Lecture 4

Identifying cell populations

Physalia course 2024

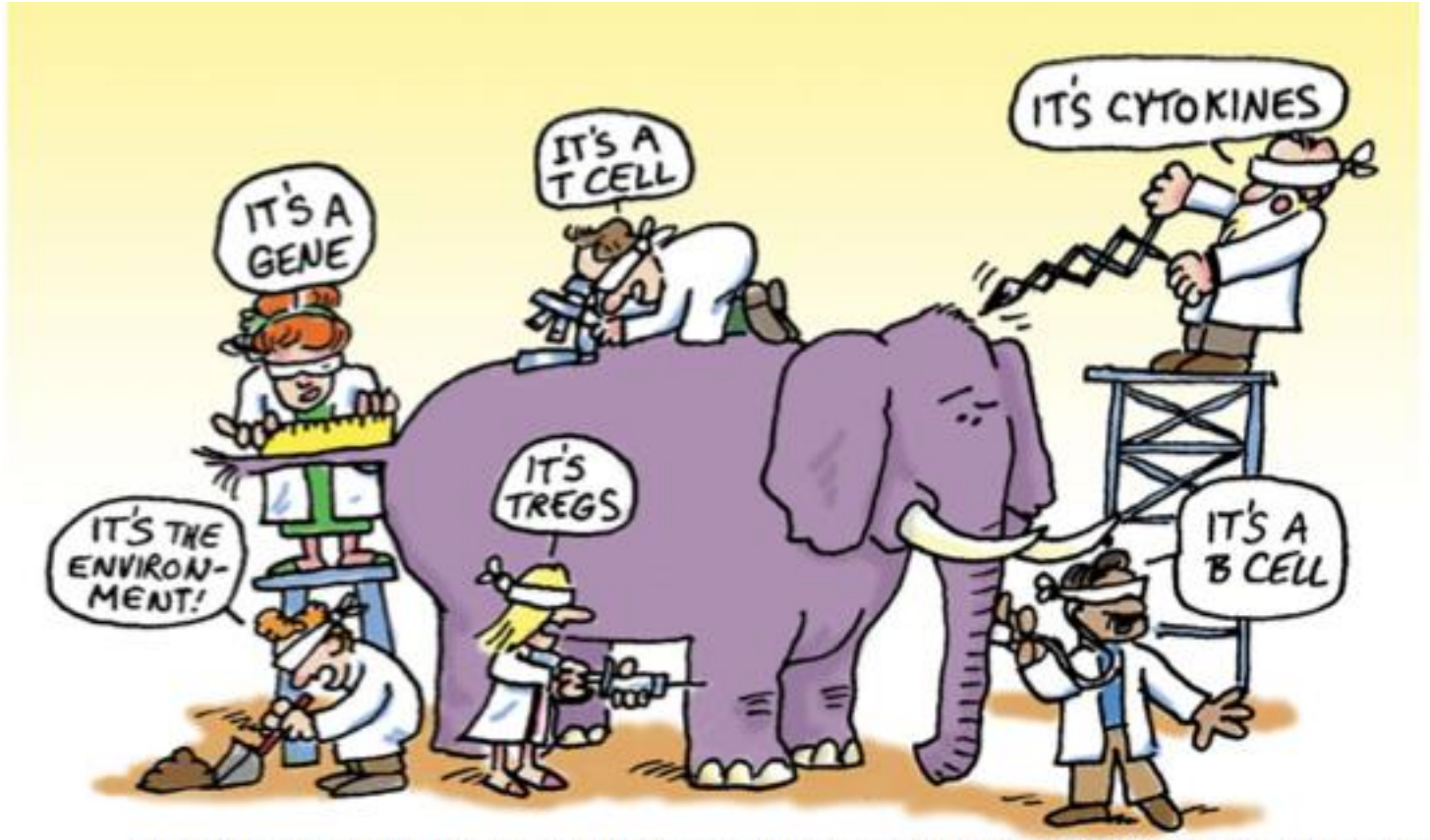
—

Single-cell RNA-seq with R/Bioconductor

Instructors: Orr Ashenberg, Jacques Serizay, Fabrício Almeida-Silva

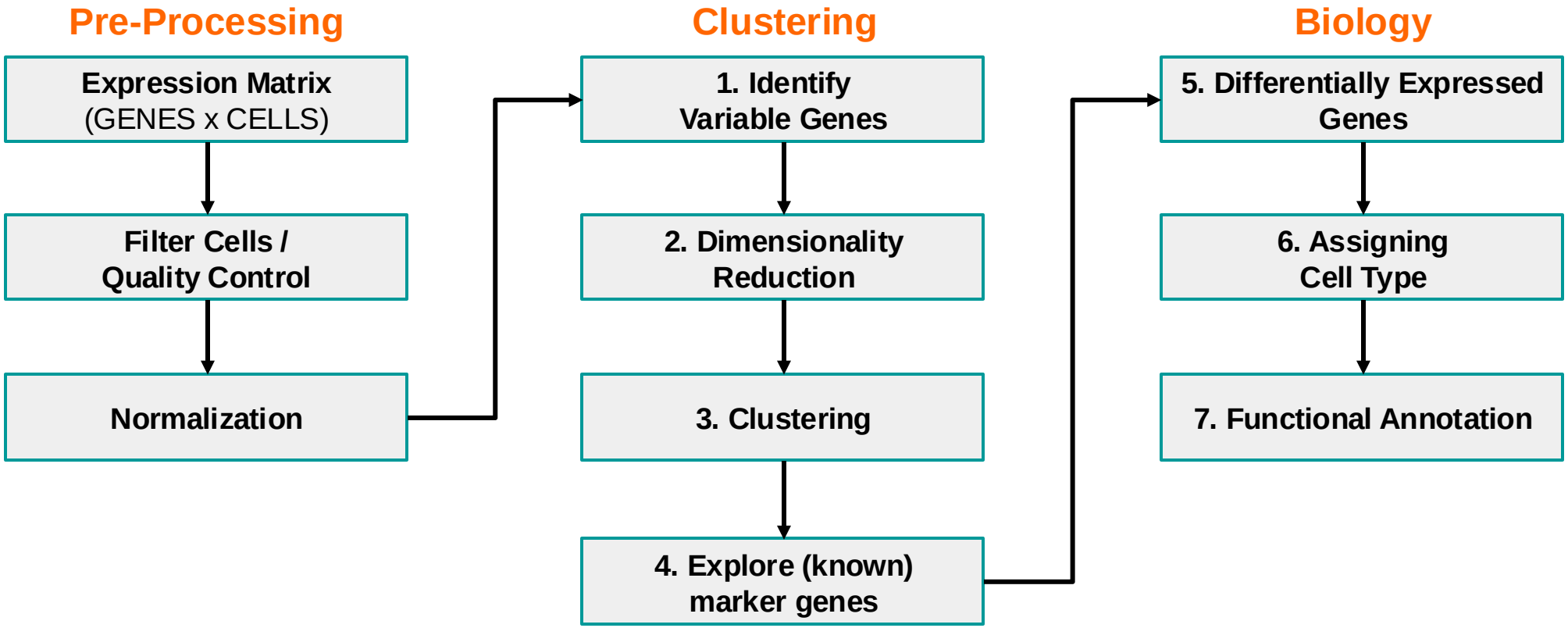
How Do We Define Cellular Identity?

A cell participates in multiple processes/contexts.



Herold, K. C., & Bluestone, J. A. (2011). Type 1 diabetes immunotherapy: is the glass half empt or half full?. *Science translational medicine*, *3*(95), 95fs1-95fs1.

Analysis workflow



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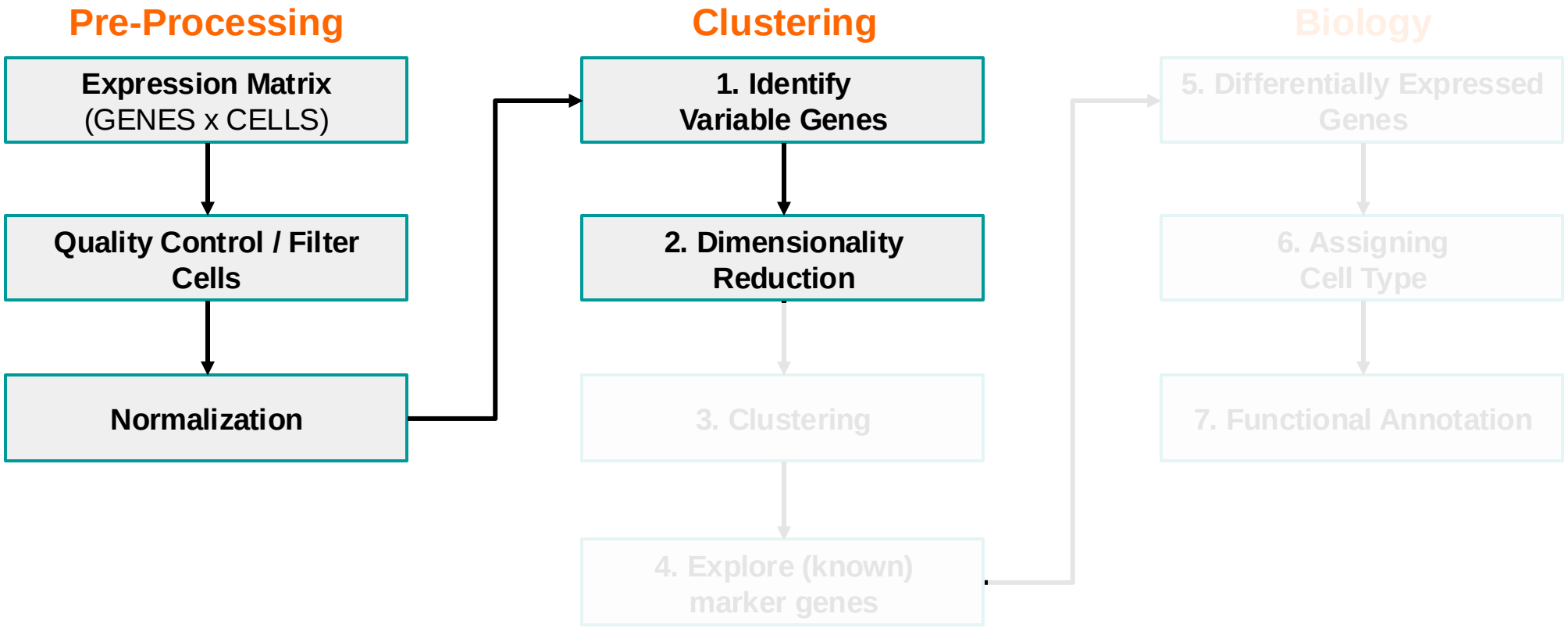

Dimensional reduction in scRNAseq studies

High-dimensional data can be difficult to interpret.

One approach to simplification is to **assume that the data of interest lies within lower-dimensional space**. If the data of interest is of low enough dimension, the data can be visualised in the low-dimensional space.

- A scRNA seq starts with many measurements (features, genes).
- We want to reduce it to fewer informative dimensions.
- We have starting doing this by using only highly variable genes.
- We can further reduce dimension with linear or non-linear approaches.

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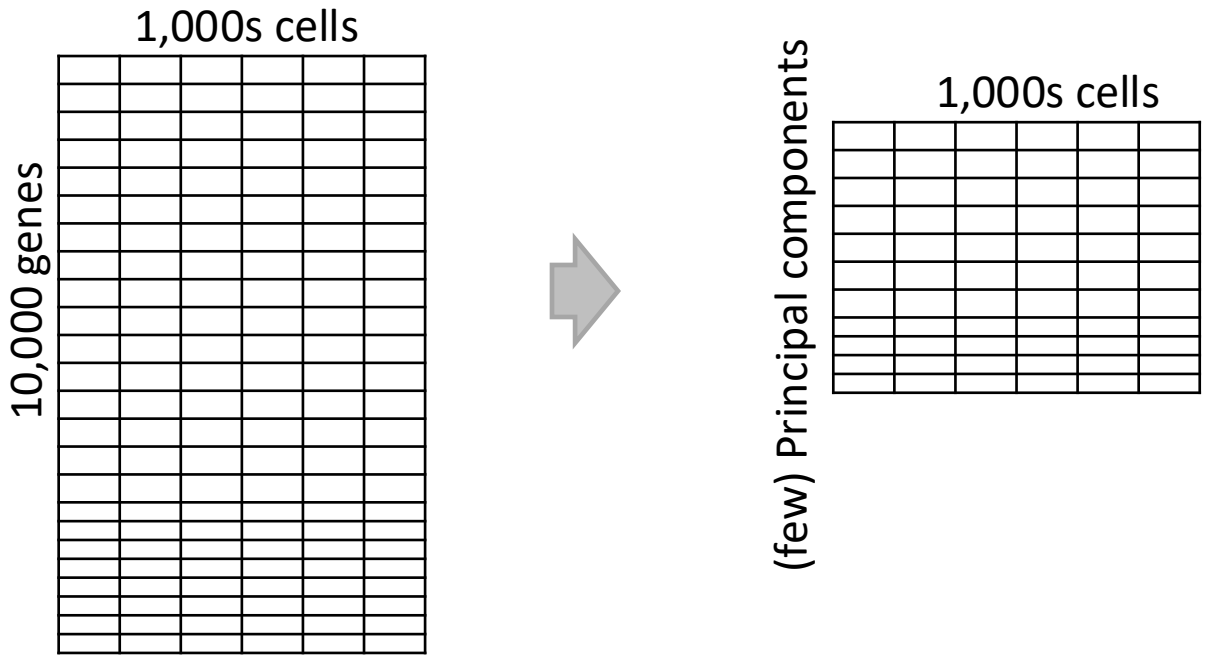
Common Techniques

- Principal Component Analysis (PCA)
- Independent Component Analysis (ICA)
- Multidimensional Scaling (MDS)
- Non-negative Matrix Factorization (NMF)
- Probabilistic Modeling (e.g. Latent Dirichlet Allocation - LDA)

Principal Component Analysis

- PCA is a dimensionality reduction method that transforms a **set of features** into a set of **linearly uncorrelated variables** called principal components

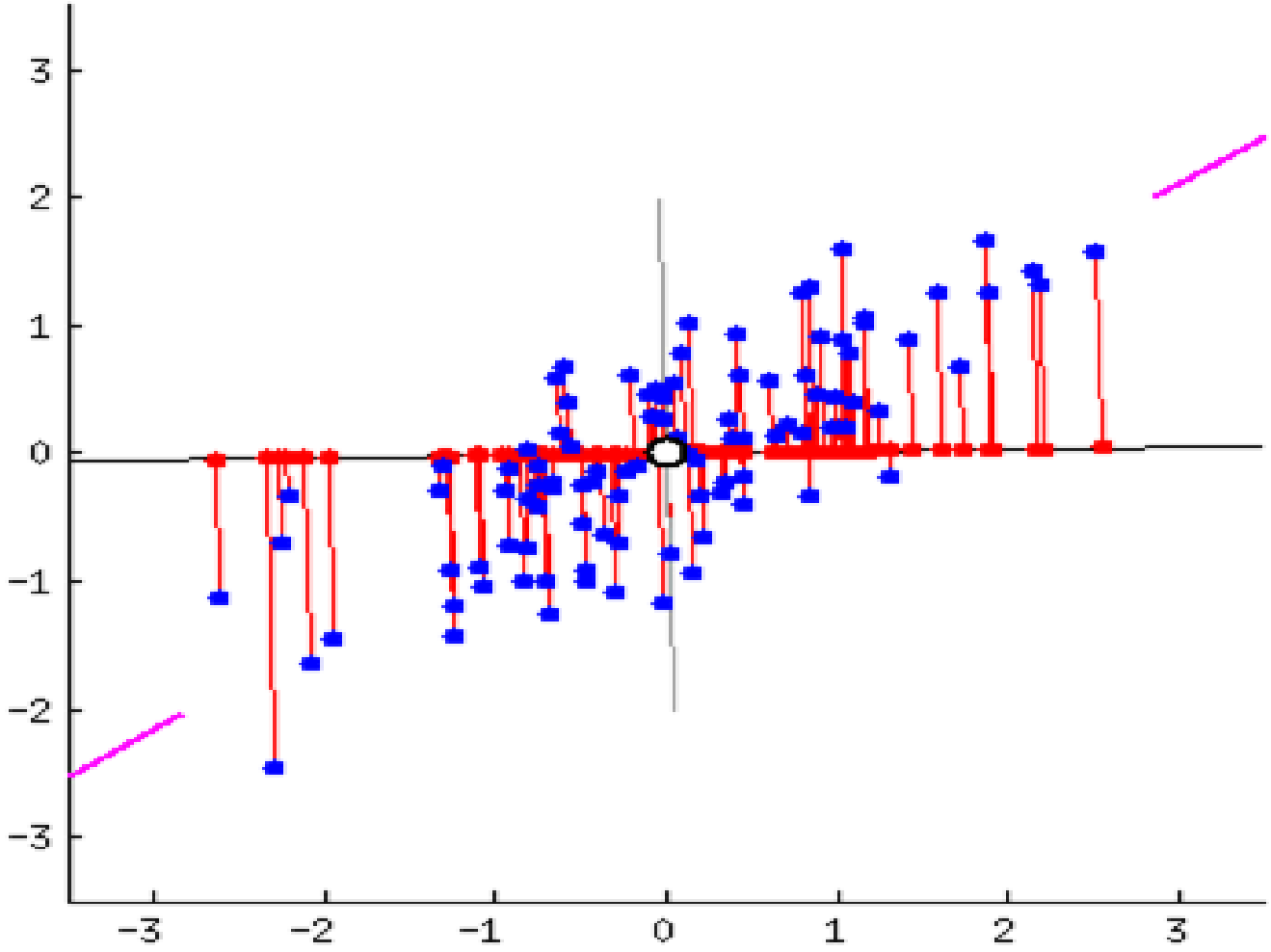


https://stats.stackexchange.com/questions/2691/making-sense-of-principal-component-analysis-eigenvectors-eigenvalues

Principal Component Analysis

- PCA is a dimensionality reduction method that transforms a **set of features** into a set of **linearly uncorrelated variables** called principal components

- The first principal component contains the most variance, and each component after contains as much variance while still being orthogonal to other components



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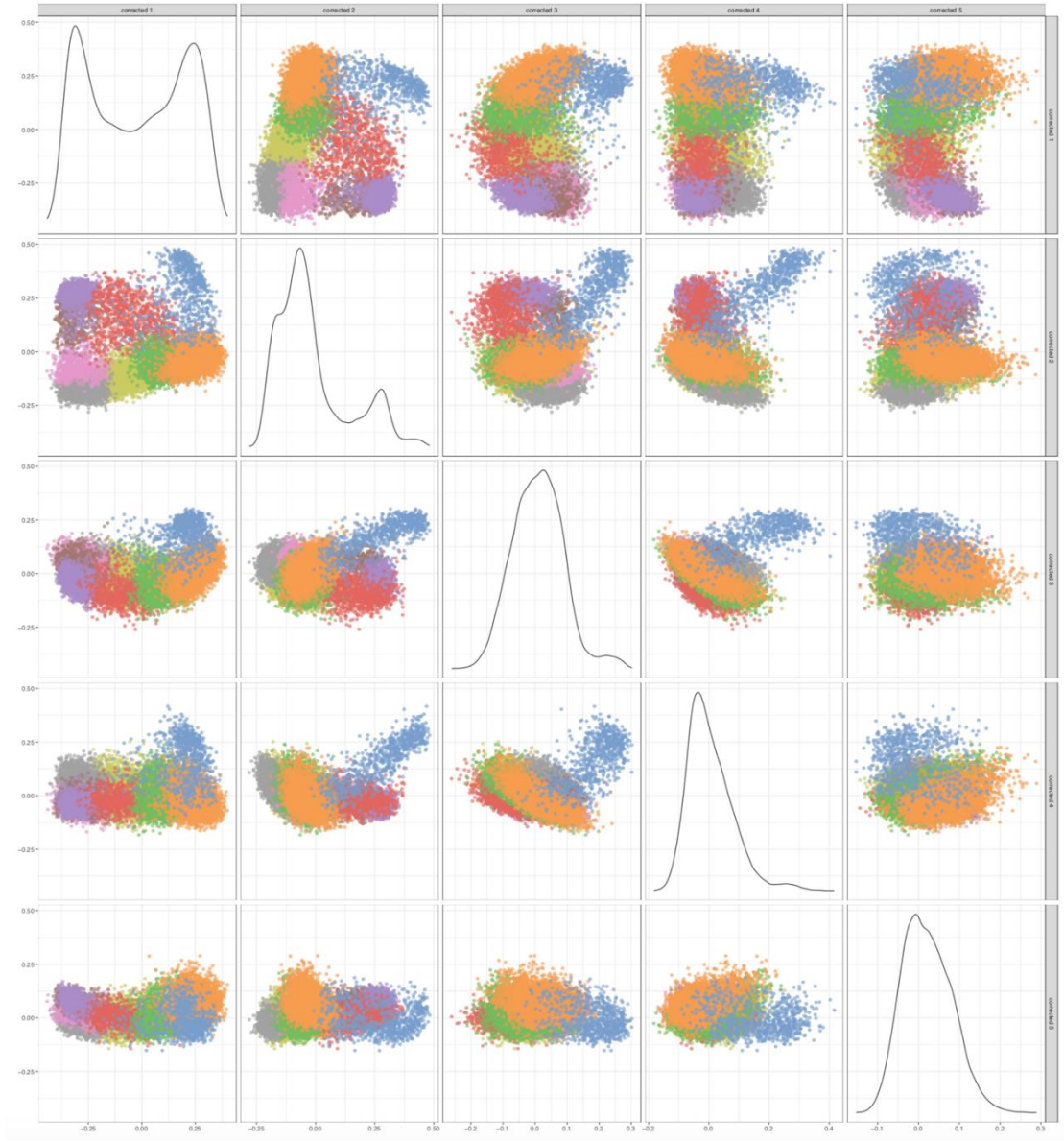
Principal Component Analysis: assessing lower dimensions

Notice how lower PCs look more and more "spherical" - this loss of structure indicates that the variation captured by these PCs mostly reflects noise.



