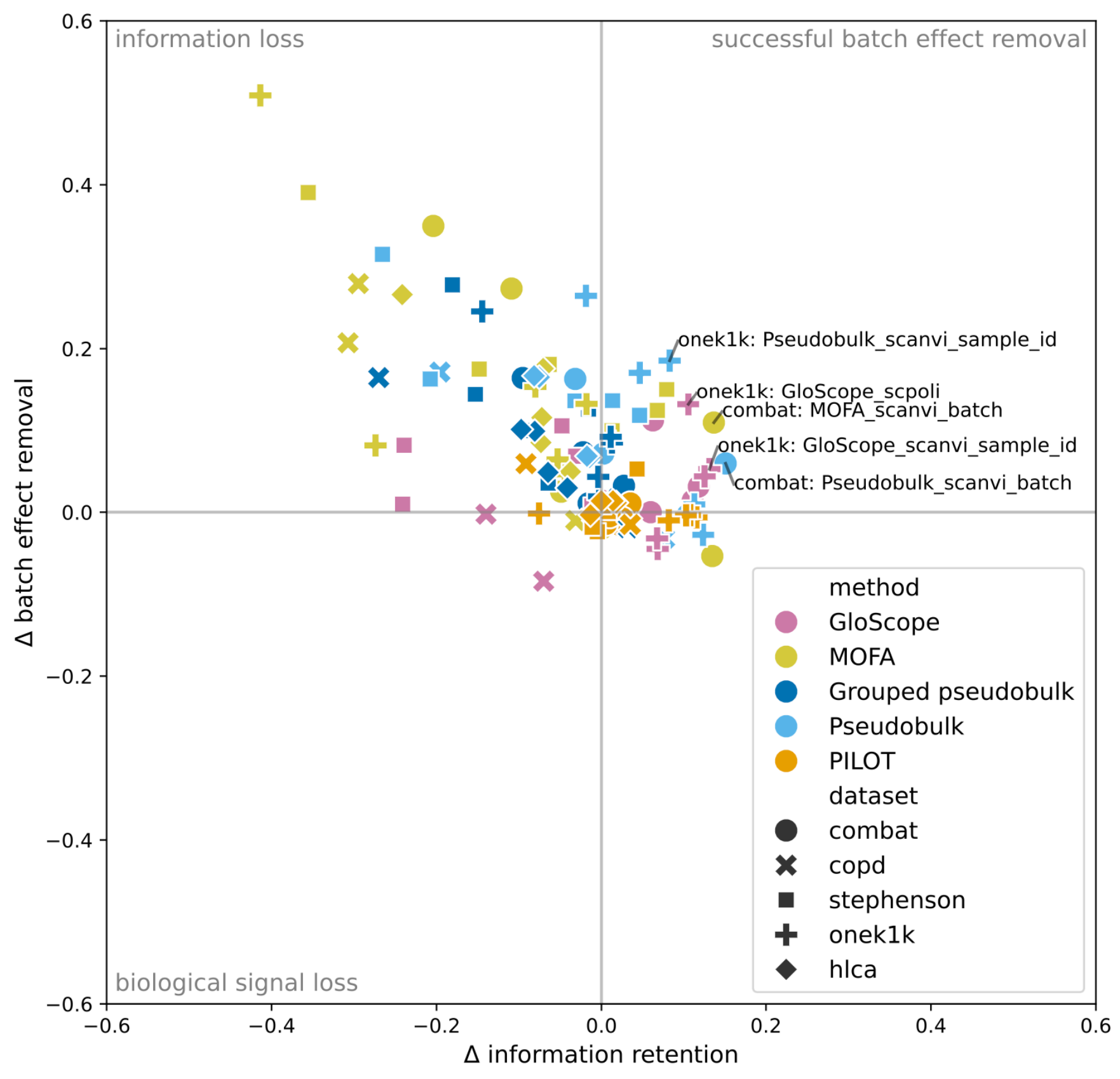
Top **3 best** and **1 worst representation** per dataset according to the total score. Representation names consist of input space (where applicable) and sample representation method. sc[AN]VI$_{b/s}$ refers to a sc[AN]VI model trained with the batch covariate “batch” or "sample" to integrate the data. Total score is weighted average with the Batch removal score weighted half as much as others.

