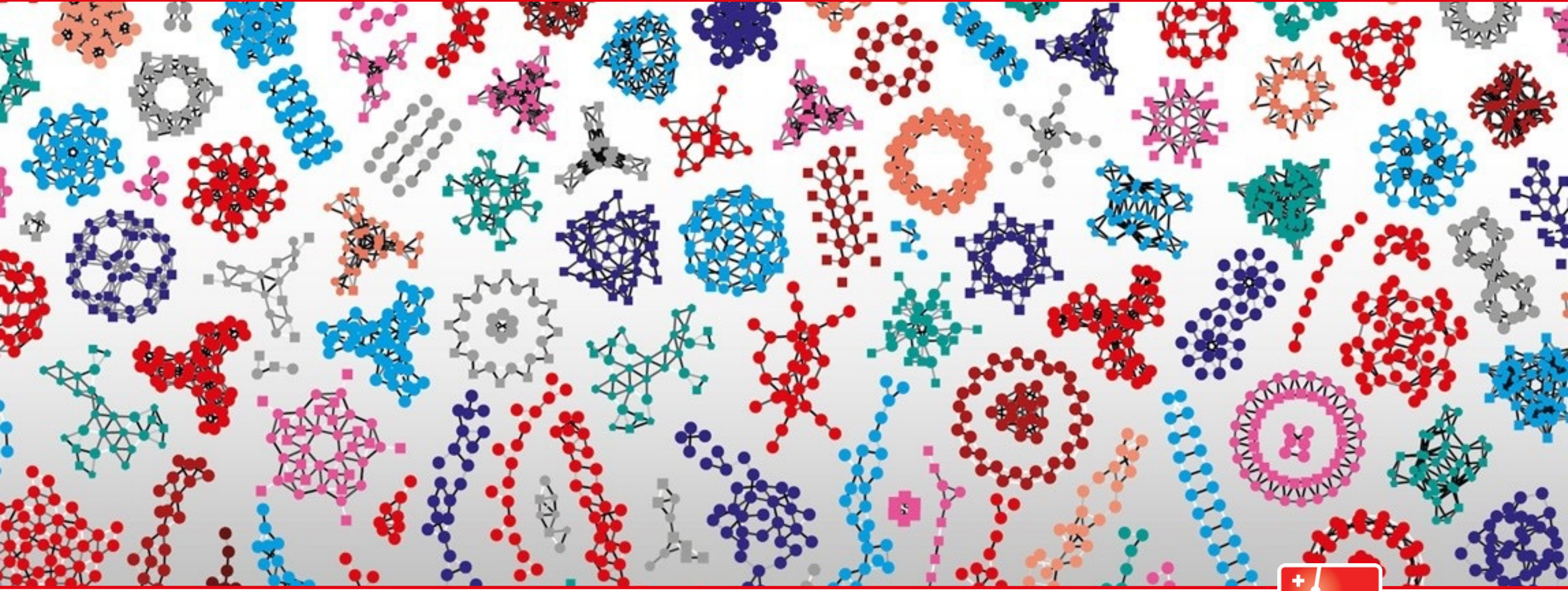
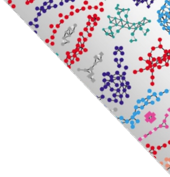


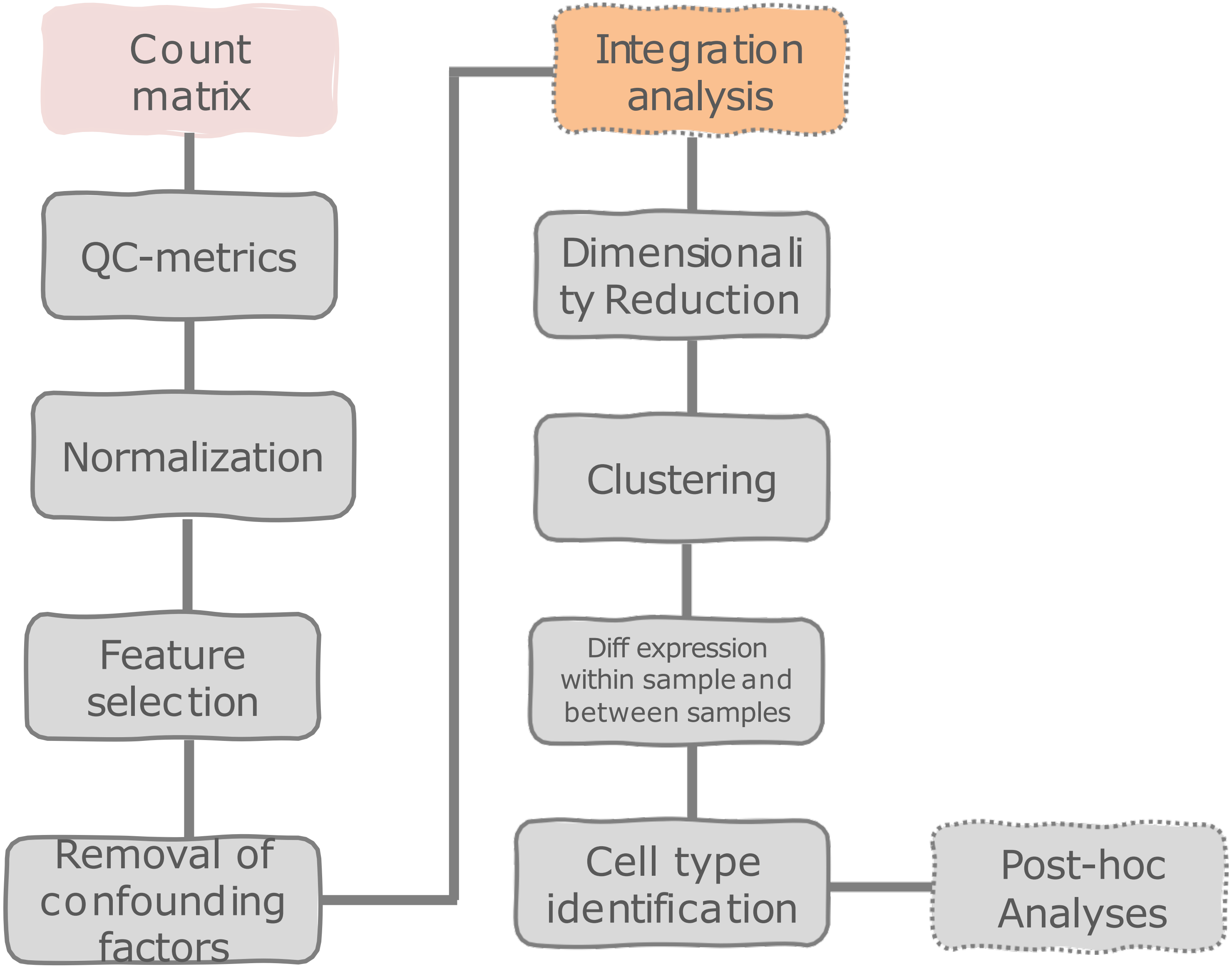
Swiss Institute of Bioinformatics

Day 2 : Single cell RNA sequencing: The bioinformatic downstream analysis

Geert van Geest, Rachel Marcone, Tania Wyss

www.sib.swiss





Count matrix

QC-metrics

Normalization

Feature selection

Removal of confounding factors

Integration analysis

Dimensionality Reduction

Clustering

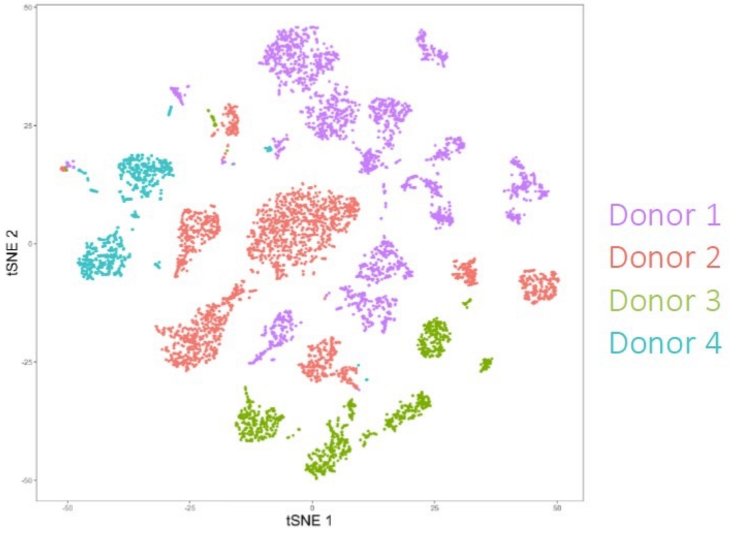
Diff expression within sample and between samples

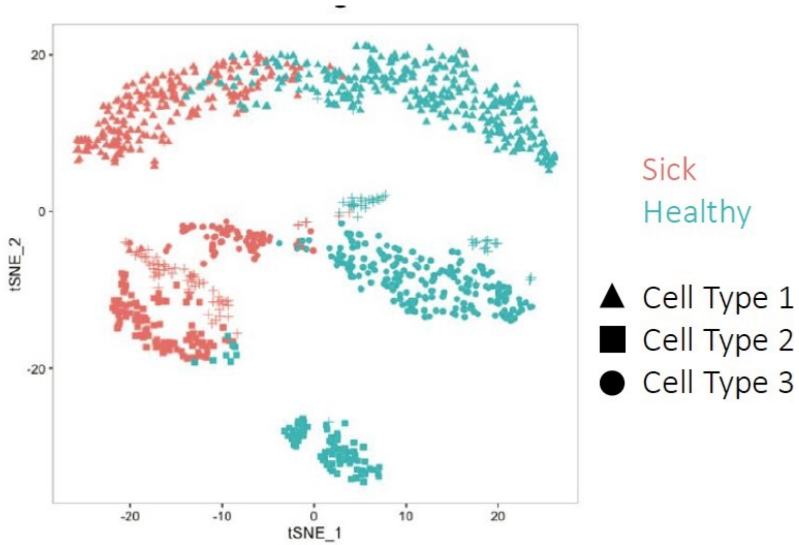
Cell type identification

Post-hoc Analyses

Integration analysis

- Why do we integrate?





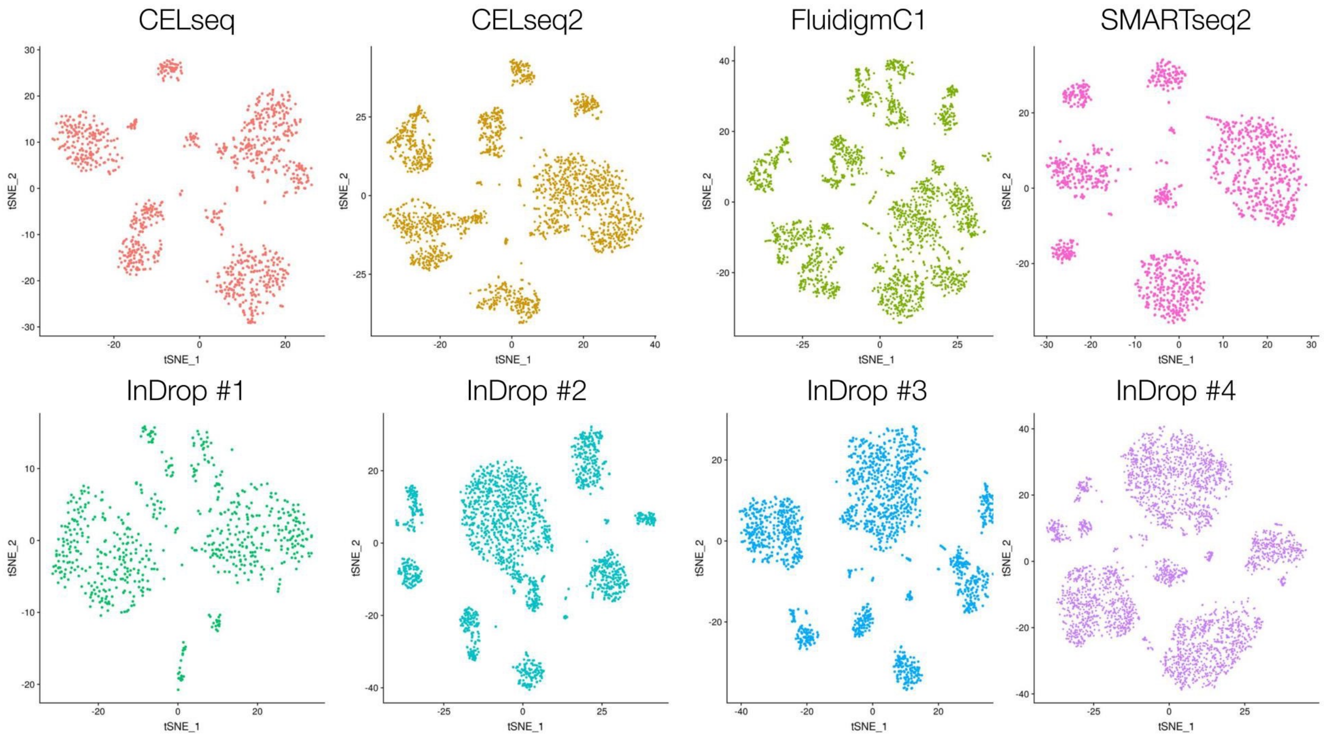
Same tissue from different donors

Cross condition comparisons

Courtesy form Ahmed Mahfouz

Integration analysis

- 8 maps from the human pancreas (Seurat tutorial)



Baron et al. 2016, *Cell Syst.*

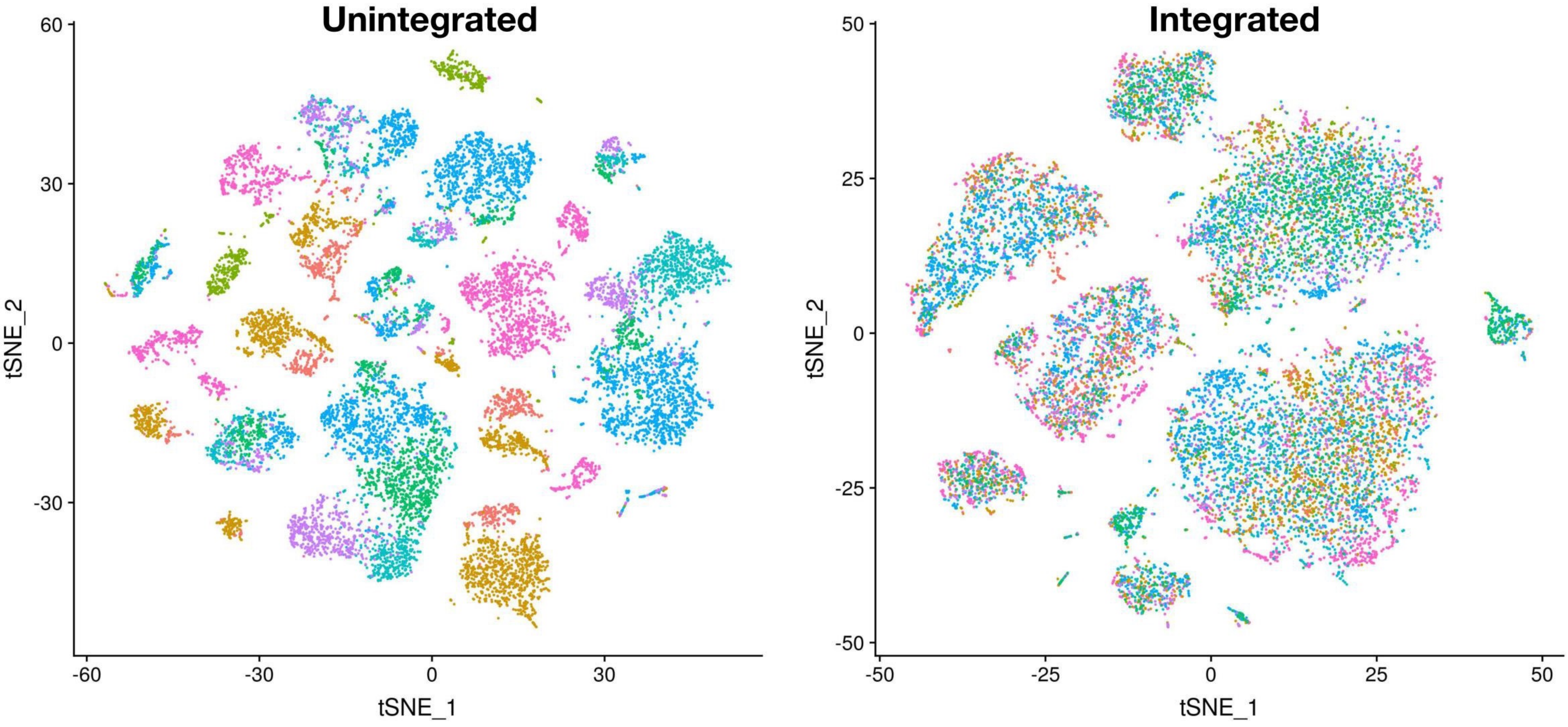
Lawlor et al. 2017, *Genome Res.*

Grun et al. 2016, *Cell Stem Cell*

Muraro et al. 2016, *Cell Syst.*

Integration analysis

- 8 maps from the human pancreas (Seurat tutorial)



Integration analysis:
Confounders and batch effect

1. Technical variability
   - Changes in sample quality/processing
   - Library prep or sequencing technology

Technical 'batch effects' confound downstream analysis

2. Biological variability
   - Patient differences
   - Evolution! (cross-species analysis)

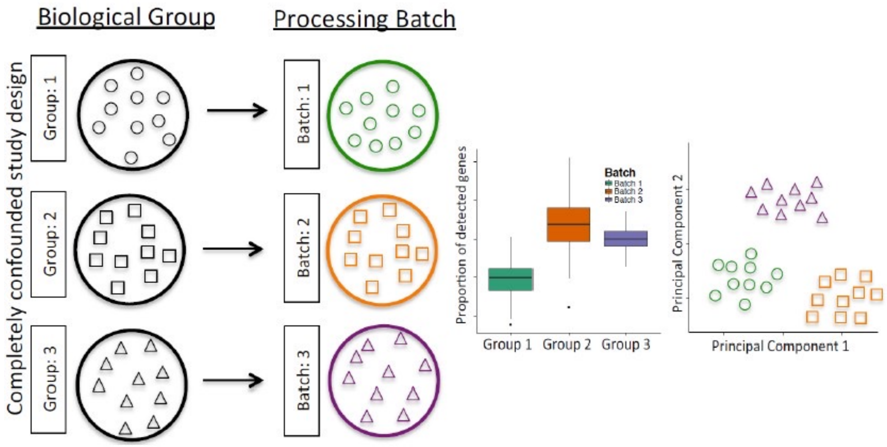
Biological 'batch effects' confound comparisons of scRNA-seq data

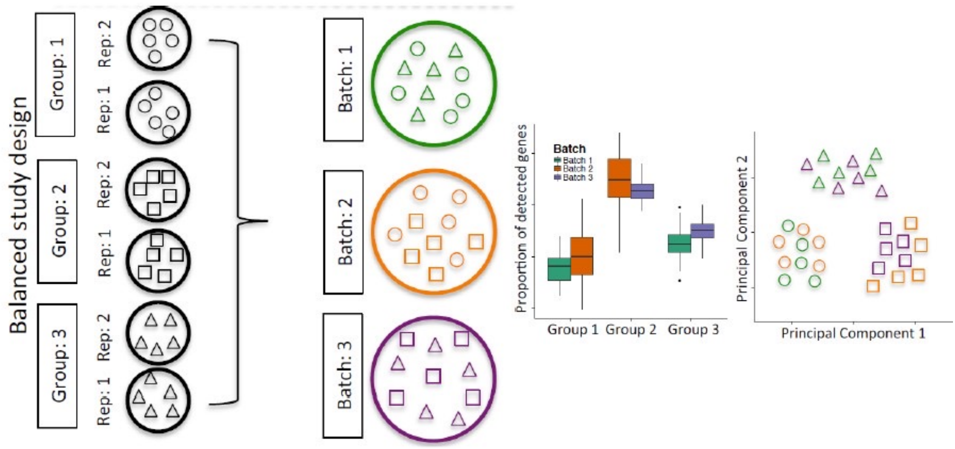
Shaham et al. (https://doi.org/10.1093/bioinformatics/btx196)

Integration analysis: Confounders and batch effect

Confounded design

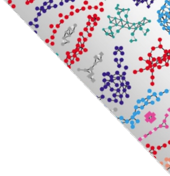
Not confounded design





Good experimental design *does not remove batch effects*, it prevents them from biasing your results.

