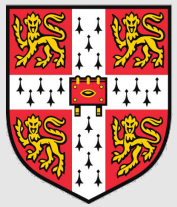


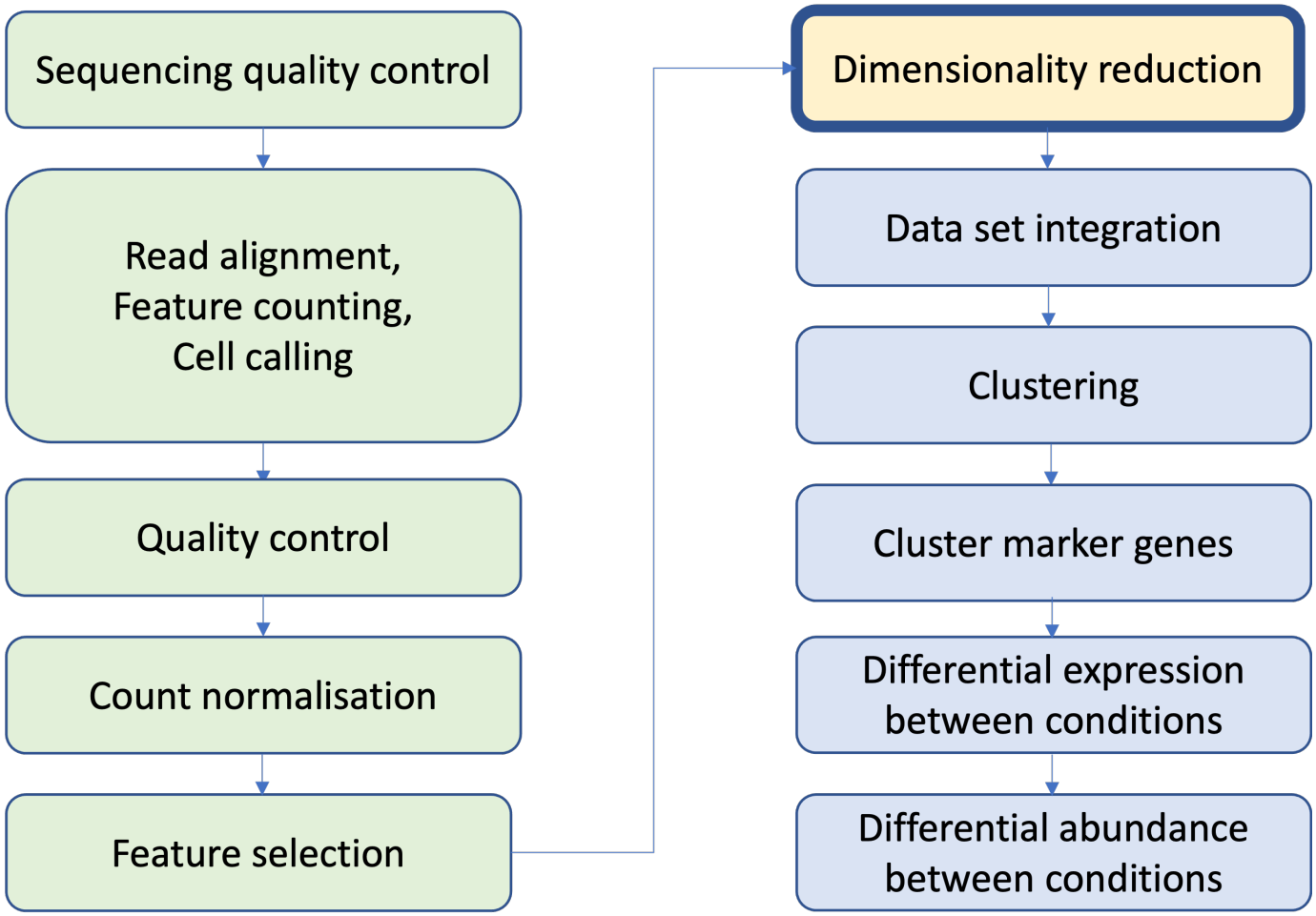
UNIVERSITY OF CAMBRIDGE

CANCER RESEARCH UK | Cambridge Institute

Feature Selection and Dimensionality Reduction

September 2022

Single Cell RNAseq Analysis Workflow

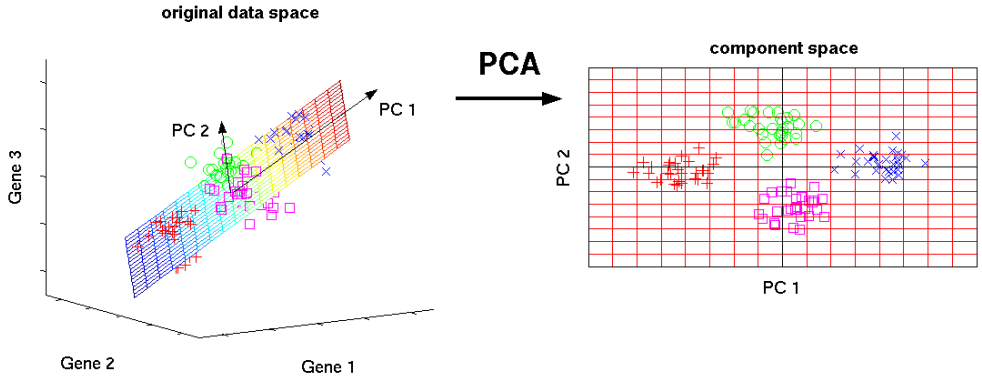


Why do high-dimensional data pose a problem?

In single-cell data we typically have thousands of genes across thousands (or millions!) of cells.

- Interpretation/visualisation beyond 2D is hard.
- As we increase the number of dimensions, our data becomes more sparse.
- High computational burden for downstream analysis (such as cell clustering)

Solution: collapse the number of dimensions to a more manageable number, while preserving information.



(Image source)

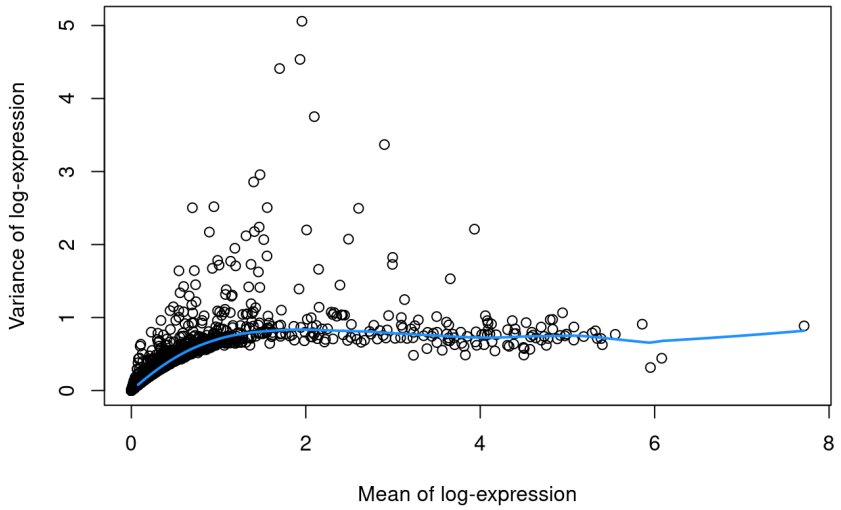
There are many dimensionality reduction algorithms

PCA	linear	Matrix Factorization		
ICA	linear	Matrix Factorization		
MDS	non-linear	Matrix Factorization		
Sparce NNMF	non-linear	Matrix Factorization	2010	https://pdfs.semanticscholar.org/664d/40258f12ad28ed0b7d4c272935ad72a150db.pdf