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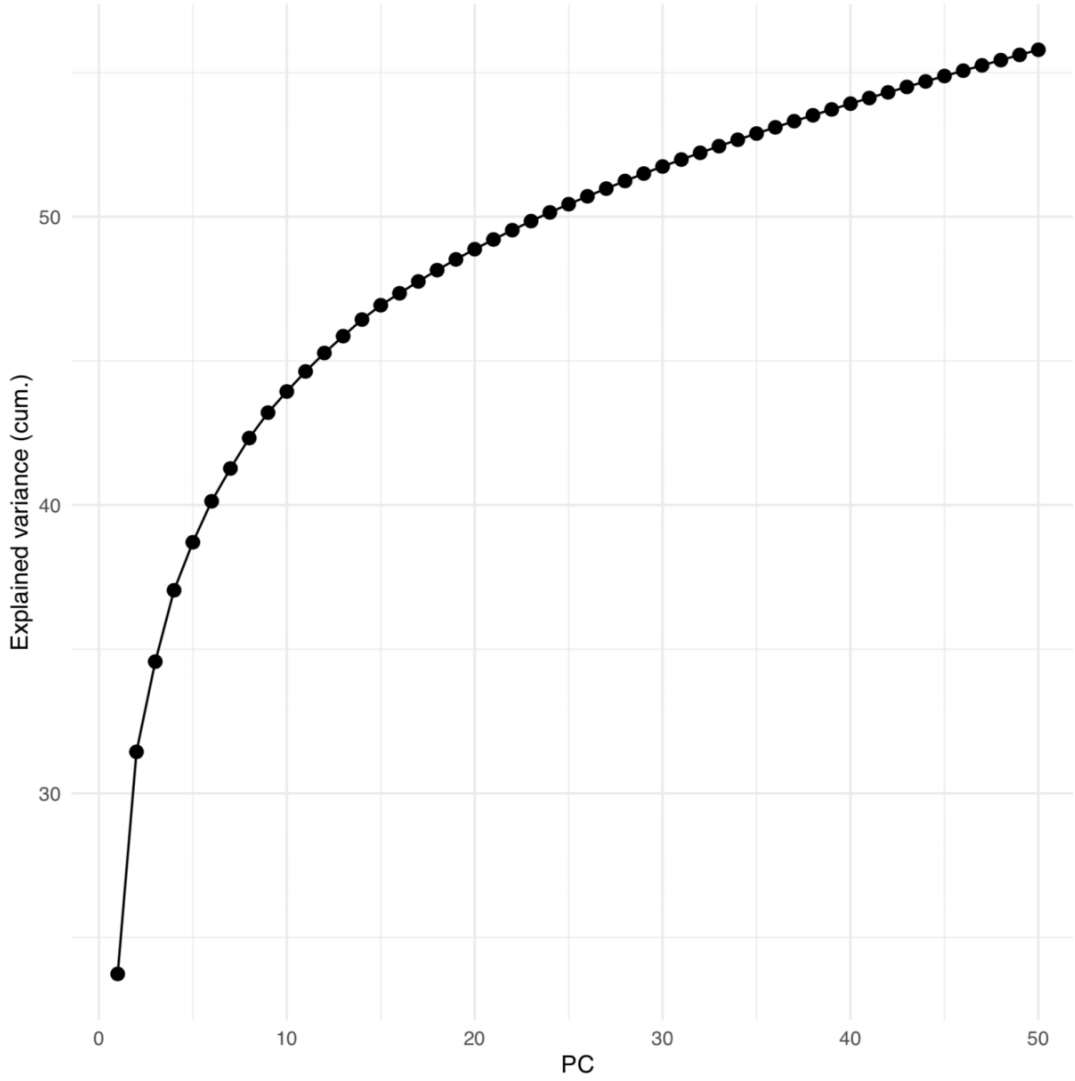
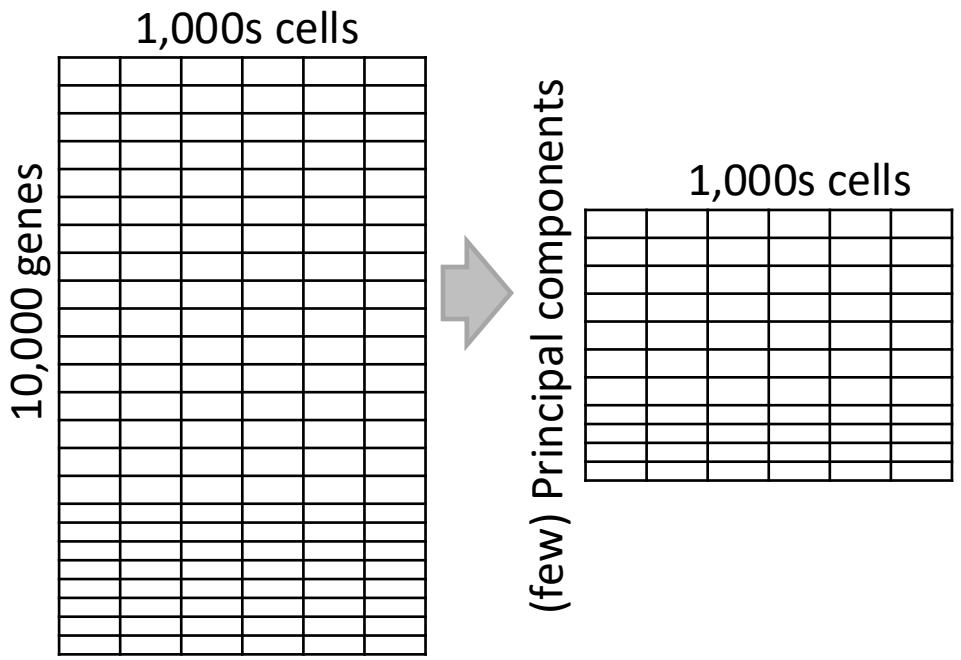
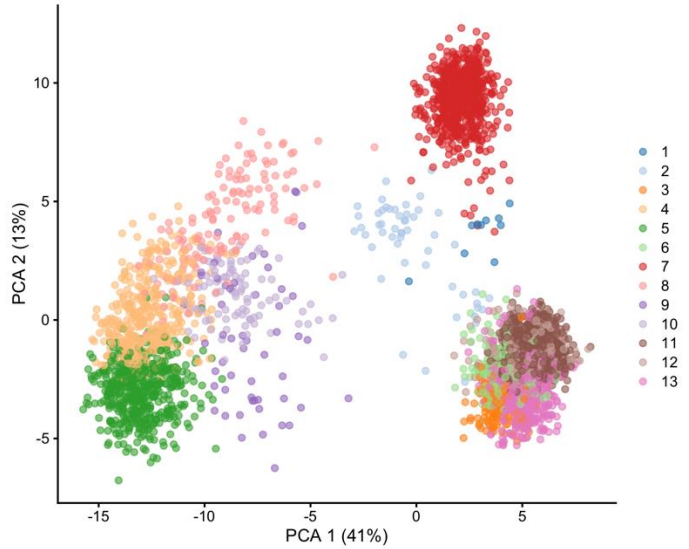
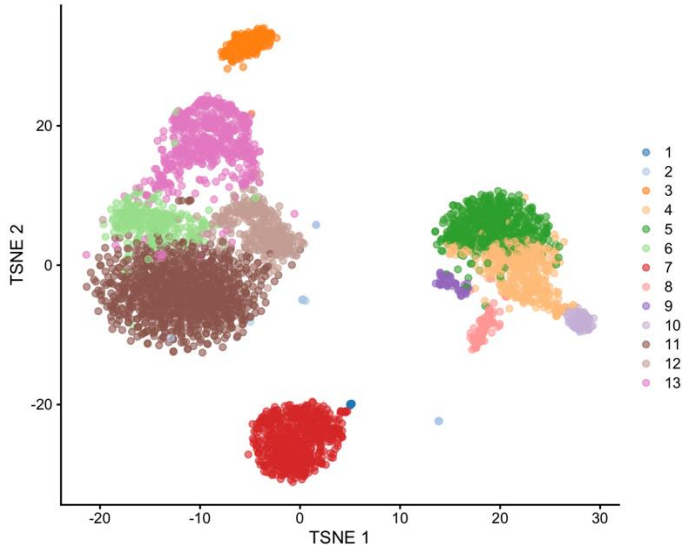


Going further: non-linear dimensional reductions

In a t-SNE projection, similar objects (cells) are modeled by nearby two-(three)dimensional points and dissimilar objects are modeled by distant points with high probability.







Caution with tSNE visualization

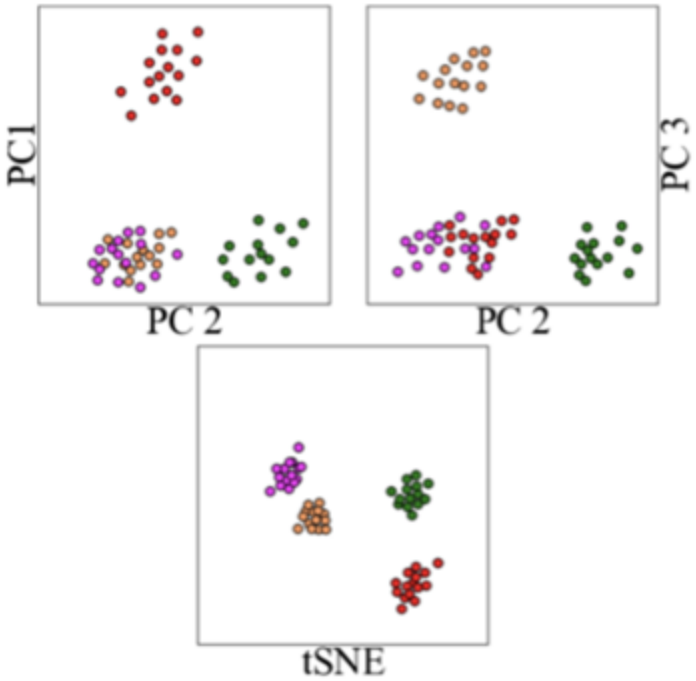
Nonlinear--optimized for local distance

Caveats to be aware of:
Distances between clusters may not mean anything—large distances do not necessarily reflect large dissimilarity

Big clusters can just mean more cells

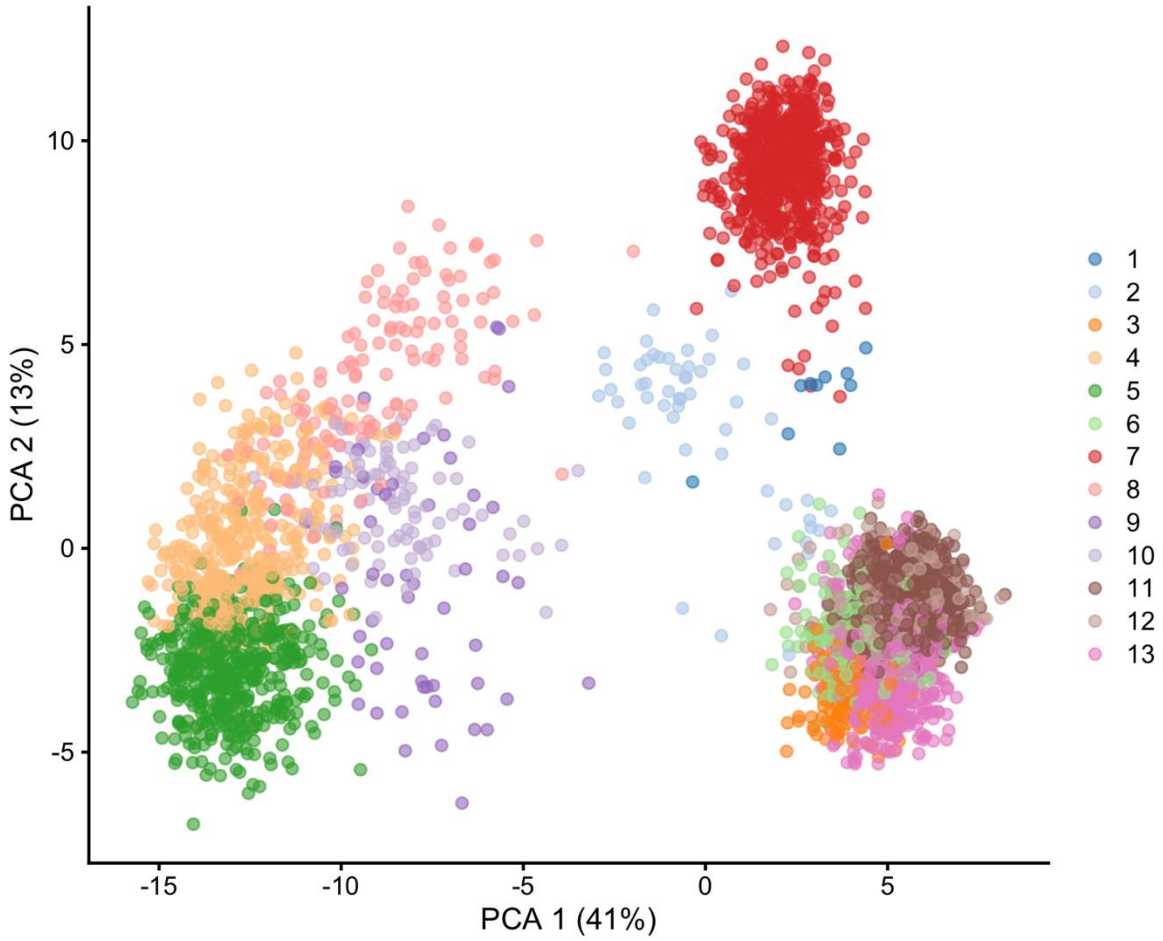
Perplexity parameter or expected number of neighbors (default 30 in Seurat) can make it hard to find very rare subpopulations (5 cells or less).

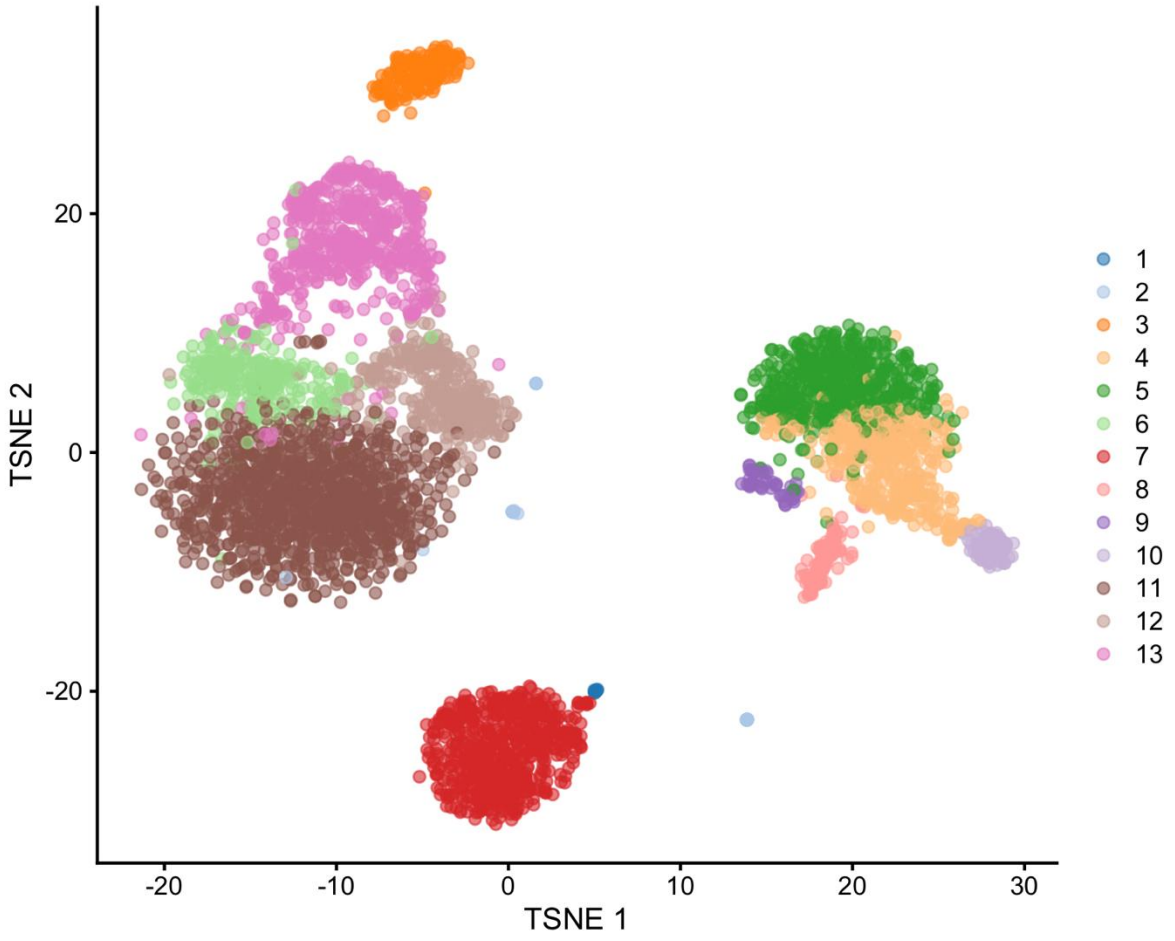
Number of iterations run will also affect final visualization



A great tSNE resource! https://distill.pub/2016/misread-tsne/

Caution with tSNE visualization





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Other non-linear dimensional reduction approaches

- Force-directed graph embedding
- UMAP
- Diffusion Maps
- Non-negative Matrix Factorization
- Probabilistic (topic models/Latent Dirichlet Allocation (LDA))

BE AWARE!!