Hicks et al. (https://doi.org/10.1093/biostatistics/kxx053)



Integration analysis: Batch correction method

- MNNcorrect (https://doi.org/10.1038/nbt.4091)
- CCA +anchors (Seurat v3) (https://doi.org/10.1101/460147)
- CCA +dynamic time warping (Seurat v2) (https://doi.org/10.1038/nbt.4096)
- LIGER (https://doi.org/10.1101/459891)
- Harmony (https://doi.org/10.1101/461954)
- Conos (https://doi.org/10.1101/460246)
- Scanorama (https://doi.org/10.1101/371179)
- scMerge (https://doi.org/10.1073/pnas.1820006116)

Integration analysis:
Batch correction method

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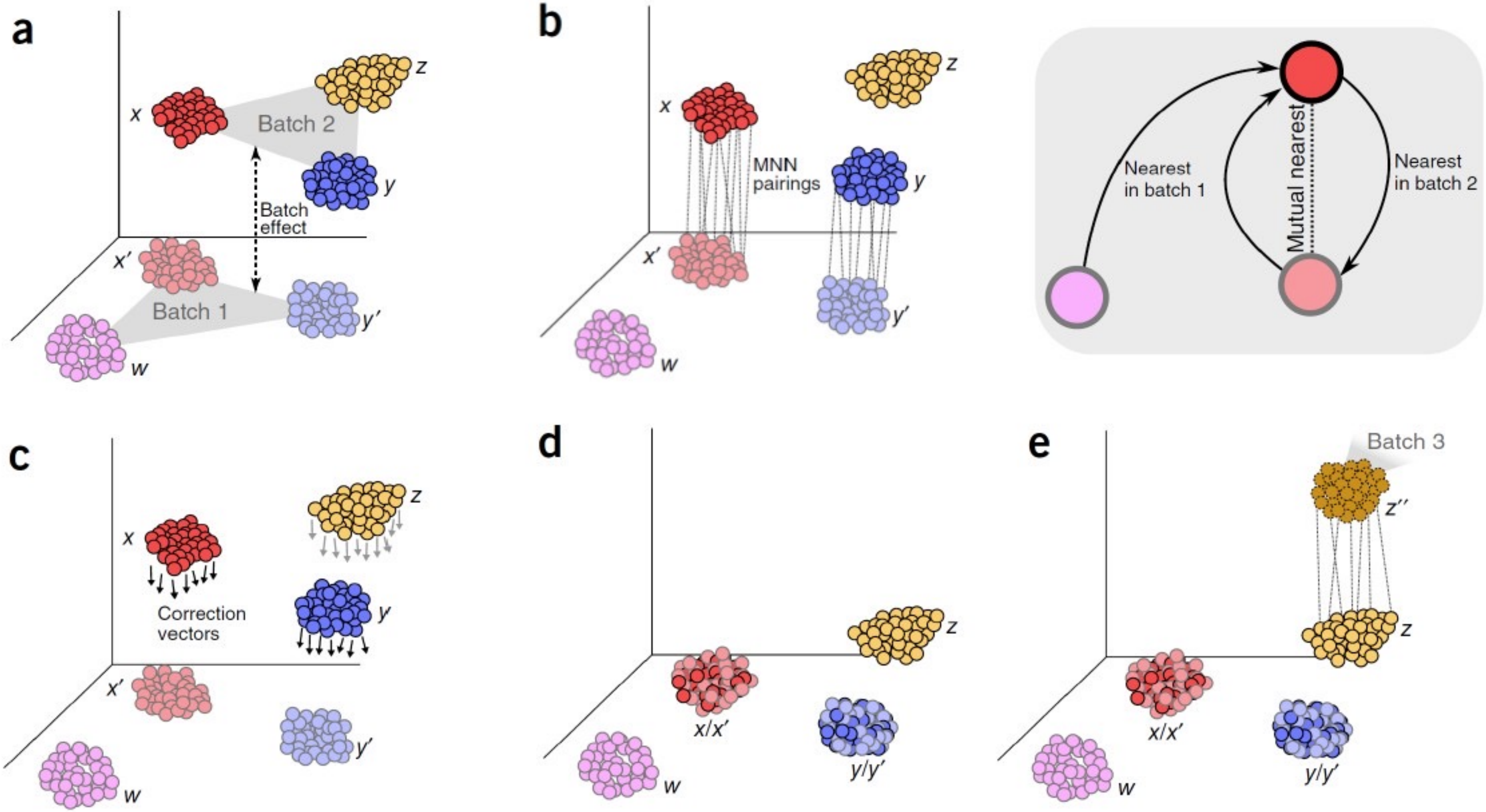
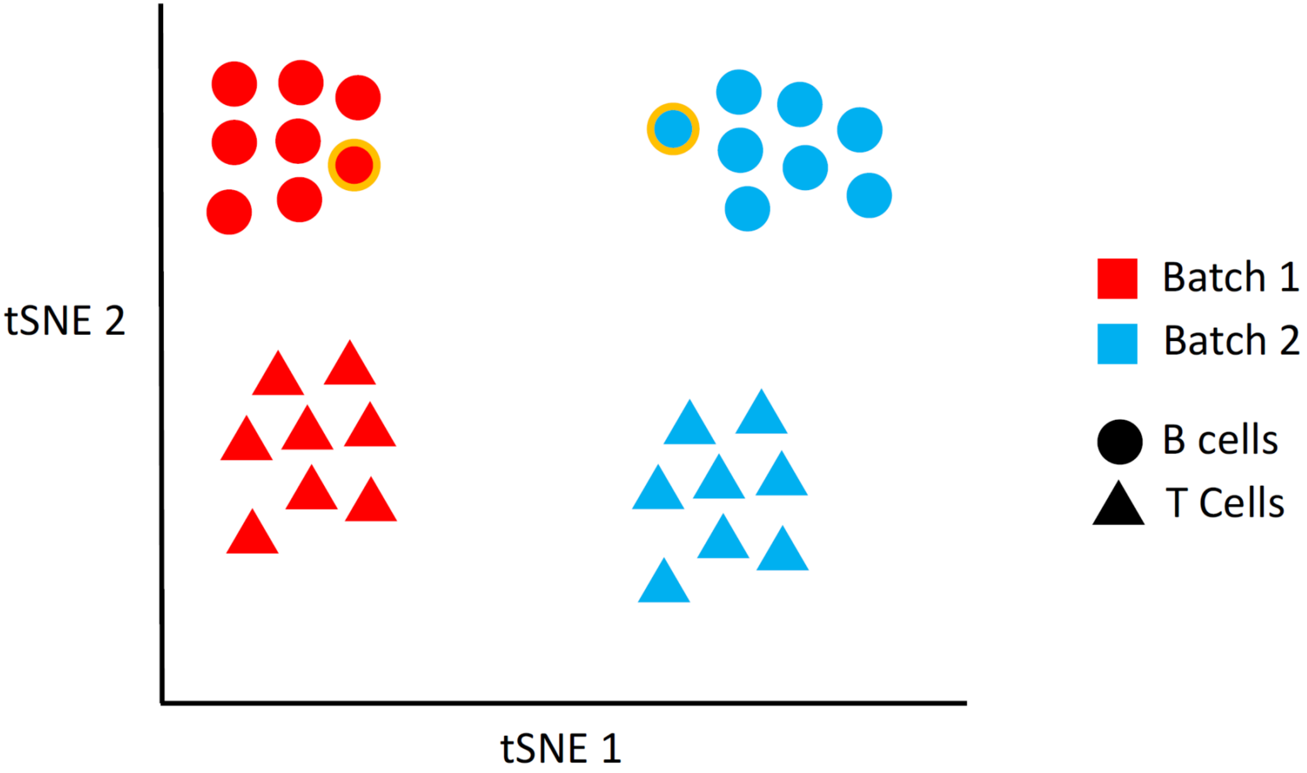
Integration analysis: Mutual Nearest Neighbors (MNN)

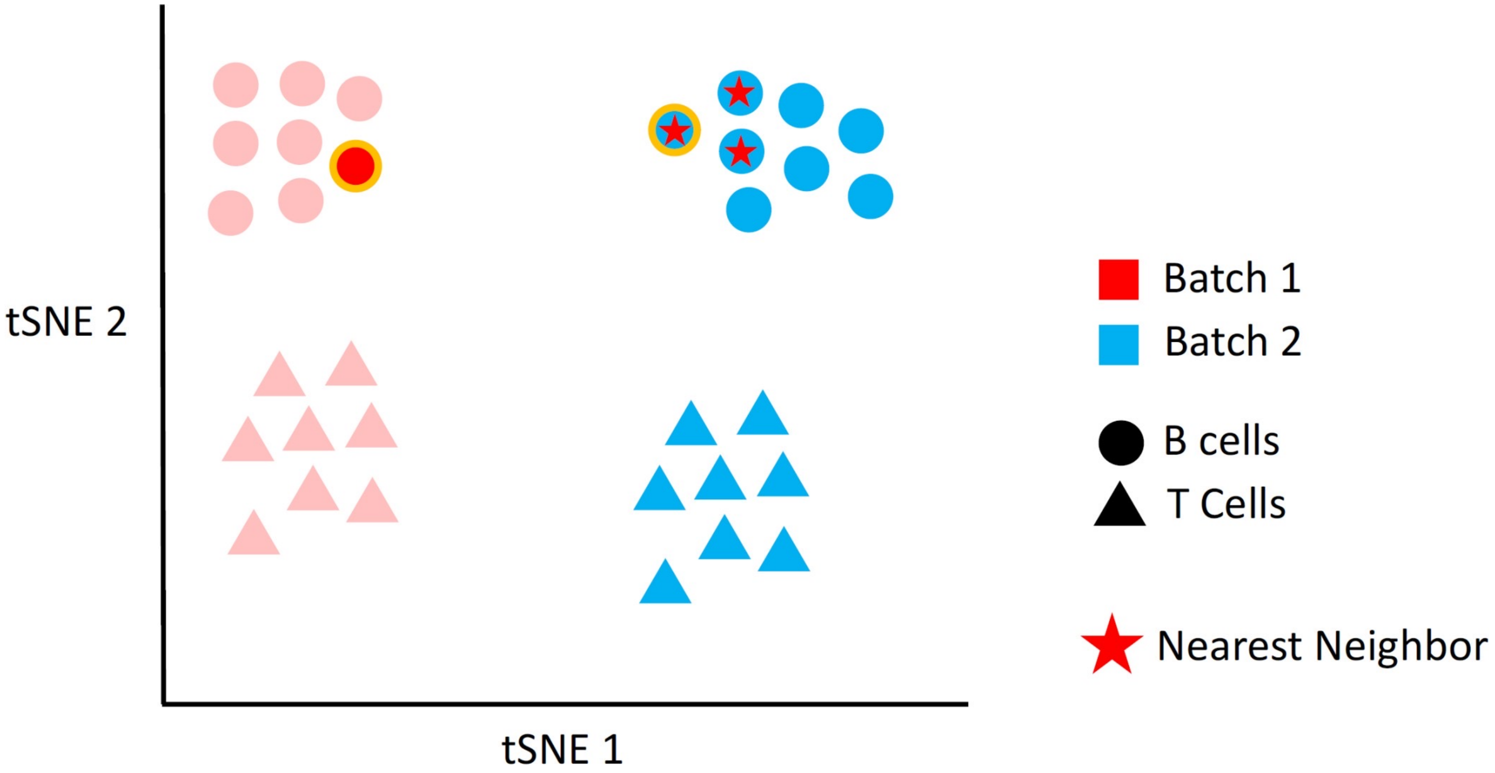


Haghverdi (https://doi.org/10.1038/nbt.4091)

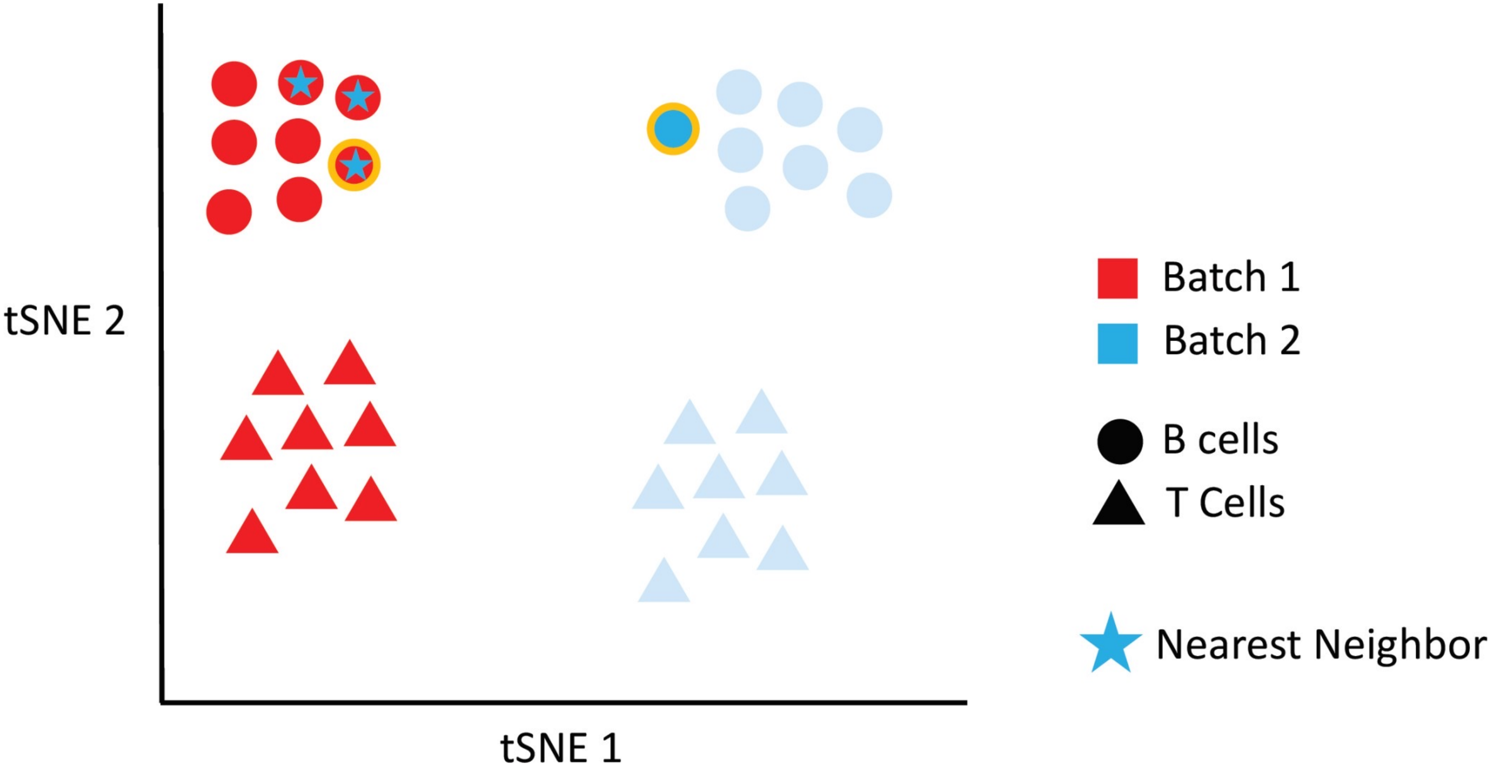
Integration analysis: Mutual Nearest Neighbors (MNN)



