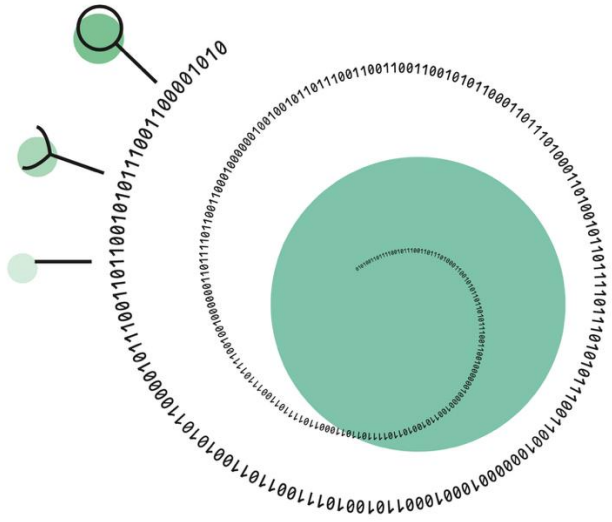
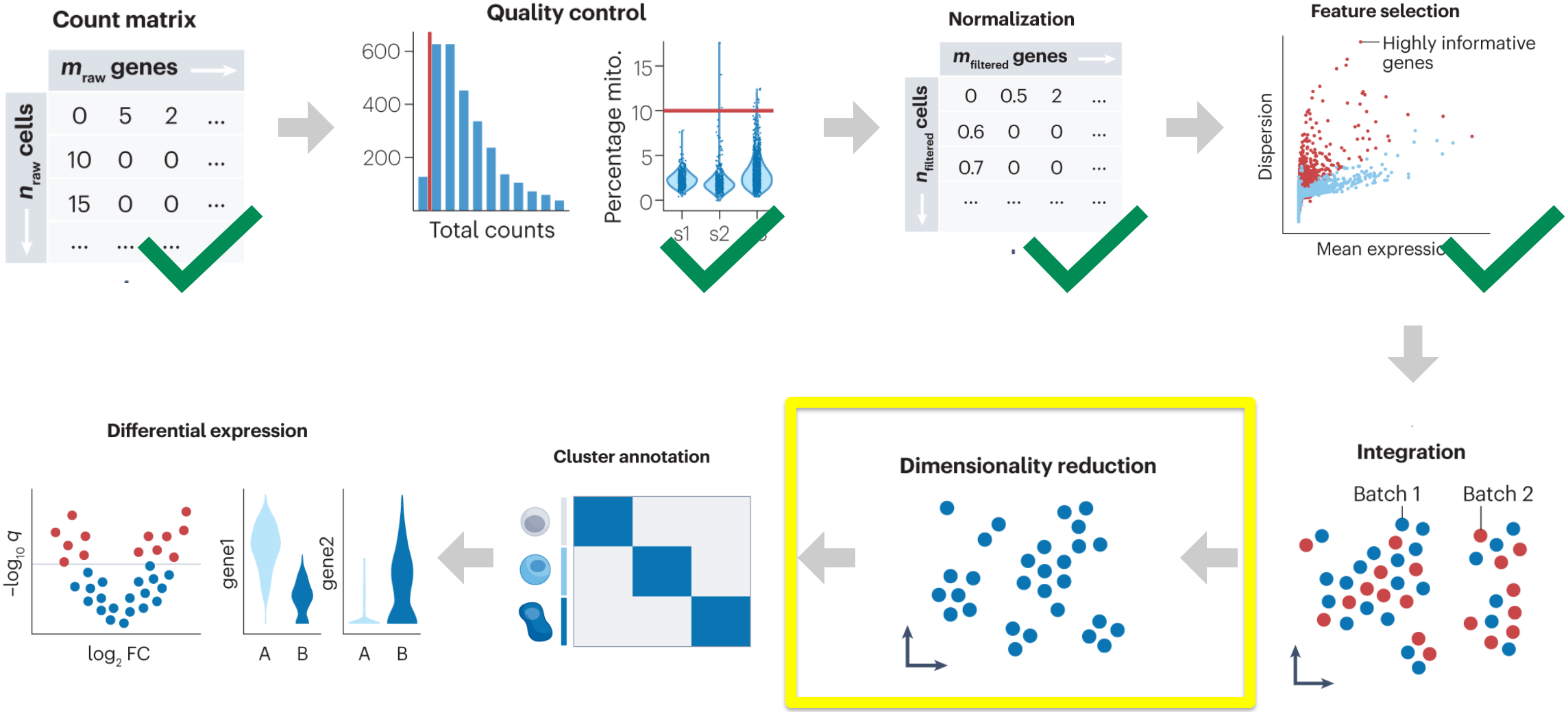


Single Dell Data Analysis Roundtable

Lecture 3 – Dimension reduction, clustering & visualization of high-dimensional data

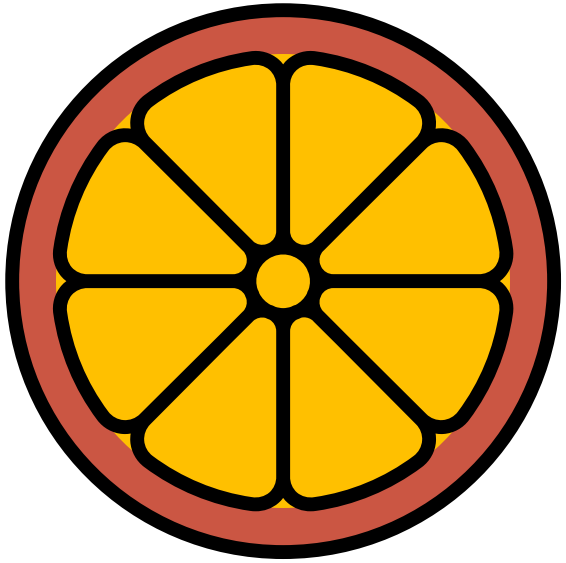
Lisa Buchauer
Professor of Systems Biology of Infectious Diseases
Department of Infectious Diseases and Intensive Care
Charité - Universitätsmedizin Berlin

Today



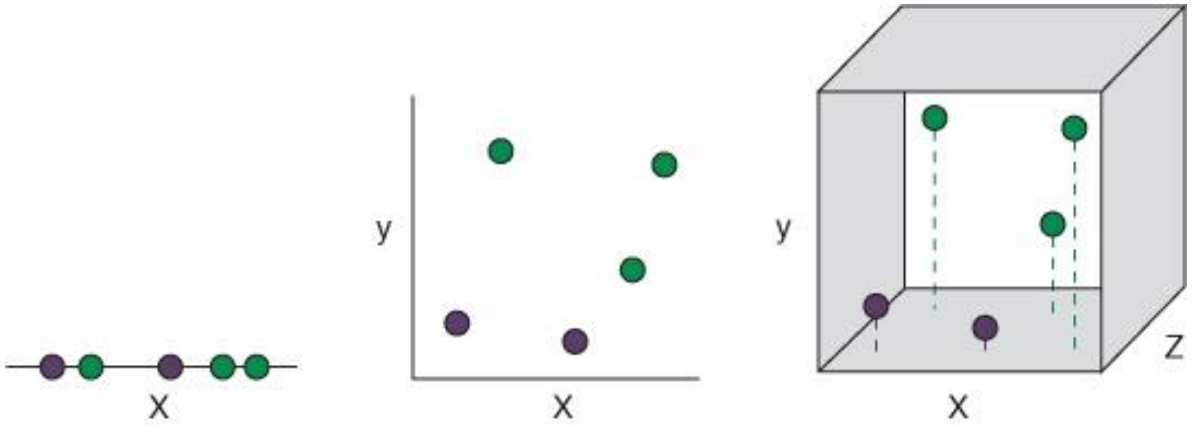
Heumos, L., Schaar, A.C., Lance, C. et al. Best practices for single-cell analysis across modalities. Nat Rev Genet 24, 550–572 (2023). https://doi.org/10.1038/s41576-023-00586-w

Losing intuition in high dimensions: a “fun fact”



In a high-dimensional orange, most of the mass is in the skin, not in the pulp

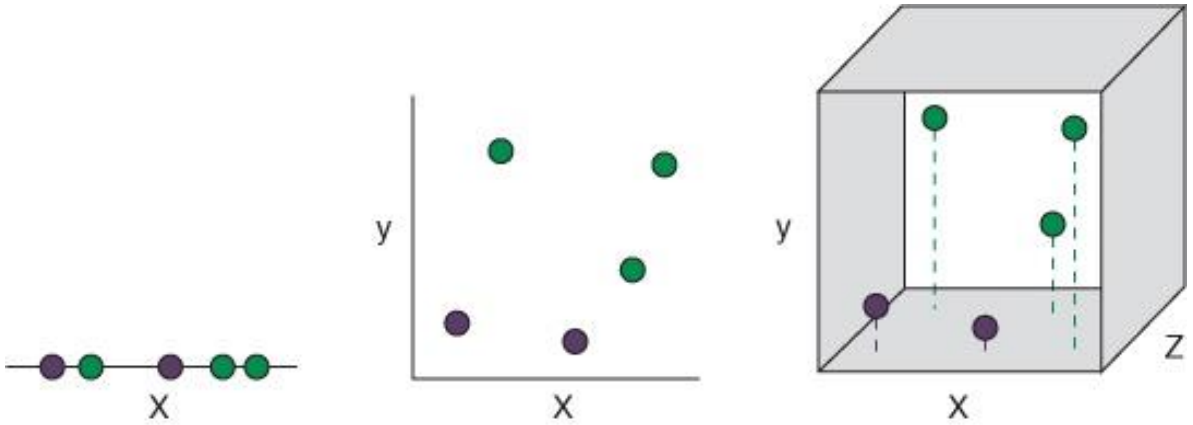
“The curse of dimensionality”



data sparsity
most of the high-dim space is empty

https://livebook.manning.com/concept/r/curse

“The curse of dimensionality”



data sparsity
most of the high-dim space is empty

distances may lose meaning

computational cost/ algorithmic efficiency

noise dimensions

visualization challenges

…

https://livebook.manning.com/concept/r/curse

Reducing dimensionality

Step 1: Feature selection

scanpy

```python
sc.pp.highly_variable_genes(adata, n_top_genes=2000, batch_key="sample")
```