- **Some are linear, some other are not.**
- **While PCA is a general "one-size-fits-all" approach, others will yield more specific outputs, targeting a particular question.**

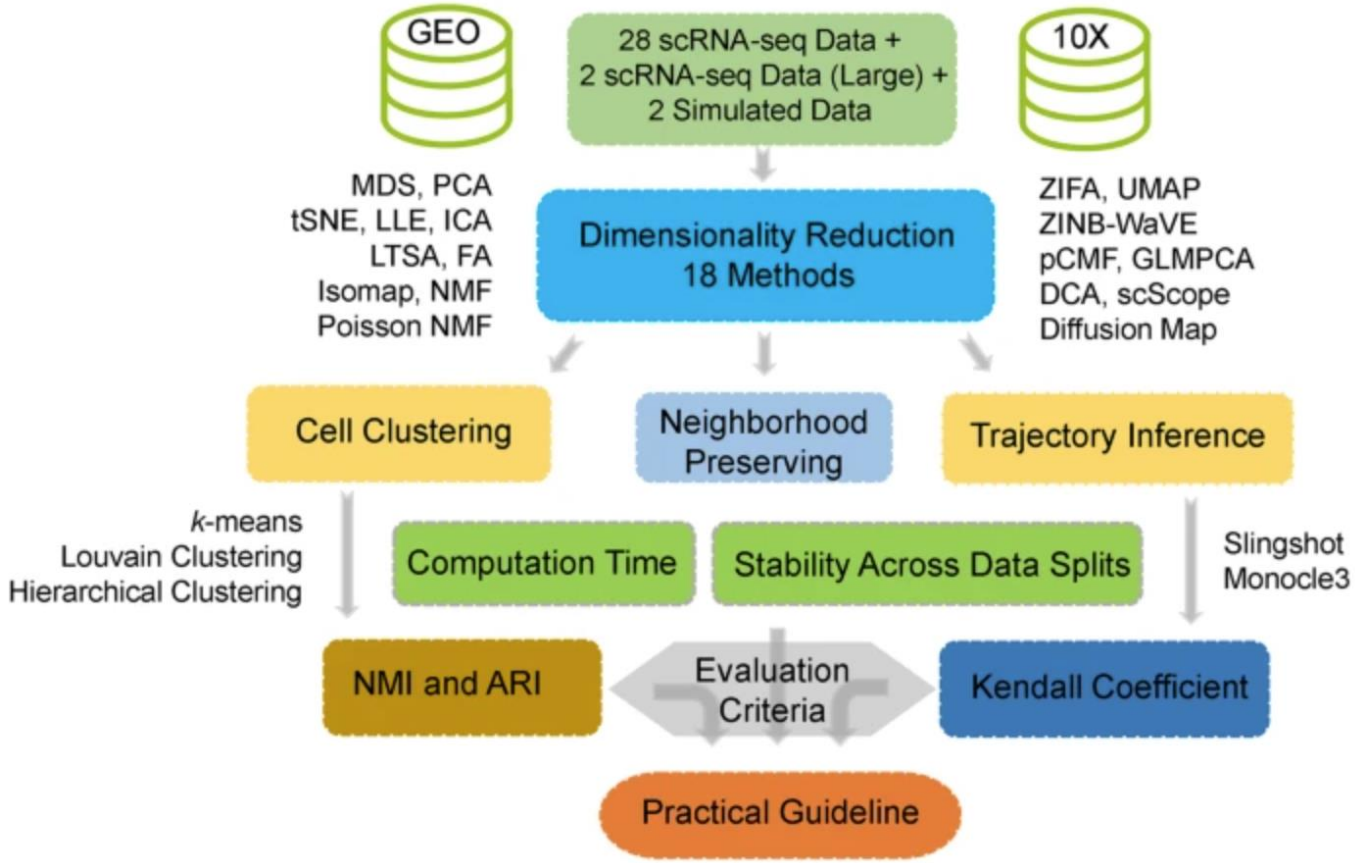
Other non-linear dimensional reduction approaches

Research | Open Access | Published: 10 December 2019

Accuracy, robustness and scalability of dimensionality reduction methods for single-cell RNA-seq analysis

Shiquan Sun, Jiaqiang Zhu, Ying Ma & Xiang Zhou

Genome Biology **20**, Article number: 269 (2019)



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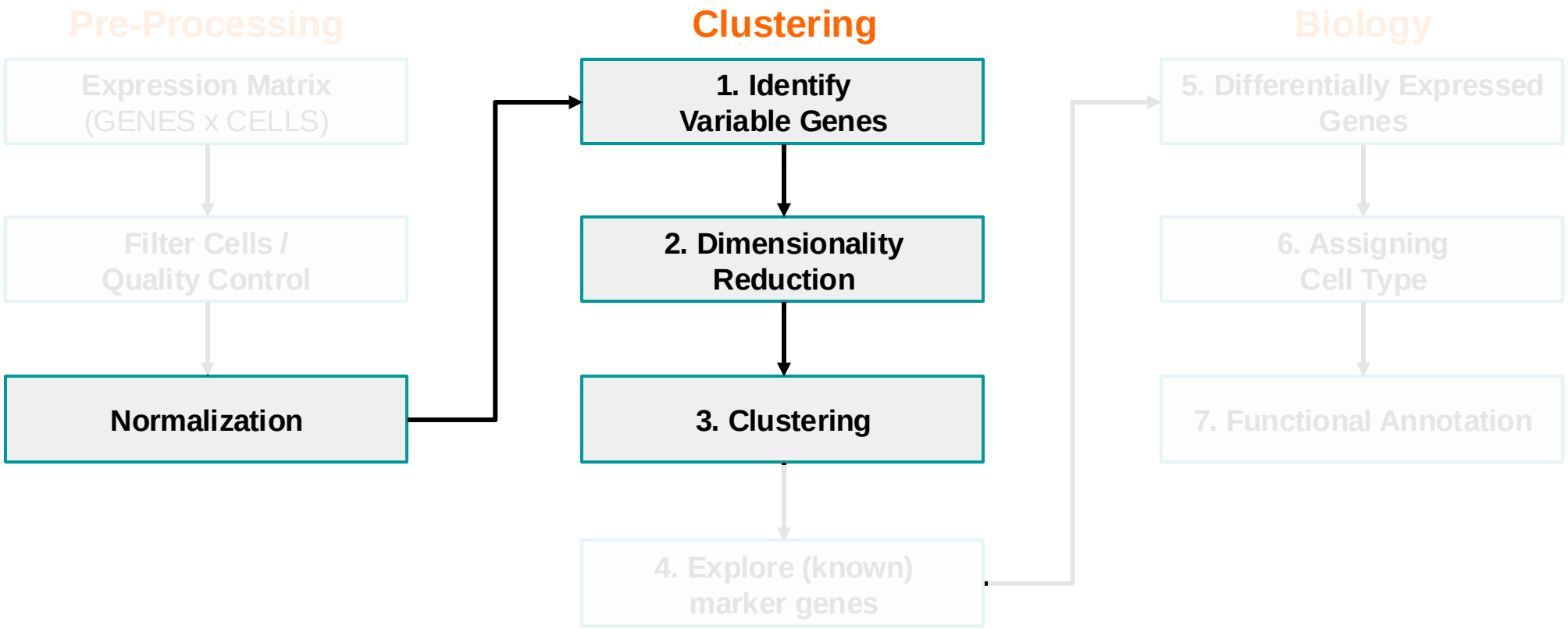
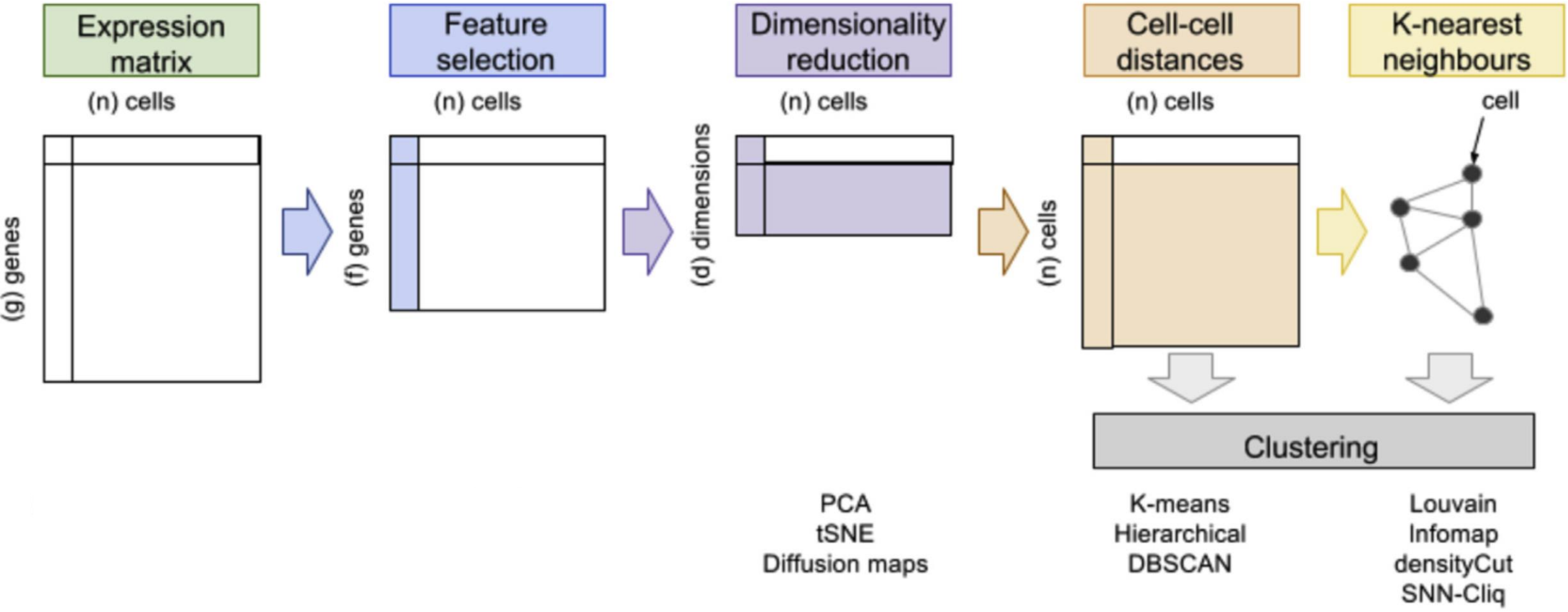
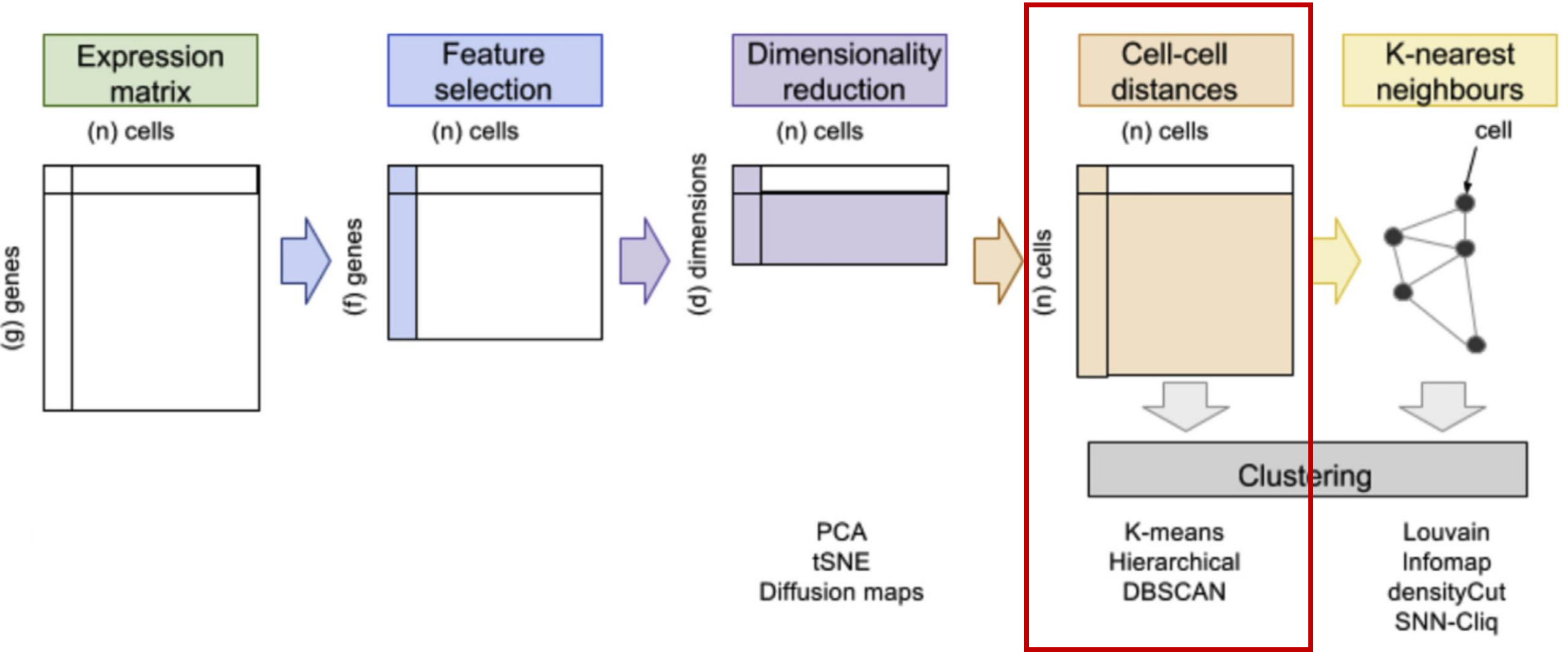


Different methodologies for clustering



Andrews et al., 2018

”Traditional” clustering



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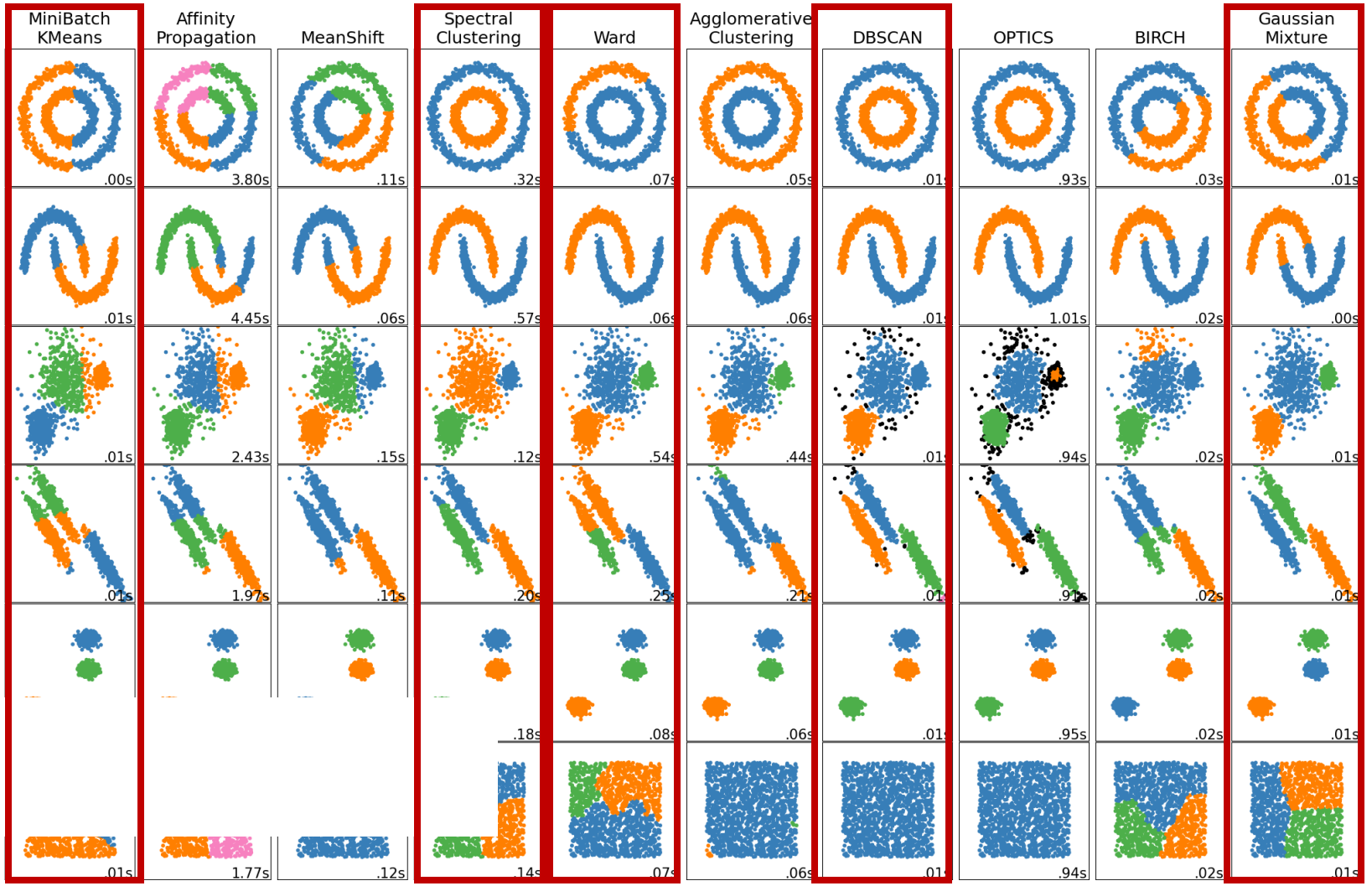
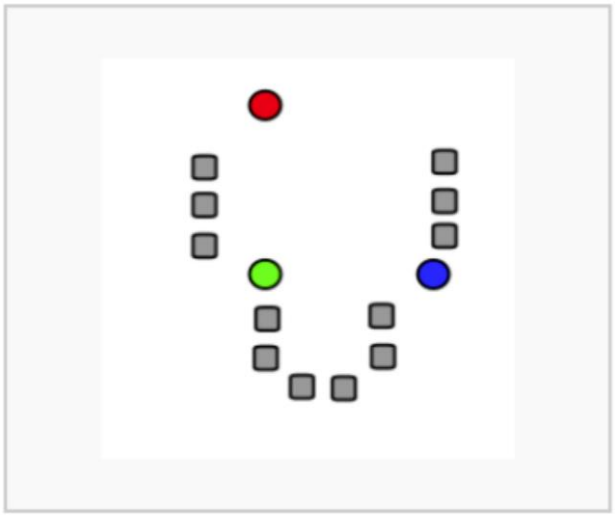
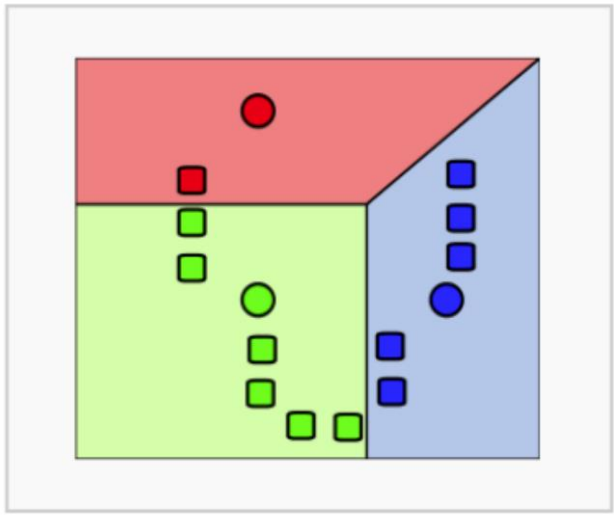
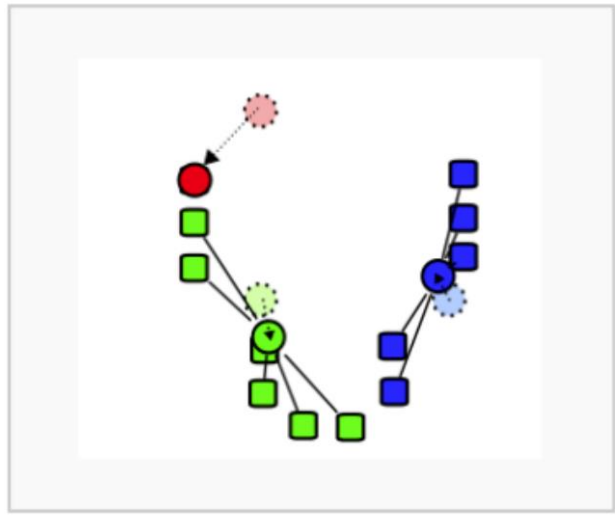
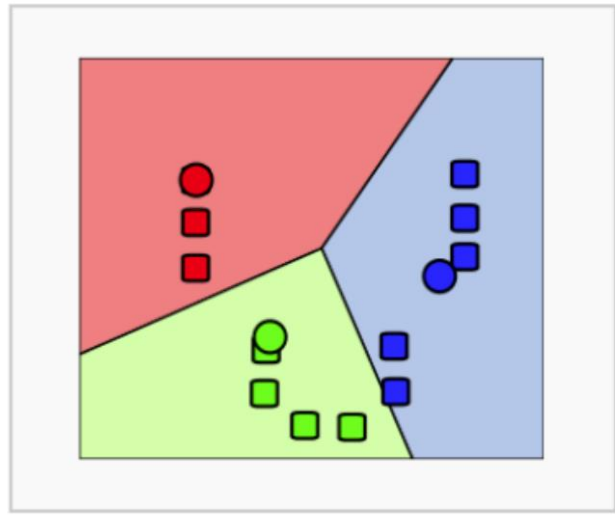


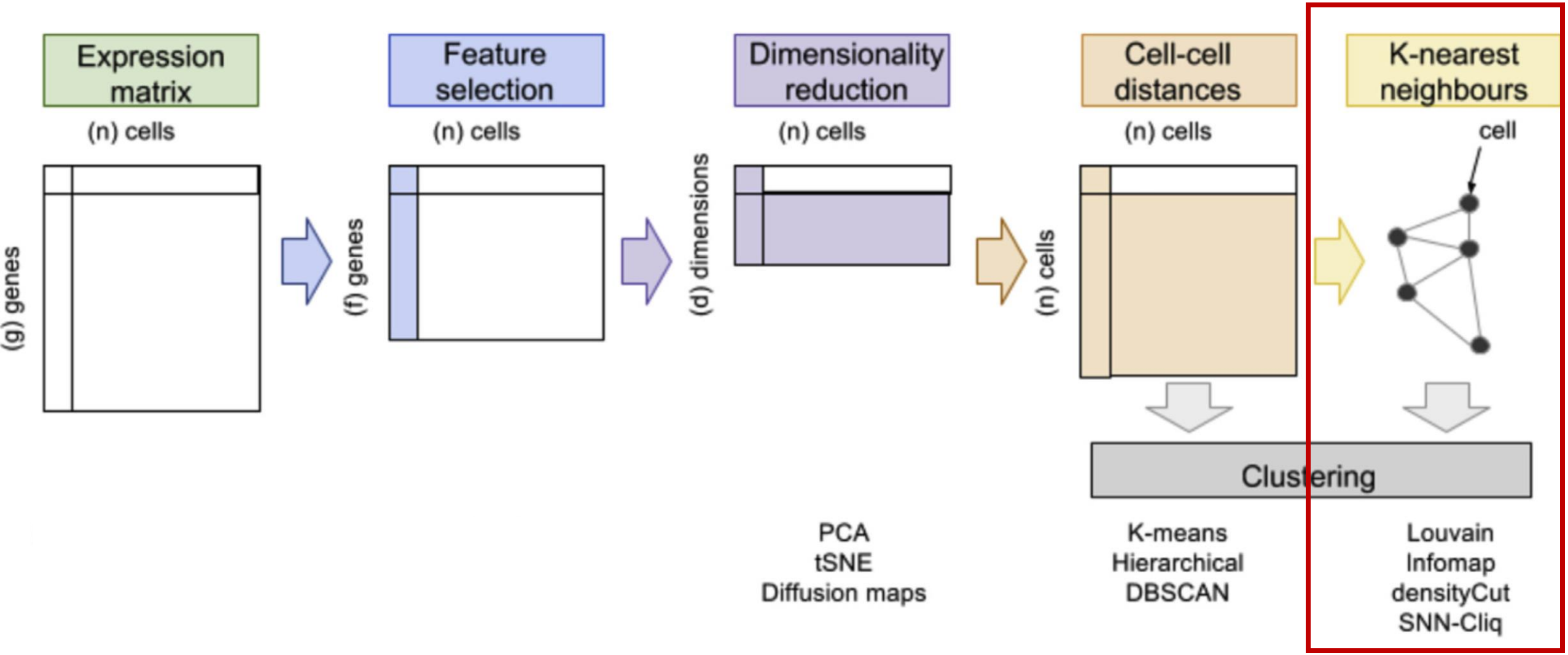
https://scikit-learn.org/stable/modules/clustering.html#overview-of-clustering-methods

”Traditional” clustering

K-means algorithm is both fast and generally reliable, as a first approach.