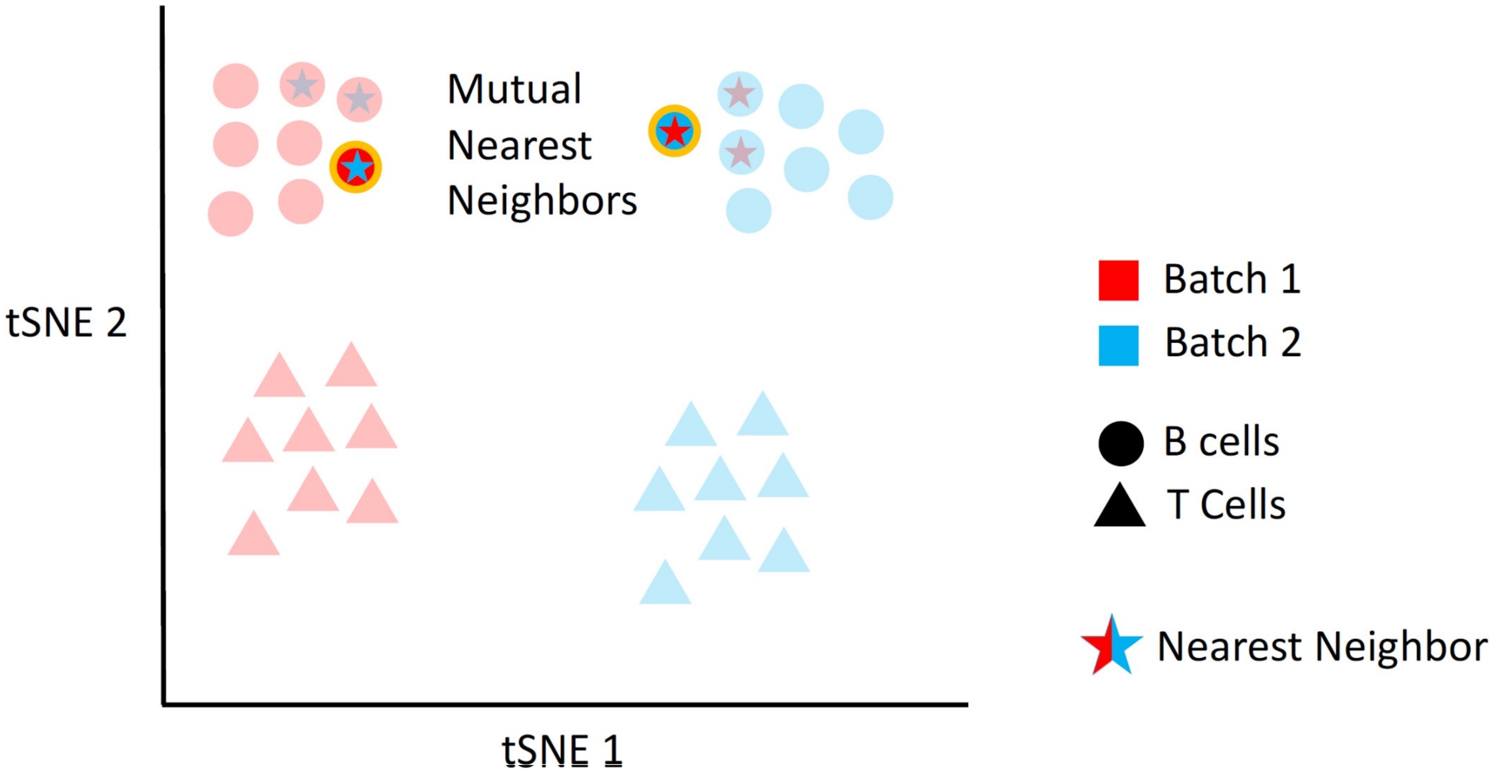
Integration analysis:
Mutual Nearest Neighbors (MNN)



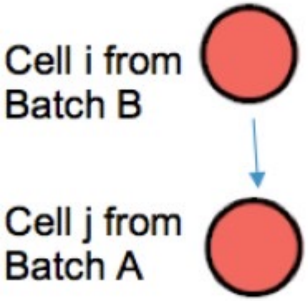
Integration analysis: Mutual Nearest Neighbors (MNN)



Integration analysis: Mutual Nearest Neighbors (MNN)



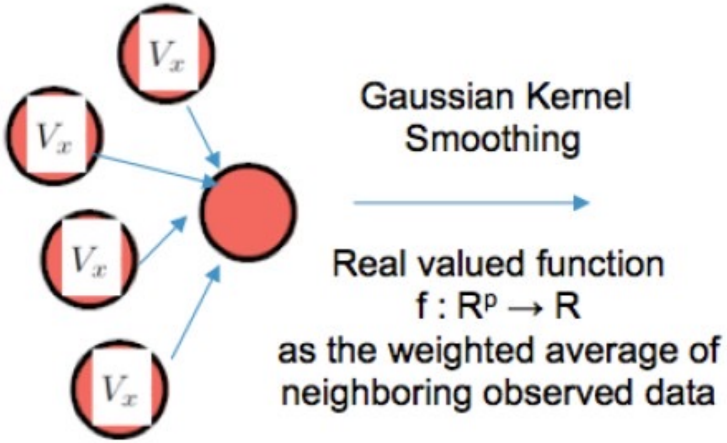
Integration analysis: Mutual Nearest Neighbors (MNN)



1) For each MNN pair, a pair-specific batch-correction vector is computed as the vector difference between the expression profiles of the paired cells.

$$V_x = \begin{pmatrix} gene1_a - gene1_b \\ gene2_a - gene2_b \\ gene3_a - gene3_b \\ \dots \\ geneN_a - geneN_b \end{pmatrix}$$

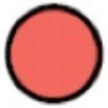
2) A cell-specific batch-correction vector is then calculated as a weighted average of these pair-specific vectors, as computed with a Gaussian kernel.

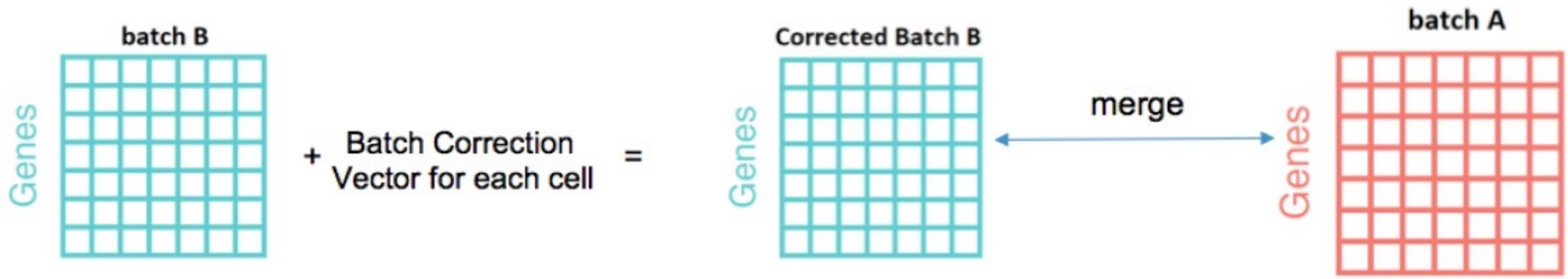


Gaussian Kernel Smoothing

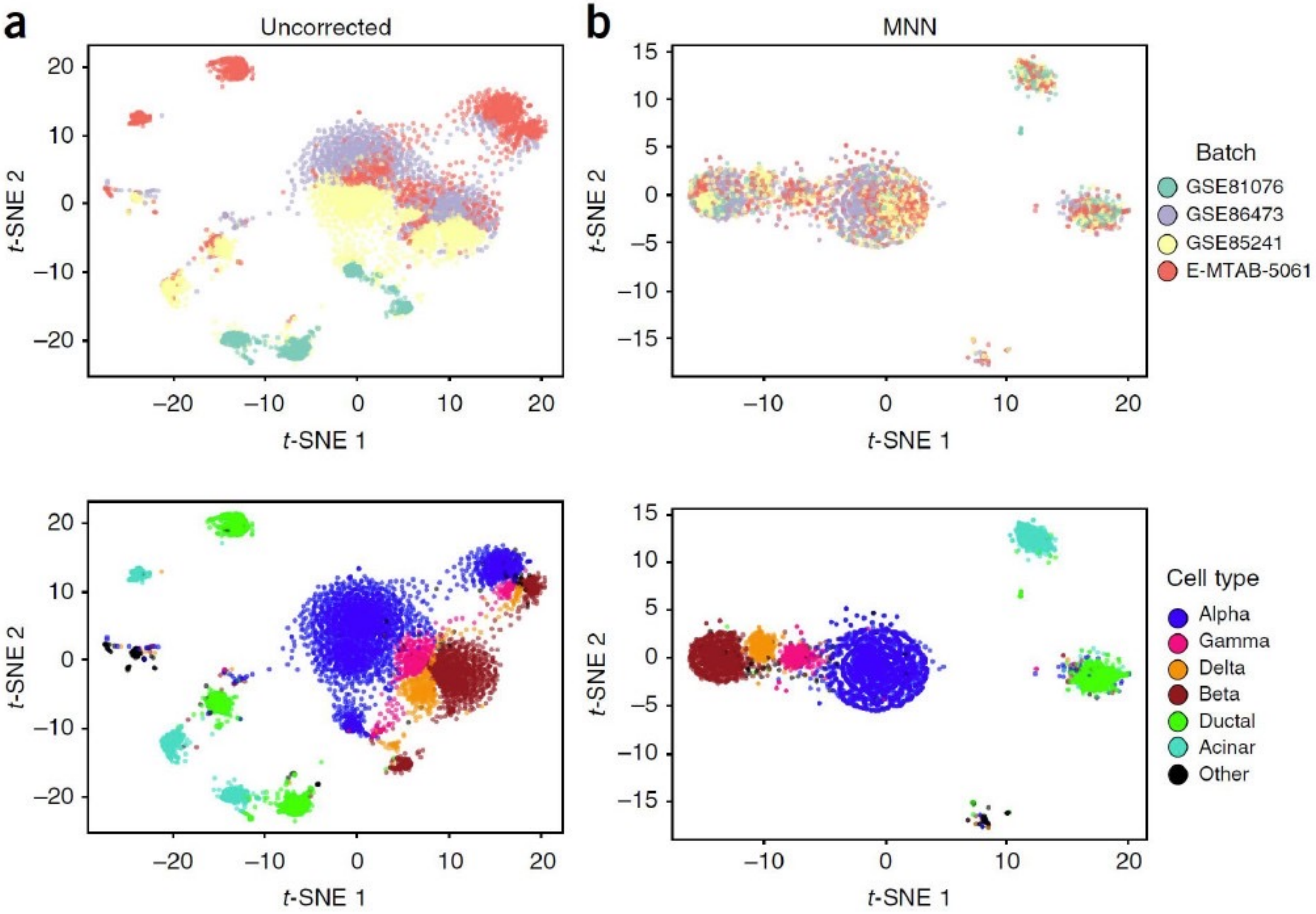
Real valued function
f : $R^p \rightarrow R$
as the weighted average of neighboring observed data

Batch Correction vector for each cell





Integration analysis: Mutual Nearest Neighbors (MNN)



Haghverdi (https://doi.org/10.1038/nbt.4091)

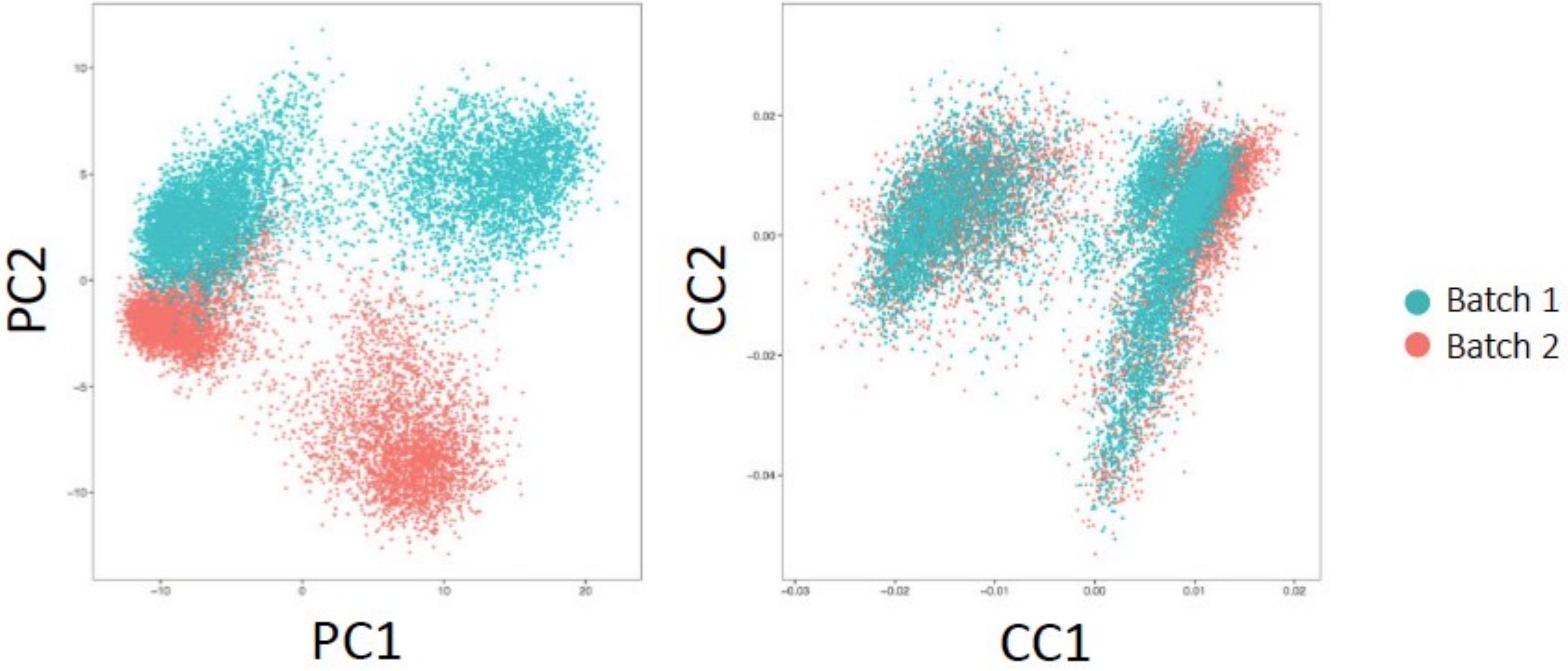
Integration analysis:
CCA +anchors (Seurat v3)

1. Find corresponding cells across datasets
2. Compute a data adjustment based on correspondences between cells
3. Apply the adjustment

Stuart et al. (https://doi.org/10.1101/460147)

Integration analysis:
CCA +anchors (Seurat v3)

1. Find corresponding cells across datasets

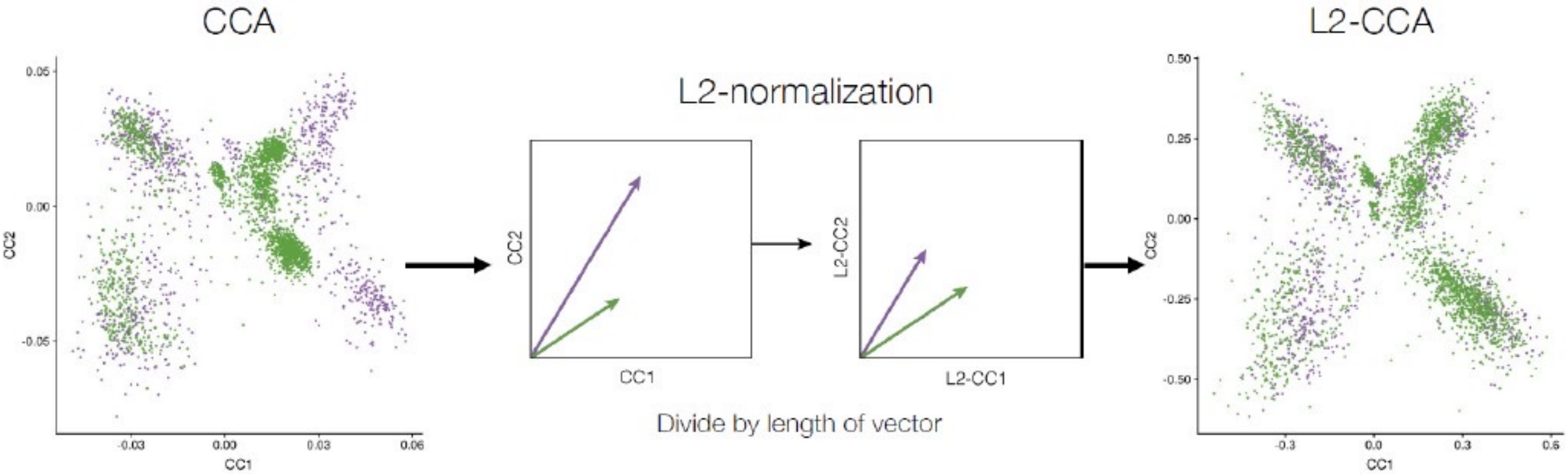


CCA captures correlated sources of variation between two datasets

Stuart et al. (https://doi.org/10.1101/460147)

Integration analysis: CCA +anchors (Seurat v3)

1. Find corresponding cells across datasets

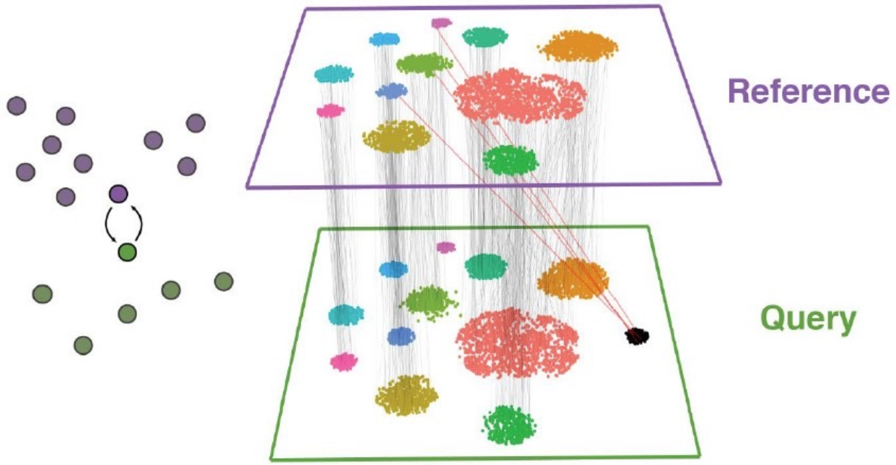


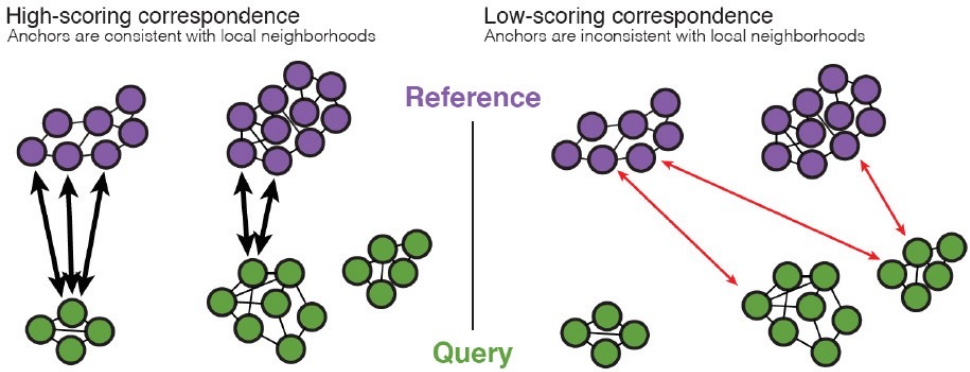
L2-normalization corrects for differences in scale

