First steps

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Overview

- 10x scRNAseq output data
- Seurat Object
- PCA
- Shared nearest neighbourgh graph
- Louvain clustering

Standard 10x scRNAseq output

- matrix.mtx -> feature(gene) x cell counts
- genes.tsv -> gene annotation (ensembl ID - > gene symbol)
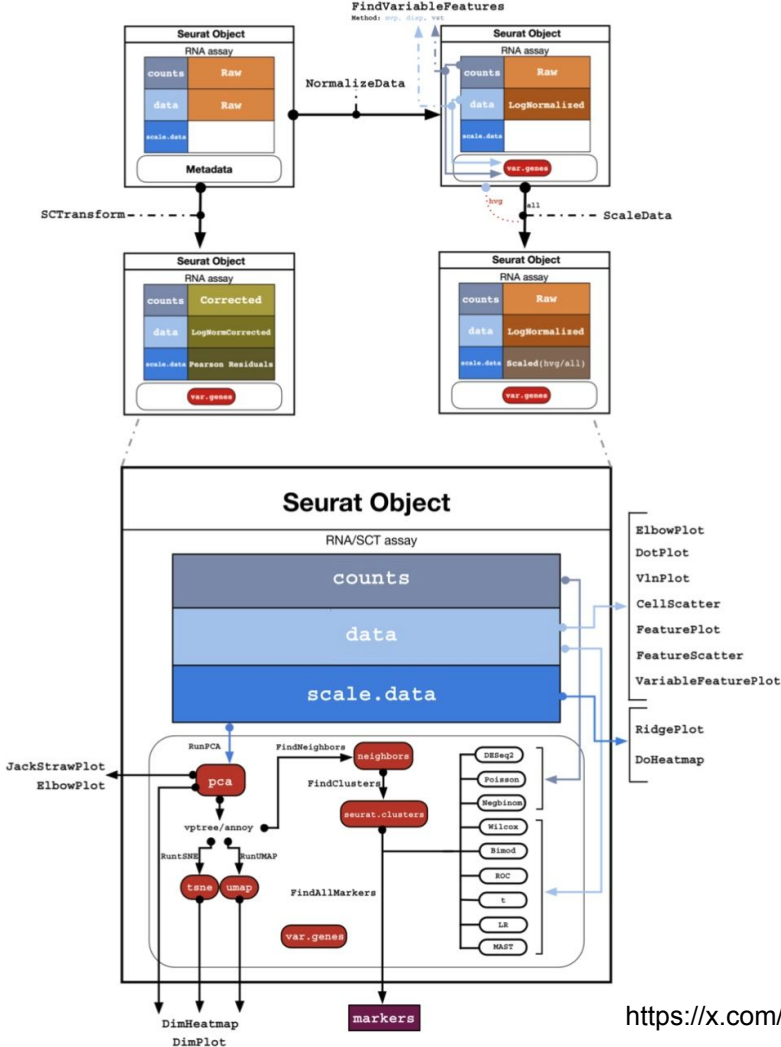- barcodes.tsv -> barcode list
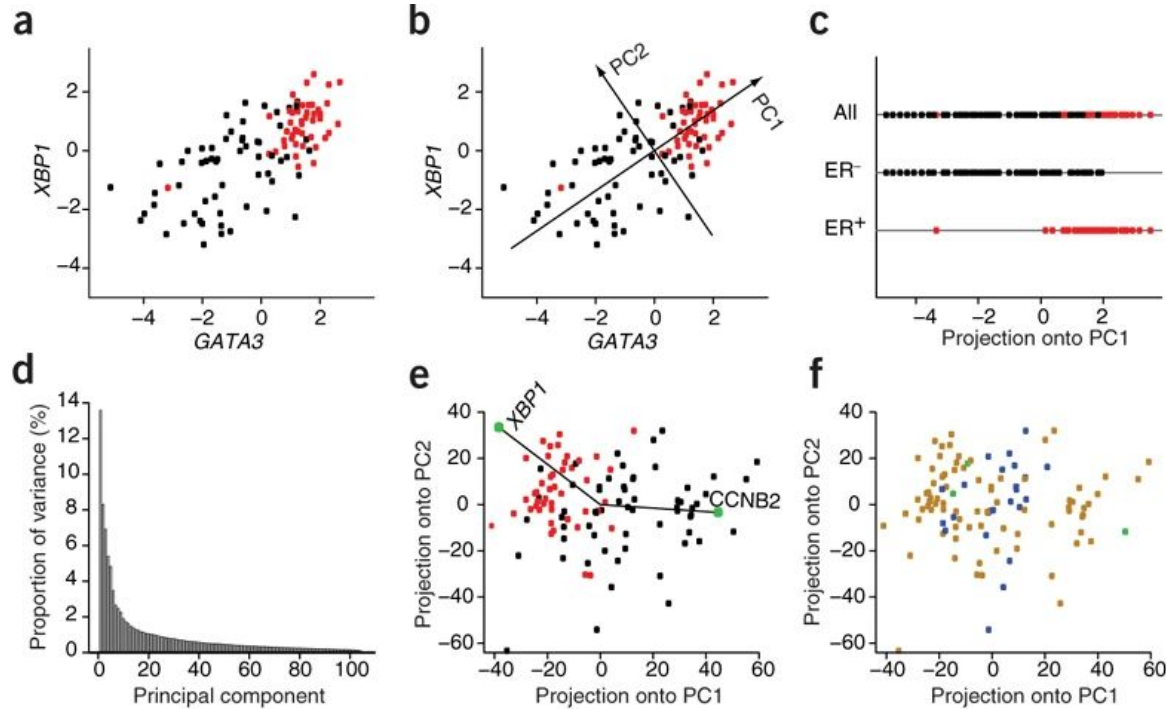
Seurat Object