Graph-based clustering



Andrews et al., 2018

Why do we need graph-based clustering?

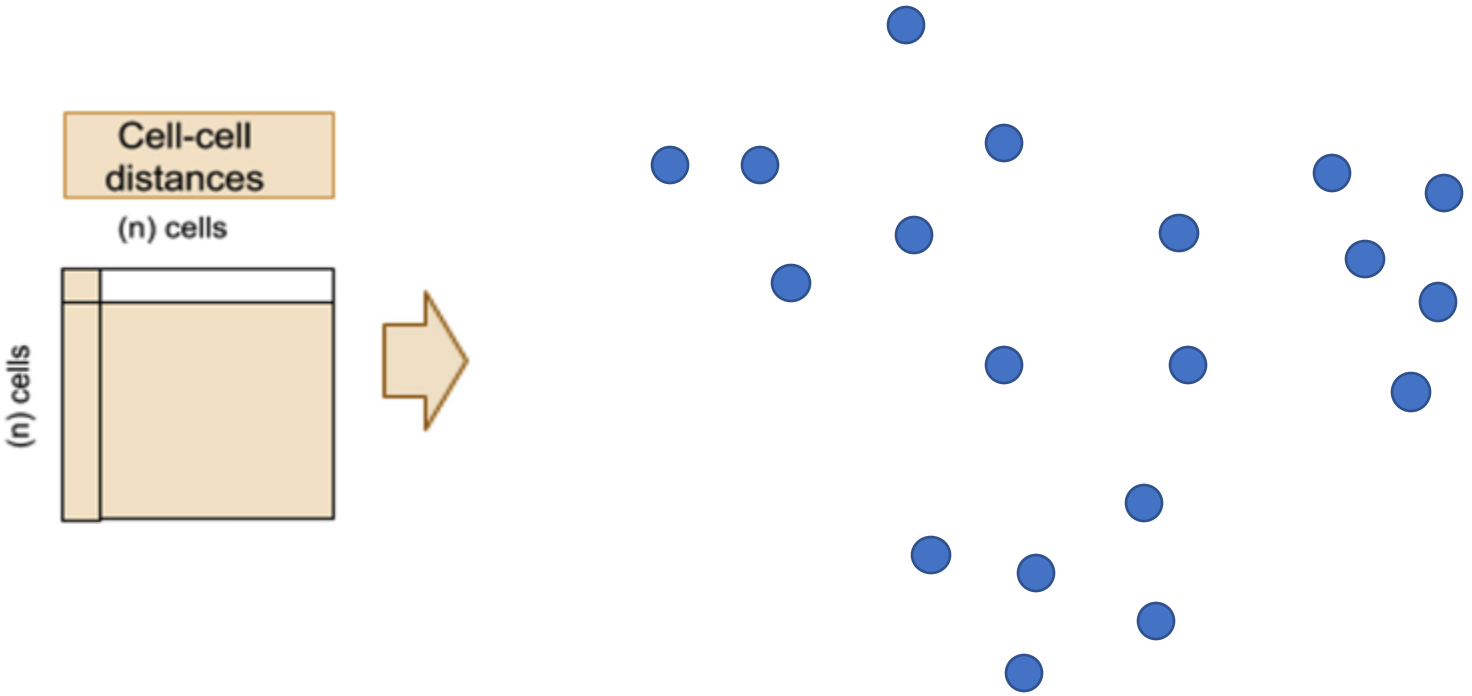
Curse of dimensionality:

"All data become sparse in high-dimensional space and therefore similarities measured by Euclidean distances etc are generally low between all objects."

There is no point performing a hierarchical clustering of 10,000 cells if 90% of the pairwise distances are null !!

Andrews et al., 2018

Building a k-Nearest Neighbors graph (with k = 4)



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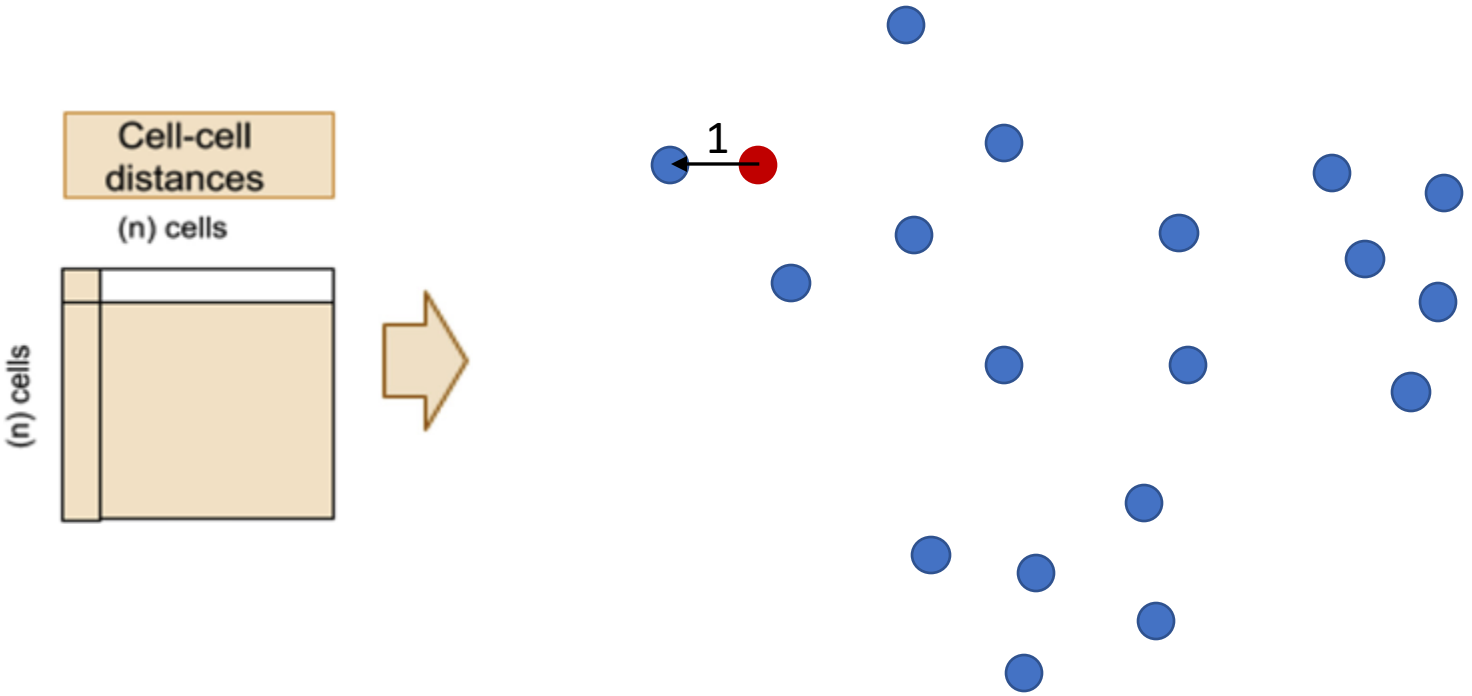

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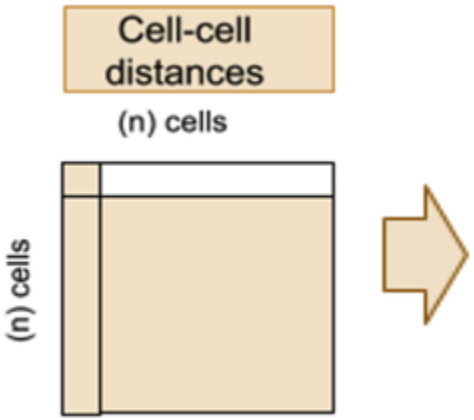

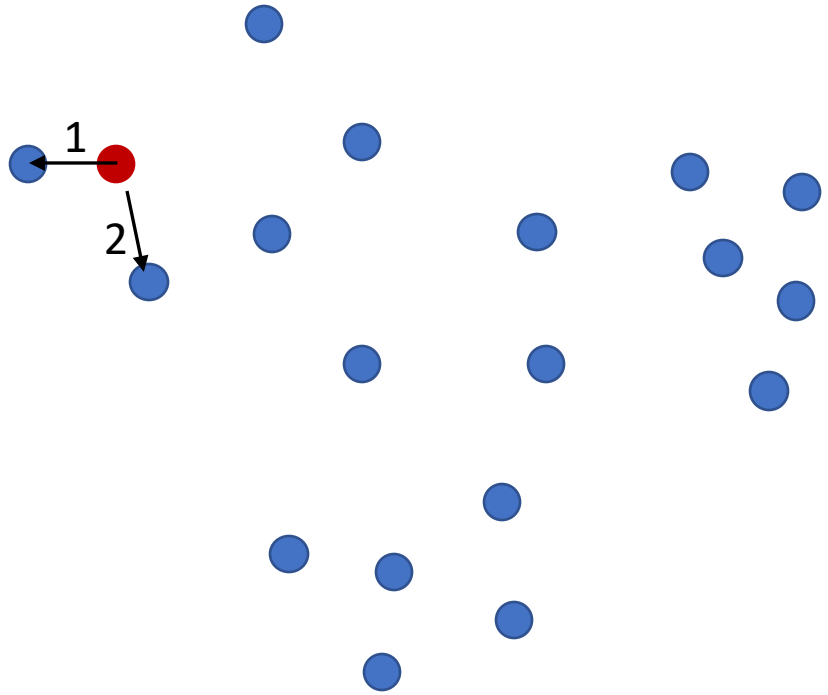

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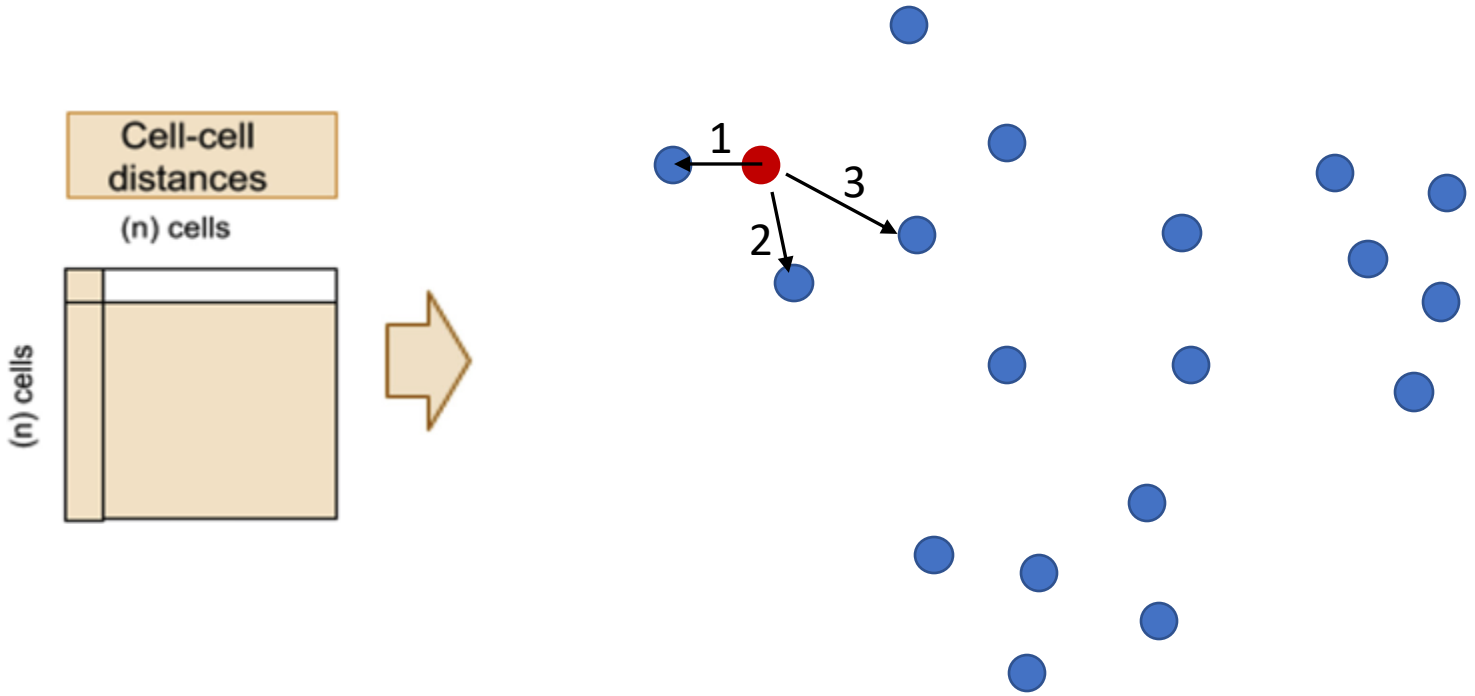

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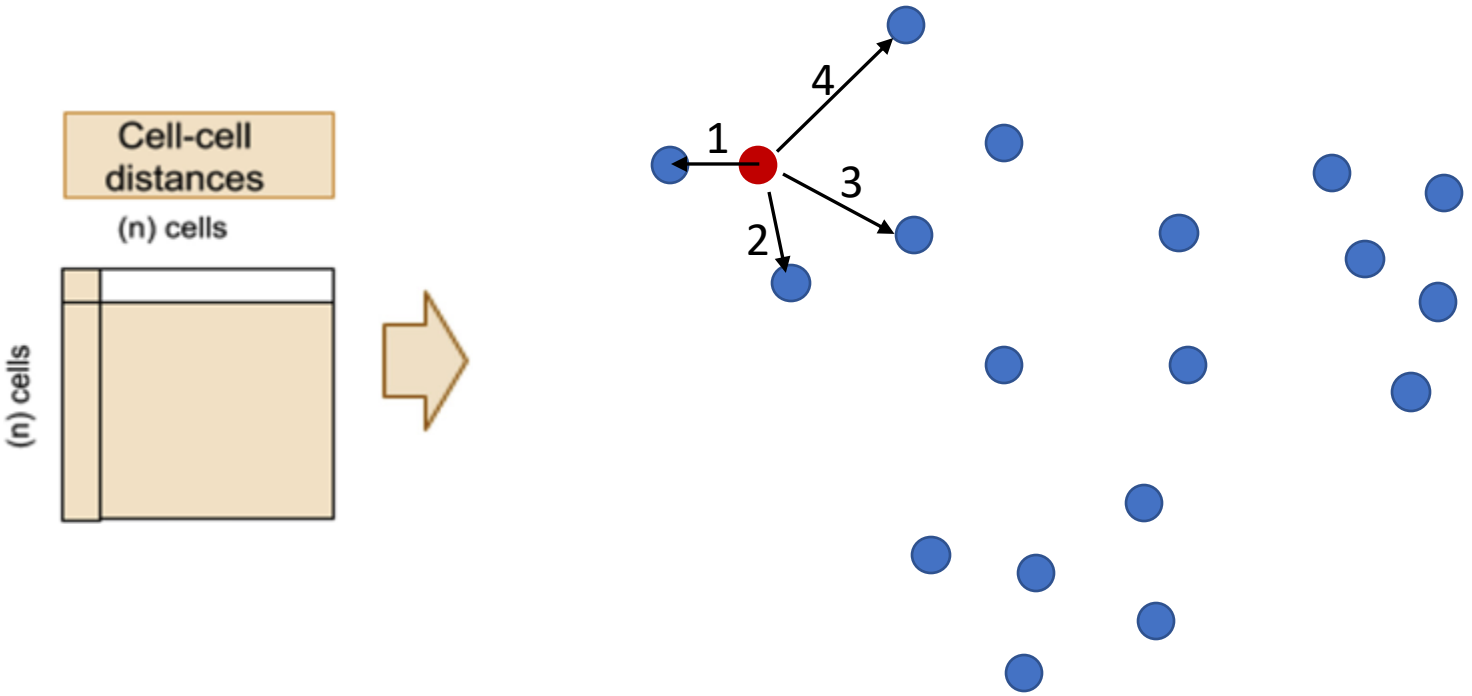

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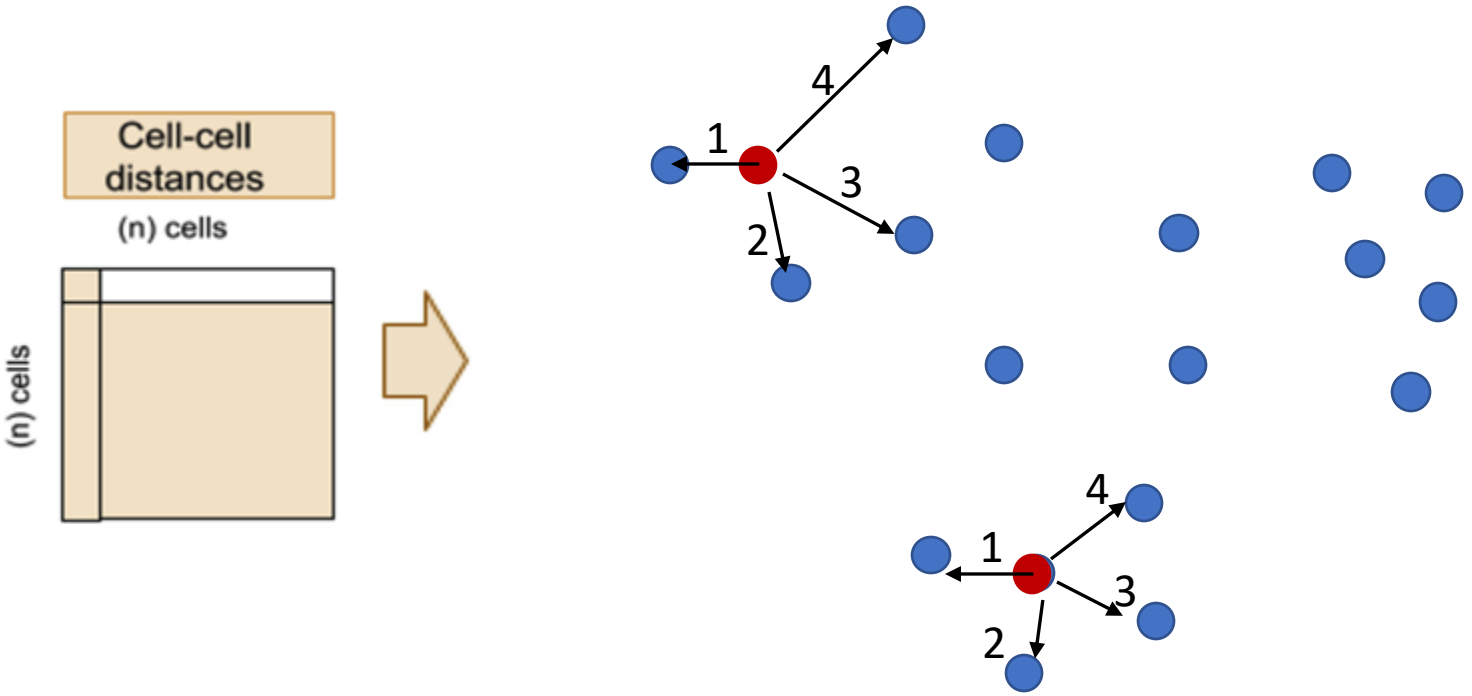