cPCA	non-linear	Matrix Factorization	2018	https://doi.org/10.1038/s41467-018-04608-8
ZIFA	non-linear	Matrix Factorization	2015	https://doi.org/10.1186/s13059-015-0805-z
ZINB-WaVE	non-linear	Matrix Factorization	2018	https://doi.org/10.1038/s41467-017-02554-5
Diffusion maps	non-linear	graph-based	2005	https://doi.org/10.1073/pnas.0500334102
Isomap	non-linear	graph-based	2000	10.1126/science.290.5500.2319
t-SNE	non-linear	graph-based	2008	https://lvdmaaten.github.io/publications/papers/JMLR_2008.pdf
- BH t-SNE	non-linear	graph-based	2014	https://lvdmaaten.github.io/publications/papers/JMLR_2014.pdf
- Flt-SNE	non-linear	graph-based	2017	arXiv:1712.09005
LargeVis	non-linear	graph-based	2018	arXiv:1602.00370
UMAP	non-linear	graph-based	2018	arXiv:1802.03426
PHATE	non-linear	graph-based	2017	https://www.biorxiv.org/content/biorxiv/early/2018/06/28/120378.full.pdf
scvis	non-linear	Autoencoder (MF)	2018	https://doi.org/10.1038/s41467-018-04368-5
VASC	non-linear	Autoencoder (MF)	2018	https://doi.org/10.1016/j.gpb.2018.08.003

Which genes should we use for downstream analysis?