Integration analysis: CCA +anchors (Seurat v3)

1. Find corresponding cells across datasets

Anchors: Mutual nearest neighbors

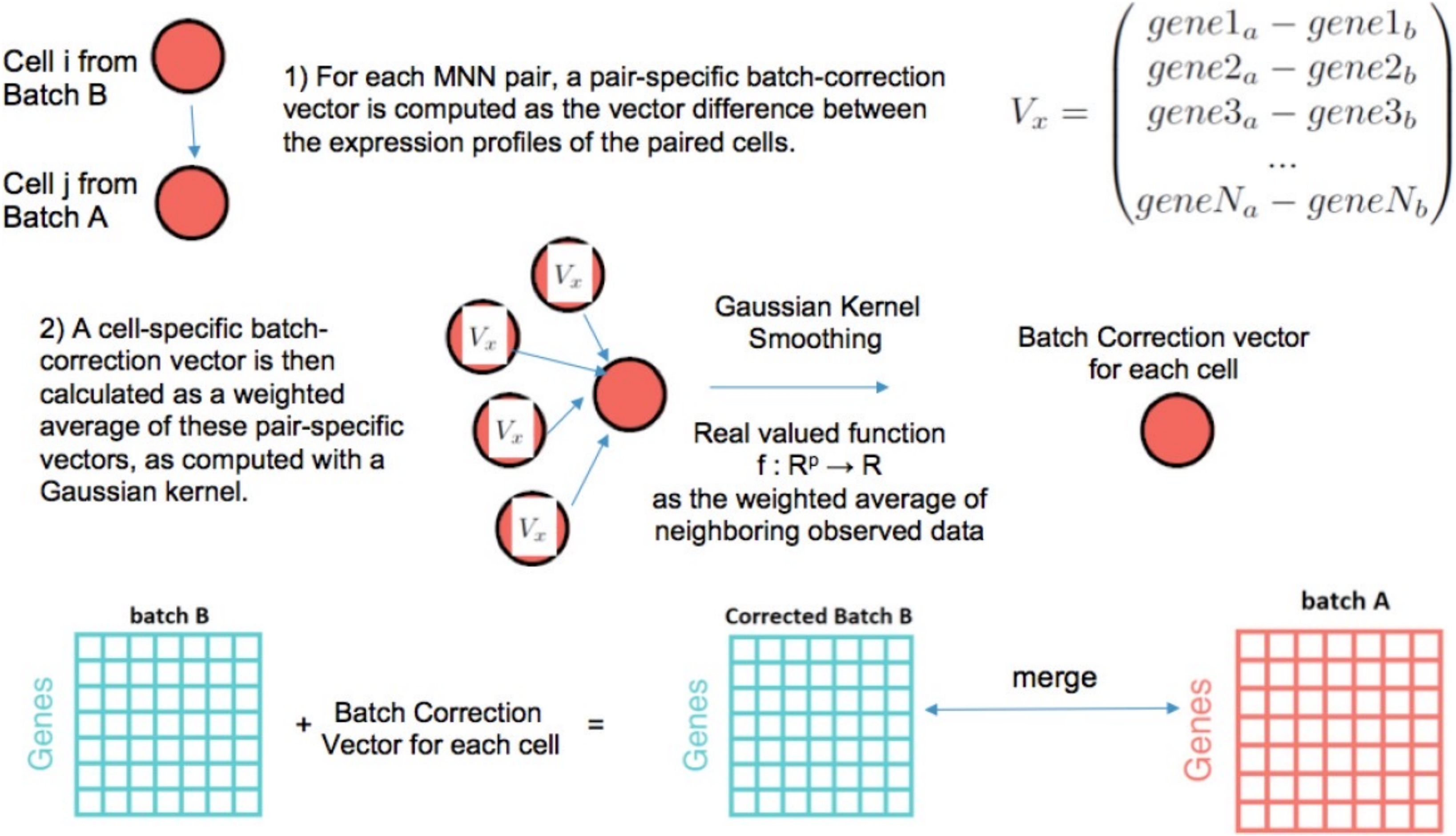



High-scoring correspondence
Anchors are consistent with local neighborhoods
Low-scoring correspondence
Anchors are inconsistent with local neighborhoods
Reference
Query

FindIntegrationAnchors()

Integration analysis: CCA +anchors (Seurat v3)

2. Data integration



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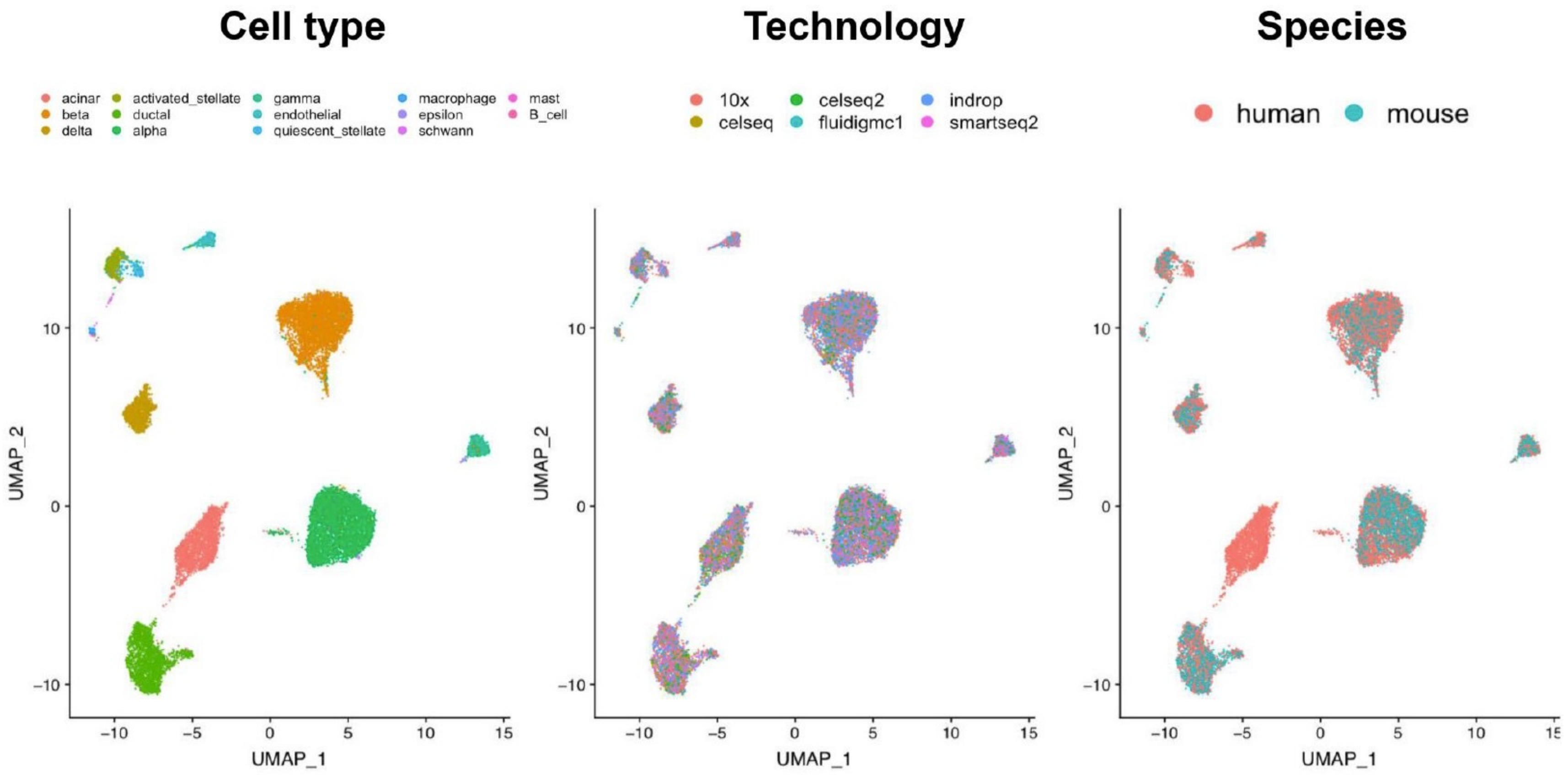
2) A cell-specific batch-correction vector is then calculated as a weighted average of these pair-specific vectors, as computed with a Gaussian kernel.

Real valued function f : $R^p \rightarrow R$ as the weighted average of neighboring observed data

+ Batch Correction Vector for each cell =

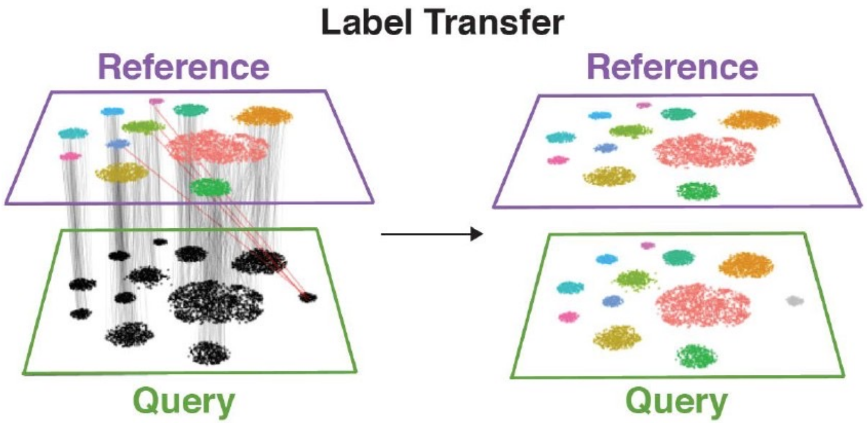
IntegrateData()

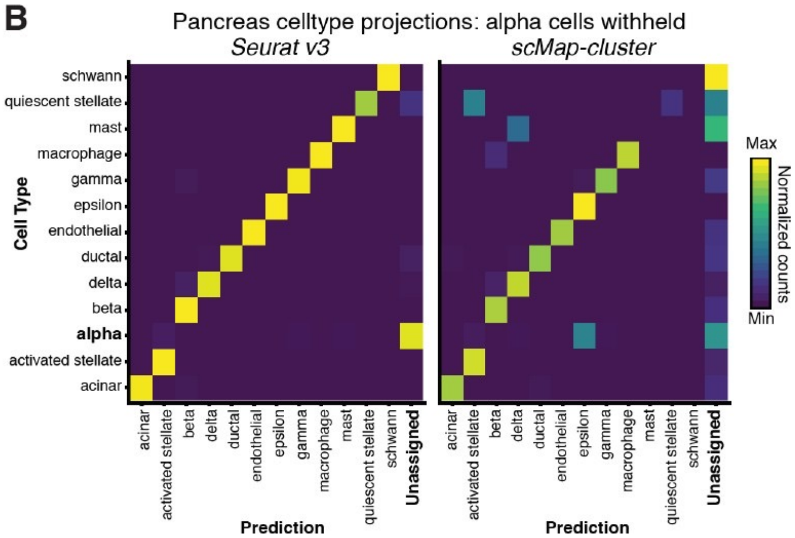
Integration analysis: CCA +anchors

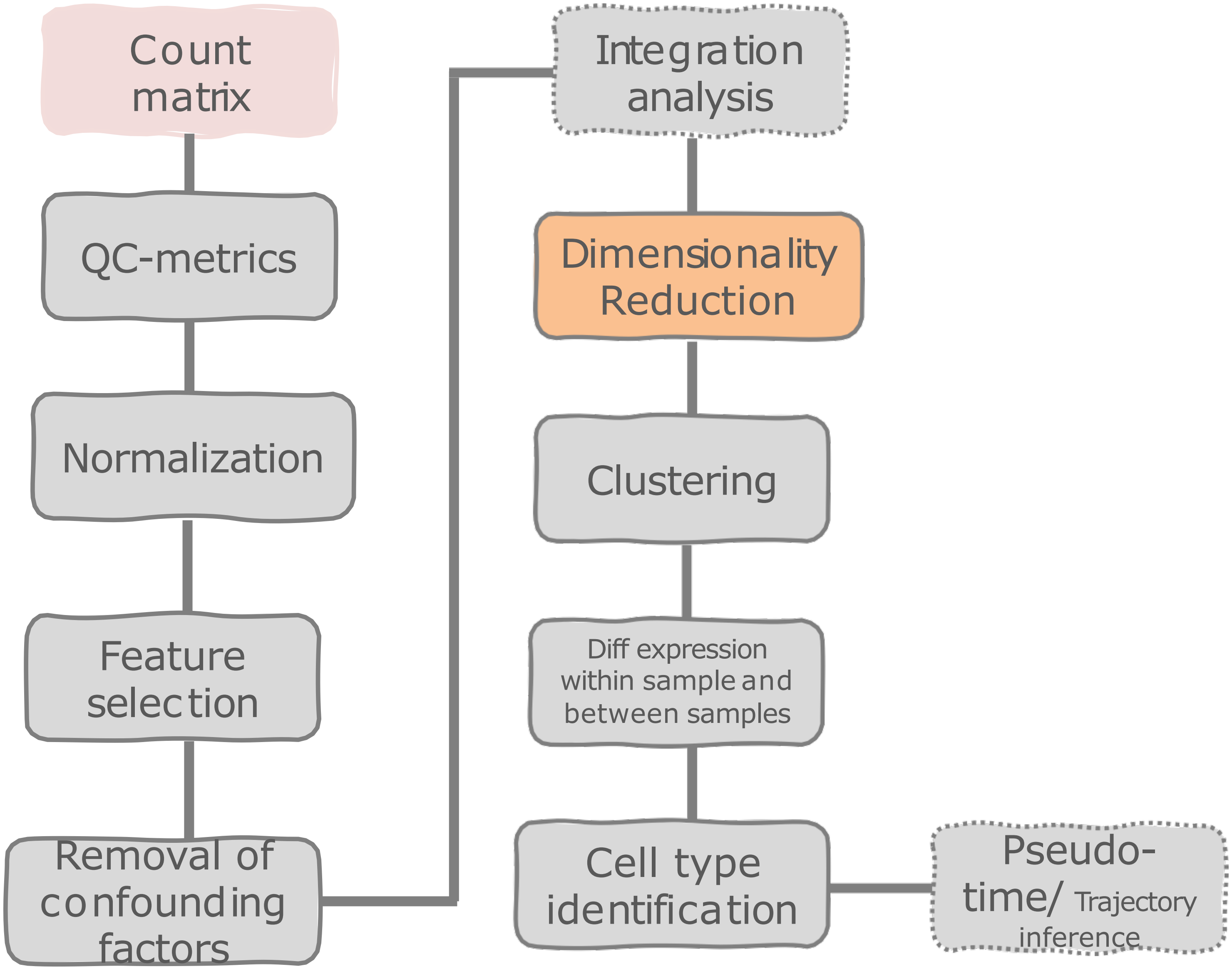


Retinal bipolar datasets: 51K cells, 6 technologies, 2 Species

Label transfer:
CCA +anchors







Count matrix

QC-metrics

Normalization

Feature selection

Removal of confounding factors

Integration analysis

Dimensionality Reduction

Clustering

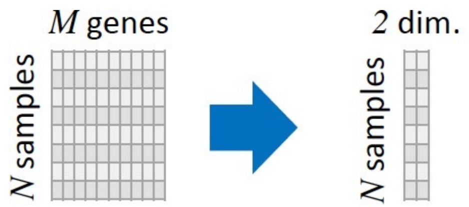
Diff expression within sample and between samples

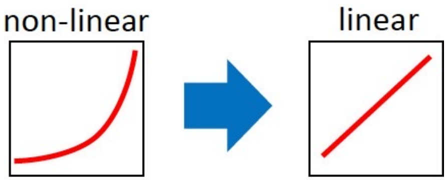
Cell type identification

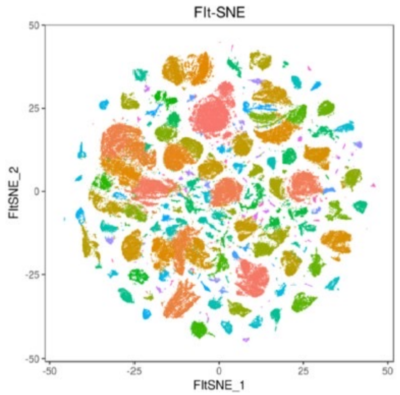
Pseudo-time/ Trajectory inference

Dimensionality Reduction

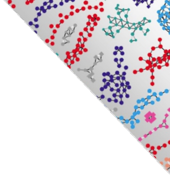
- **Simplify complexity**, so it becomes easier to work with.
  - Reduce number of features (genes)
  - In some: Transform non-linear relationships to linear
- “Remove” **redundancies** in the data
- Identify the **most relevant** information (find and filter noise)
- Reduce **computational time** for downstream procedures
- **Facilitate clustering**, since some algorithms struggle with too many dimensions
- Data **visualization**