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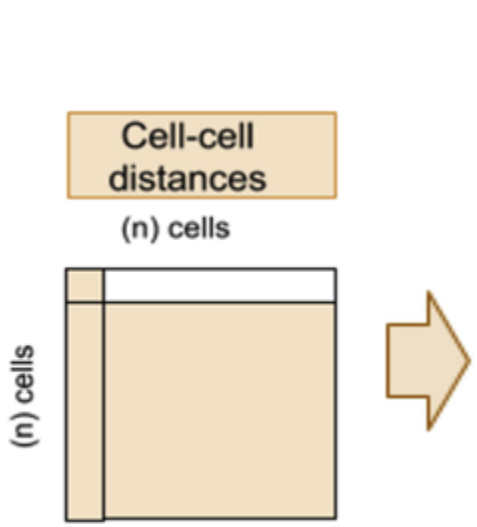
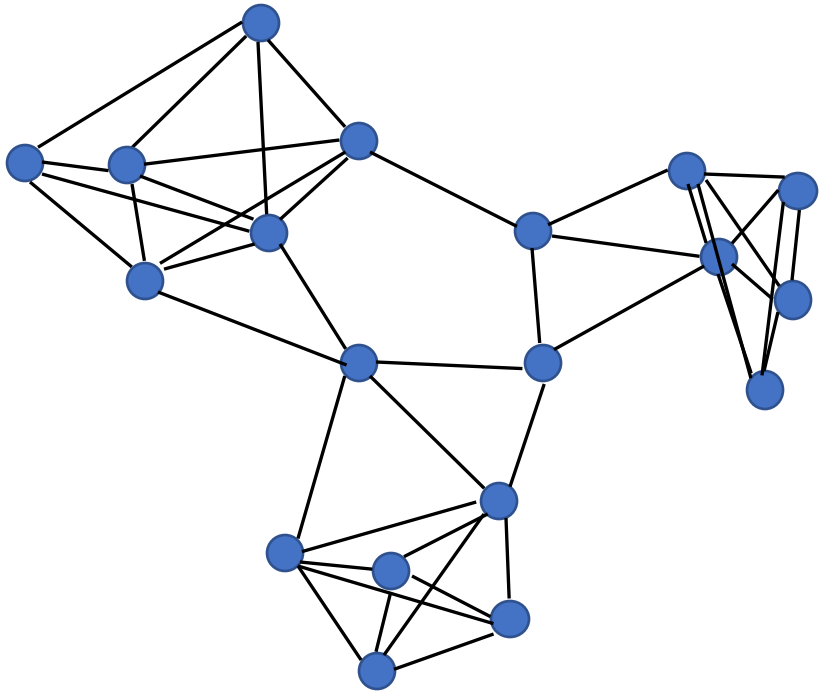
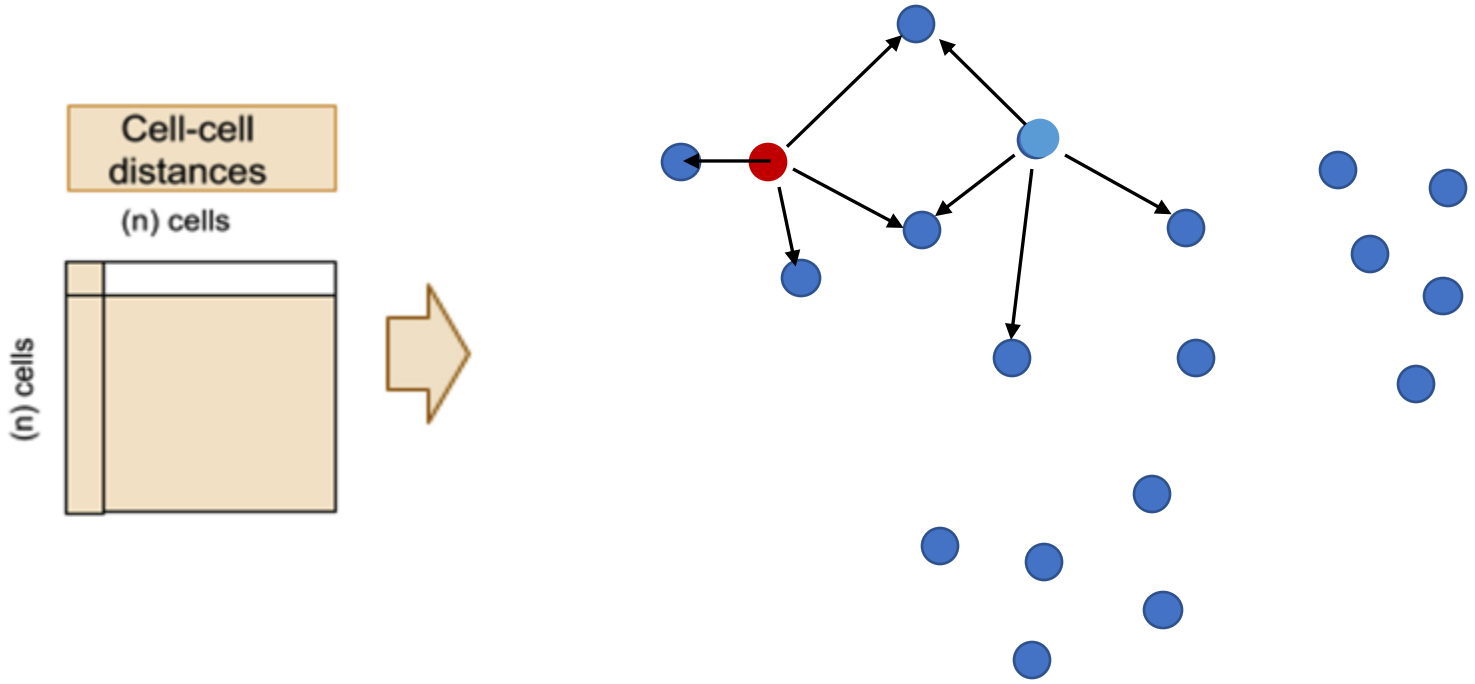


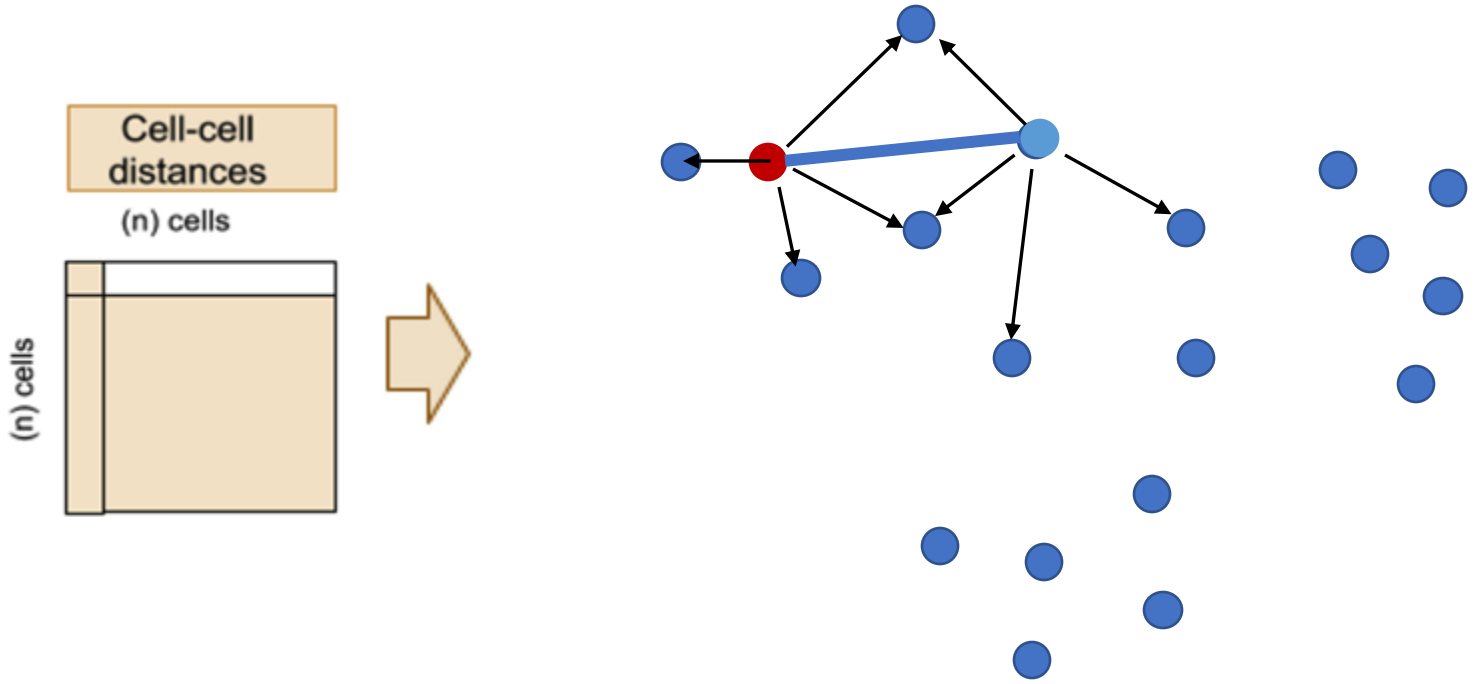


Extending KNN to SNN graphs (Shared Nearest Neighbors) (still with k = 4)



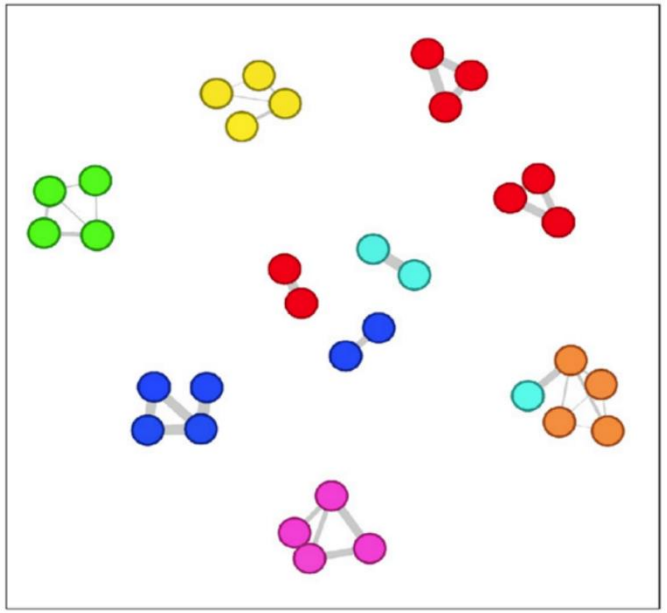
Extending KNN to SNN graphs (Shared Nearest Neighbors) (still with k = 4)

Two cells are connected by an edge if any of their nearest neighbors are shared.

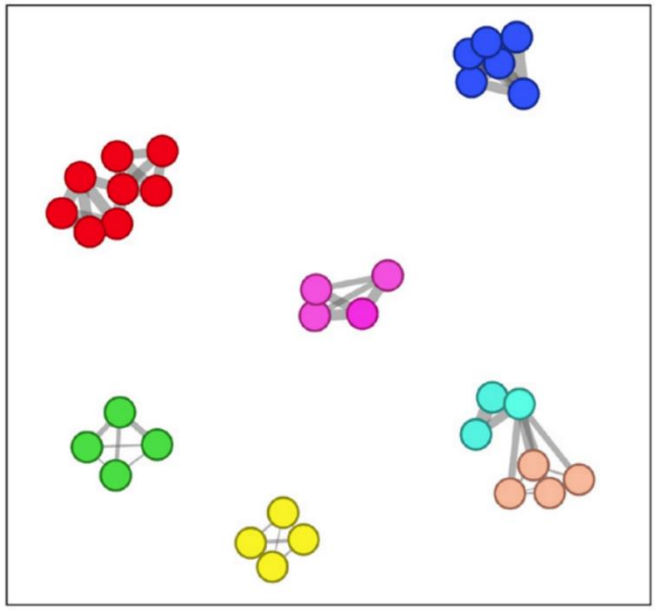


Extending KNN to SNN graphs (Shared Nearest Neighbors) (still with k = 4)

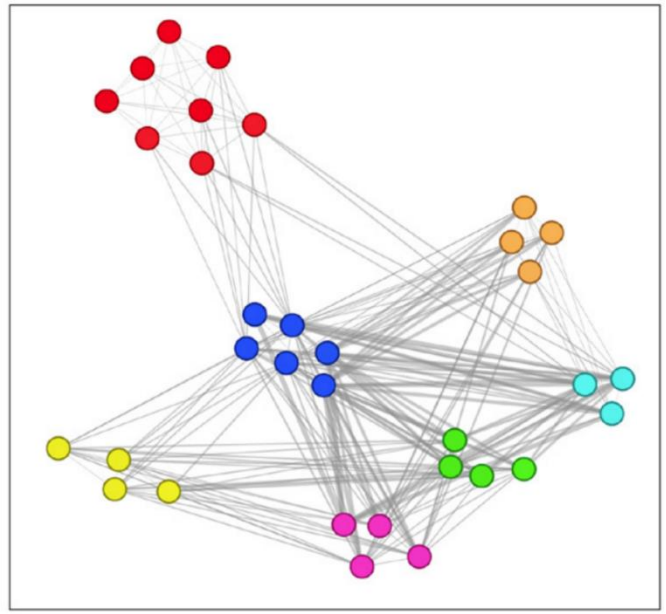
K is important when building KNN or SNN graphs !



(a) Parameter $K = 2$

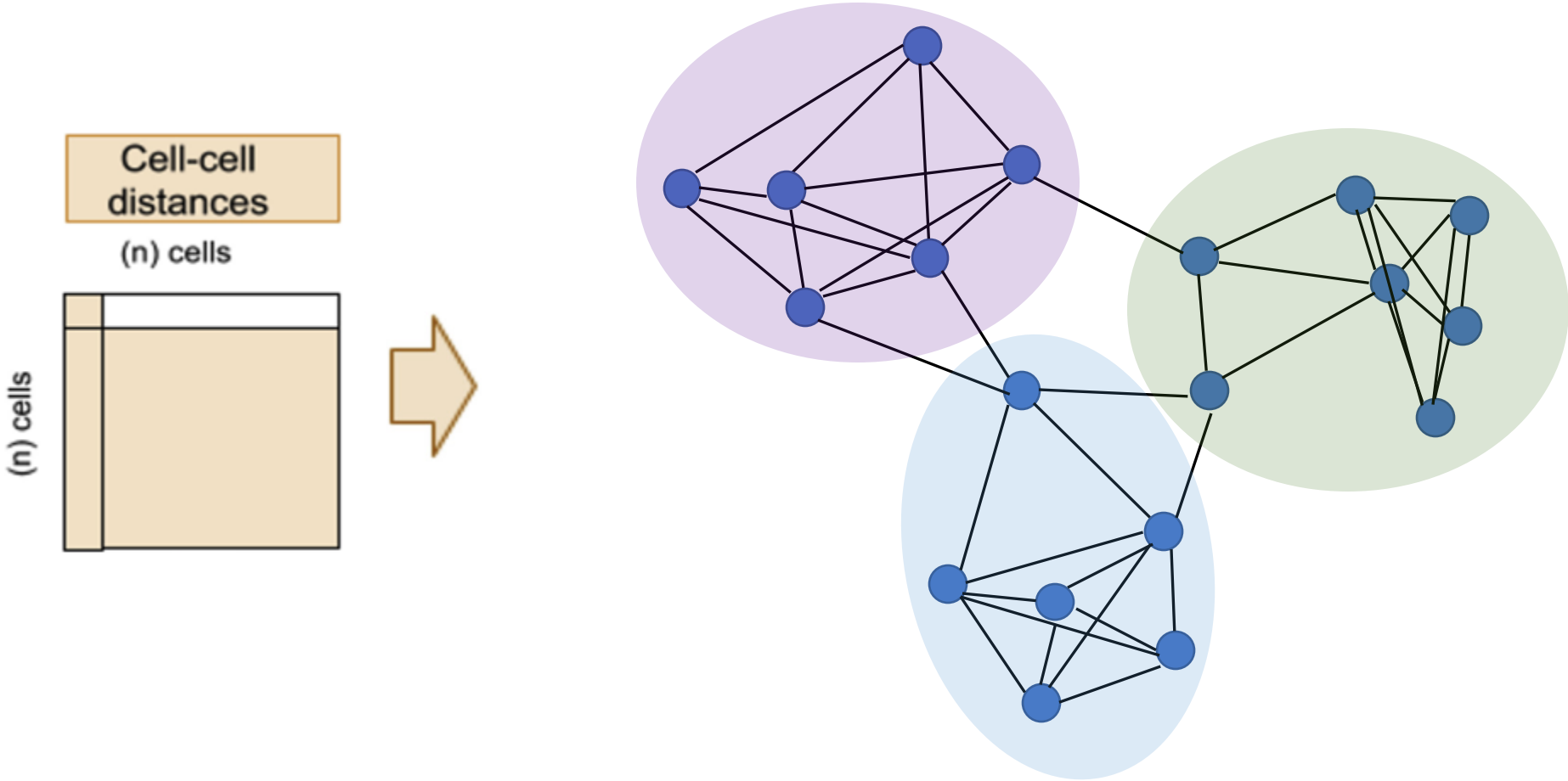


(b) Parameter $K = 3$



(c) Parameter $K = 6$

Graph-based clustering is nothing more than **community detection** (an “old” field from ’00s).

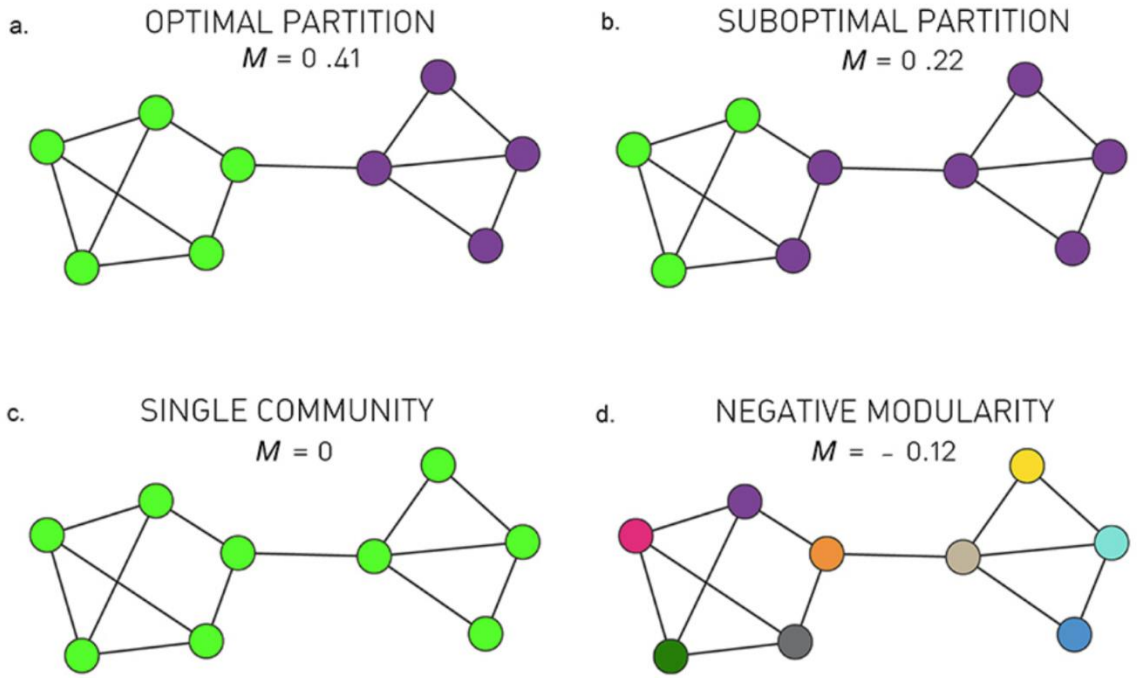


Graph-based clustering is nothing more than **community detection** (an “old” field from ’00s).