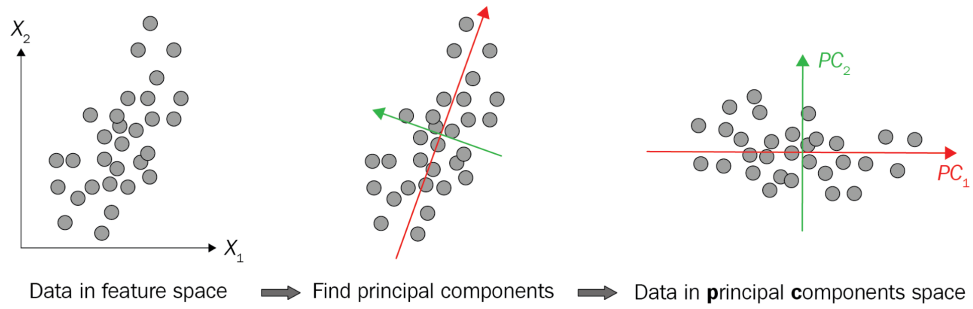
Select genes which capture biologically-meaningful variation, while reducing the number of genes which only contribute to technical noise

(Image Source)



- Model the gene-variance relationship across all genes to define a data-driven “technical variation threshold”
- Select **highly variable genes** (HVGs) for downstream analysis (e.g. PCA and clustering)

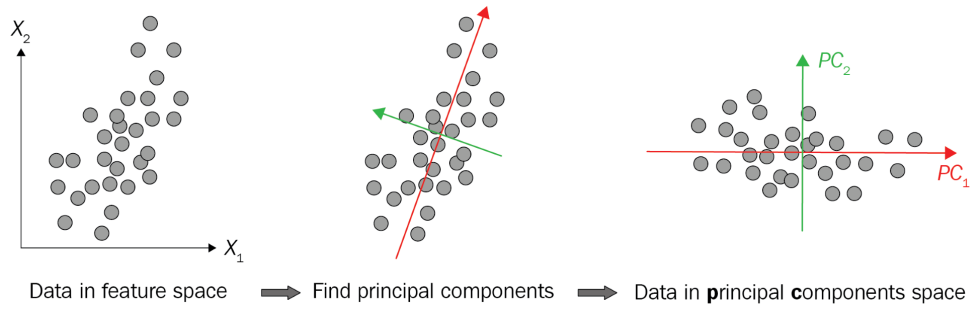
Principal Components Analysis (PCA)



(Image Source)

- It's a linear algebraic method of dimensionality reduction
- Finds principal components (PCs) of the data
  - Directions where the data is most spread out (highest variance)
  - PC1 explains most of the variance in the data, then PC2, PC3, etc.
  - PCA is primarily a dimension reduction technique, but it is also useful for visualization
  - A good separation of dissimilar objects is provided
  - Preserves the global data structure

Principal Components Analysis (PCA)

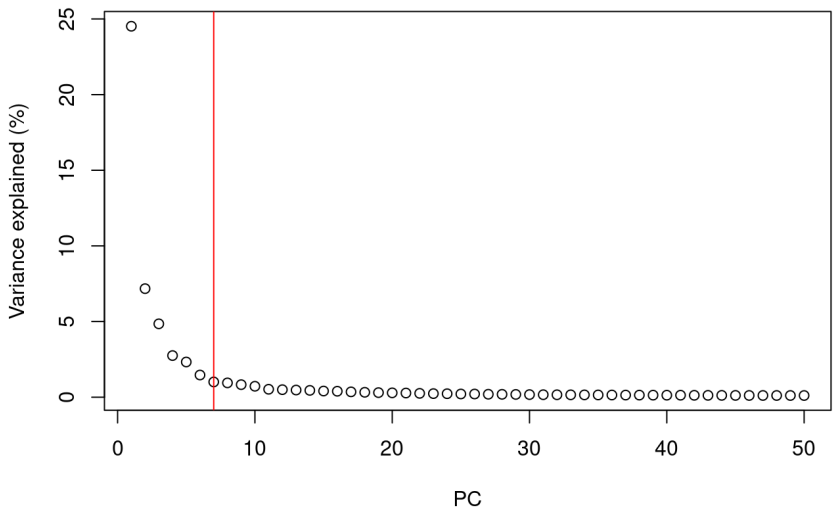


(Image Source)