Dimentionality 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

PCA- Principal component analysis

-PCA is based on variance

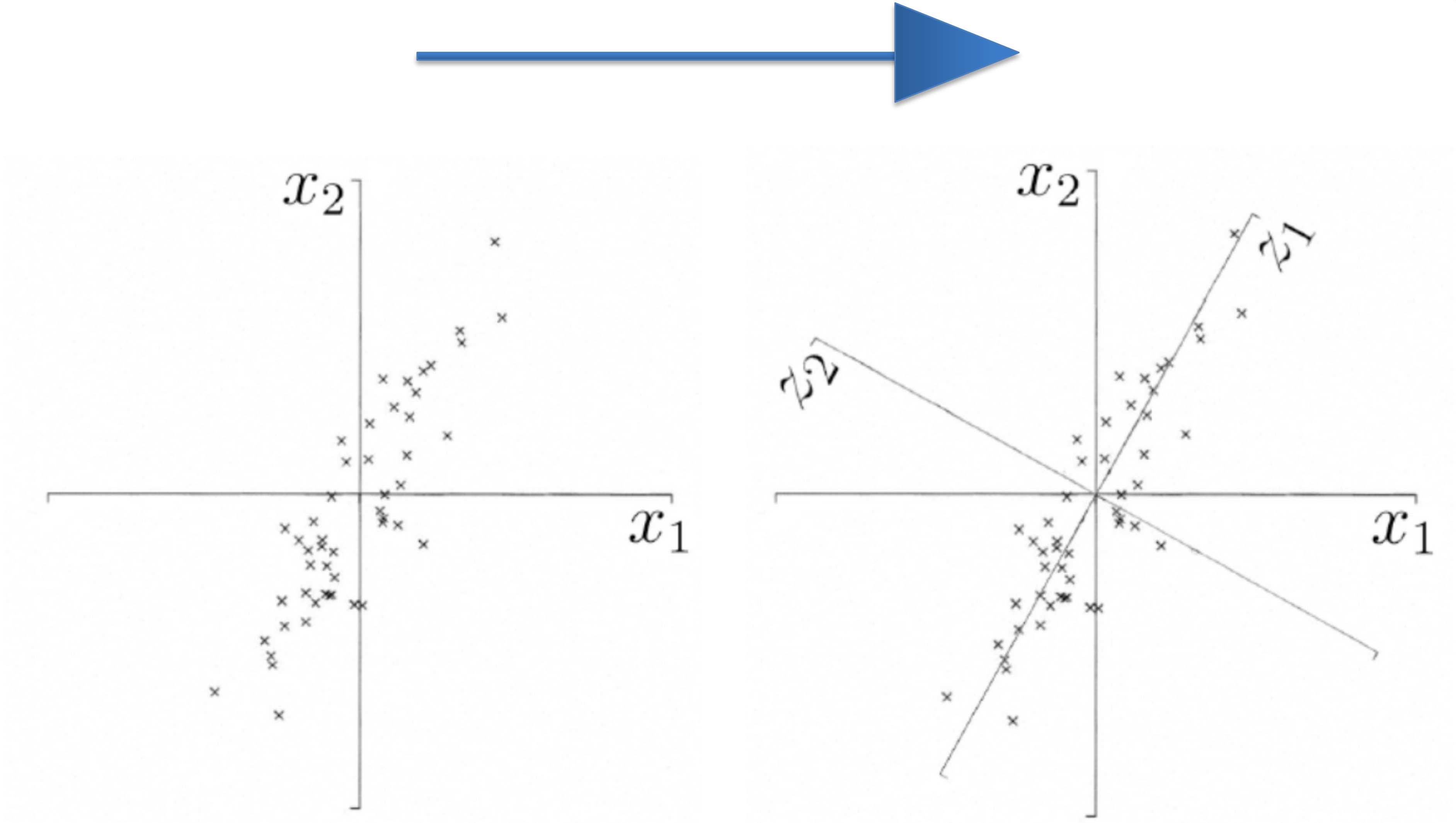
-PCA is the best angle to see and evaluate the data

-New axis that are linear combination of the original axes

PCA- Principal component analysis

Which and how ?

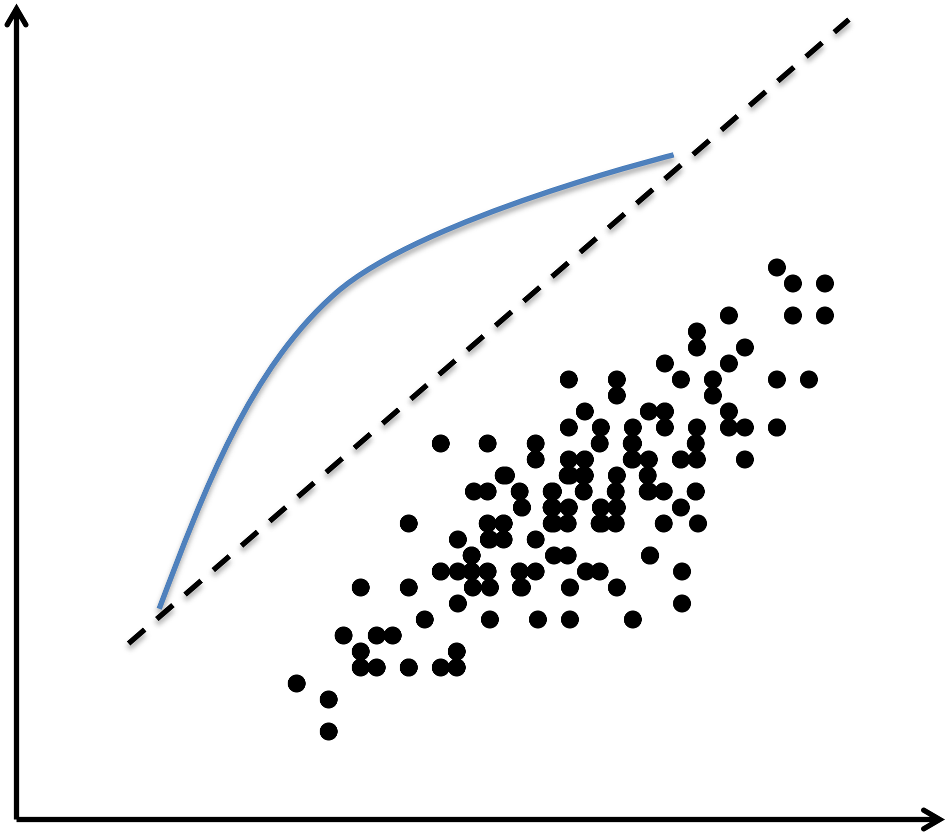
PCA- Principal component analysis



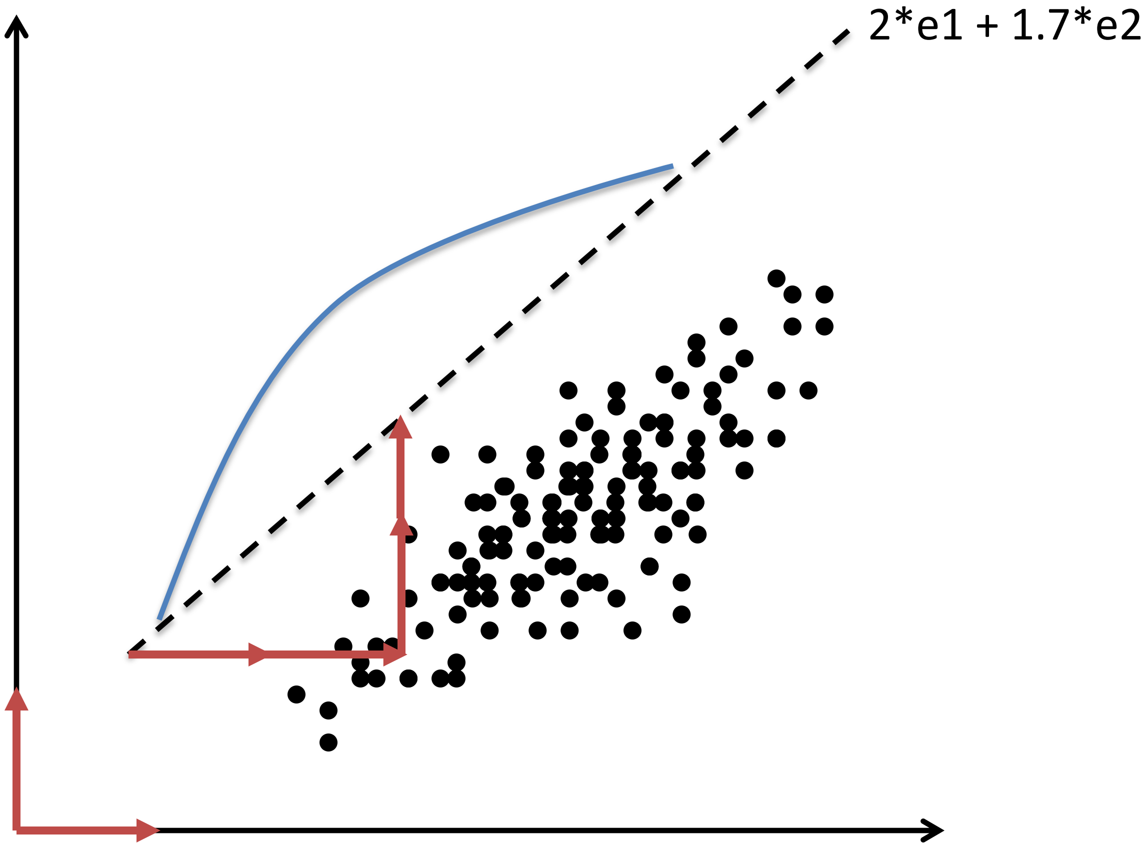
PCA- Principal component analysis

1. Largest variance first

PCA- Principal component analysis



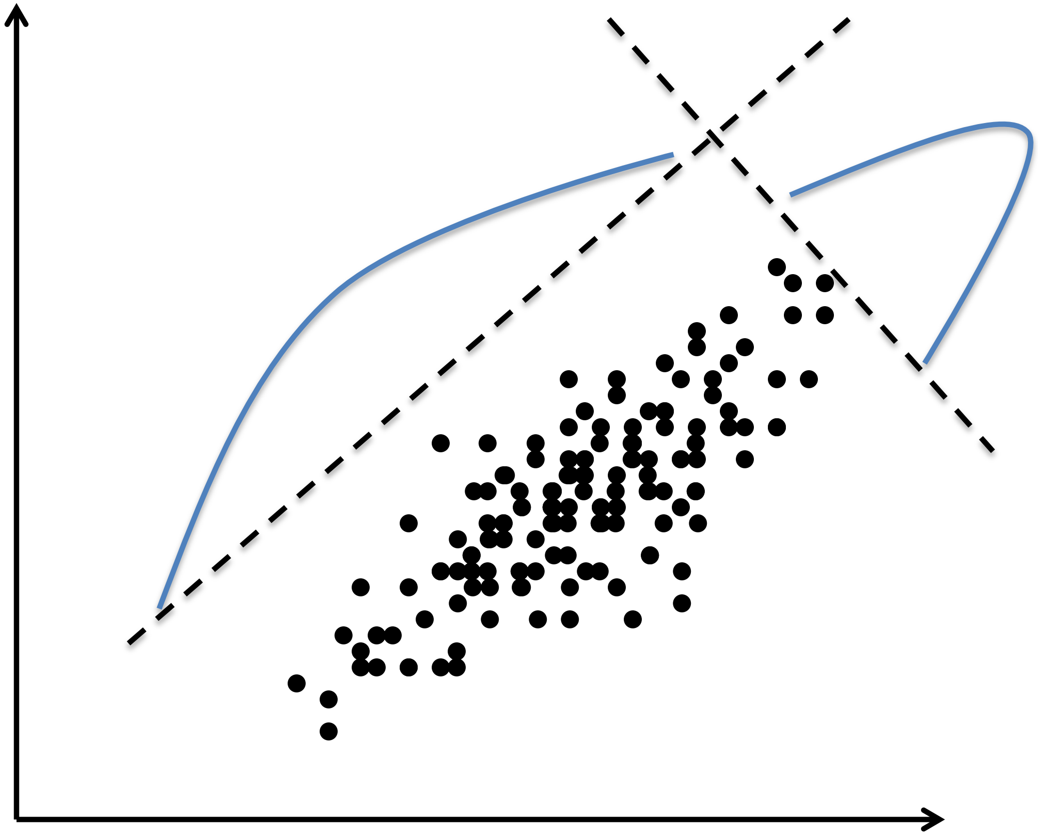
PCA- Principal component analysis



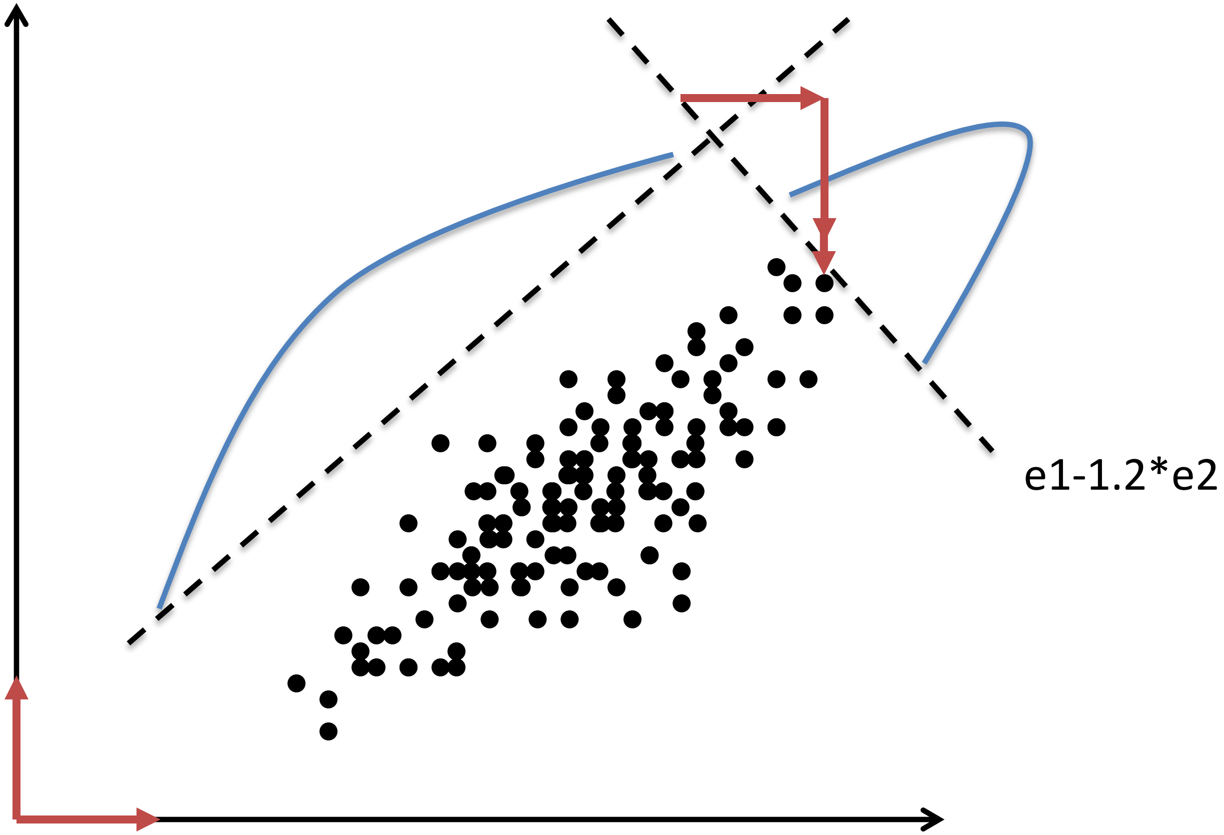
PCA- Principal Component Analysis

2. Select uncorrelated principal axis (orthogonal)

PCA- Principal Component Analysis



PCA- Principal Component Analysis



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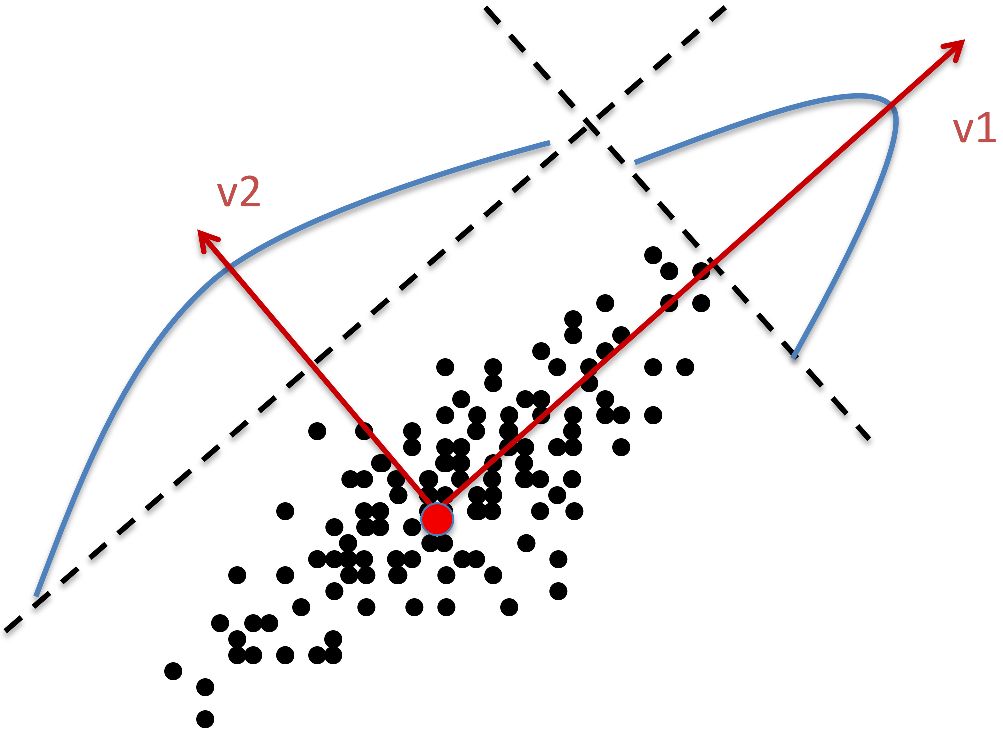
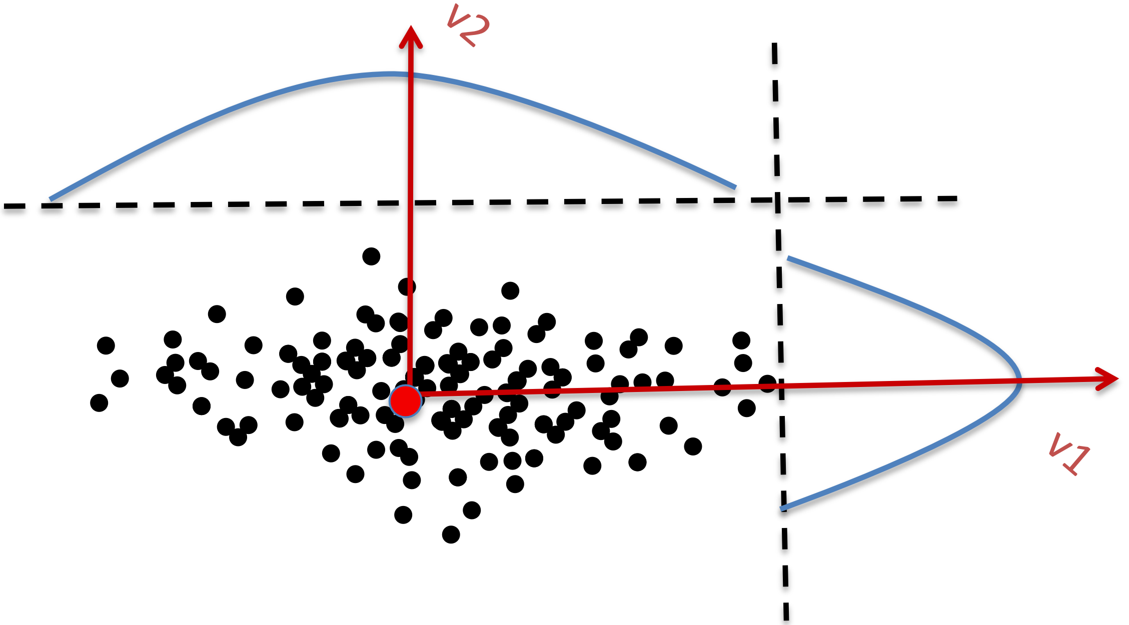