Many different algorithms for community detection:

- Louvain (heuristic)
- Infomap
- Walktrap
- …

Most of them are based on modularity maximization



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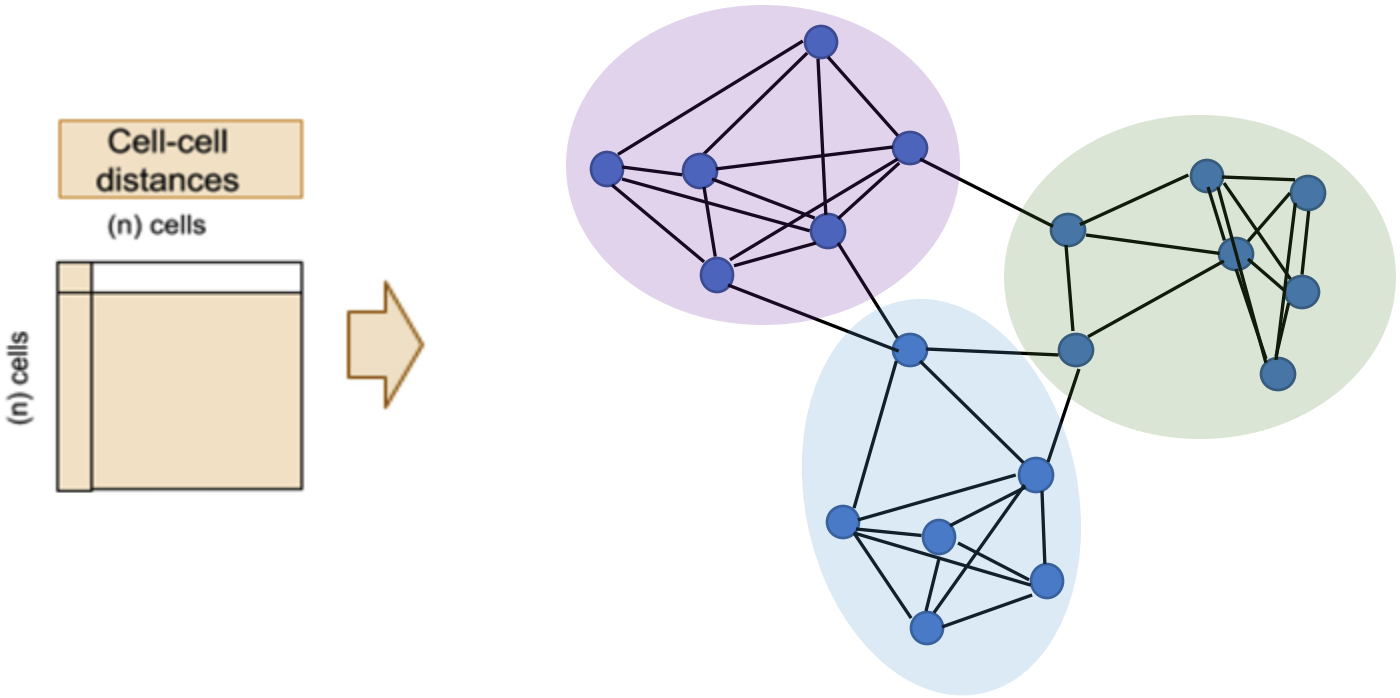
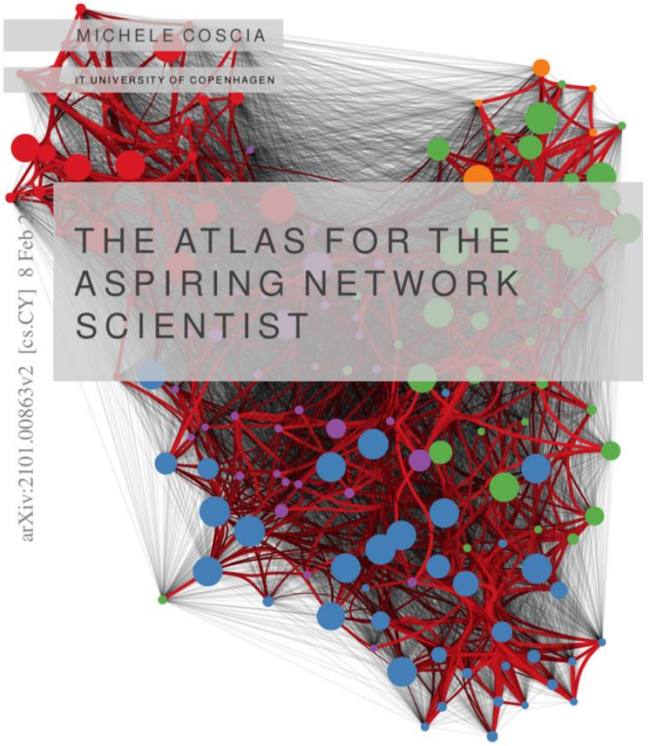




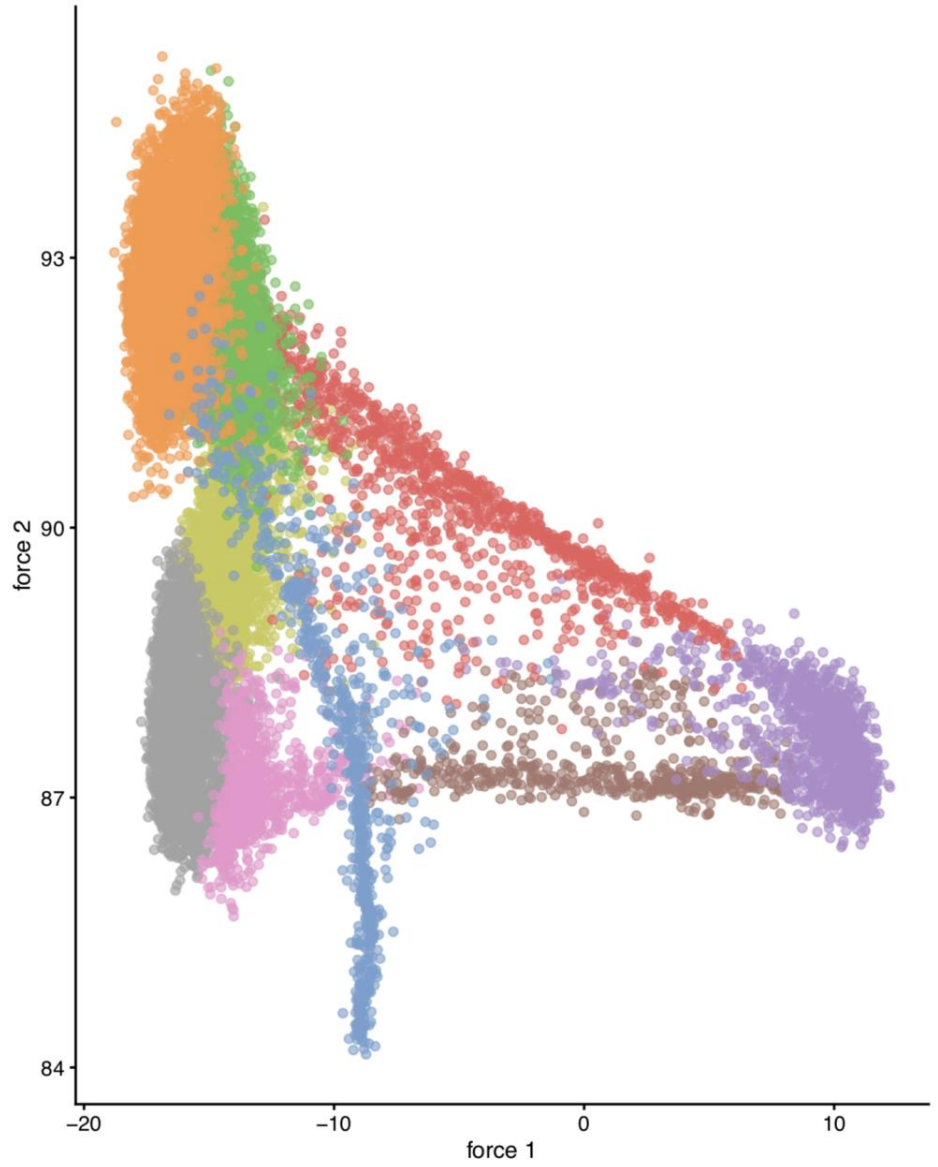
The Atlas for the Aspiring Network Scientist, Michele Coscia 2021

CAREFUL!

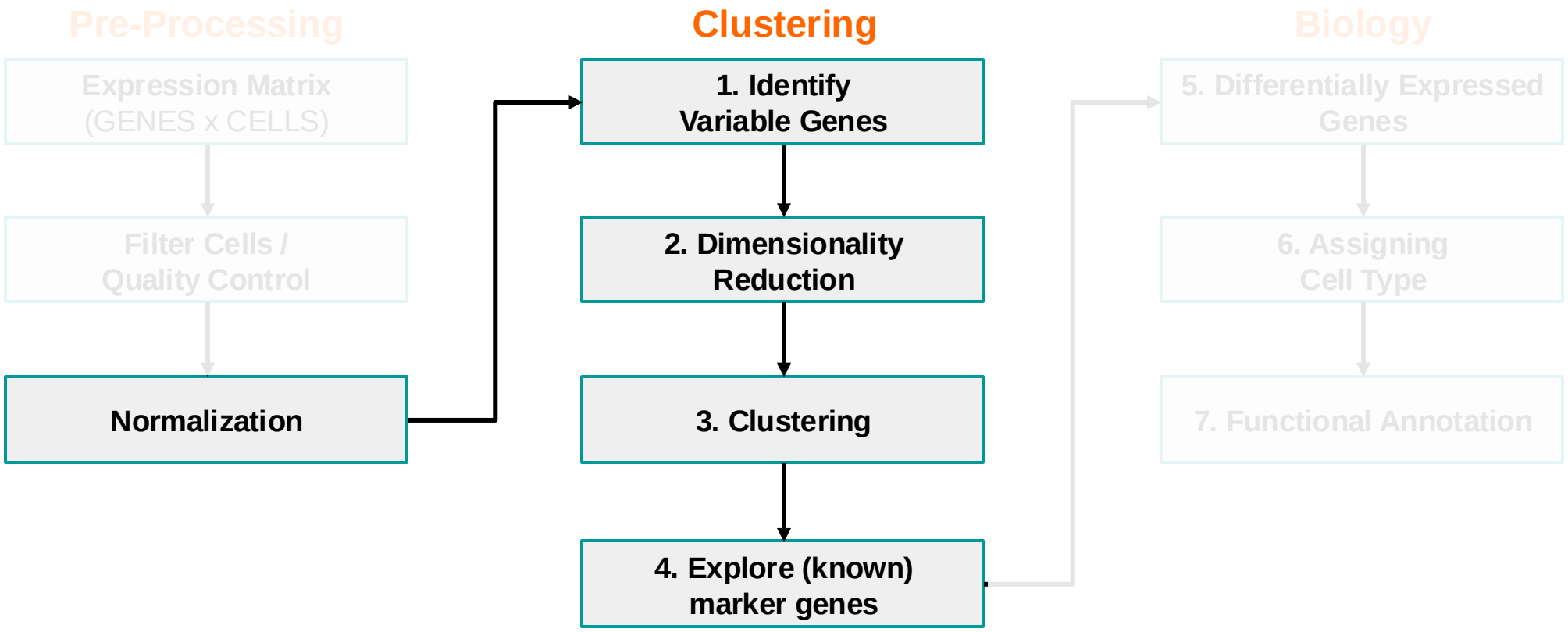
A graph can be **visualized** (i.e. embedded) in 2D, but the graph-based clustering step (i.e. **community finding**) is not done on its 2D embedding!!

"Do not let the tail (of visualization) wag the dog (of quantitative analysis)"

-- A. Lun



Analysis workflow



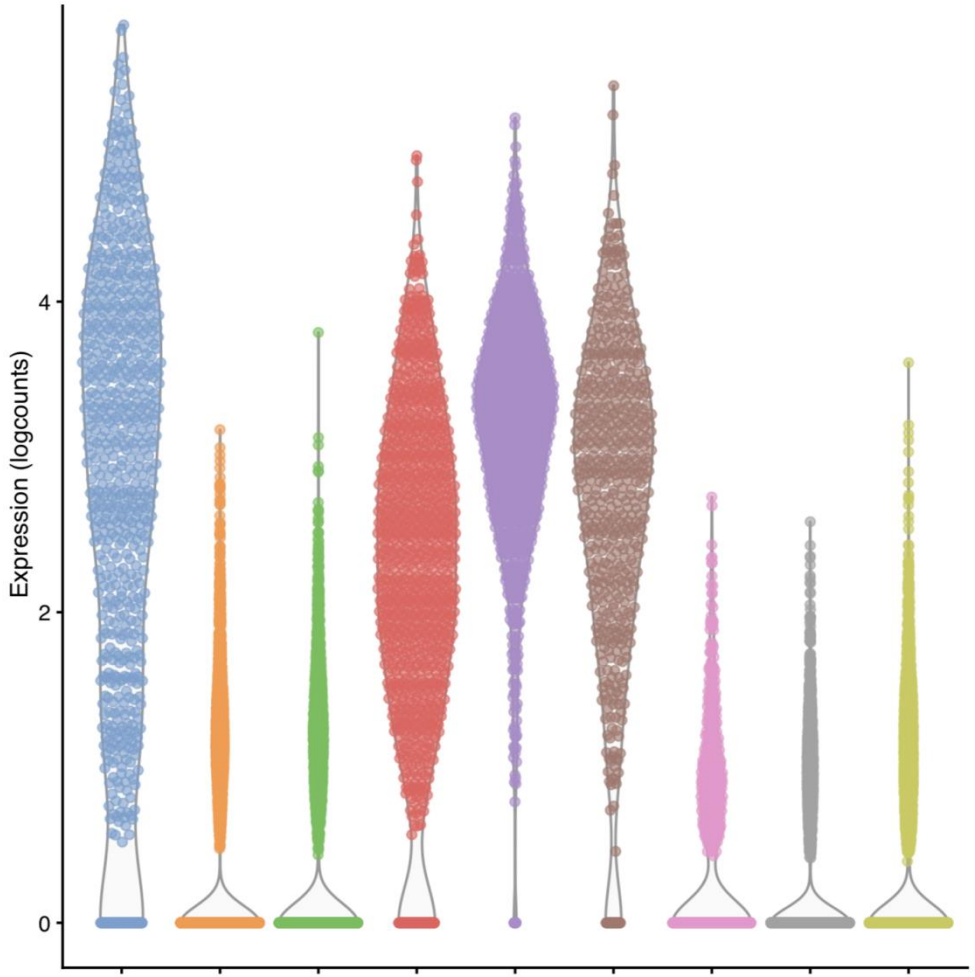
Visualizing expression of a gene of interest

On the dataset embedding:

Visualizing expression of a gene of interest

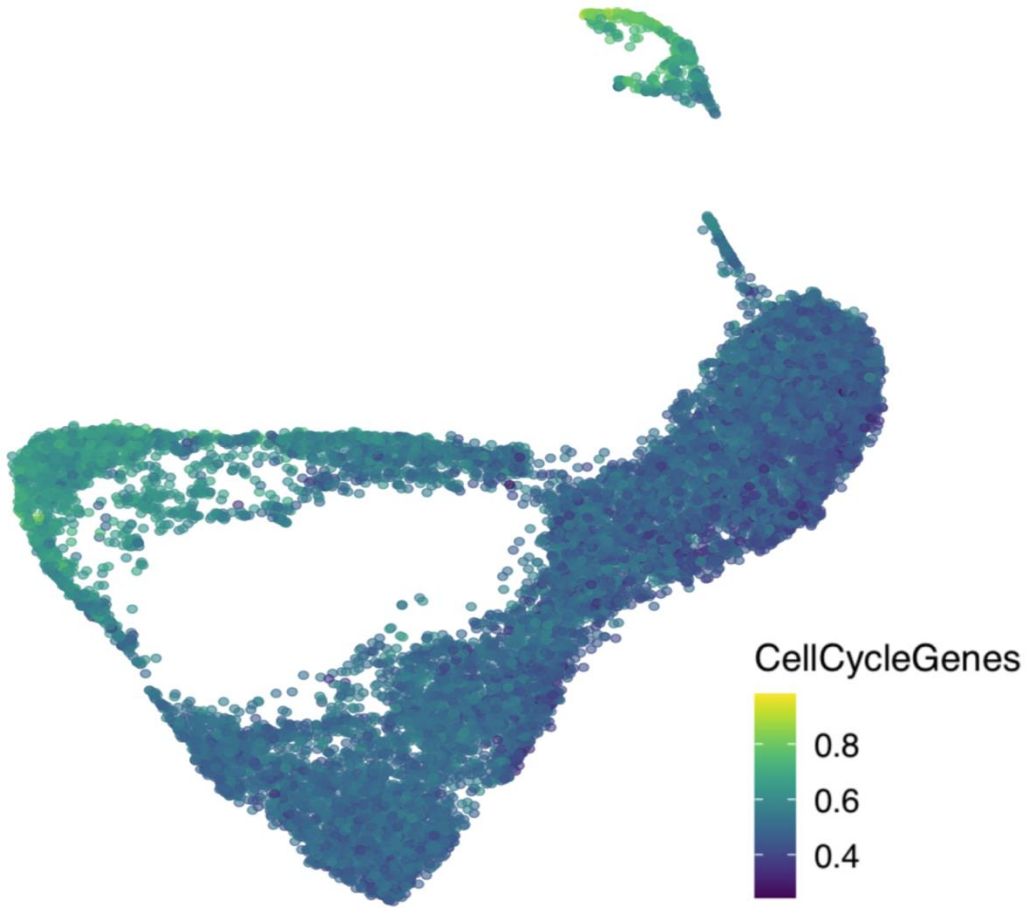
On the dataset embedding:

By clusters:

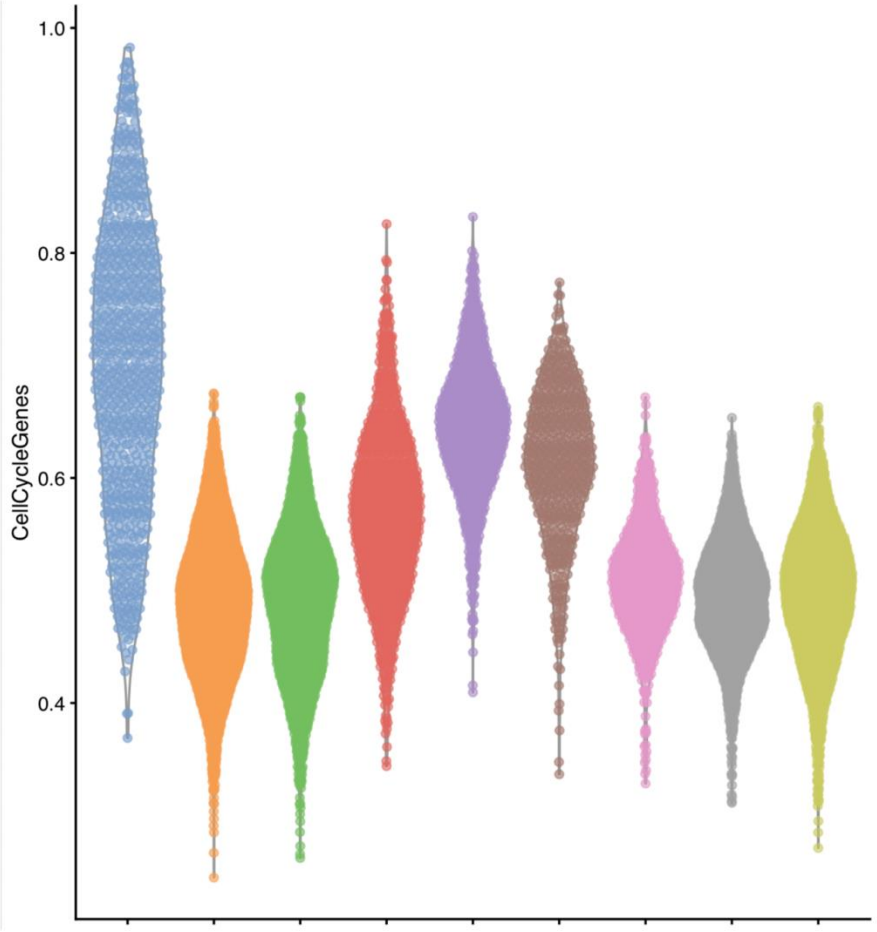


Visualizing expression of a module of interest

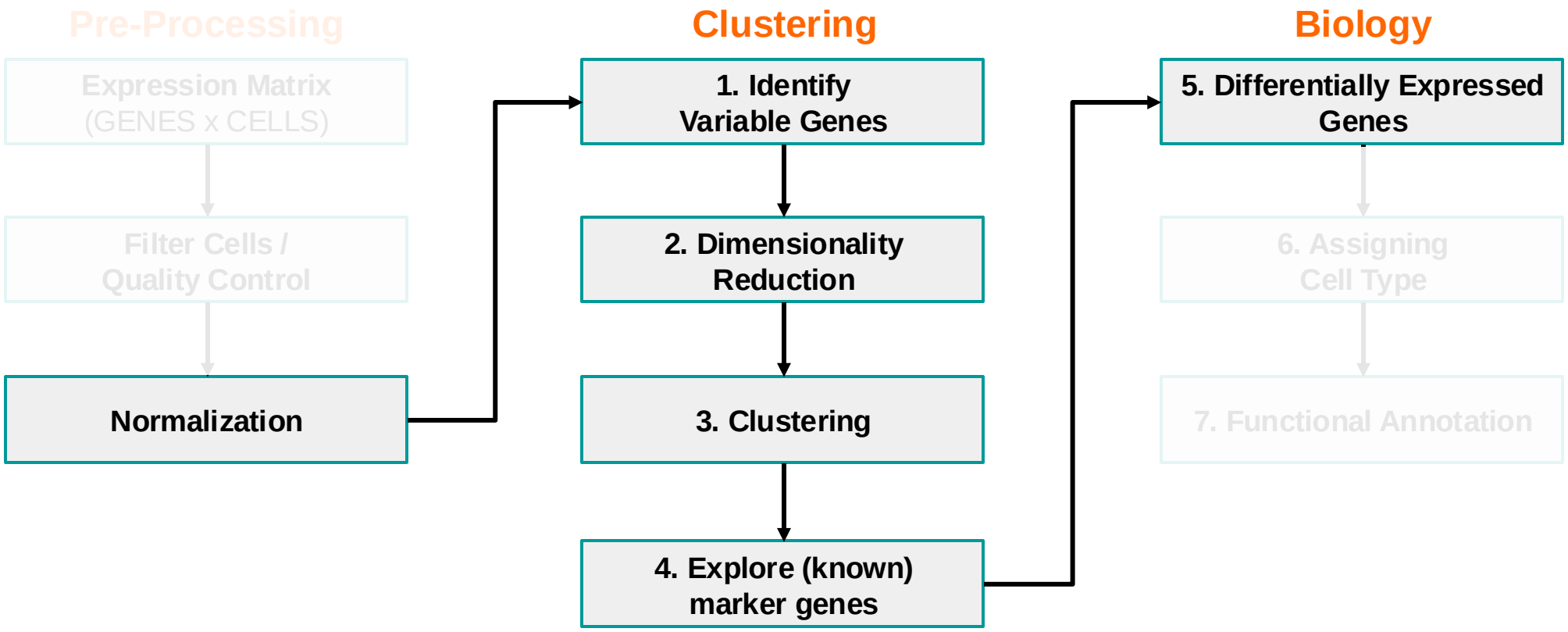
On the dataset embedding:



By clusters:



Analysis workflow



Differential Expression Testing

In scRNA-seq we often do not have a defined set of experimental conditions.