- When data is very highly-dimensional, we can select the most important PCs only, and use them for downstream analysis (e.g. clustering cells)
  - This reduces the dimensionality of the data from ~20,000 genes to maybe 20-50 PCs
  - Each PC represents a robust 'metagene' that combines information across a correlated gene set
- Prior to PCA we scale the data so that genes have equal weight in downstream analysis and highly expressed genes don't dominate

How many principal components for downstream analysis?

After PCA we are still left with as many dimensions in our data as we started



(Image Source)

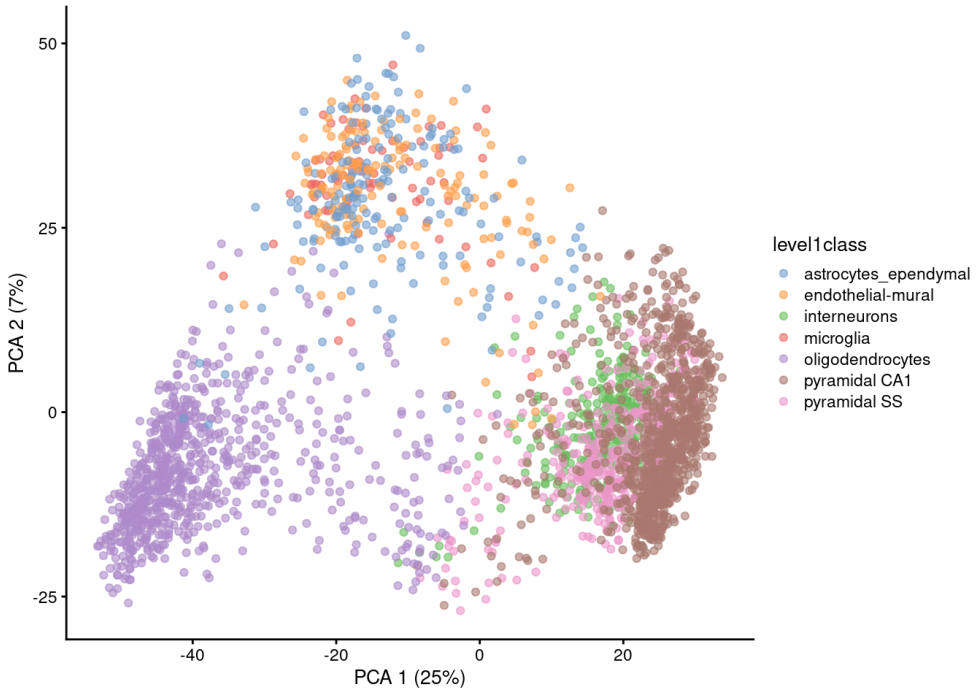
But our principal components progressively capture less variation in the data

How do we select the number of PCs to retain for downstream analysis?

- Using the “Elbow” method on the scree plot
- Using the model of technical noise (shown earlier)
- Trying downstream analysis with different number of PCs (10, 20, or even 50)

Visualizing PCA results: PC scores

Because PC1 and PC2 capture most of the variance of the data, it is common to visualise the data projected onto those two new dimensions.

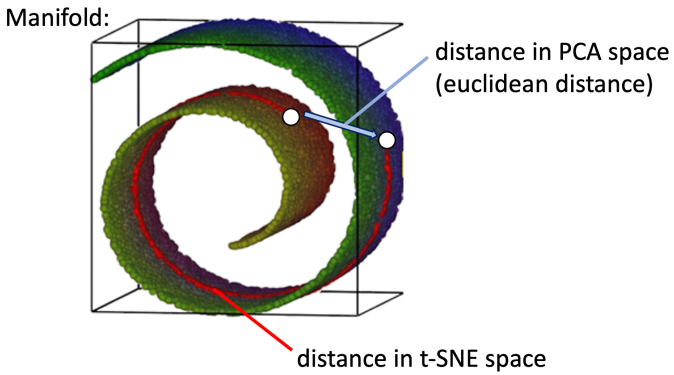


Gene expression patterns will be captured by PCs → PCA can separate cell types

Note that PCA can also capture other things, like sequencing depth or cell heterogeneity/complexity!