Accessors & accessor functions:
- obj@meta.data
- obj[[assay]]@layers$slot
- LayerData()
- FetchData()

- Layers()
- Assays()
- Features()
- Cells()

- Embeddings()

https://satijalab.org/seurat/articles/essential_commands#seurat-object-data-access

PCA: a simple example

5

PCA: multiple dimensions

13714 features $\longrightarrow$ 50 PCs
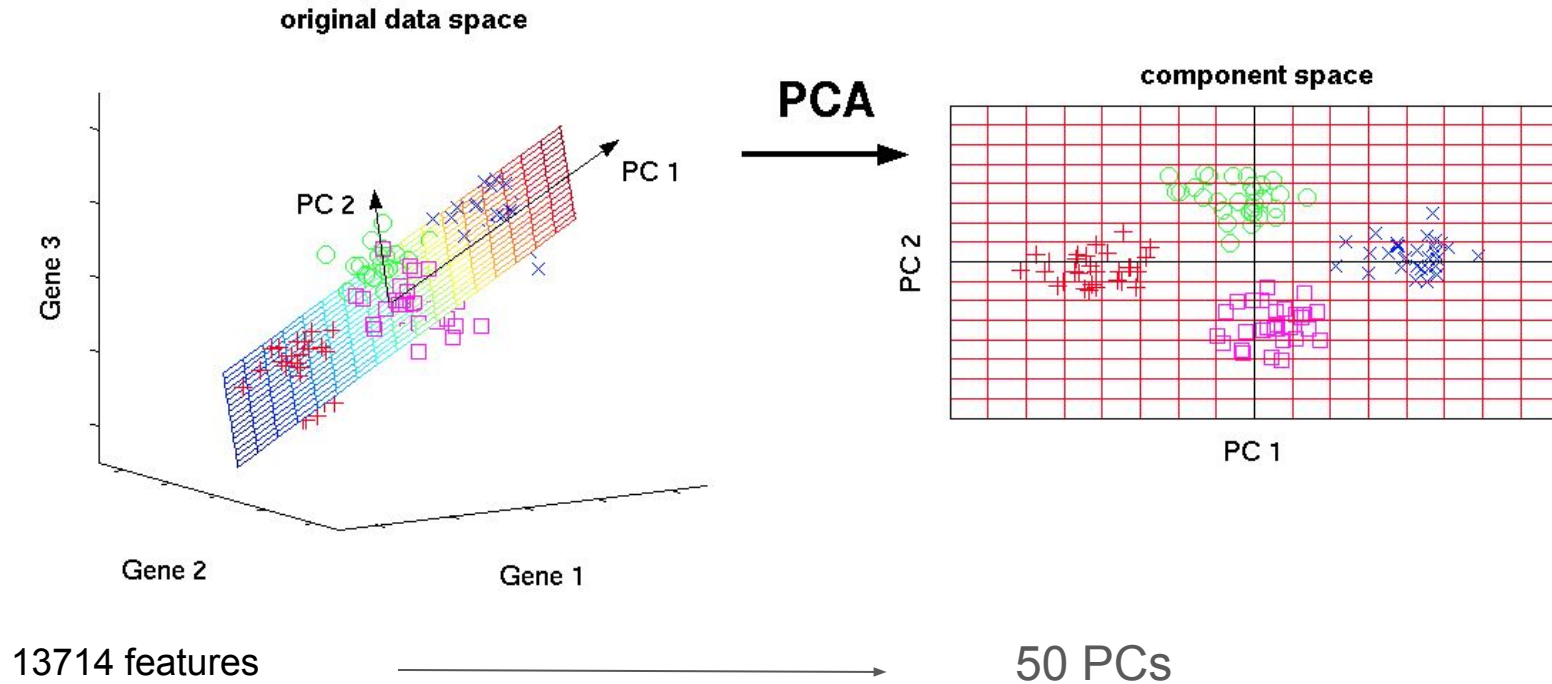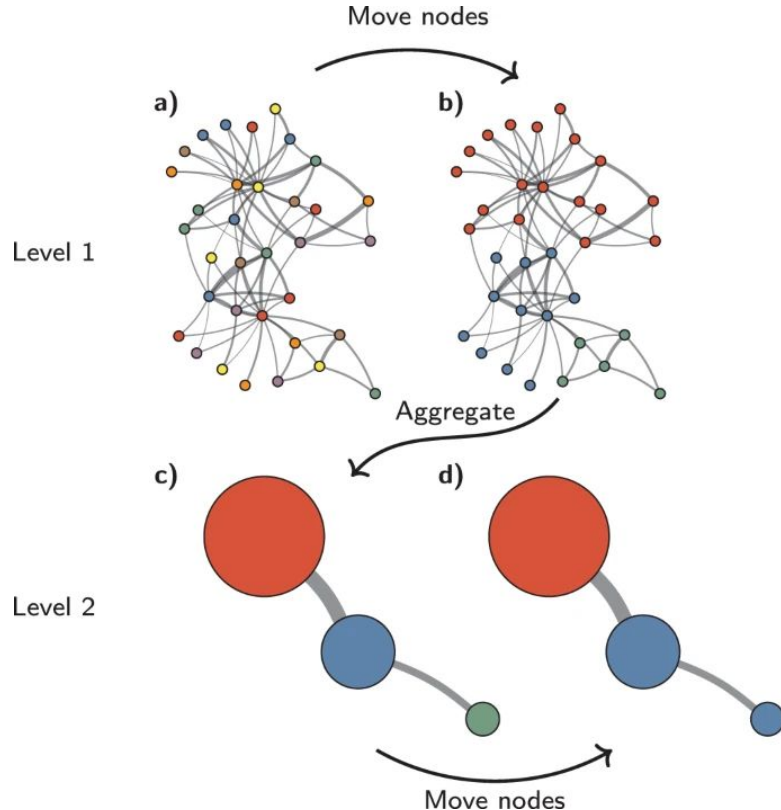
PCA on a scRNAseq dataset