Instead, we can perform **pairwise comparisons** of gene expression, **between pairs of cell clusters**, using some of the following tests:

- "wilcox" : Wilcoxon rank sum test (default)
- t" : Student's t-test
- "poisson" : Likelihood ratio test assuming an underlying poisson distribution. Use only for UMI-based datasets
- "negbinom" : Likelihood ratio test assuming an underlying negative binomial distribution. Use only for UMI-based datasets
- Others…

Differential Expression Testing

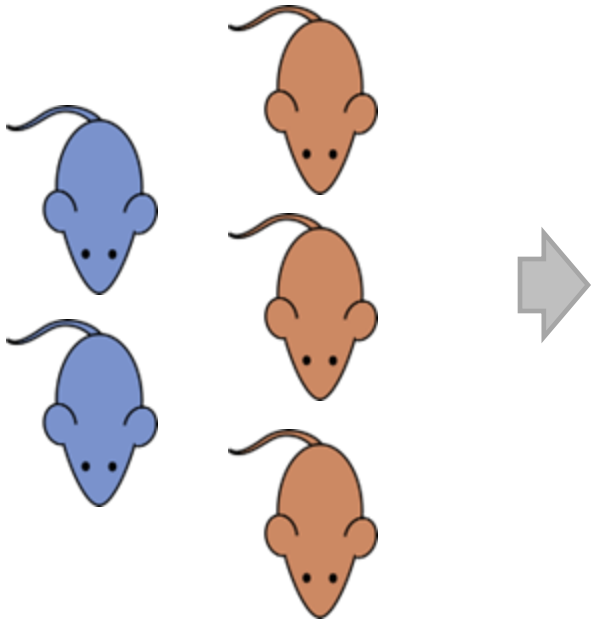
In scRNA-seq we often do not have a defined set of experimental conditions.

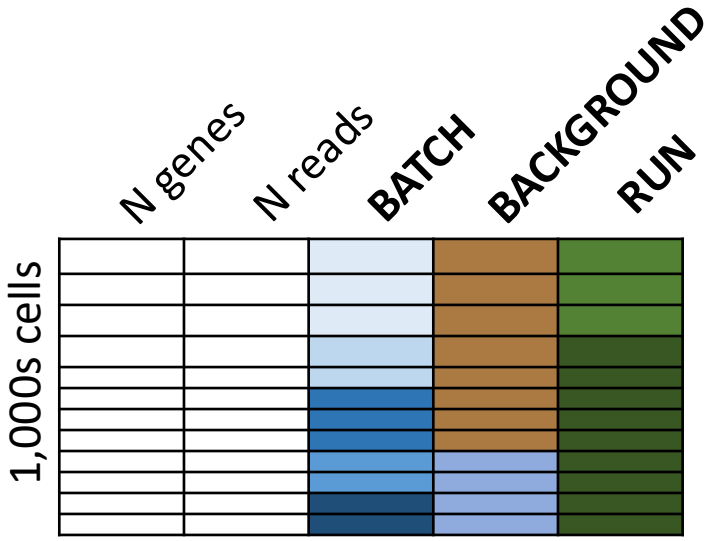
Instead, we can perform **pairwise comparisons** of gene expression, **between pairs of cell clusters**, using some of the following tests:

See Seurat::FindMarkers() and scran::findMarkers() for more info…

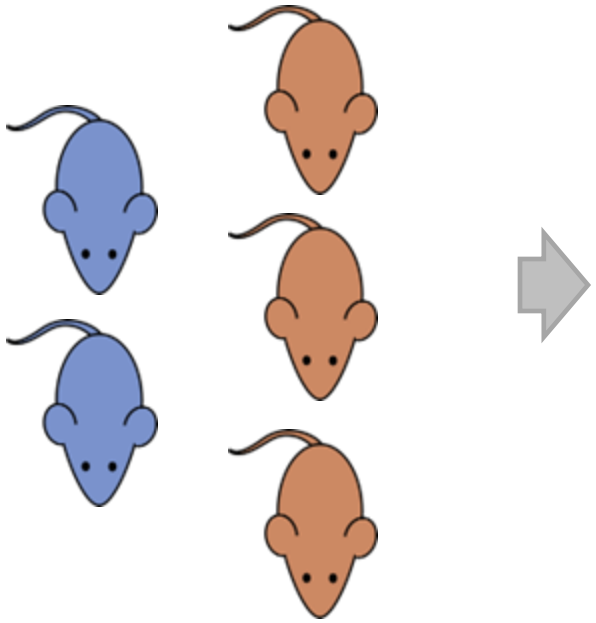
```
markers <- scran::findMarkers(
  sce,
  groups = sce$cluster,
  test.type = "t"
)
```

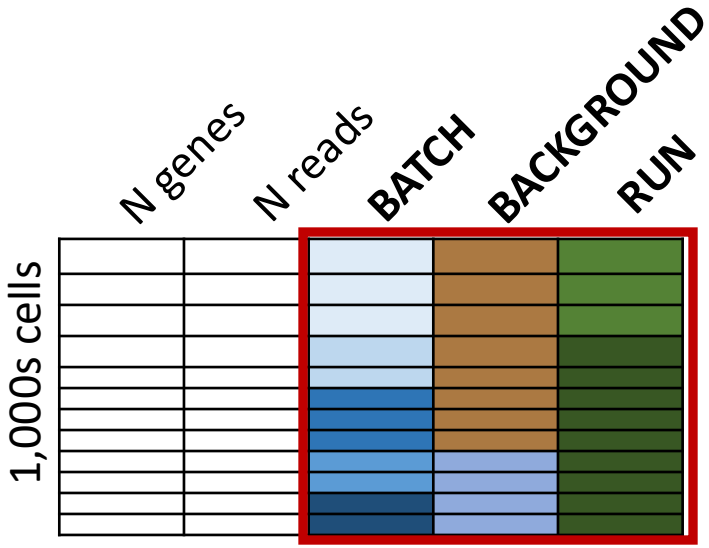
Think about your experimental design!!!





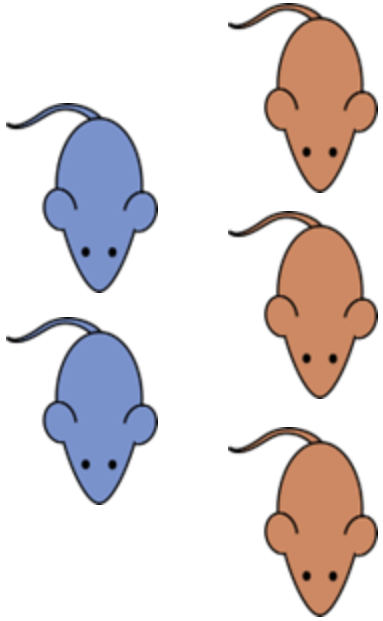
Think about your experimental design!!!

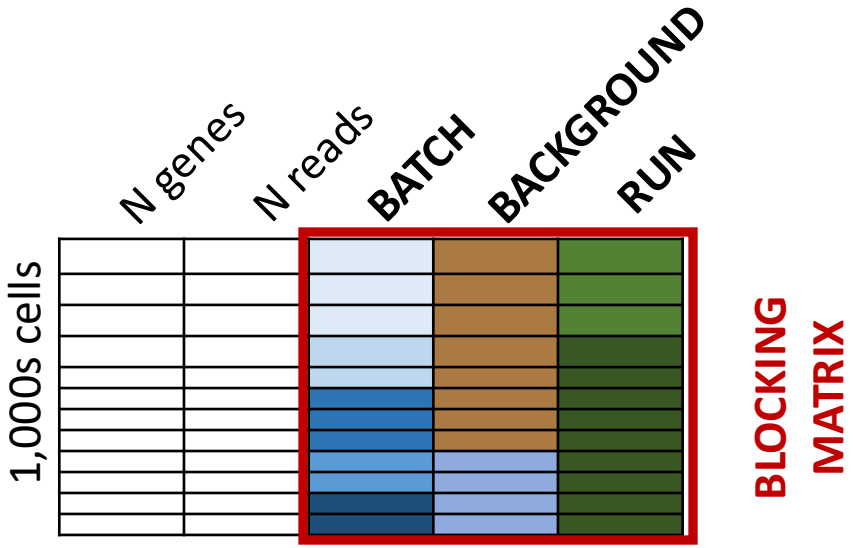




Not all the cells are the same: there are confounding variables.

Think about your experimental design!!!





Not all the cells are the same: there are confounding variables.

```
markers <- scran::findMarkers(
  sce,
  groups = sce$cluster,
  test.type = "t",
  block = <BLOCKING MATRIX>
)
```

Differential Expression Testing: many different assays…

Again, different tests are available and depending on your study case, might be more/less appropriate.

I would recommend going with t-test as default.

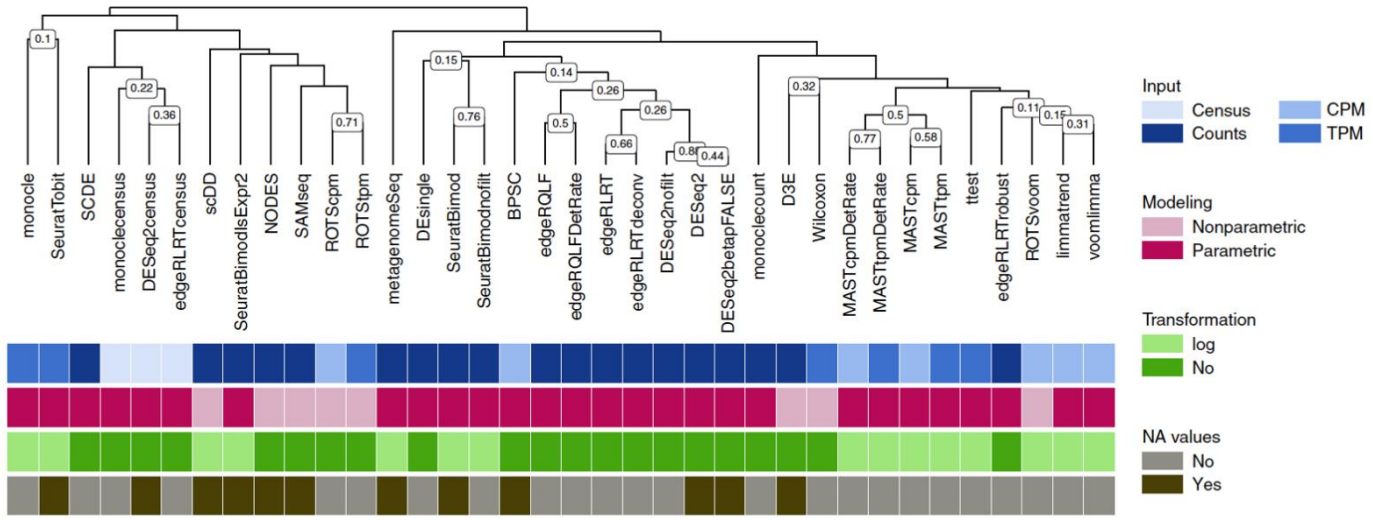
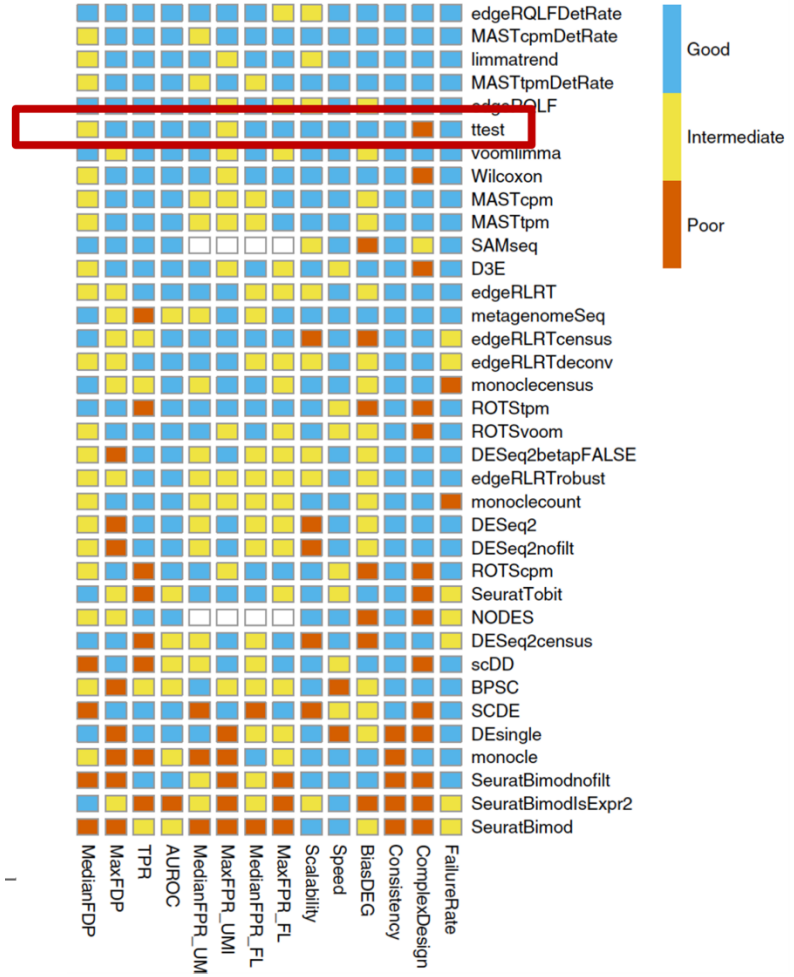
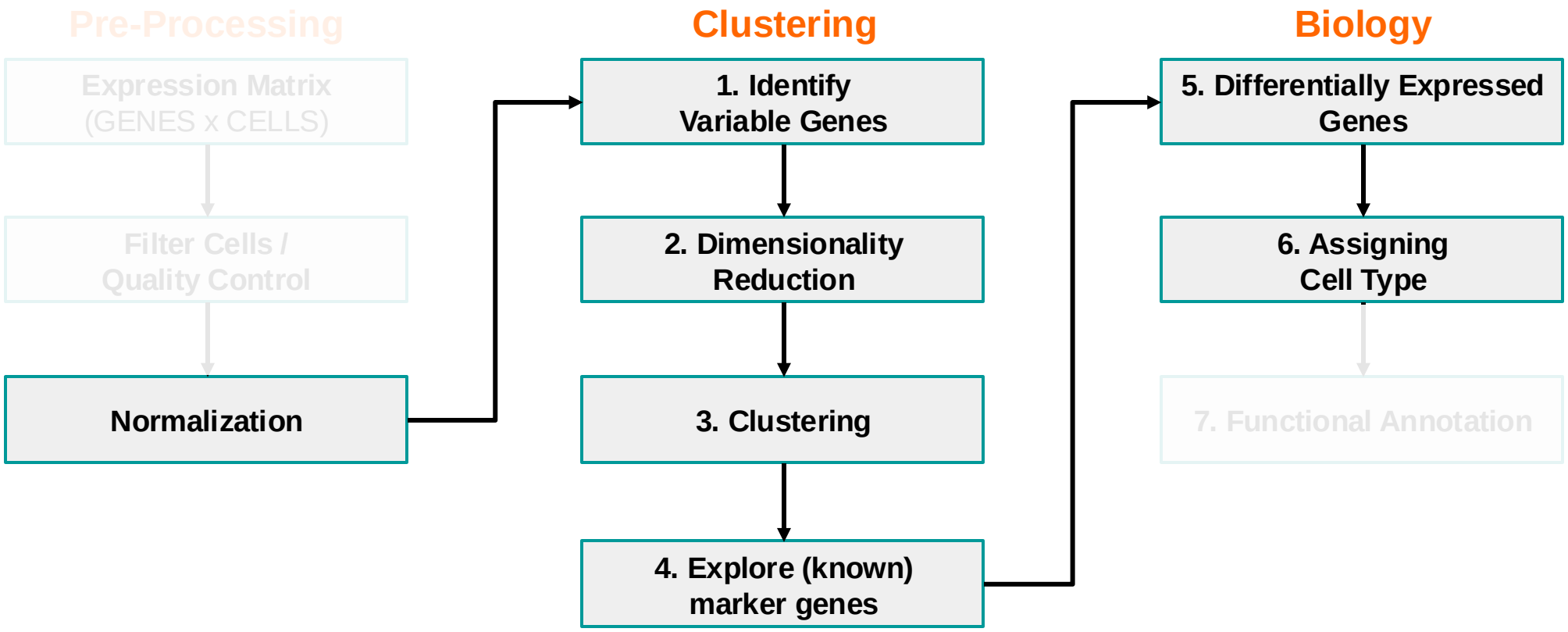


Figure 3 | Average similarities between gene rankings obtained by the evaluated DE methods. The dendrogram was obtained by complete-linkage hierarchical clustering based on the matrix of average AUCC values across all data sets. The labels of the internal nodes represent their stability across data sets (fraction of instances where they are observed). Only nodes with stability scores of at least 0.1 are labeled. Colored boxes represent method characteristics.