Seurat

```r
pbmc <- FindVariableFeatures(pbmc, selection.method = "vst", nfeatures = 2000)
```

https://cofactorgenomics.com/curse-of-dimensionality-wk-16/

From PCA to clusters and UMAP in basic tutorials

1

```
sc.tl.pca(adata)
```

2

```
sc.pp.neighbors(adata)
```

3

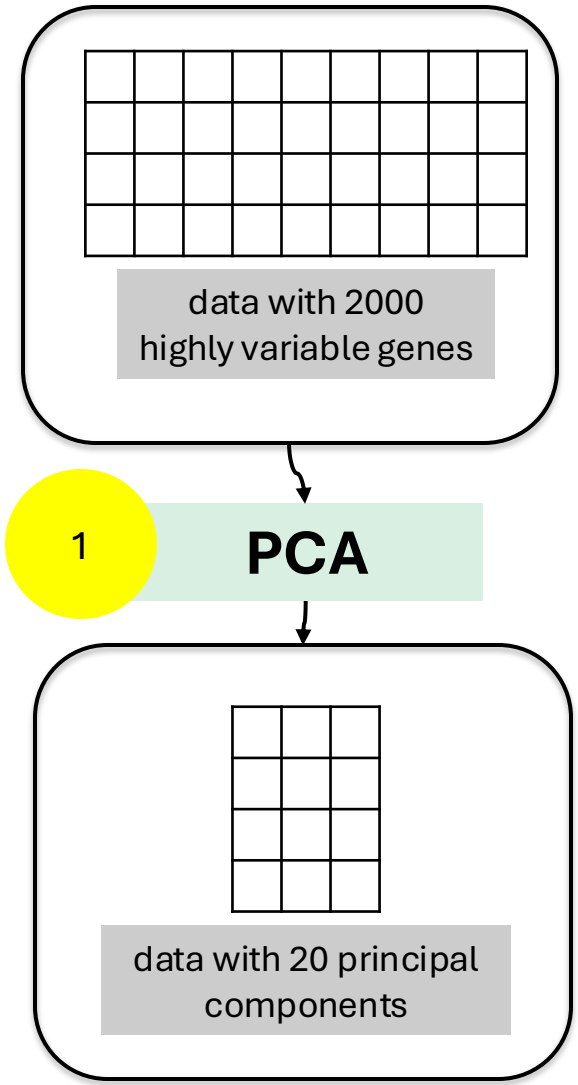
```
# Using the igraph implementation and a fixed number of iterations can be significantly fast
sc.tl.leiden(adata, flavor="igraph", n_iterations=2)
```

4

```
sc.tl.umap(adata)
```

https://scanpy.readthedocs.io/en/stable/tutorials/basics/clustering.html

Data types along the processing path



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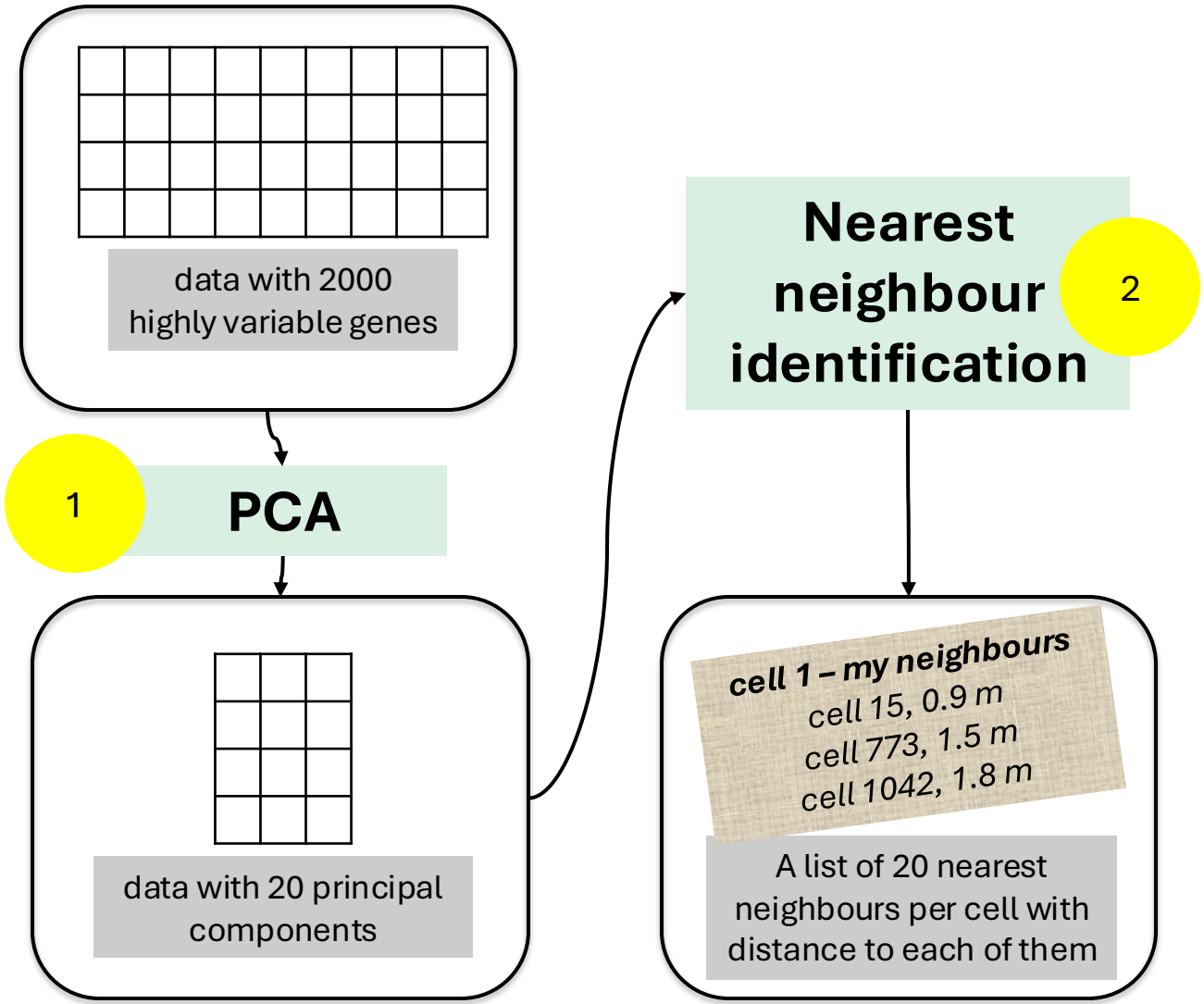

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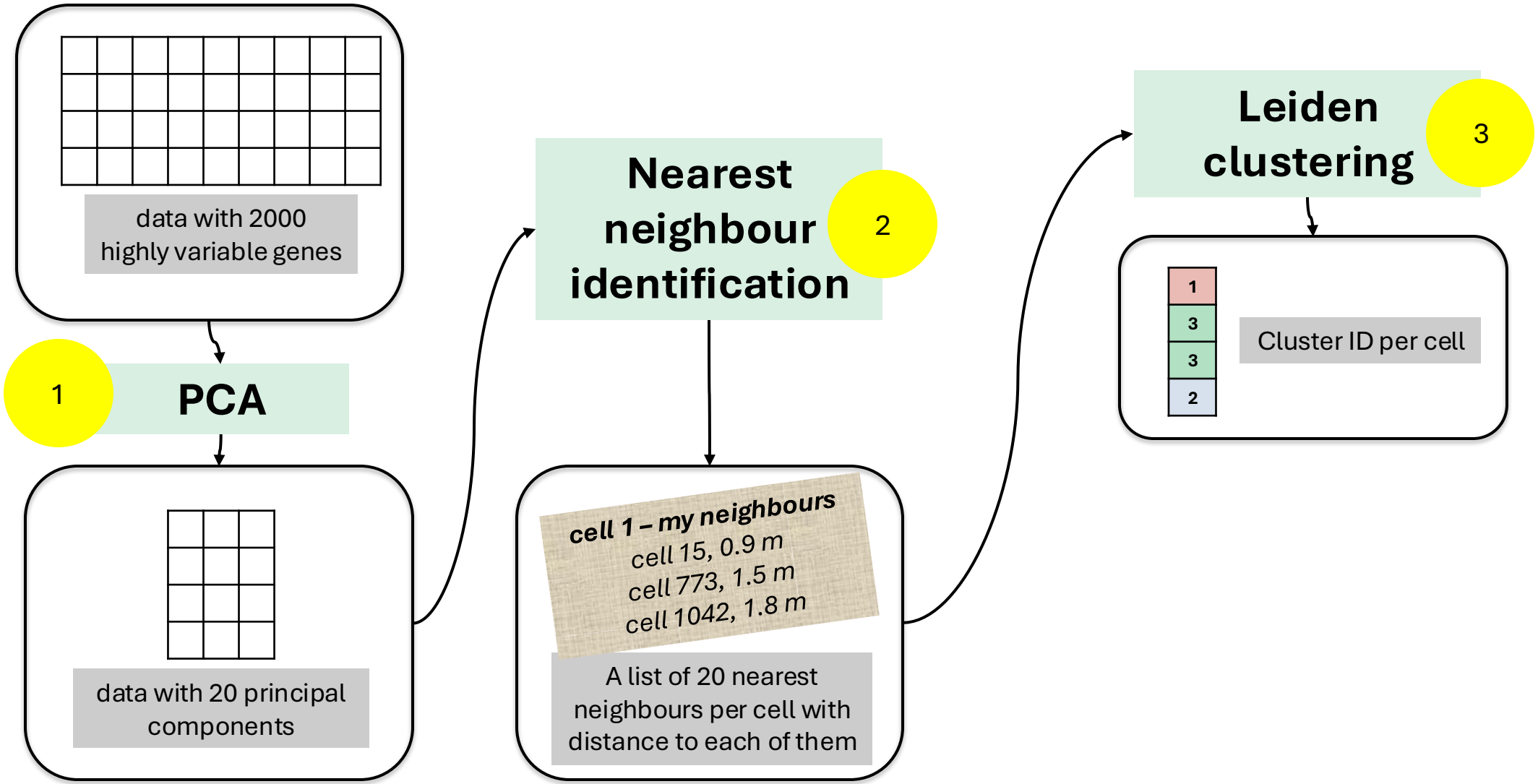