## There is a trade-off between Information retention and Batch removal



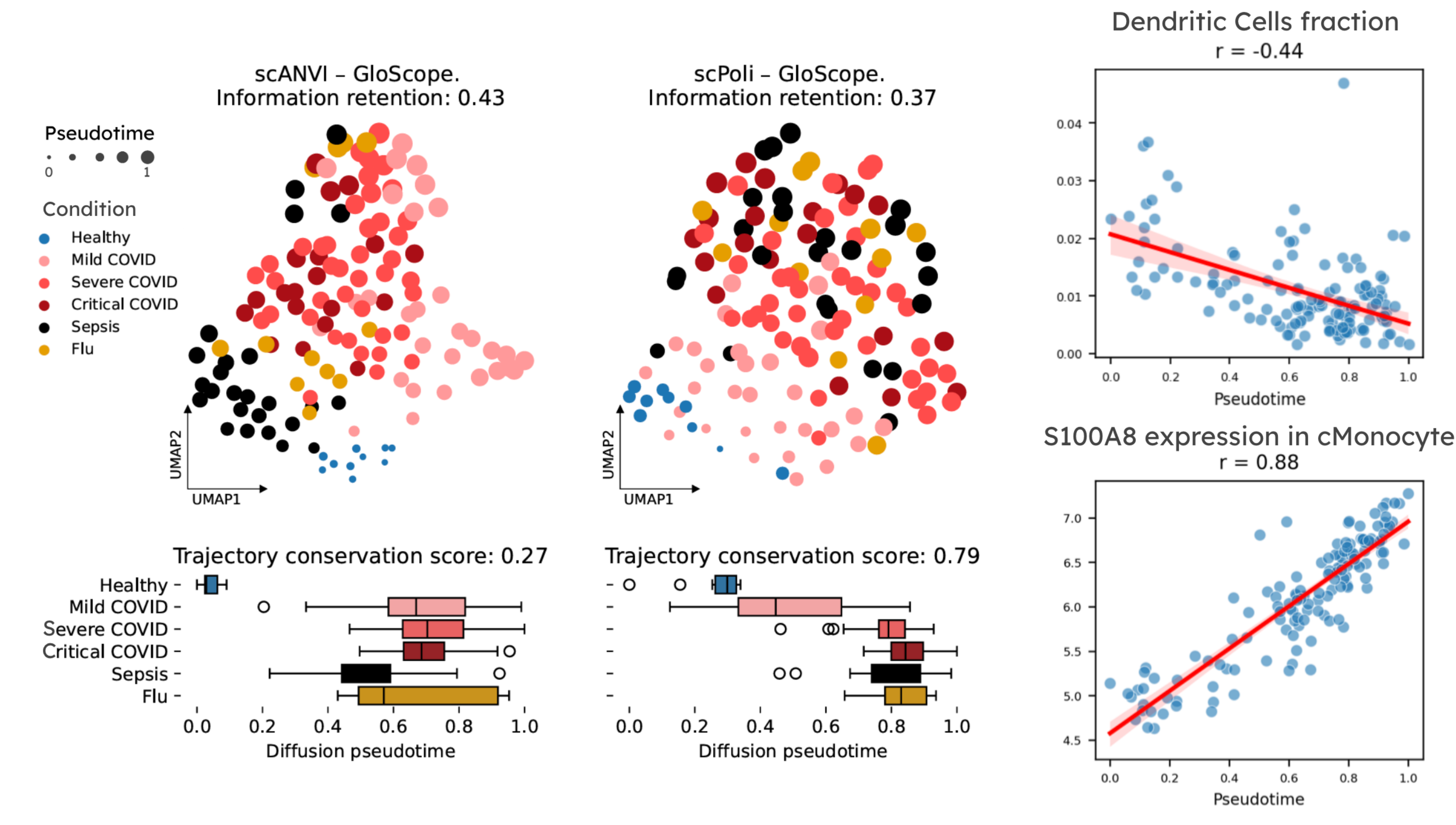
Mean and standard deviation across datasets are shown for the best sample representation from each method

## Batch correction improves sample representation



Differences in information retention and batch removal scores for each method in comparison to PCA-based representation with the same method.

## Best COMBAT representation reveals markers of COVID-19 severity



Left: best representation according to the Information retention score, middle: best representation according to the Trajectory conservation score (and overall). Right: top pseudotime-correlated cell type fraction and gene expression in a cell type.

## Datasets

| Dataset | COMBAT | Stephenson | Onek1k | HLCA | COPD |
|---|---|---|---|---|---|
| **#donors** | 140 | 130 | **982** | 344 | 61 (72 samples) |
| **#cells** | 784k | 639k | 1.25M | **1.68M** | 176k |
| **Tissue** | PBMC | PBMC | PBMC | Lung and airways | Lung parenchyma |
| **Relevant information** | Condition, Severity, Death in 28 days, Duration | Condition, Severity, Outcome, Duration | Age | Tissue anatomical location, Condition, Smoking status | Severity, Lung function tests, Progression |
| **Technical covariates** | Institute, Pool ID | Site | Sex | Suspension type, Fresh or frozen, Sequencing platform, Assay | Batch, Lung lobe, Cancer |

## Code and paper

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