However, PC1 + PC2 are usually not enough to visualise all the diversity of cell types in single-cell data (usually we need to use PC3, PC4, etc…) → not so good for visualisation, so…

Non-linear dimensionality reduction methods

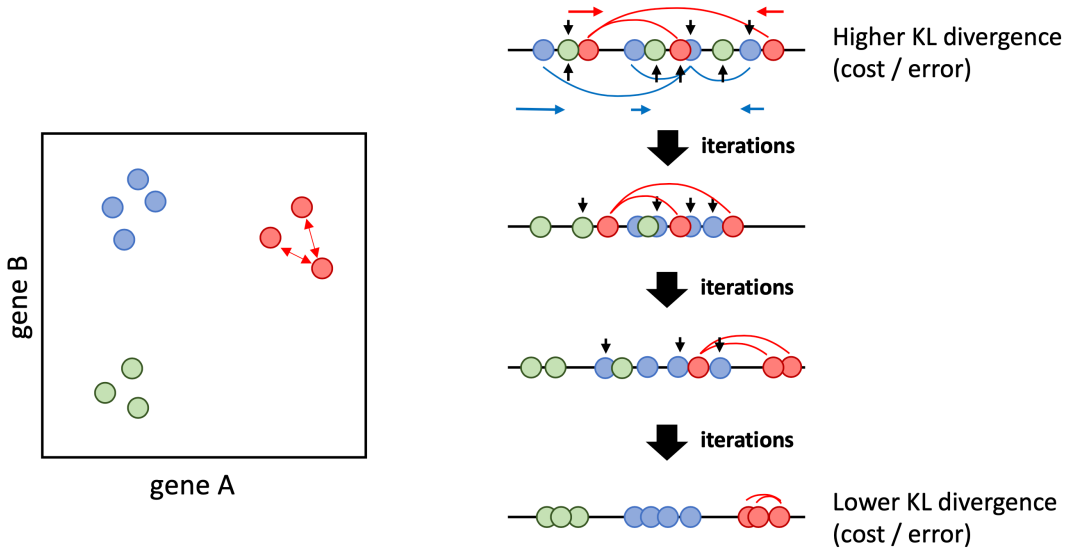


Graph-based, non-linear methods: **UMAP** and **t-SNE**

These methods can run on the output of the PCA, which speeds their computation and can make the results more robust to noise

t-SNE and UMAP should only be used for visualisation, not as input for downstream analysis

t-Distributed Stochastic Neighbor Embedding (t-SNE)



It has a stochastic step (results vary every time you run it)

Only local distances are preserved, while distances between groups are not always meaningful

Some parameters dramatically affect the resulting projection (in particular “perplexity”)