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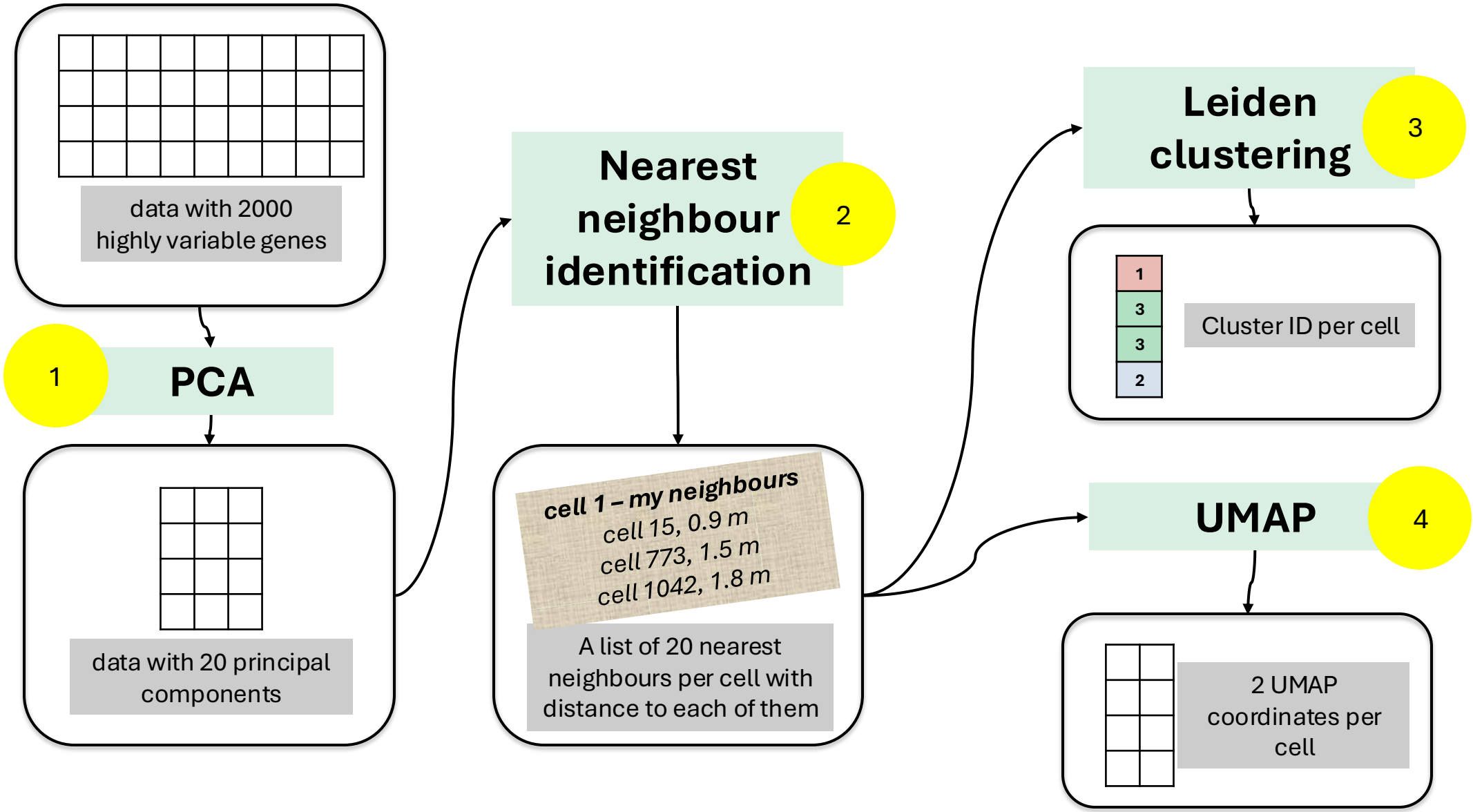

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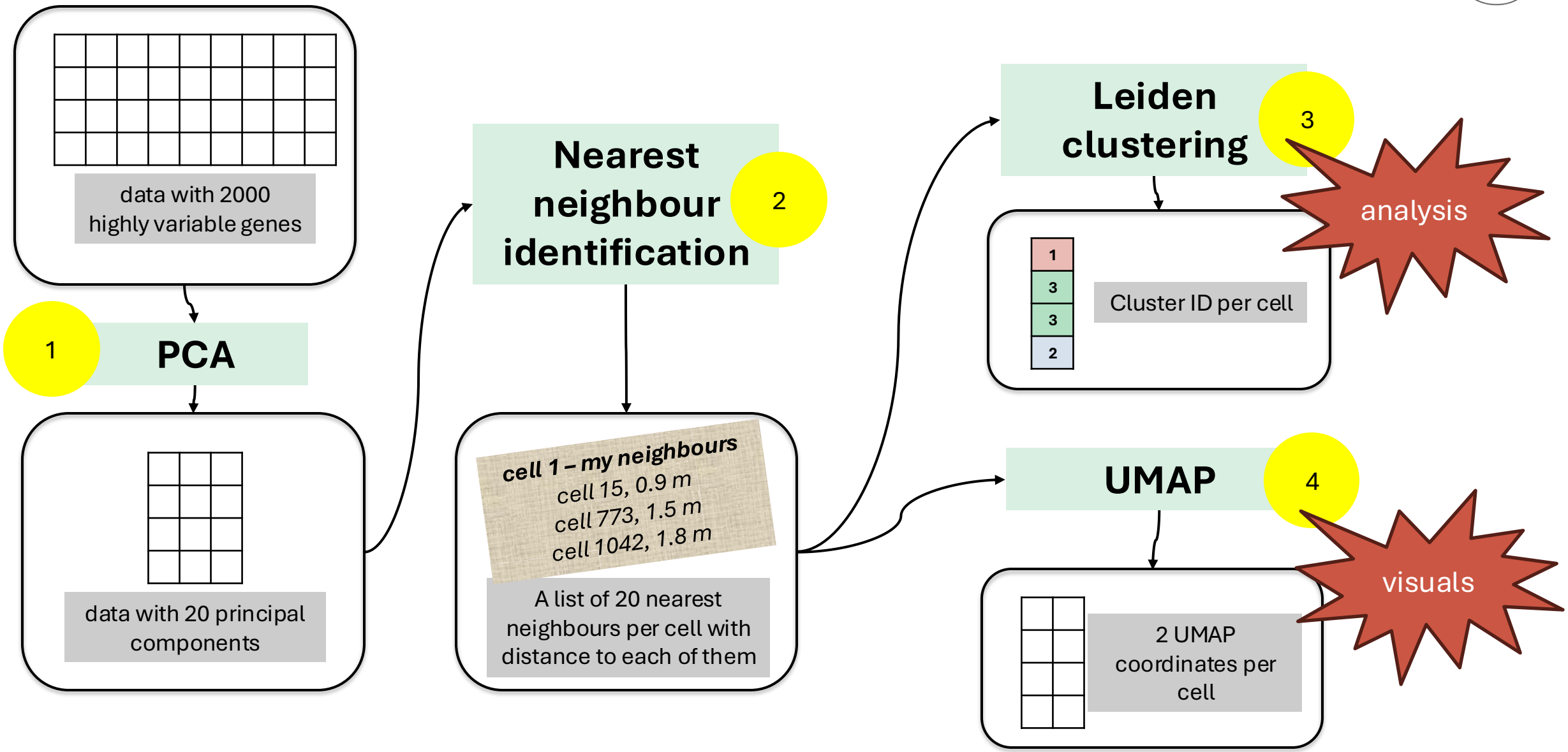

1

Principal Component Analysis

python

```
sc.tl.pca(adata)
```

R

```
pbmc <- RunPCA(pbmc, features = VariableFeatures(object = pbmc))
```

https://satijalab.org/seurat/articles/pbmc3k_tutorial
https://scanpy.readthedocs.io/en/stable/tutorials/basics/clustering.html

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Principal Component Analysis

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Motivation

Less dimensions are better for downstream performance and interpretability

Many genes have correlated behaviour – not every original dimension adds value

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Motivation

Less dimensions are better for downstream performance and interpretability

Many genes have correlated behaviour – not every original dimension adds value

Goal

Move as much information as possible into as few dimensions as possible

https://satijalab.org/seurat/articles/pbmc3k_tutorial
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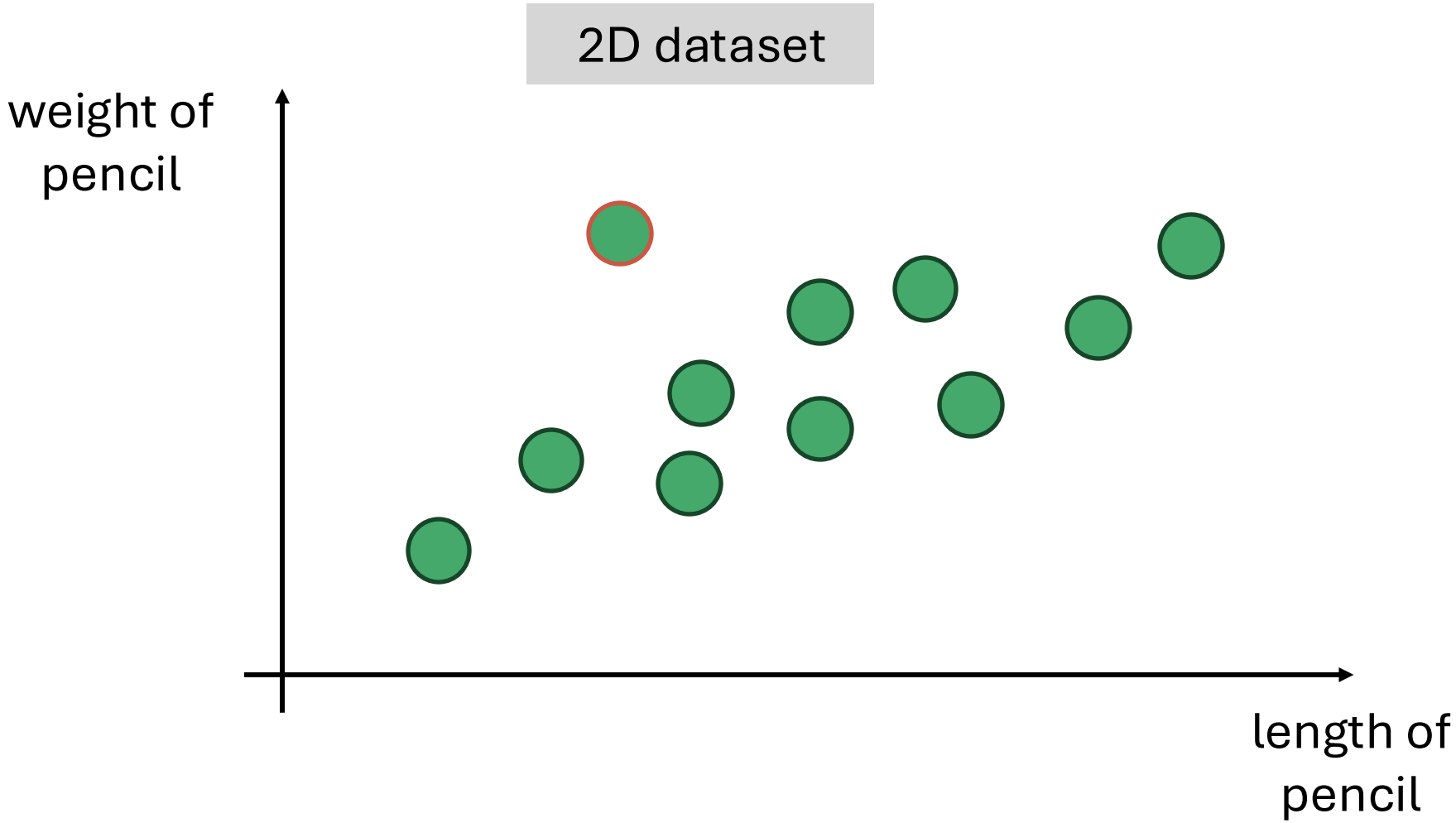
1