scater::plotReducedDim()
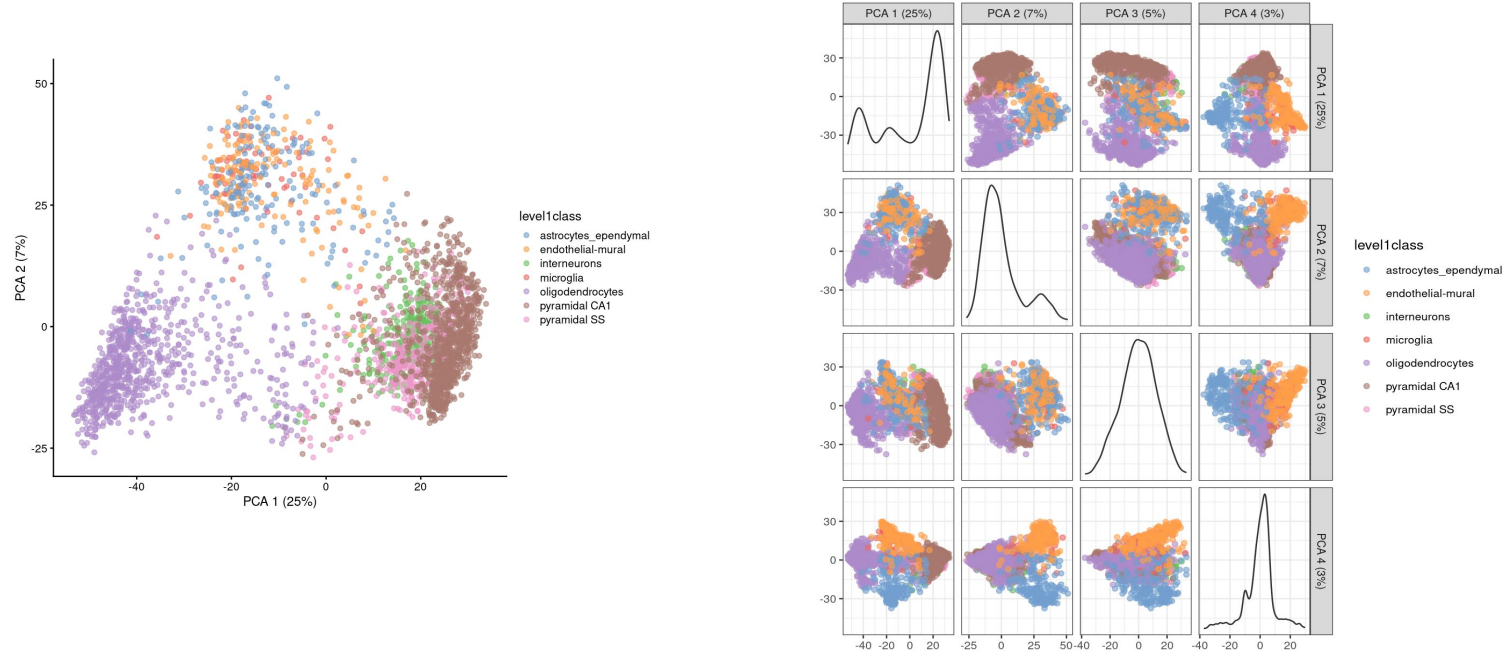
Shared nearest neighbour graph construction
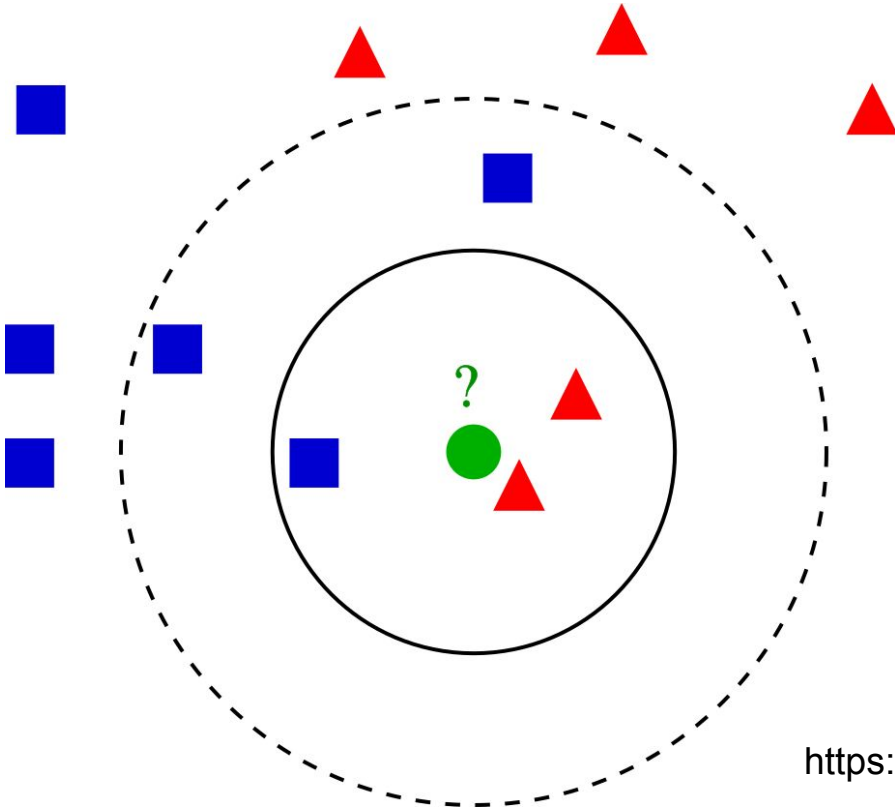
1. find k nearest neighbours for each cell
2. calculate neighbourhood overlap (Jaccard index)
3. construct SNN graph

https://en.wikipedia.org/wiki/K-nearest_neighbors_algorithm

Louvain/Leiden clustering on the SNN graph

Resolution parameter:
Higher resolutions lead to more communities, while lower resolutions lead to fewer communities.

https://www.nature.com/articles/s41598-019-41695-z