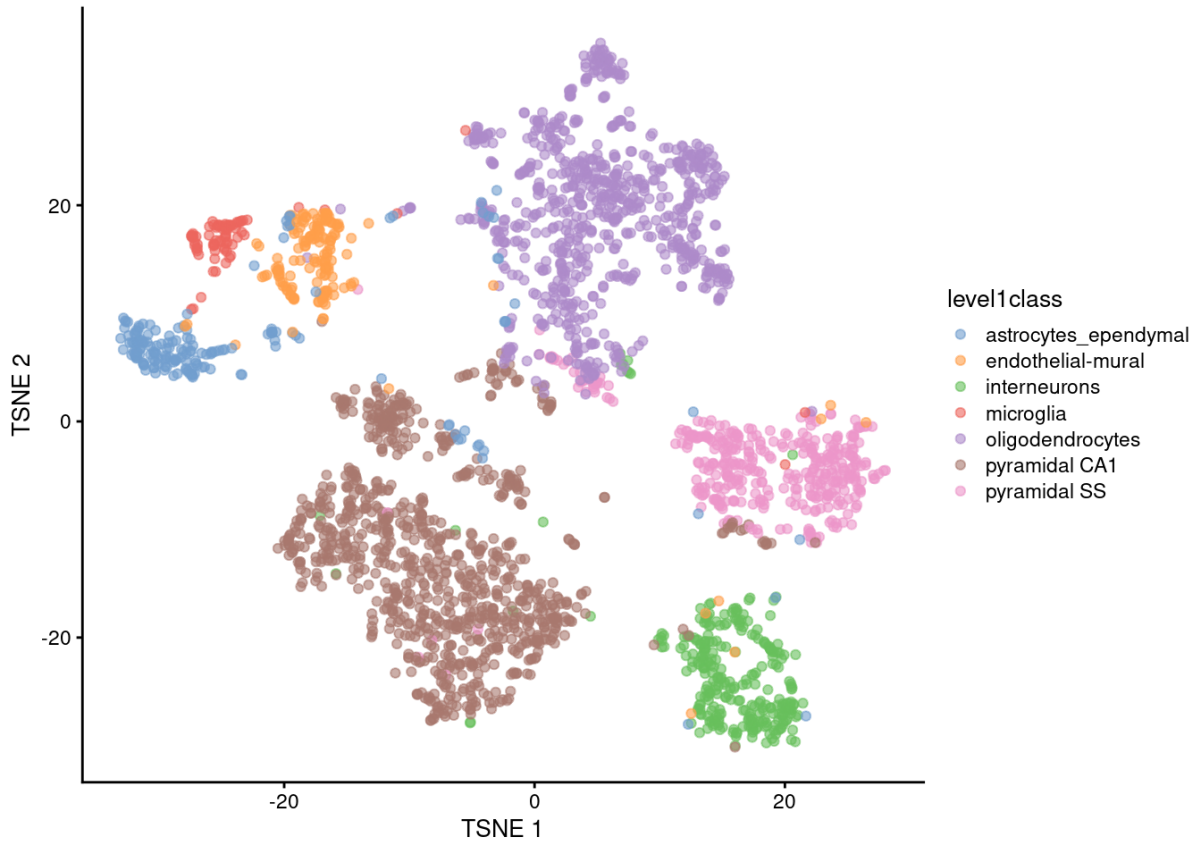
Learn more about how t-SNE works from this video: StatQuest: t-SNE, Clearly Explained

t-SNE

Main parameter in t-SNE is the **perplexity** (~ number of neighbours each point is “attracted” to)

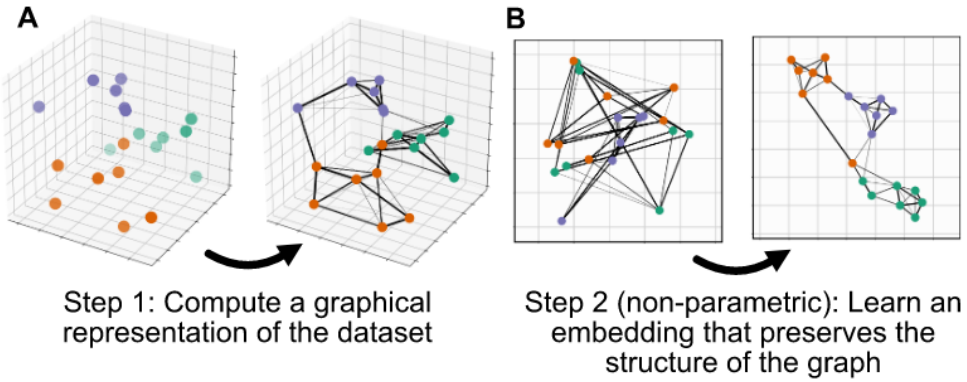
- Balance between preserving local vs global structure
- Higher values usually result in more compact clusters
- But too high can lead to overlap of clusters, making them harder to distinguish

Exploring different perplexity values that best represent the biological diversity of cells is recommended.