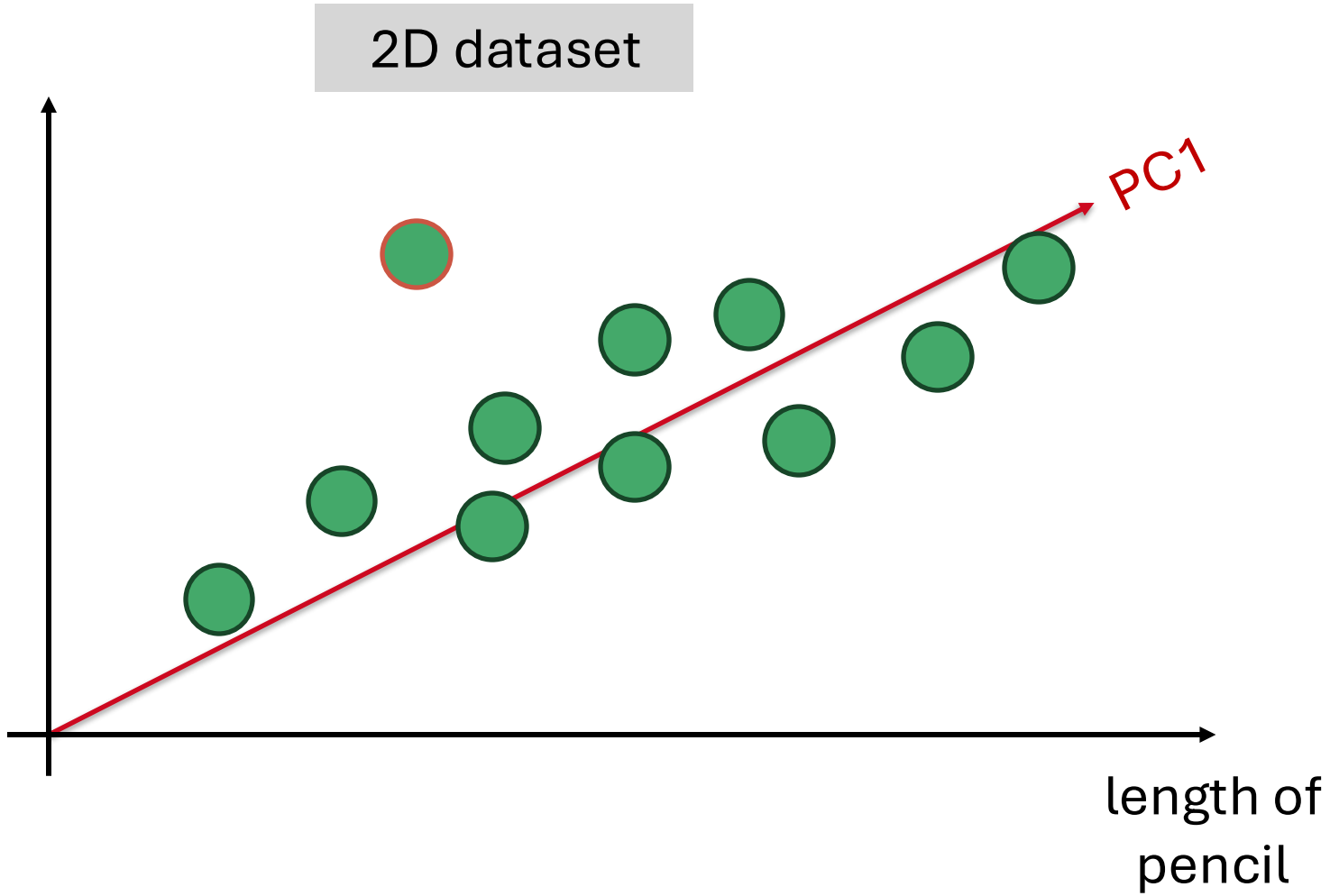
Principal Component Analysis



1

Principal Component Analysis

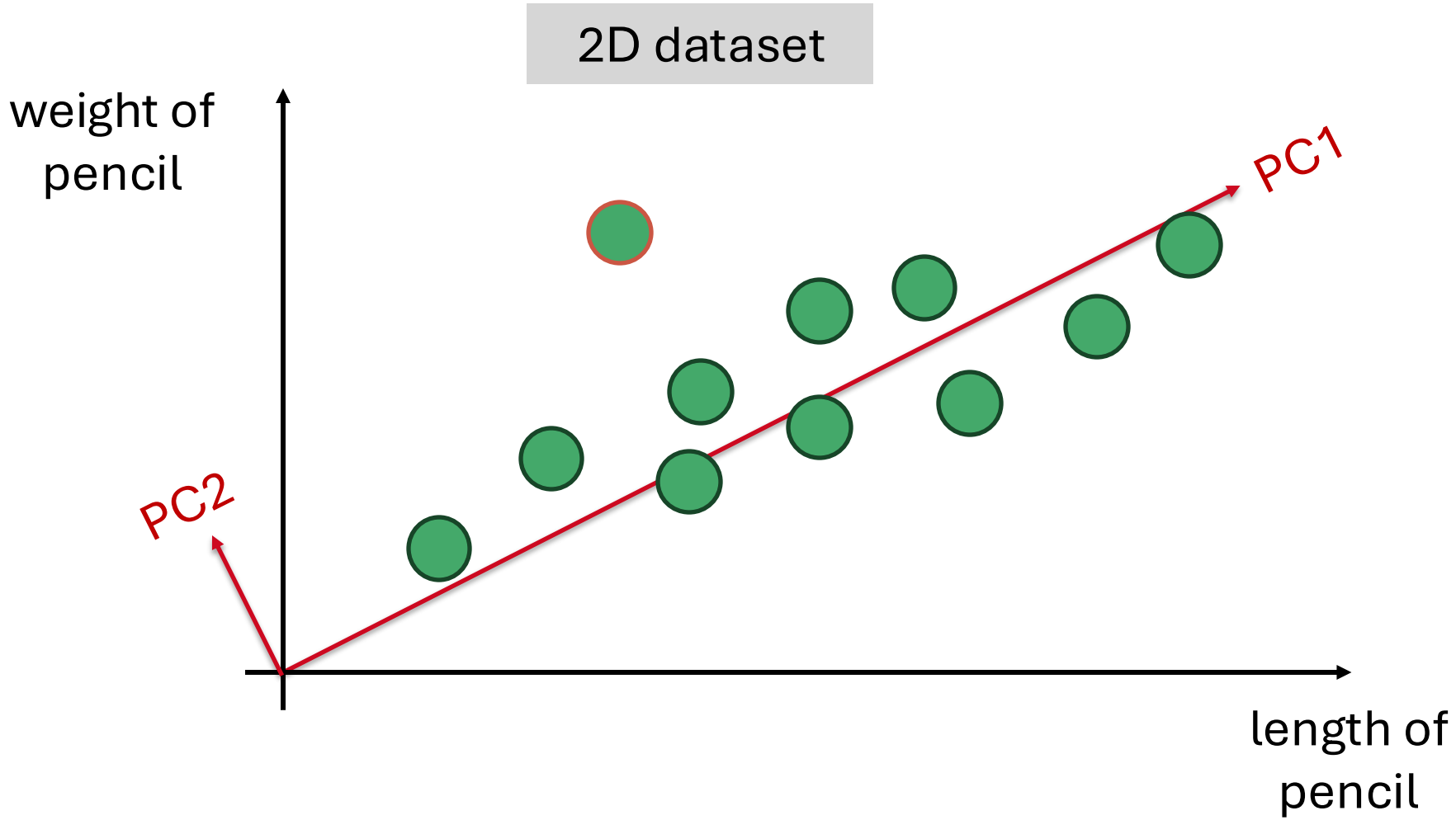
Find the dimension which captures the most variability