PCA- Principal Component Analysis

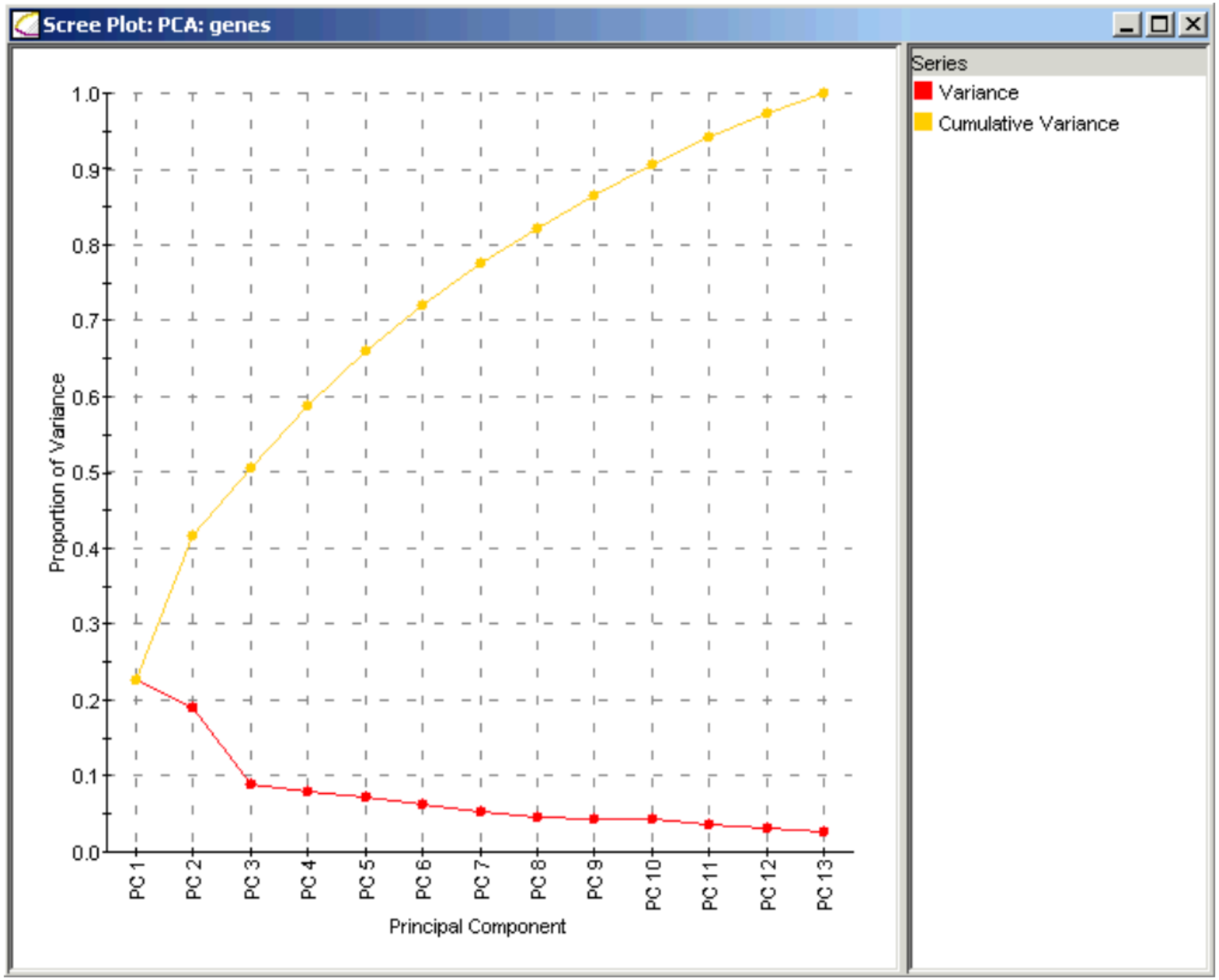


Mathematically

Calculate the eigenvectors of the **Covariance matrix** are *the directions of the axes where there is the most variance (this is something you can prove mathematically!)*

eigenvalues are the coefficients attached to eigenvectors, which give the *amount of variance carried in each Principal Component*.

After having the principal components, to compute the percentage of variance (information) accounted for by each component, we divide the eigenvalue of each component by the sum of eigenvalues.

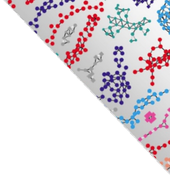


Scree Plot for Genetic Data. (Source.)

https://towardsdatascience.com/a-one-stop-shop-for-principal-component-analysis-5582fb7e0a9c

The PCA axis

- The PC are linear combination of the original axis.
- The estimated parameters of the linear combination is known and therefore we can know positively or negatively how much it goes into one direction or the other one.
- Indeed as the original axis are g1,g2,g3… and the new axis are a1g1 +a2g2…, one takes the ai that are the highest positively and negatively and therefore knows which genes are mostly representing the axis you see.
- By default, 10 highest positive and negative values are displayed in R with the seurat package.



Dimentionality reduction: PCA doesn’t fit

- It is a **LINEAR** method of dimensionality reduction
- It is an **interpretable** dimensionality reduction
- Data is usually **SCALED** prior to PCA (Z-score | see ScaleData in the Seurat)
- The **TOP** principal components contain higher variance from the data
- Can be used as **FILTERING**, by selecting only the top significant PCs
  - PCs that explain at least 1% of variance
  - Jackstraw of significant p-values
  - The first 5-10 PCs
  - Scater library describes correlation between PCs and metadata, take PCs until metadata information is covered

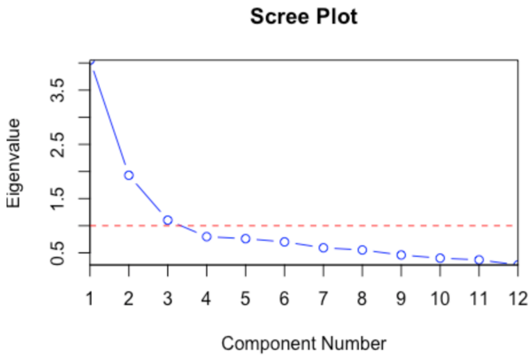
Problems:

- The two first PC in SC-RNAseq often account for only few percent of the total variance
- It performs poorly to separate cells in 0-inflated data types (because of it non-linearity nature)
- Cell sizes and sequencing depth are usually captured in the top principal components

In R, Elbow plot

RunPCA – Computes the PCA with default : 50 pcs.
Check Elbow plot to see if 50 pcs are explaining well your data.
RunPCA will output a message with the genes contributing most to the PC (positif and negatif).
Uses irlba: Fast Truncated Singular Value Decomposition and Principal Components Analysis for Large Dense and Sparse Matrices (!!Approximation of PCA).
Usually first PCs only account for few percentages of the total variance.

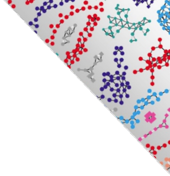
```
obj <-RunPCA( obj )
ElbowPlot(obj,ndims=50)
```



Wikipedia:
https://en.wikipedia.org/wiki/Scree_plot

T-SNE

T-SNE



T-SNE = t-distributed stochastic neighborhood embedding

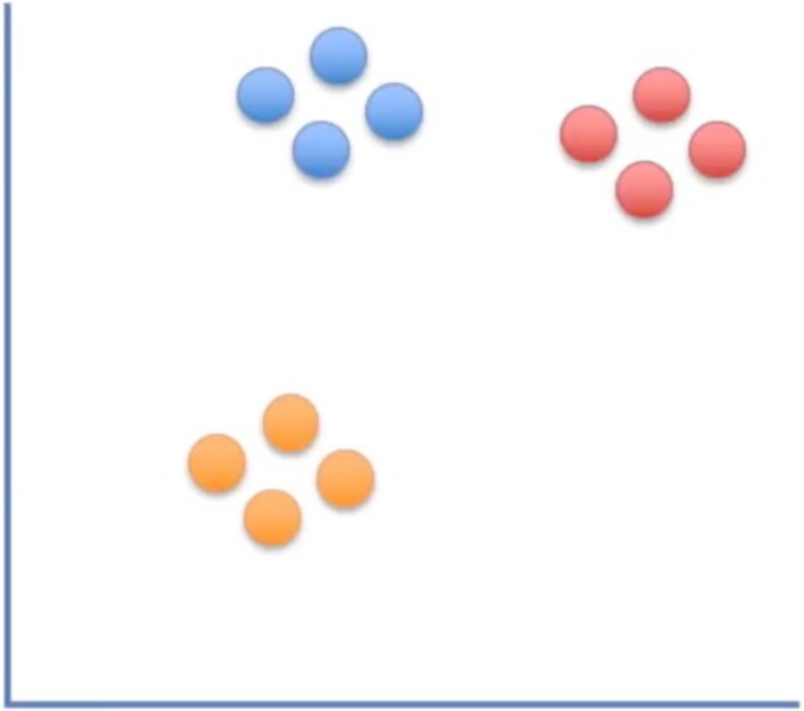
Laurens van der Maaten, Geoffrey Everest Hinton

http://www.jmlr.org/papers/volume9/vandermaaten08a/vandermaaten08a.pdf

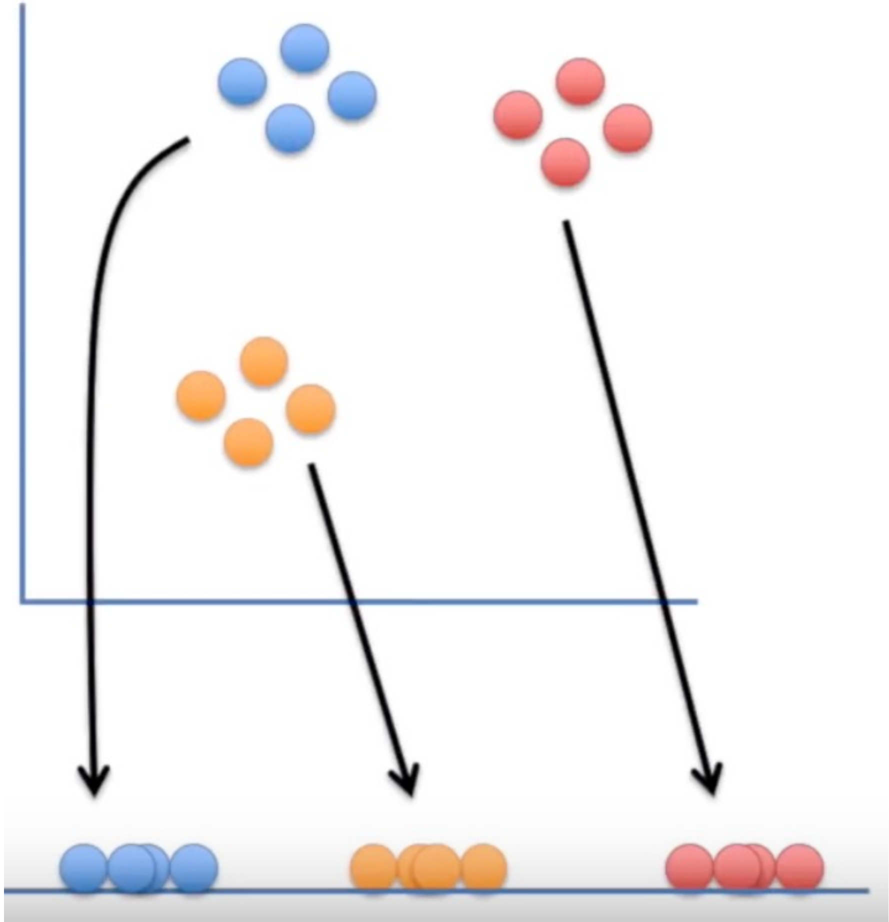
https://www.youtube.com/watch?v=NEaUSP4YerM

Many of the following figures are inspired by this youtube link check out his channel ! (StatQuestion with Josh Starmer)

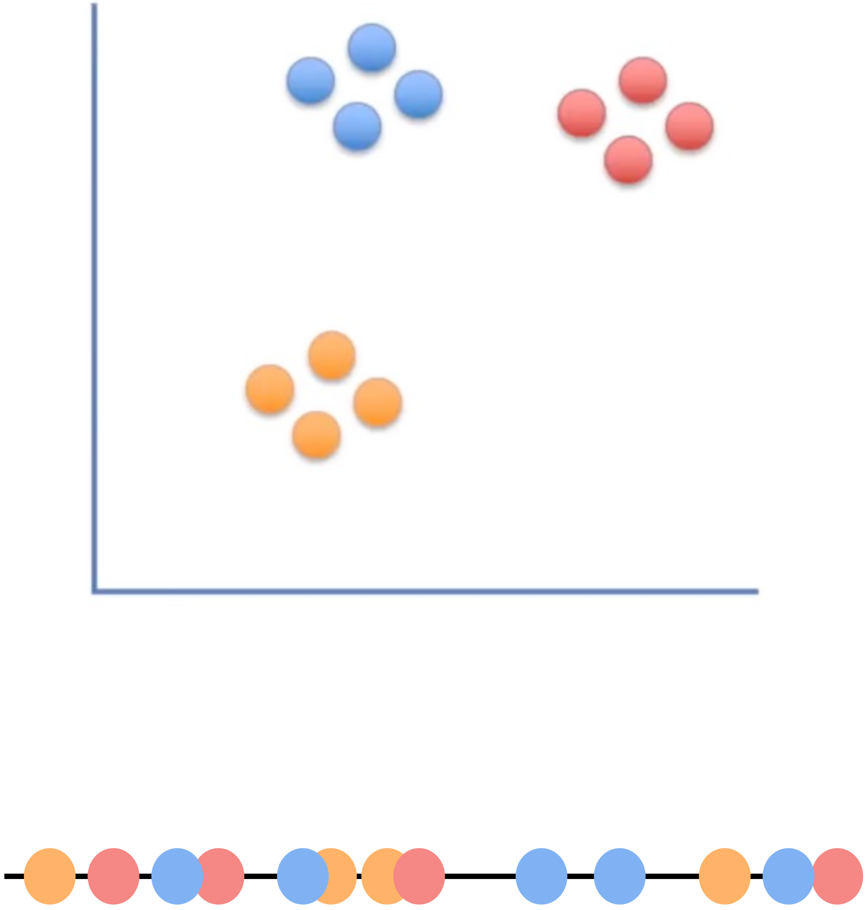
Start with a data-set



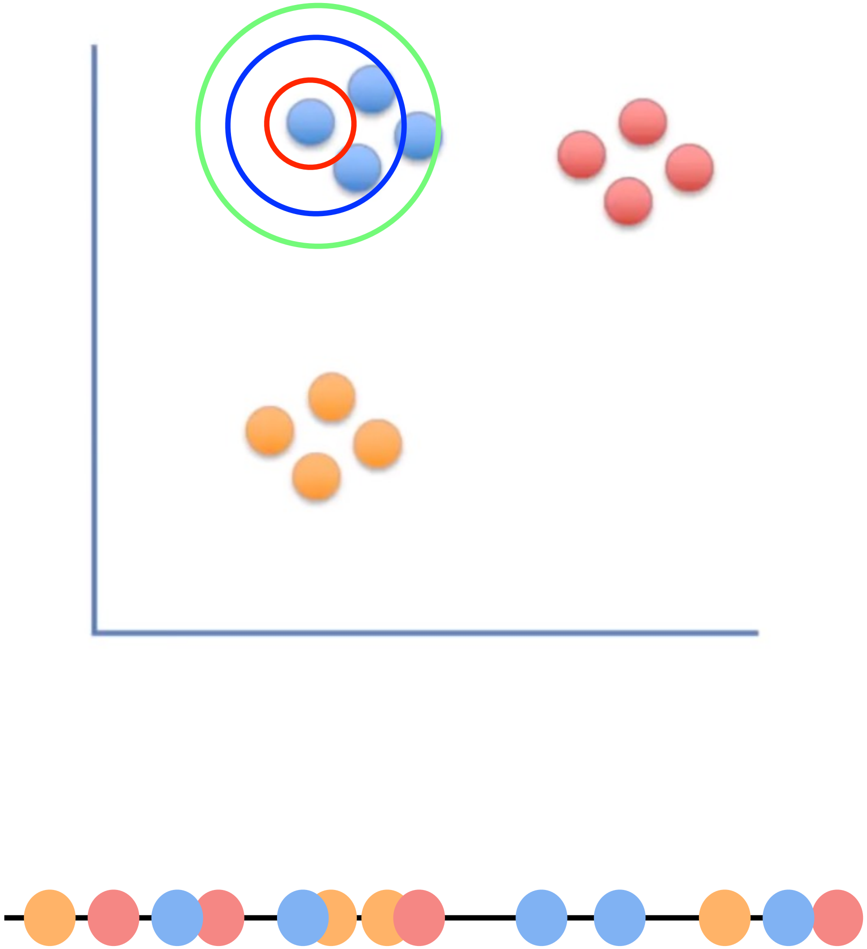
Find a right way to reduce dimension

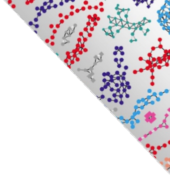


Basic idea (!! set a seed)



Normal distribution around a point





We calculate

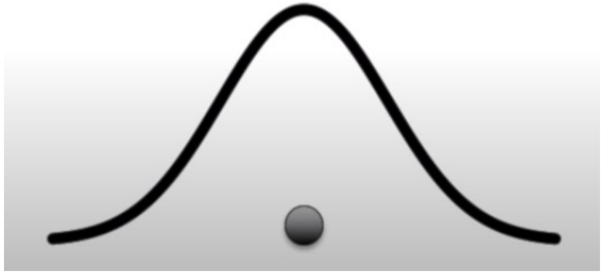
The similarity of datapoint A to datapoint B is the conditional probability, that A would pick B as its neighbor, if neighbors were picked in proportion to their probability density under a Gaussian centered at B, written p_A|B.

p_A|A = 0

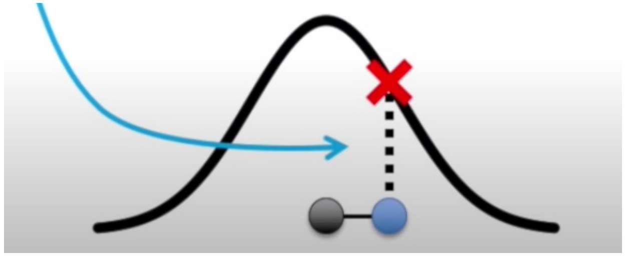
The variance of this normal distribution depends on the density around C (the more cells closer to C the lower the variance of this normal distribution will be).