UMAP

- Non-linear graph-based dimension reduction method like t-SNE
- Newer & efficient = fast
- Runs on top of PCs
- Based on topological structures in multidimensional space
- Faster and less computationally intensive than tSNE
- Preserves the global structure better than t-SNE



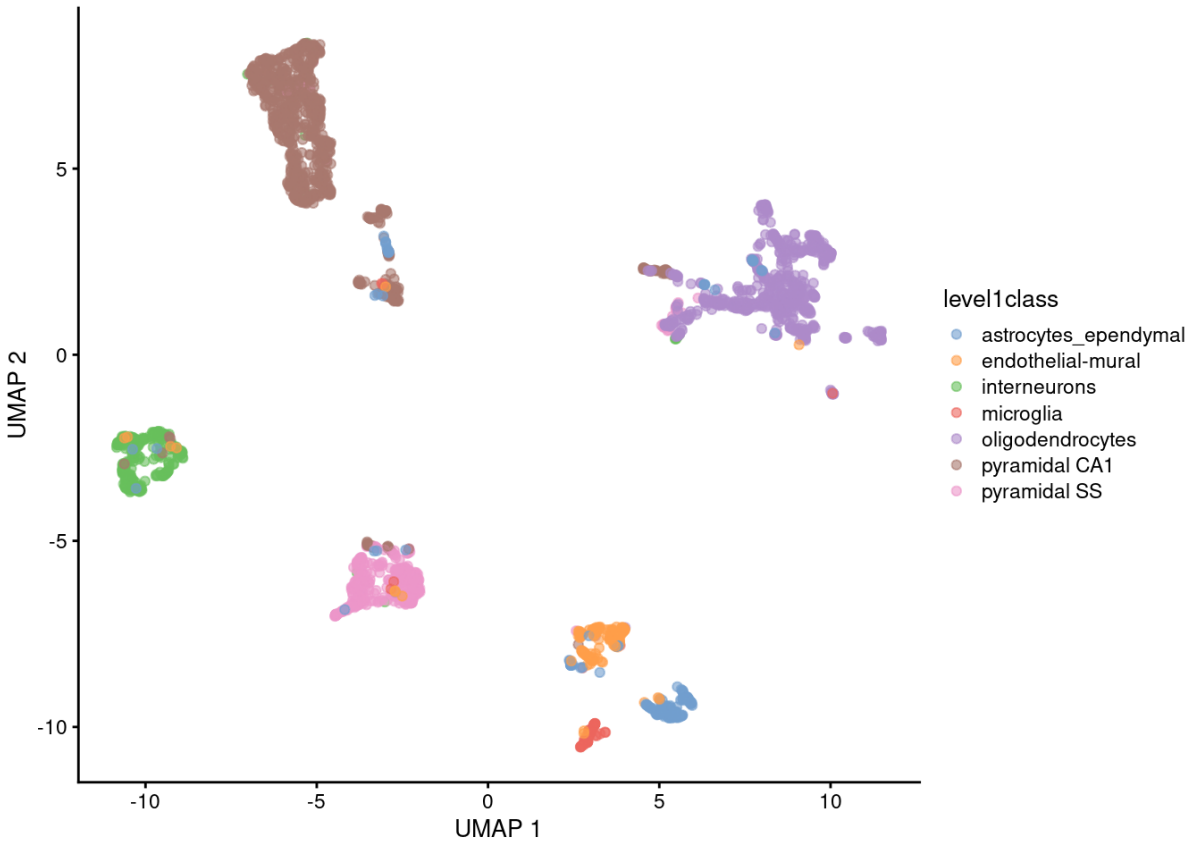
UMAP

Main parameter in UMAP is `n_neighbors` (the **number of neighbours** used to construct the initial graph).

Another common parameter is `min_dist` (**minimum distance** between points)

- Together they determine balance between preserving local vs global structure
- For practical simplicity, we usually only tweak `n_neighbors`, although playing with both parameters can be beneficial

Exploring different number of neighbours that best represent the biological diversity of cells is recommended.



Key Points

- Dimensionality reduction methods simplify high-dimensional data while preserving biological signal.
- Common methods in scRNA-seq analysis include PCA, t-SNE, and UMAP.
- PCA transforms the data linearly to capture the main variance and reduce the dimensionality from thousands of genes to a few principal components.
- PCA results can be utilized for downstream analysis like cell clustering and trajectory analysis, and as input for non-linear methods such as t-SNE and UMAP.
- t-SNE and UMAP are non-linear methods that group similar cells and separate dissimilar cell clusters.
- These non-linear methods are primarily for data visualization, not for downstream analysis.

Acknowledgments

Slides are adapted from Paulo Czarnewski and Zeynep Kalender-Atak

References (image sources):

- Orchestrating Single-Cell Analysis with Bioconductor
- Parametric UMAP embeddings for representation and semi-supervised learning