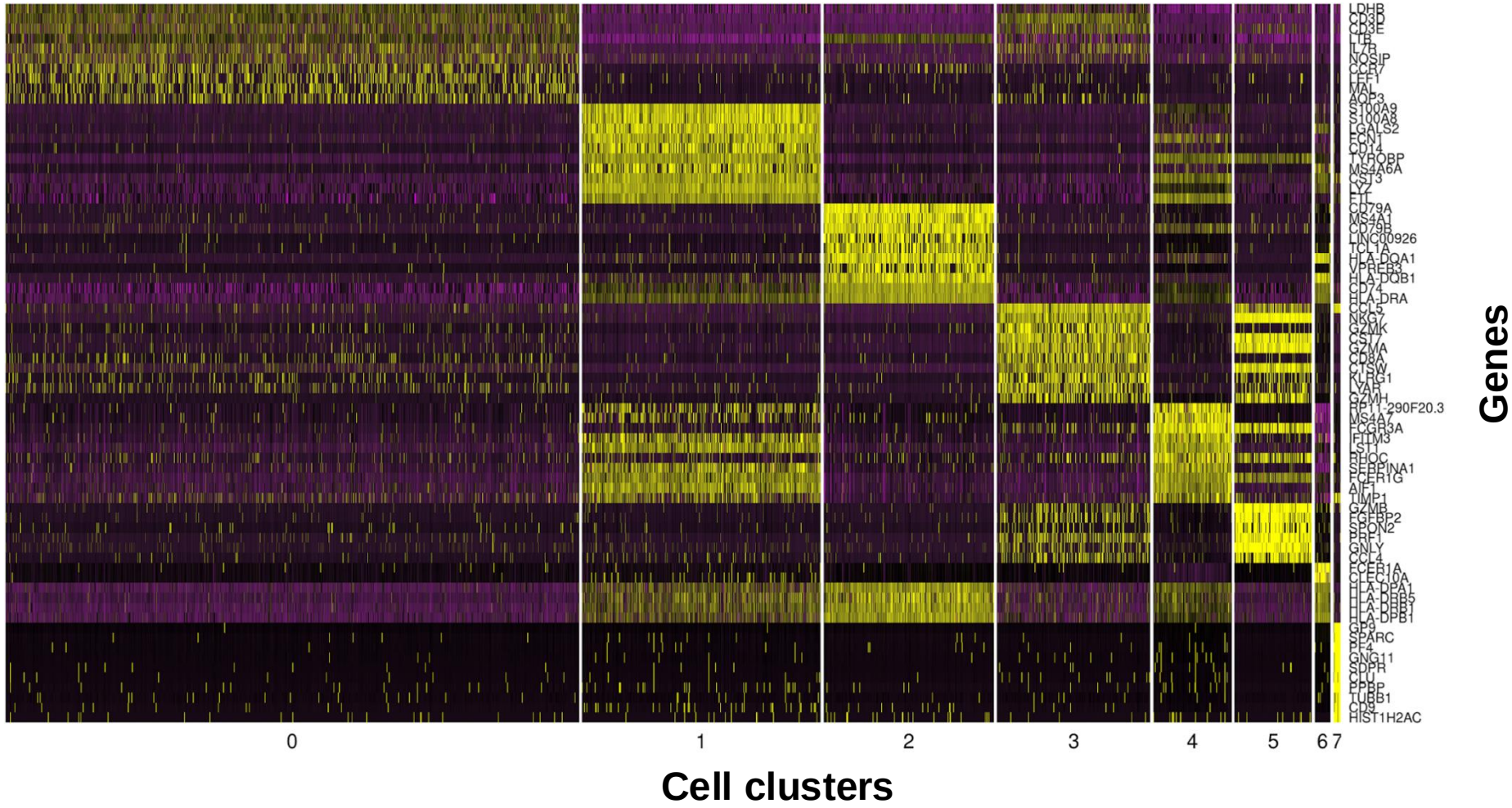


Soneson and Robinson, 2018

Analysis workflow



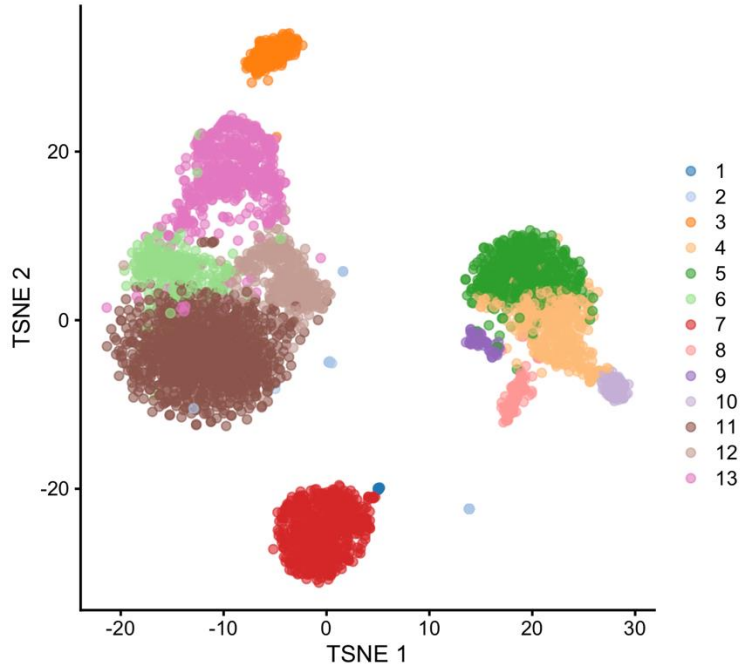
Cell type annotation using identified markers per cluster

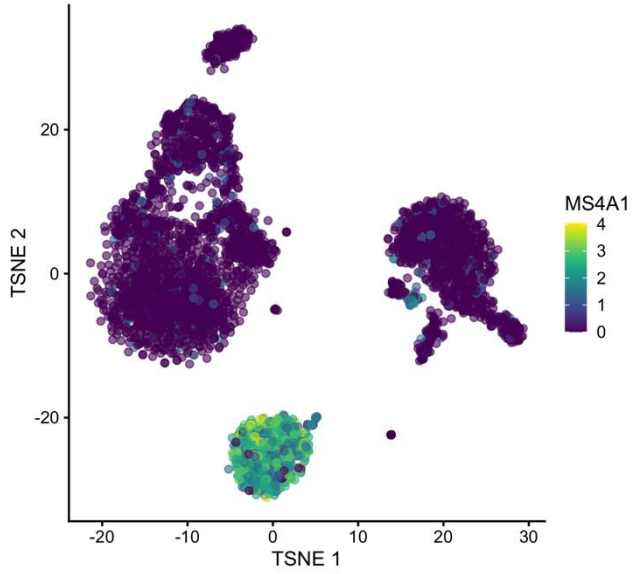


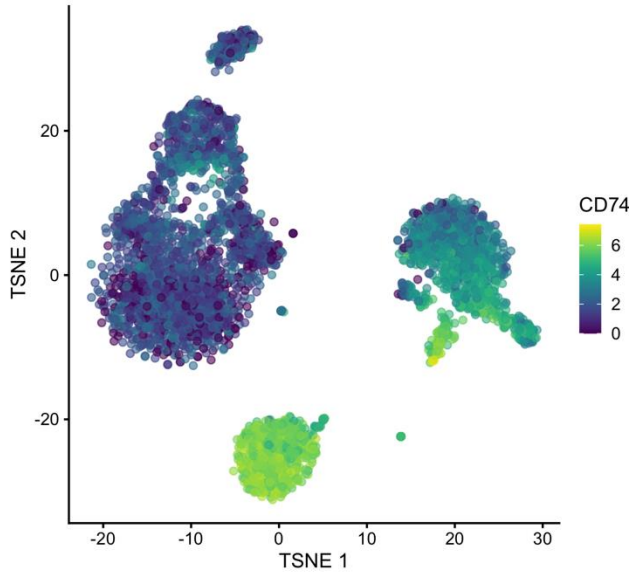
Manual cell type annotation using identified markers per cluster

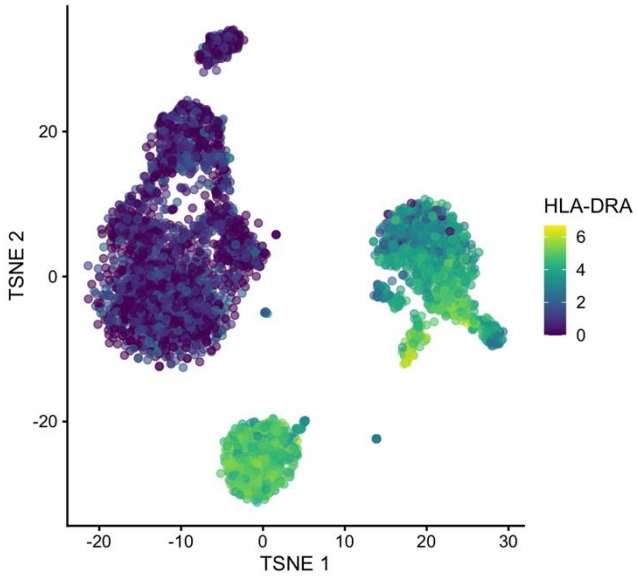
Top markers of cluster #7 in PBMCs:

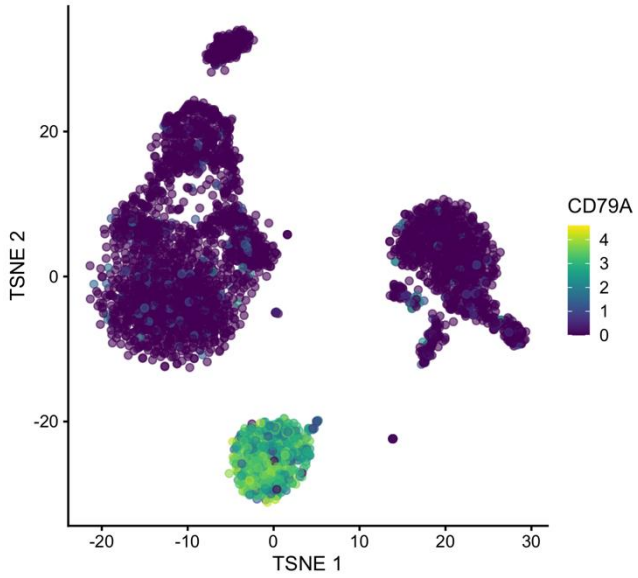
CD74
HLA-DRA
MS4A1
CD79A
HLA-DRB1
HLA-DPA1
CD79B
LTB
HLA-DQB1
TCL1A
CD52
HLA-DPB1
CD37





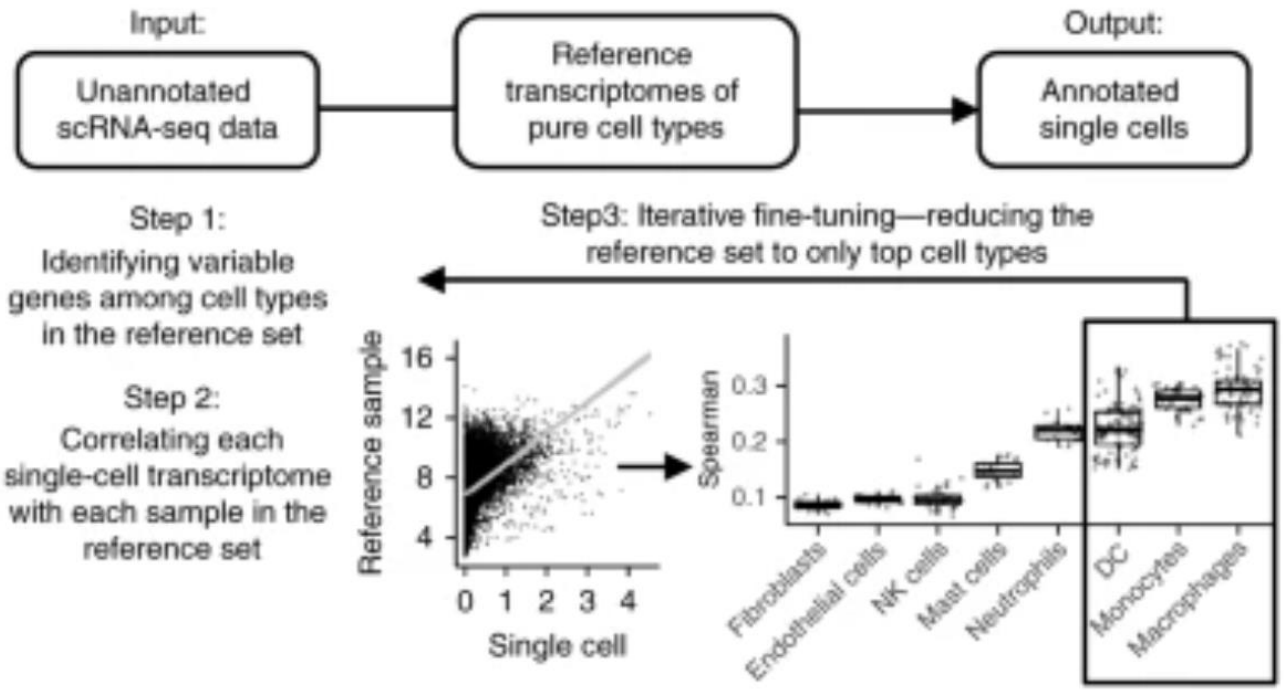






Automated cell type annotation using public marker databases

SingleR can rely on references of pure cell types to annotate individual cells within a scRNAseq dataset.



Aran et al., Nat. Immuno. 2019

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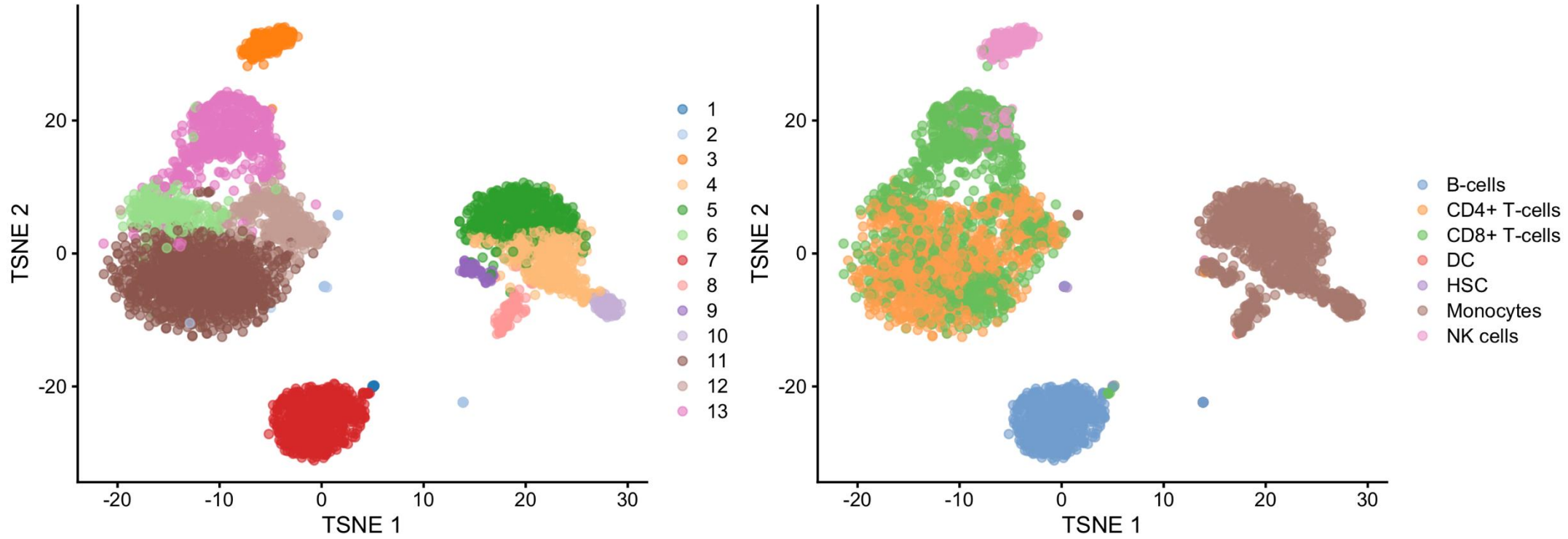

However, it is limited in sensitivity, as it can only identify cells based on the references used.

Automated cell type annotation using public marker databases

Huge (and growing!) collection of tools for automated cell annotation…

Name	Version	Language	Underlying classifier	Prior knowledge	Rejection option
Garnett	0.1.4	R	Generalized linear model	Yes	Yes
Moana	0.1.1	Python	SVM with linear kernel	Yes	No
DigitalCellSorter	GitHub version: e369a34	Python	Voting based on cell type markers	Yes	No
SCINA	1.1.0	R	Bimodal distribution fitting for marker genes	Yes	No
scVI	0.3.0	Python	Neural network	No	No
Cell-BLAST	0.1.2	Python	Cell-to-cell similarity	No	Yes
ACTINN	GitHub version: 563bcc1	Python	Neural network	No	No
LAmbDA	GitHub version: 3891d72	Python	Random forest	No	No
scmapcluster	1.5.1	R	Nearest median classifier	No	Yes
scmapcell	1.5.1	R	kNN	No	Yes
scPred	0.0.0.9000	R	SVM with radial kernel	No	Yes
CHETAH	0.99.5	R	Correlation to training set	No	Yes
CaSTLe	GitHub version: 258b278	R	Random forest	No	No
SingleR	0.2.2	R	Correlation to training set	No	No
scID	0.0.0.9000	R	LDA	No	Yes
singleCellNet	0.1.0	R	Random forest	No	No
LDA	0.19.2	Python	LDA	No	No
NMC	0.19.2	Python	NMC	No	No
RF	0.19.2	Python	RF (50 trees)	No	No
SVM	0.19.2	Python	SVM (linear kernel)	No	No
$SVM_{rejection}$	0.19.2	Python	SVM (linear kernel)	No	Yes
kNN	0.19.2	Python	kNN ($k = 9$)	No	No

Abdelaal et al., Genome Biol. 2019

Automated cell type annotation using public marker databases

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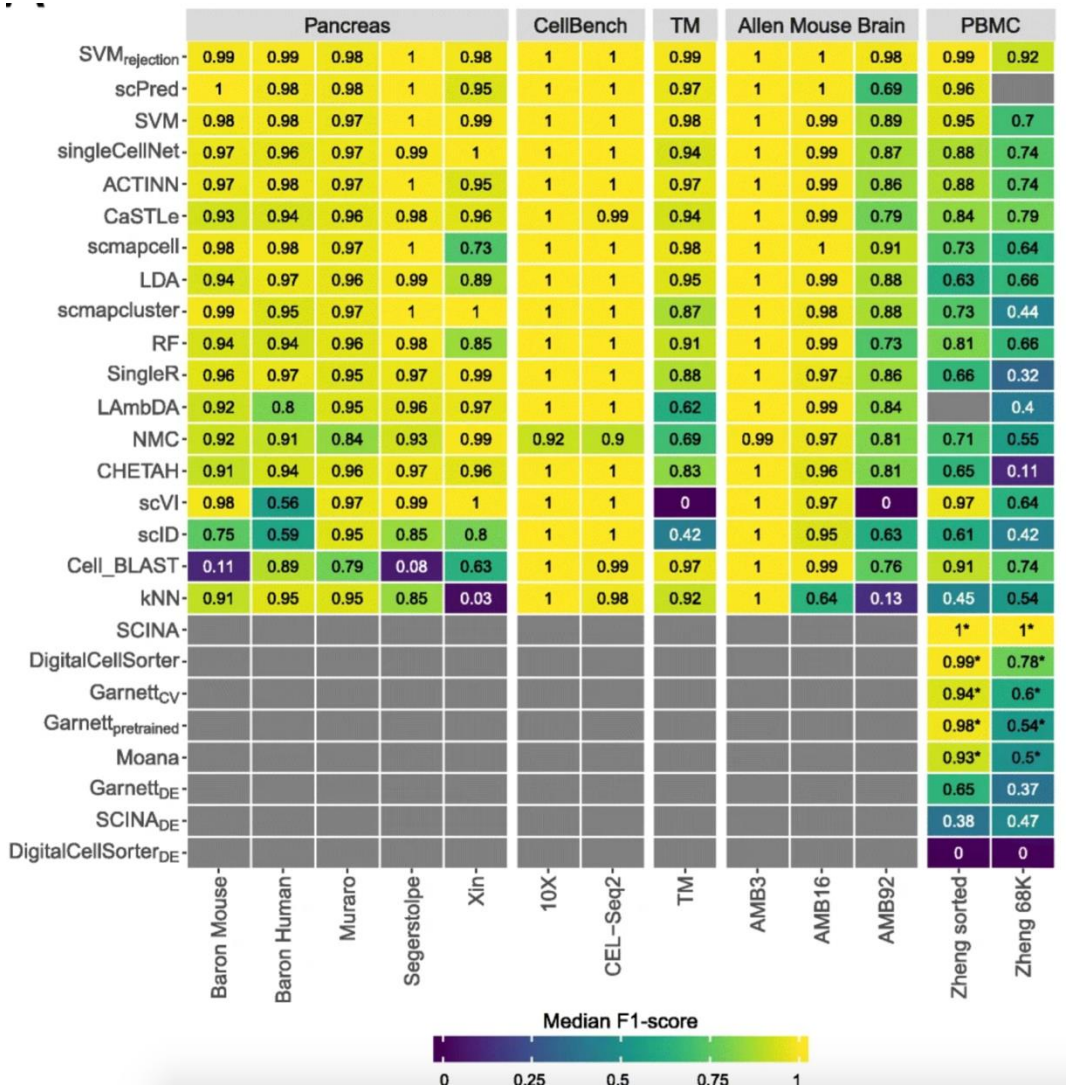

Abdelaal et al., Genome Biol. 2019