Steps

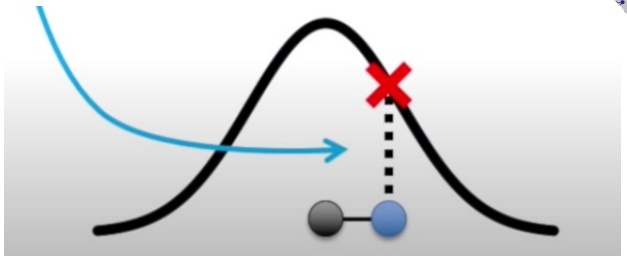
1. Take a point A.
2. Take another point B
3. Plot that point on a normal distribution distributed around A.



4. Take another point B and plot it on that distribution, this will be called the unscaled similarity.

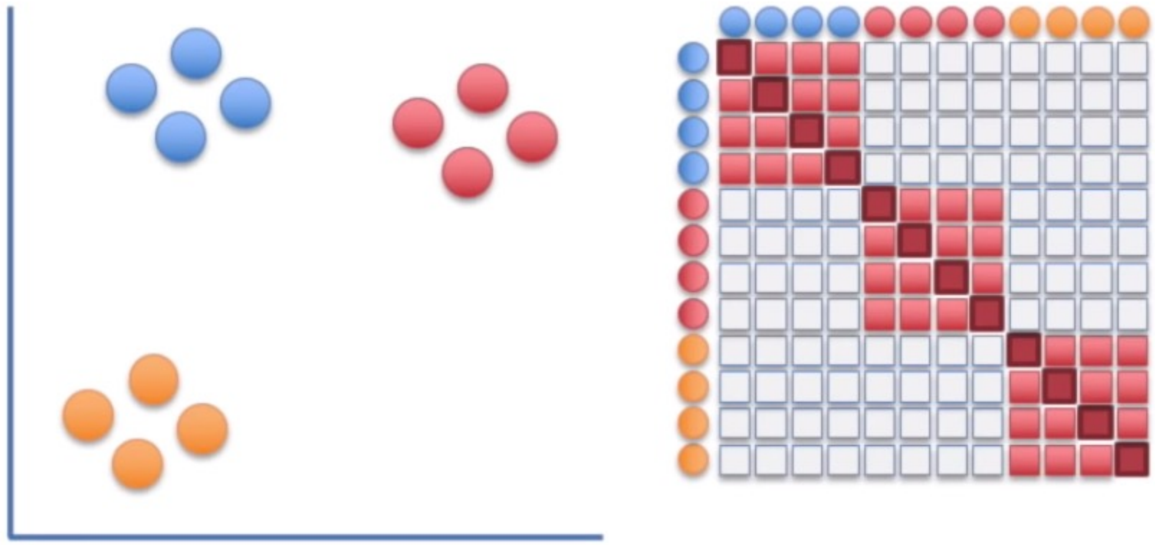


Steps



5. This is done for all the points. Distant points will have a very low similarity, whereas close points a very high similarity.
6.These unscaled similarities are then scaled so that they add up to one.
7. The similarity between A and B might be different than the similarity between B and A, so to correct for that the mean of the two values is taken.

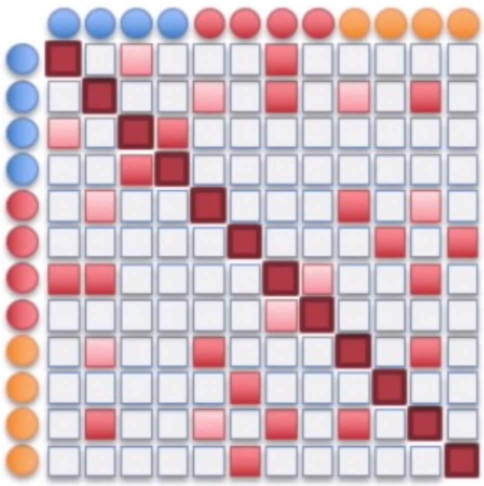
Illustration

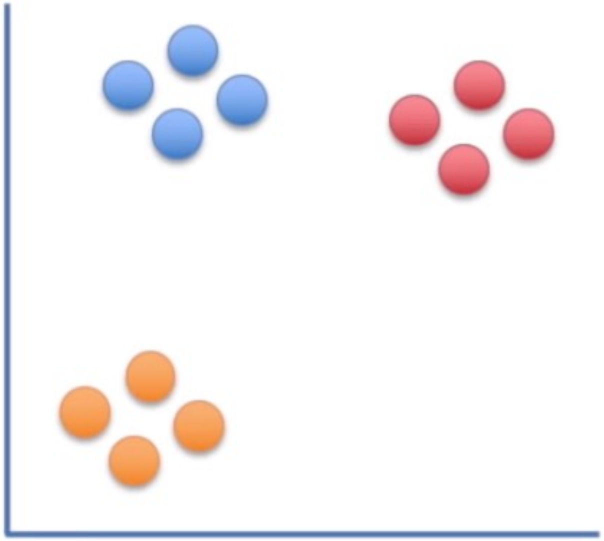


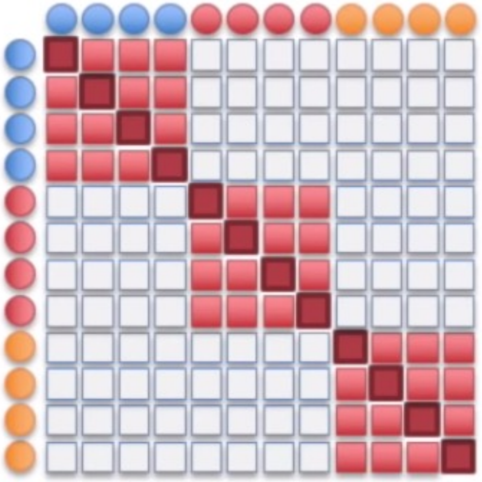
On the projection

Do the same into the randomly projected points.

Using a t-distribution instead of a normal distribution.

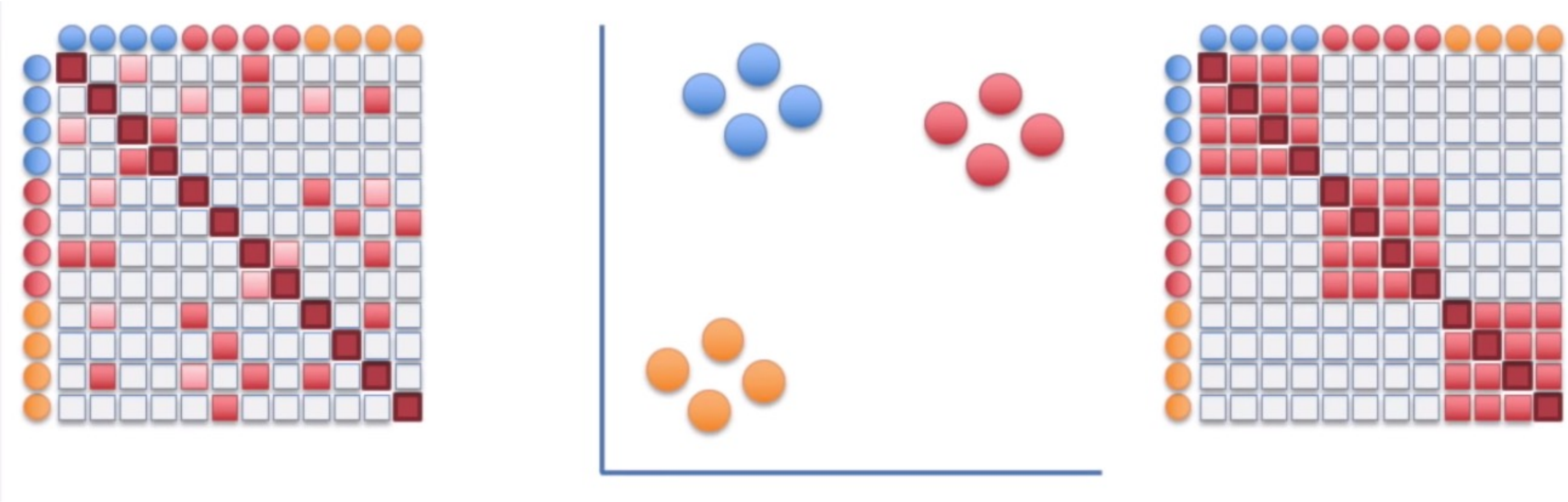






On the projection

Move points little by little and redo calculation until you are « as close as possible » to the original similarity matrix or you reach a certain number of iteration (chosen by the user).



« As close as possible »

To measure the minimization of the sum of difference of conditional probability t-SNE minimizes the sum of Kullback-Leibler divergence of overall data points using a *gradient descent method*.

In other words : tSNE minimizes the divergence between two distributions: a distribution that measures pairwise similarities of the input objects and a distribution that measures pairwise similarities of the corresponding *low*-dimensional points in the embedding

To measure the minimization of the sum of difference of conditional probability t-SNE minimizes the sum of Kullback-Leibler divergence of overall data points using a gradient descent method.

$$C = \sum_i KL(P_i||Q_i) = \sum_i \sum_j p_{j|i} \log \frac{p_{j|i}}{q_{j|i}},$$

Parameters for T-sne

perplexity = 30L => linked to parameter σi

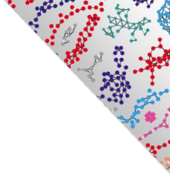
momentum = 0.5, => linked to optimisation

final_momentum = 0.8, => linked to optimisation

A cool webpage:

https://distill.pub/2016/misread-tsne/

(used to generate the figures in the next slides)



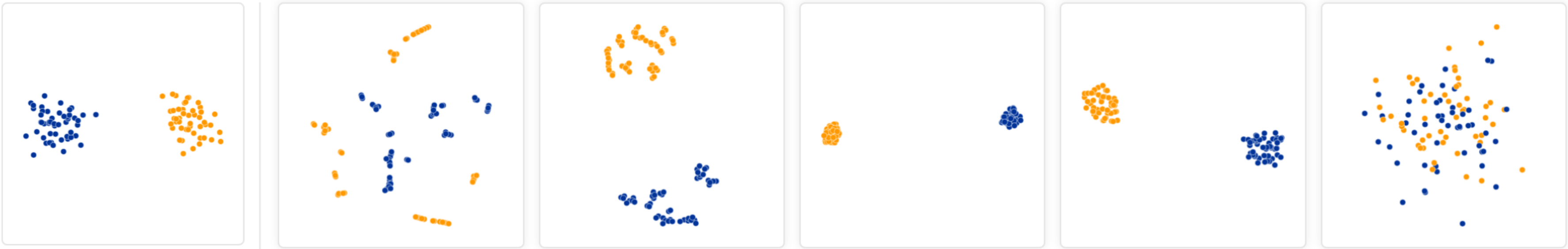
Getting the most from t-SNE may mean analyzing multiple plots with different perplexities.

The perplexity can be interpreted as a smooth measure of the effective number of neighbors

$$Perp(P_i) = 2^{H(P_i)},$$

where $H(P_i)$ is the Shannon entropy of P_i measured in bits

$$H(P_i) = -\sum_j p_{j|i} \log_2 p_{j|i}.$$



Original

Perplexity: 2
Step: 5,000

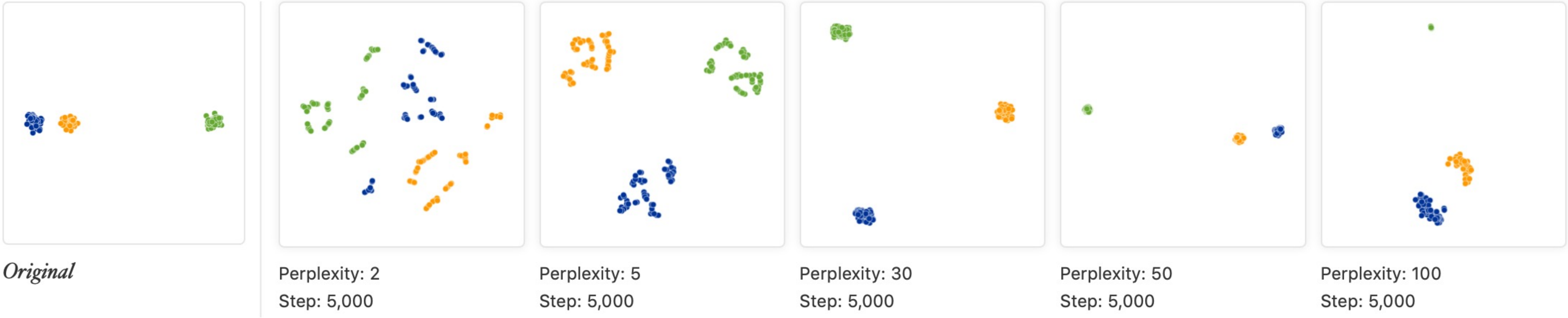
Perplexity: 5
Step: 5,000

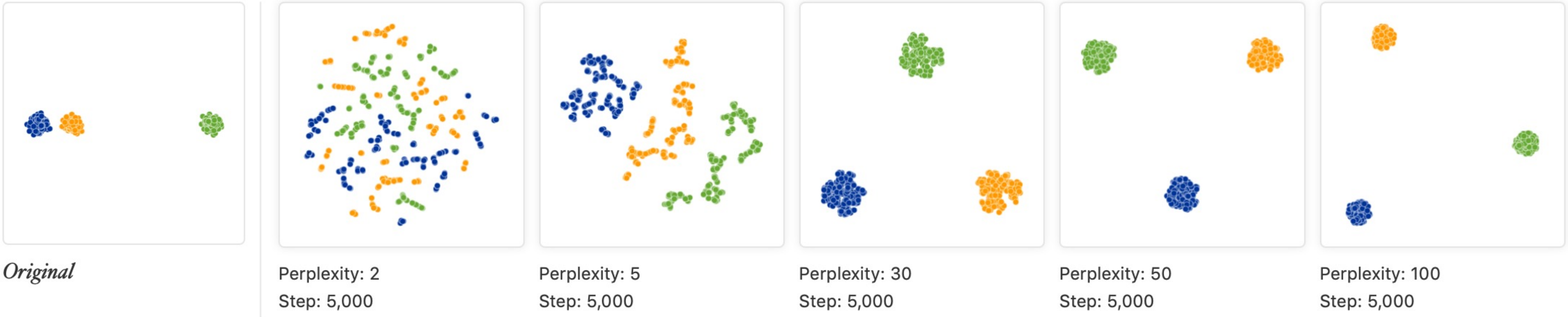
Perplexity: 30
Step: 5,000

Perplexity: 50
Step: 5,000

Perplexity: 100
Step: 5,000

Between cluster distances do not matter !