1

Principal Component Analysis

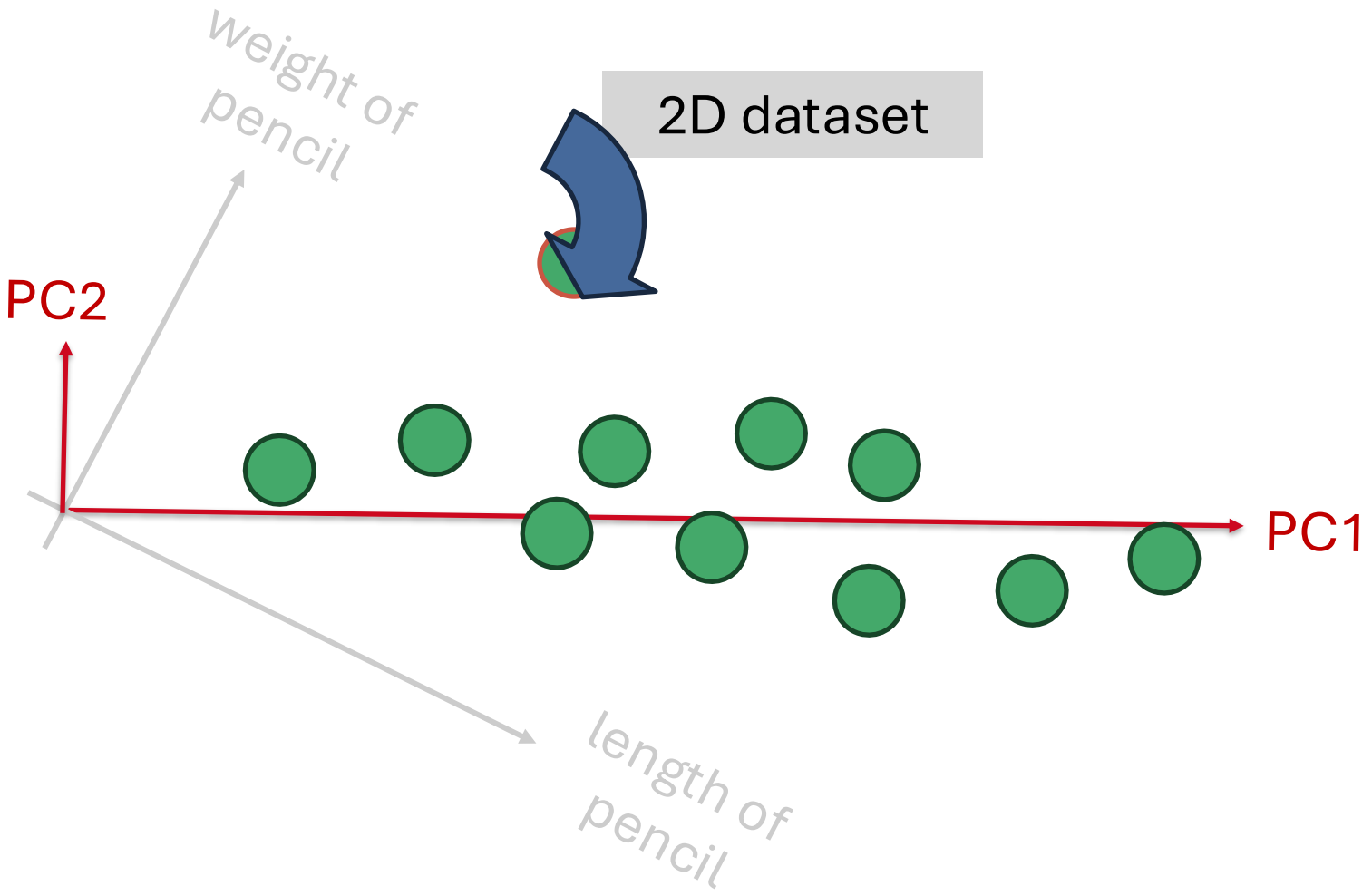
Find the dimension which captures the **2nd** most variability (etc)



1

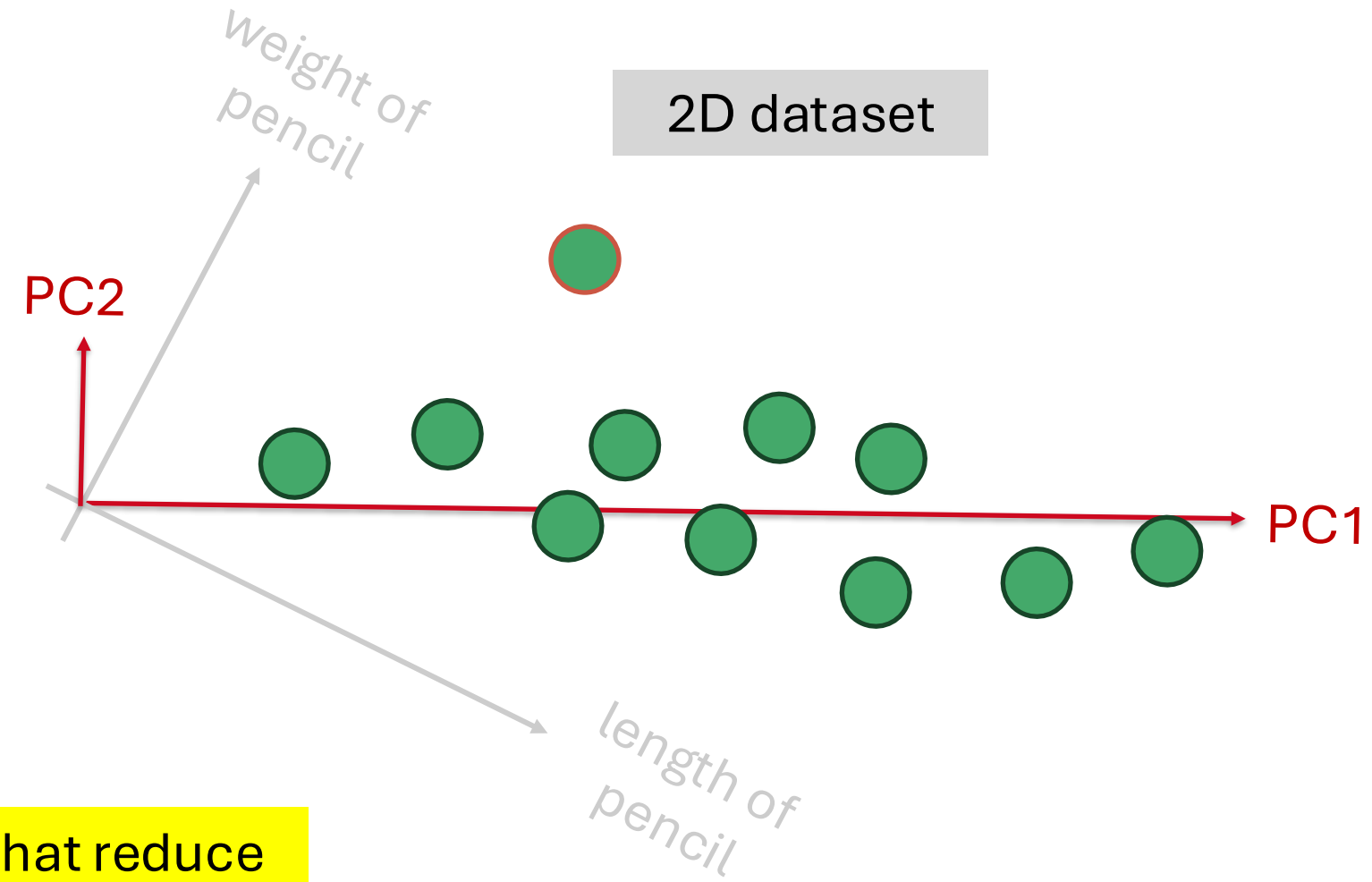
Principal Component Analysis

Describe each point in terms of the new rotated coordinates



1

Principal Component Analysis

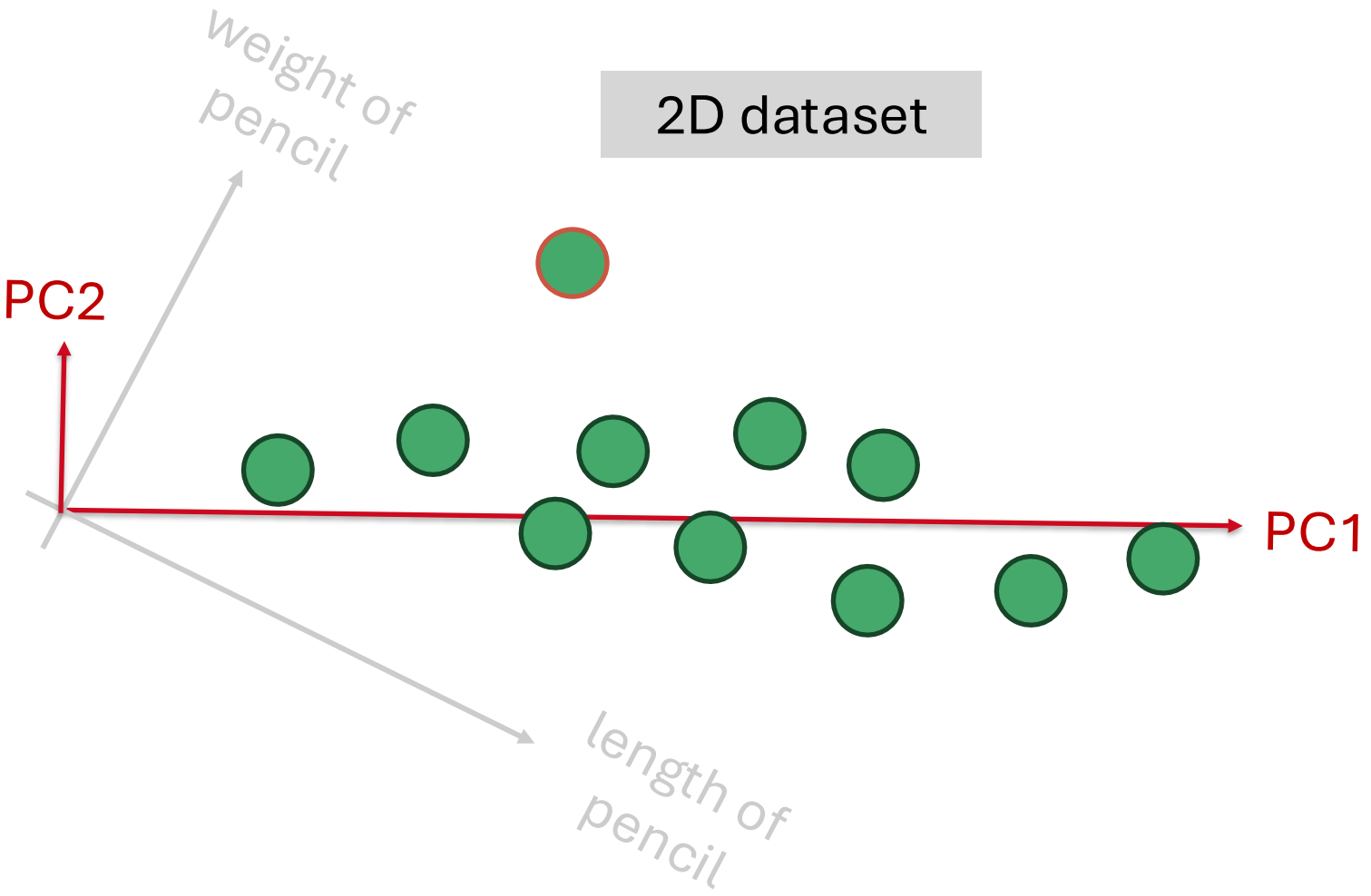


Describe each point in terms of the new rotated coordinates

?! How does that reduce dimensionality?

1

Principal Component Analysis

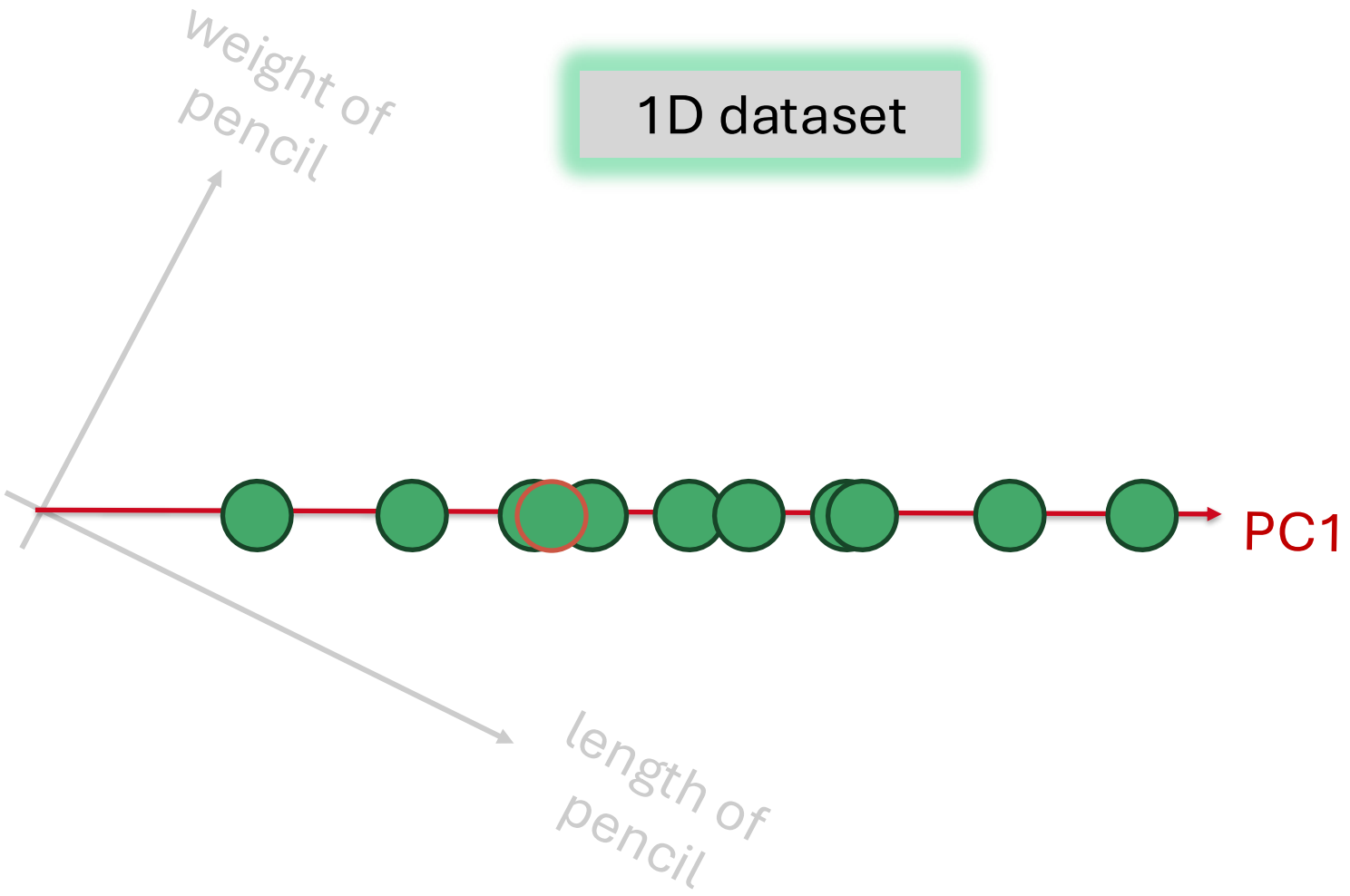


Inspect how much of the total variance is explained by each PC

	Explained variance
PC1	95%
PC2	5%

1

Principal Component Analysis



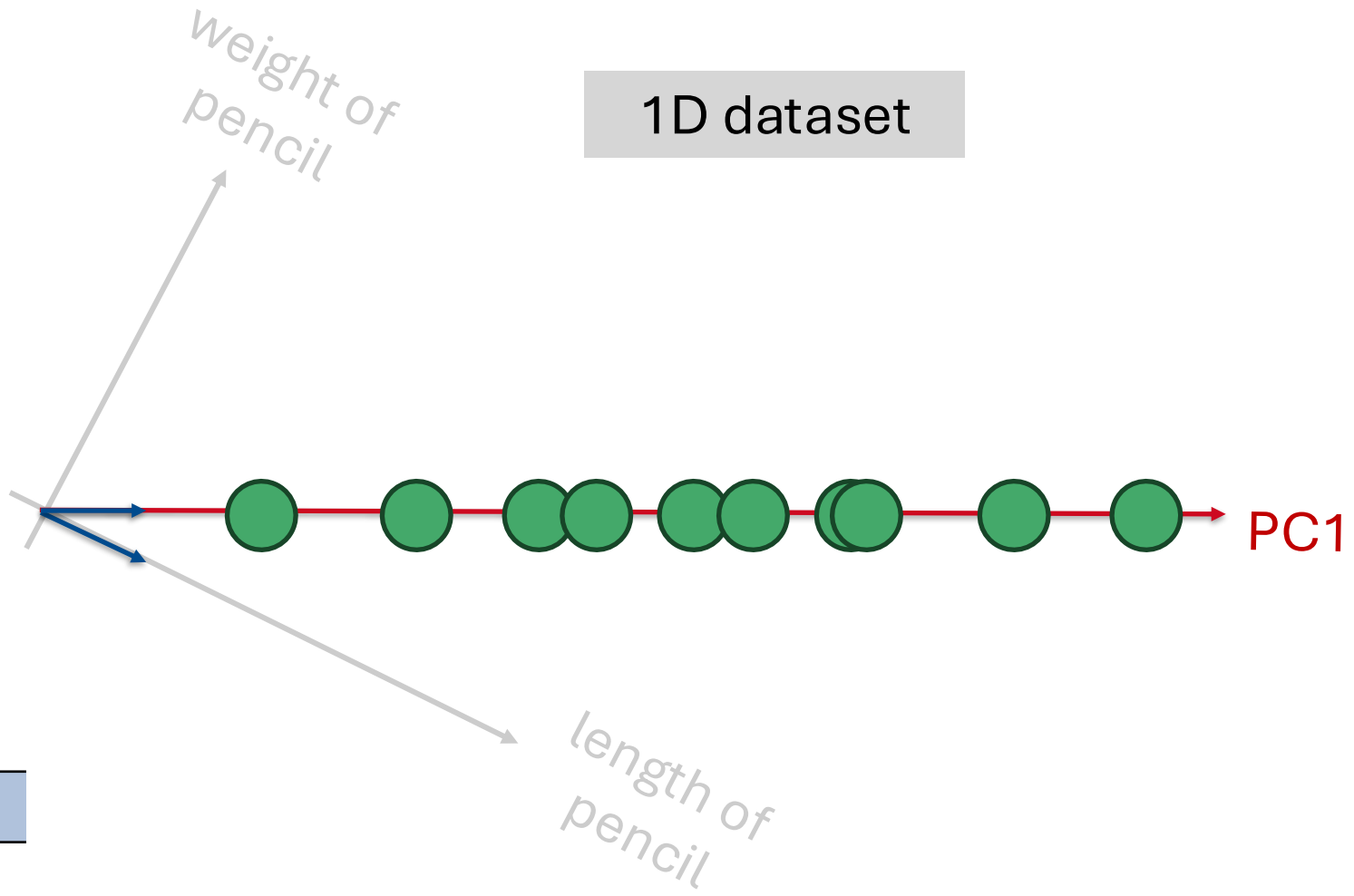
Discard lesser important dimensions

	Explained variance
PC1	95%

1

Principal Component Analysis

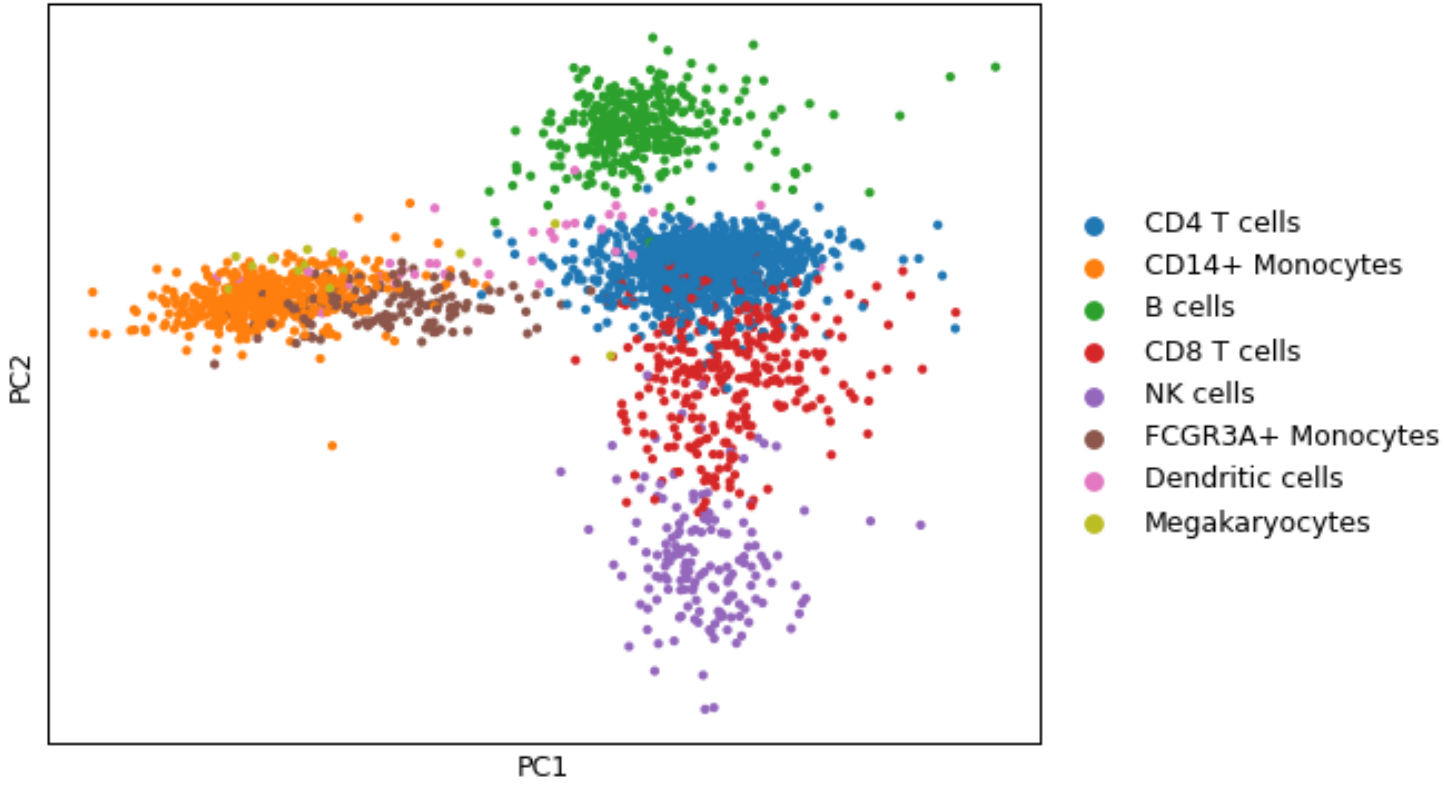
Loadings (projections of features onto PCs) help interpretability



	Loading on PC1
Length	0.9
Weight	0.2

1

Principal Component Analysis for single cell data

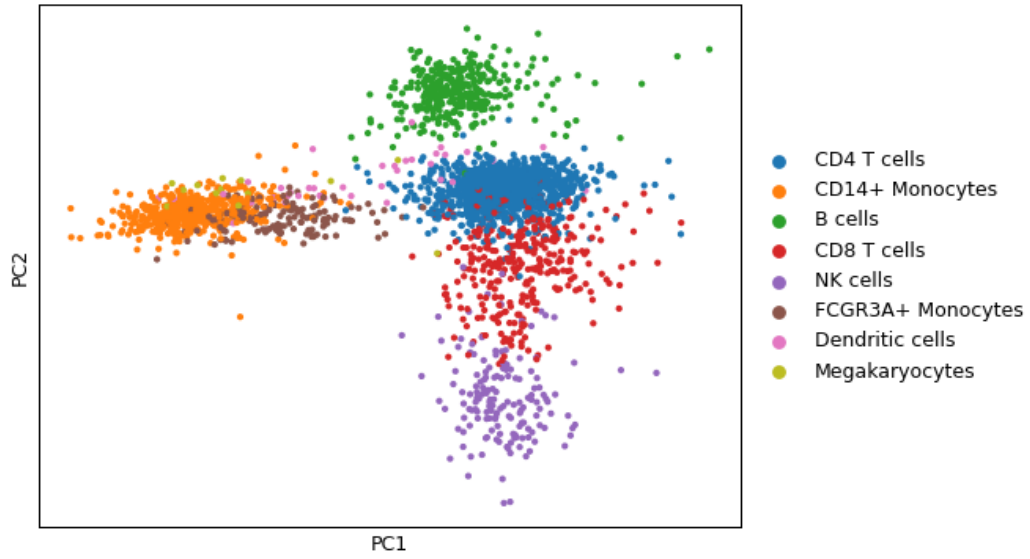


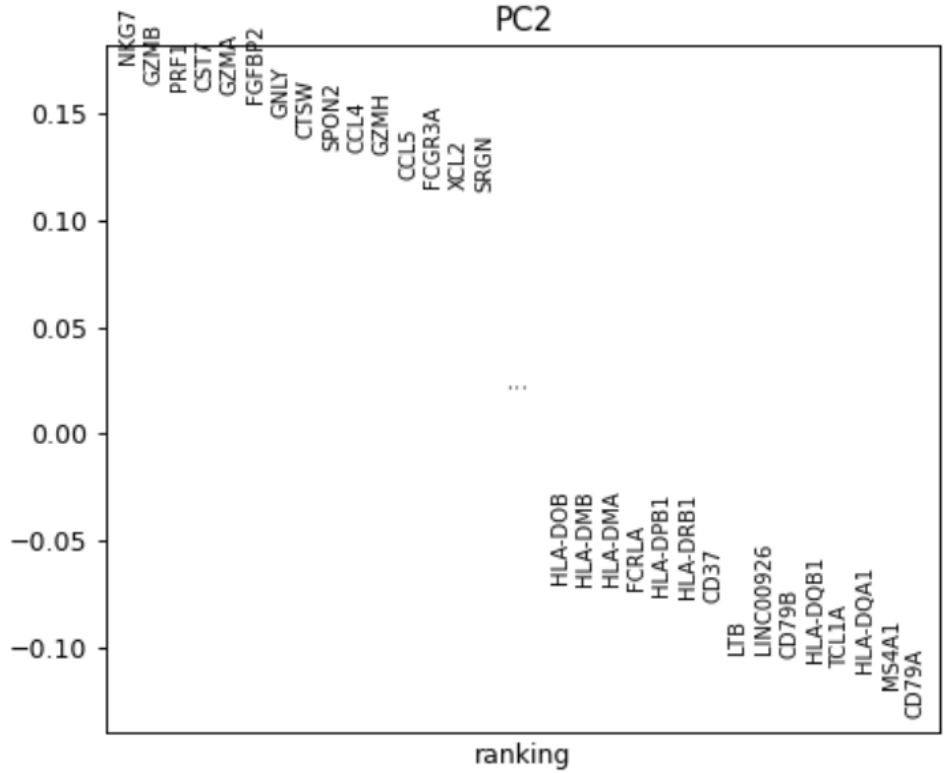
First 2 components are sufficient to separate B cells, T cells and monocytes!

https://scanpy.readthedocs.io/en/stable/api/generated/scanpy.pl.pca_overview.html

1

Principal Component Analysis for single cell data





Important loadings on PC2 include NK cell marker NKG7 and B cell marker CD79a

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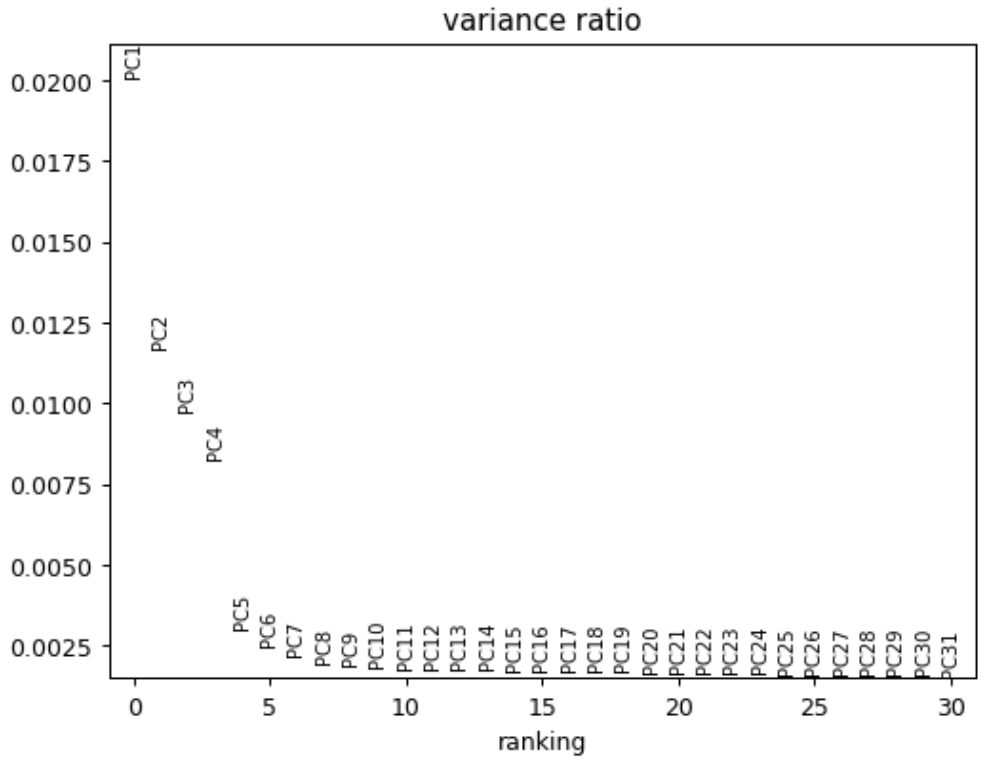
1

Principal Component Analysis for single cell data



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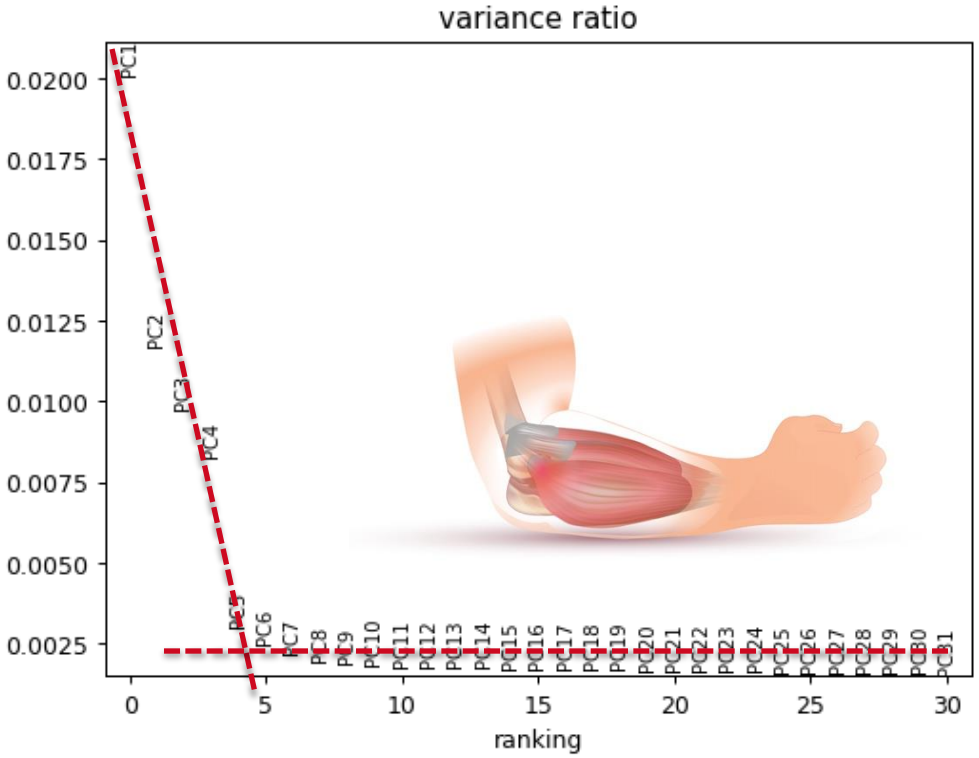

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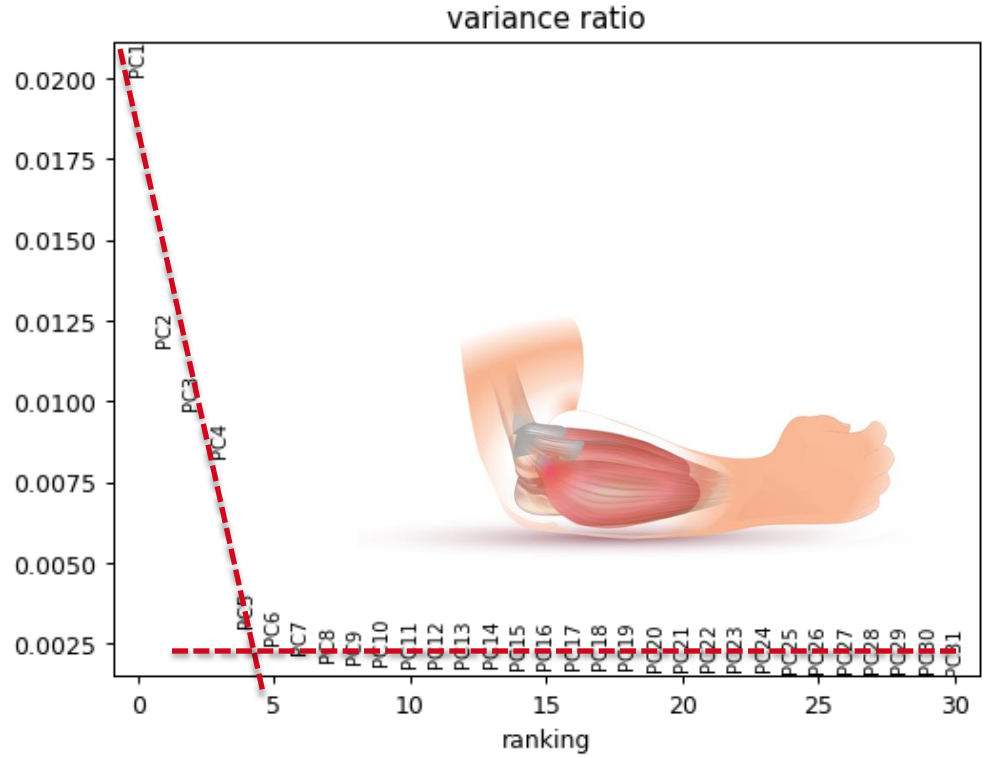
Principal Component Analysis for single cell data



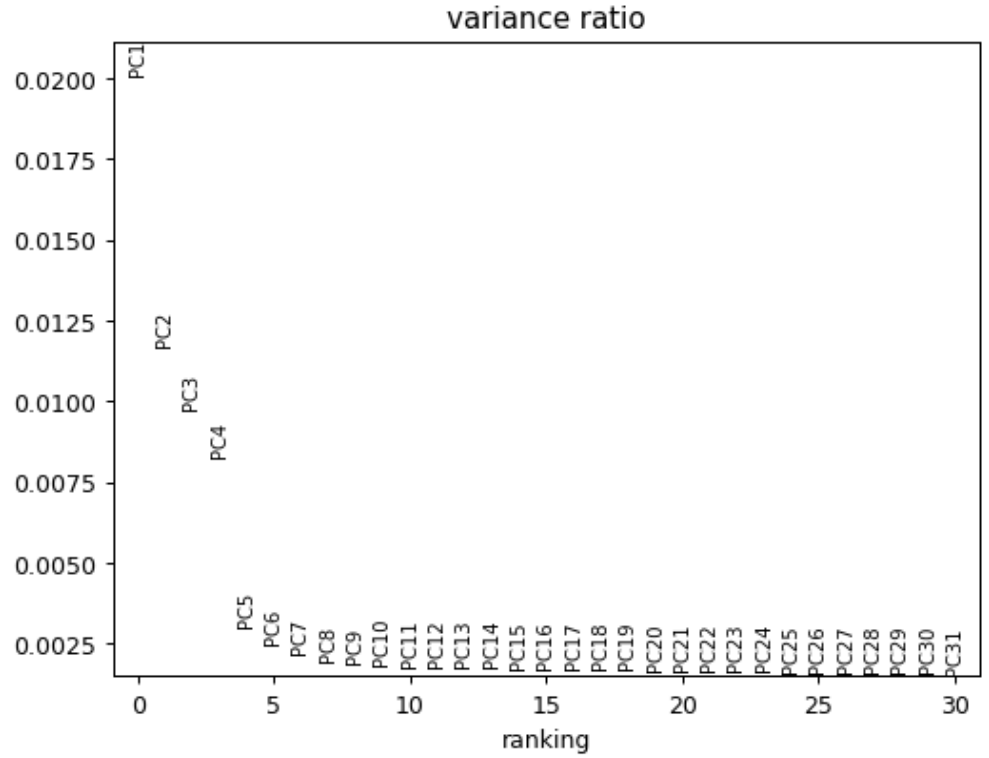
Elbow method can help to decide on the number of PCs to keep ...

- but may be too aggressive
- In this PBMC example, first 100 PCs explain 20% of variance

https://scanpy.readthedocs.io/en/stable/api/generated/scanpy.pl.pca_overview.html

1

Principal Component Analysis for single cell data



- Typically: keep 10-30 dimensions based on dataset complexity (e.g. number of cell types, conditions)
- If in doubt, keep more rather than removing too much info
- Repeat analysis with different choices of PCs

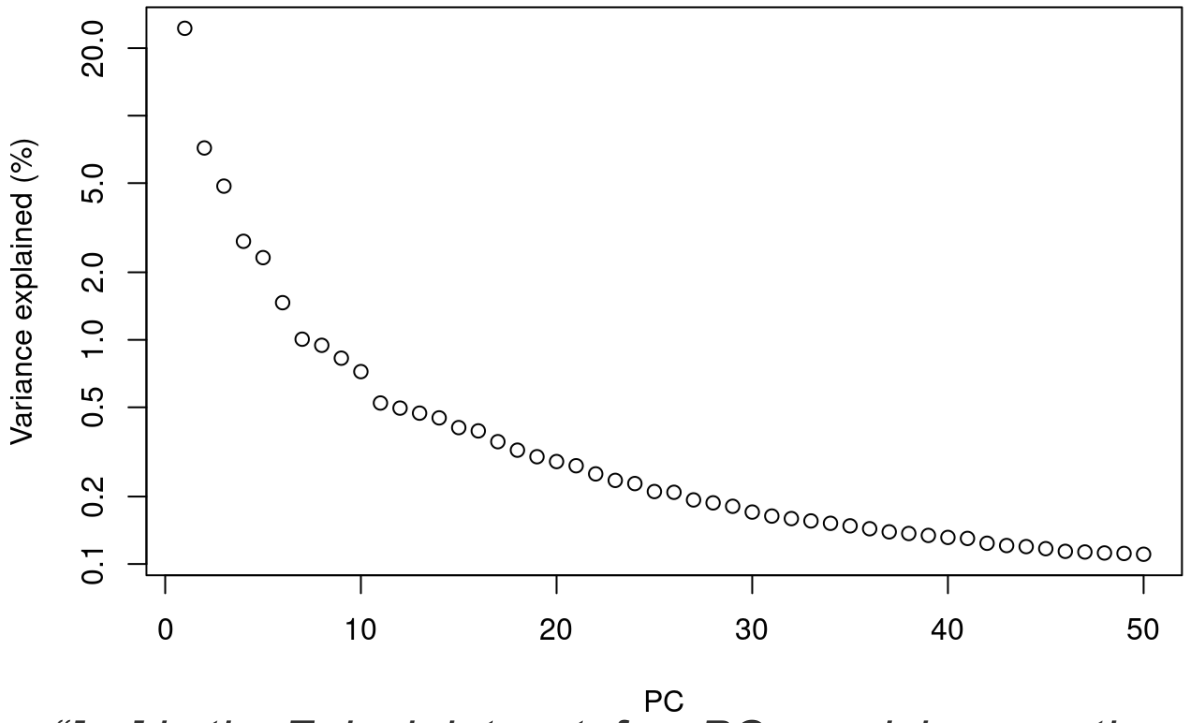
https://scanpy.readthedocs.io/en/stable/api/generated/scanpy.pl.pca_overview.html

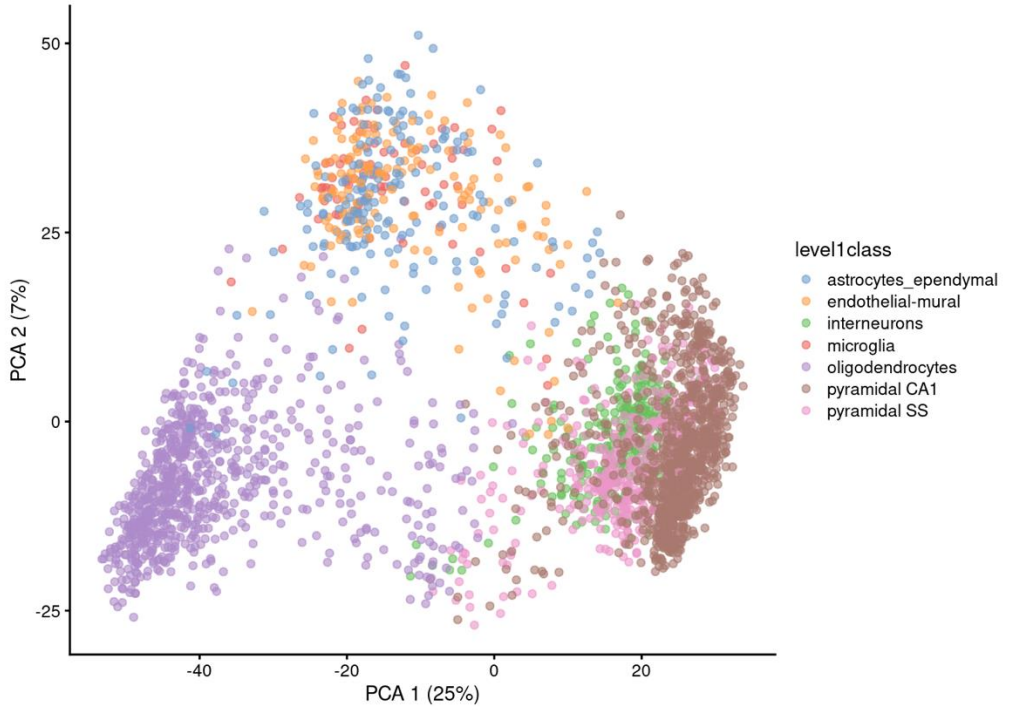
1

More diverse populations → first PCs capture more variability

Zeisel 2015 mouse brain dataset

(https://www.science.org/doi/10.1126/science.aaa1934, another classic dataset used in many tutorials)





"[…] in the Zeisel dataset, few PCs explain more than 1% of the variance in the entire dataset (Figure 4.1) and choosing between, say, 20 and 40 PCs would not even amount to four percentage points' worth of difference in variance."

https://bioconductor.org/books/3.13/OSCA.basic/dimensionality-reduction.html