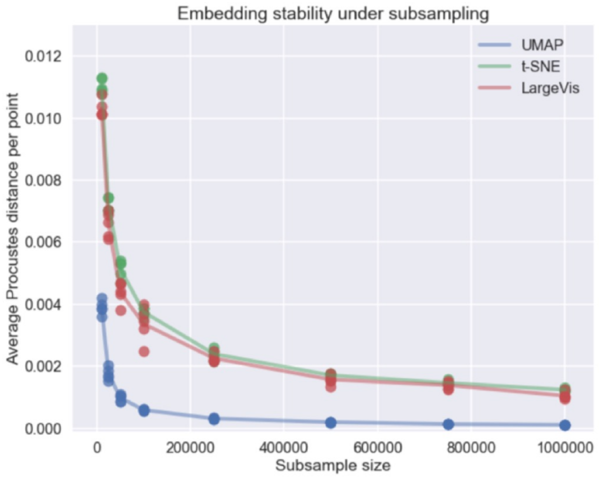


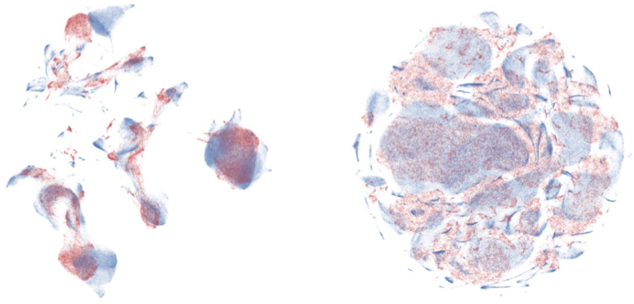


Dimentionality reduction: UMAP

UMAP: **U**niform **M**anifold **A**pproximation and **P**rojection

- It is a NON-LINEAR graph-based method of dimensionality reduction
- UMAP assumes that there is a manifold in the dataset.
- **Very efficient** - O(n)
- Can be run from the top PCs (e.g.: PC1 to PC10)
- Can use any distance metrics!
- Can integrate between different data types (text, numbers, classes)
- It is **no** longer **completely stochastic** as t-SNE
- Defines both **LOCAL** and **GLOBAL** distances
- Can be applied to **new data points**
- **Works on original data, but best on PCA reduced dimension (default in Seurat)**





(a) UMAP

(b) t-SNE

McInnes et al (2018) BioRxiv
https://umap-learn.readthedocs.io/en/latest/how_umap_works.html

UMAP

UMAP: Uniform Manifold Approximation and Projection for Dimension Reduction

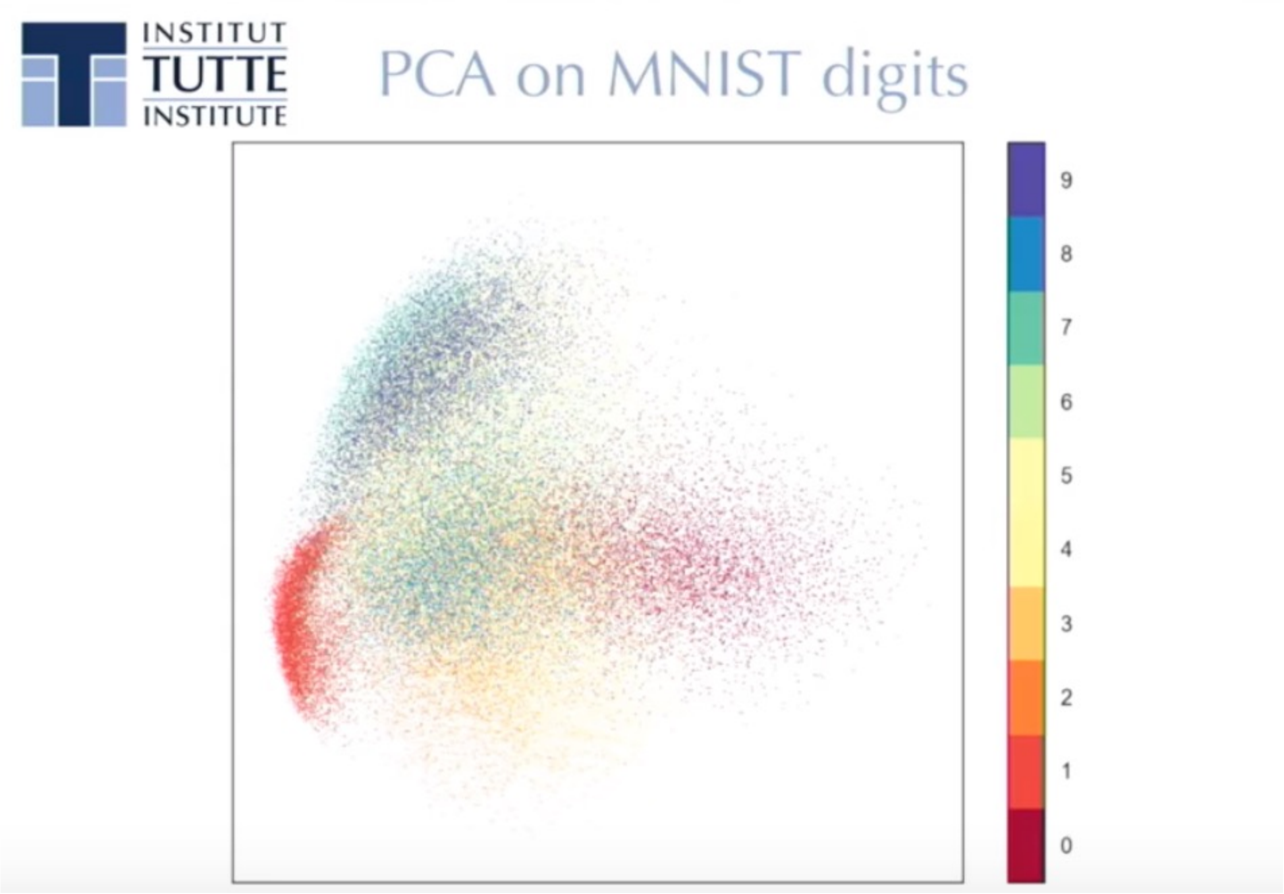
Leland McInnes (Mathematician), John Healy (Computing theorist), James Melville (Computing in R)

https://arxiv.org/abs/1802.03426

https://www.youtube.com/watch?v=nq6iPZVUxZU

https://umap.scikit-tda.org/parameters.html

PCA is good, but one can do better!



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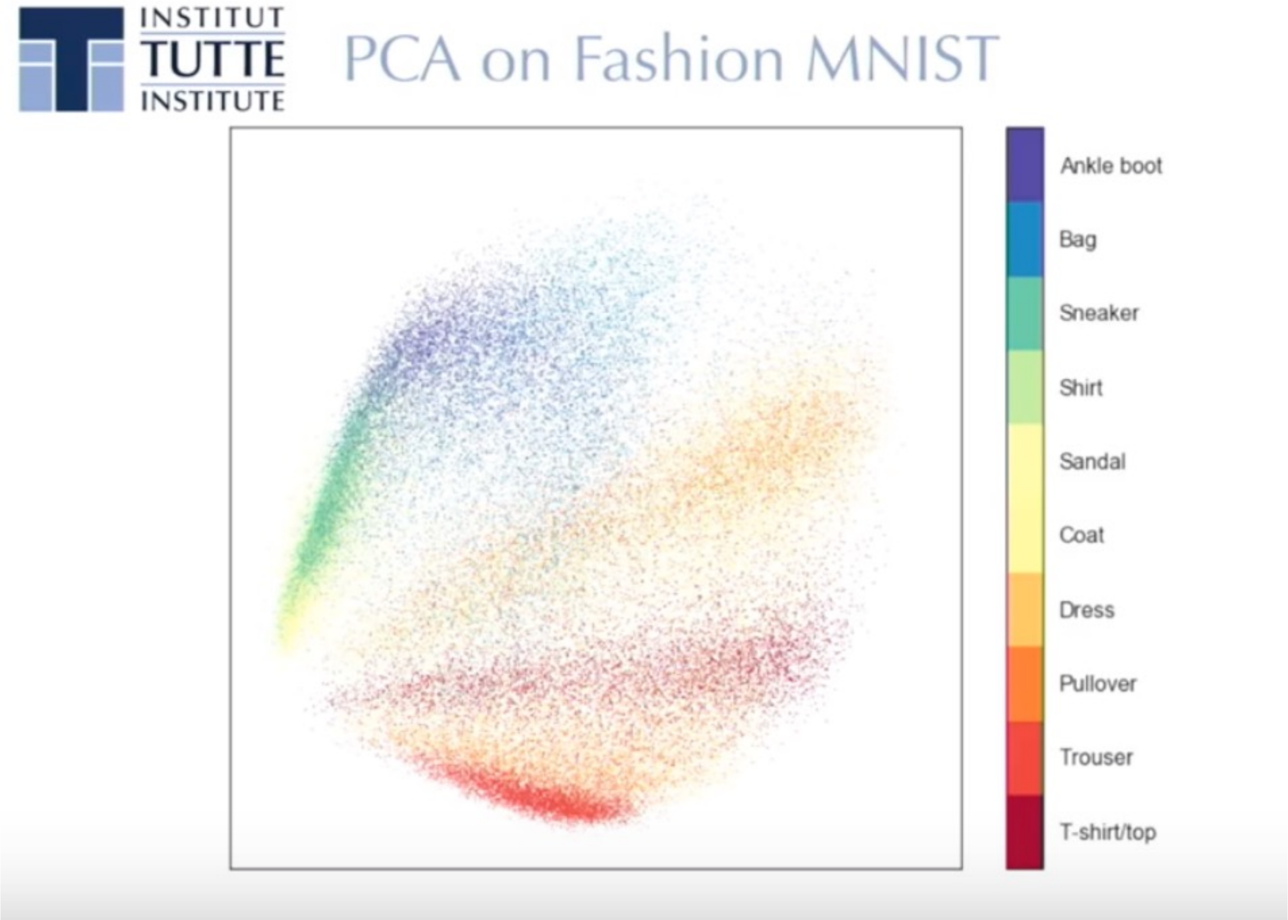
PCA on MNIST digits

From L.McInnes, SciPy 2018

PCA is good, but one can do better!

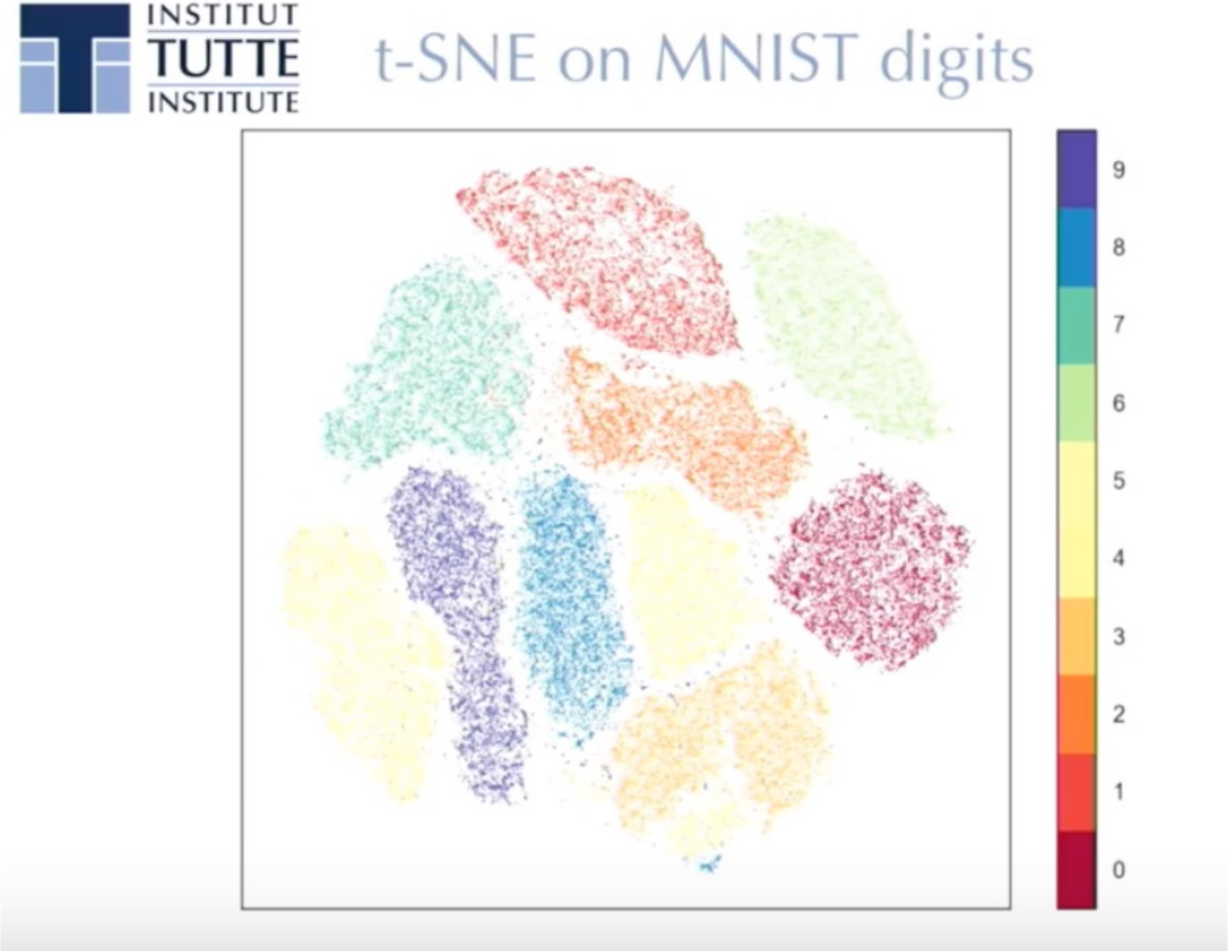


PCA on Fashion MNIST

See the
global structure
and
Interpretable axis

From L.McInnes, SciPy 2018

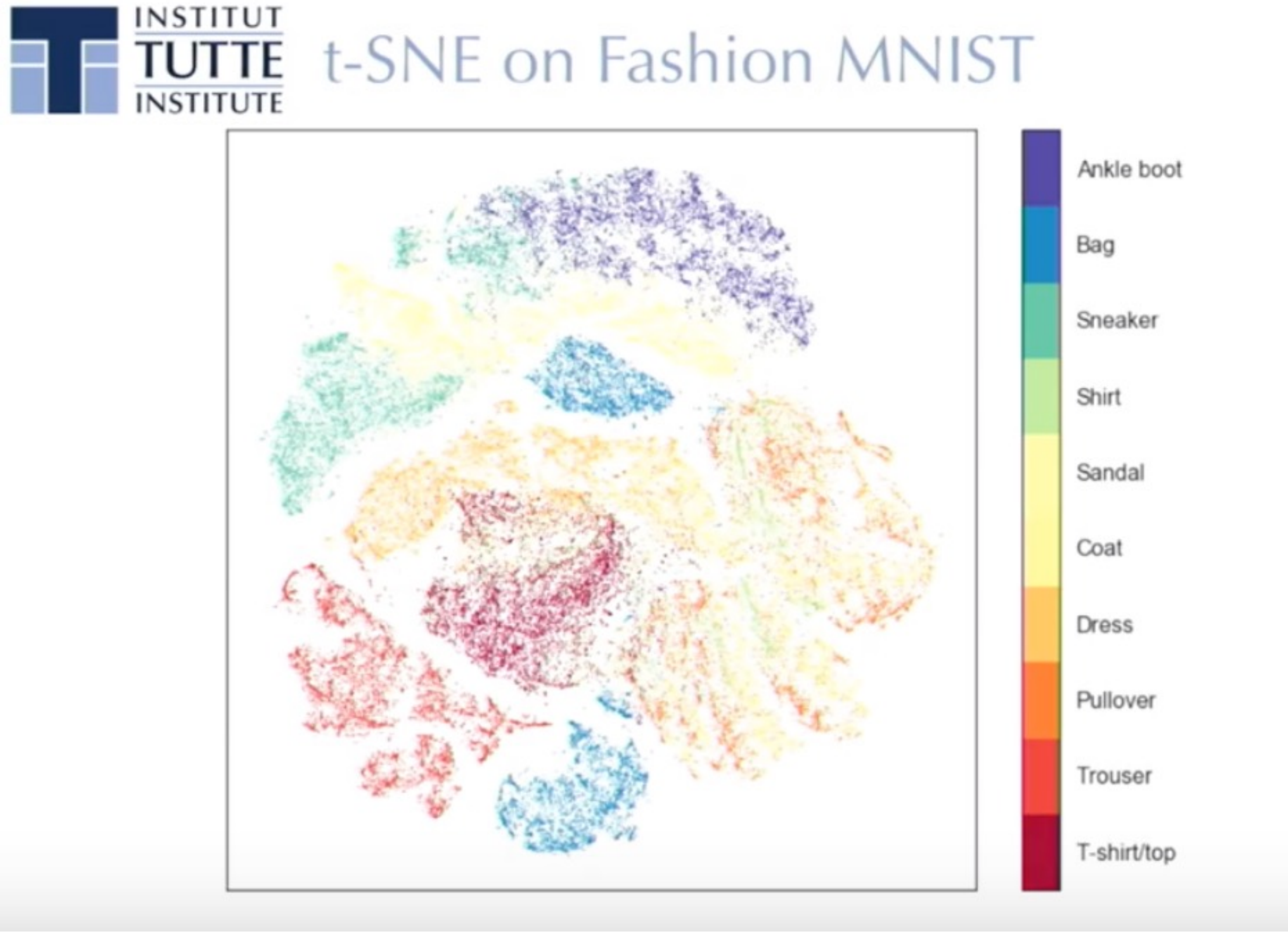
T-SNE manages to see the local structure



t-SNE on MNIST digits

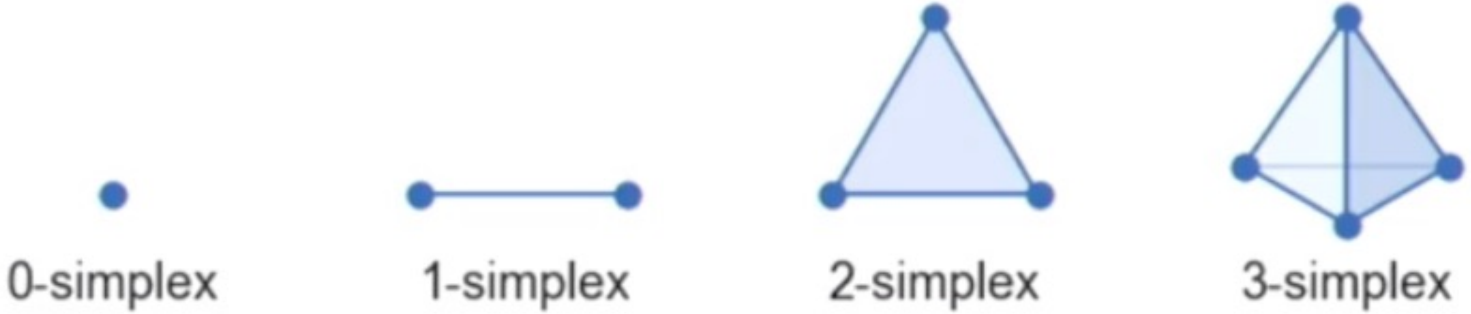
From L.McInnes, SciPy 2018

T-SNE manages to see the local structure

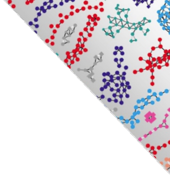


t-SNE on Fashion MNIST

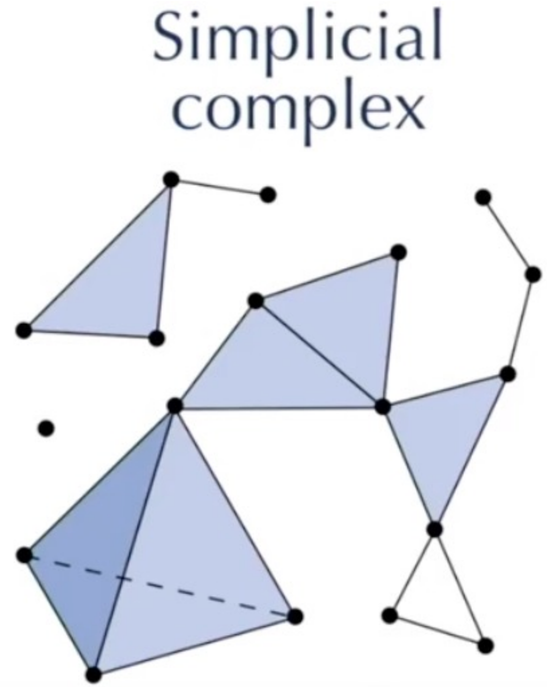
From L.McInnes, SciPy 2018



From L.McInnes, SciPy 2018

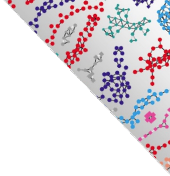


What it enables you to represent

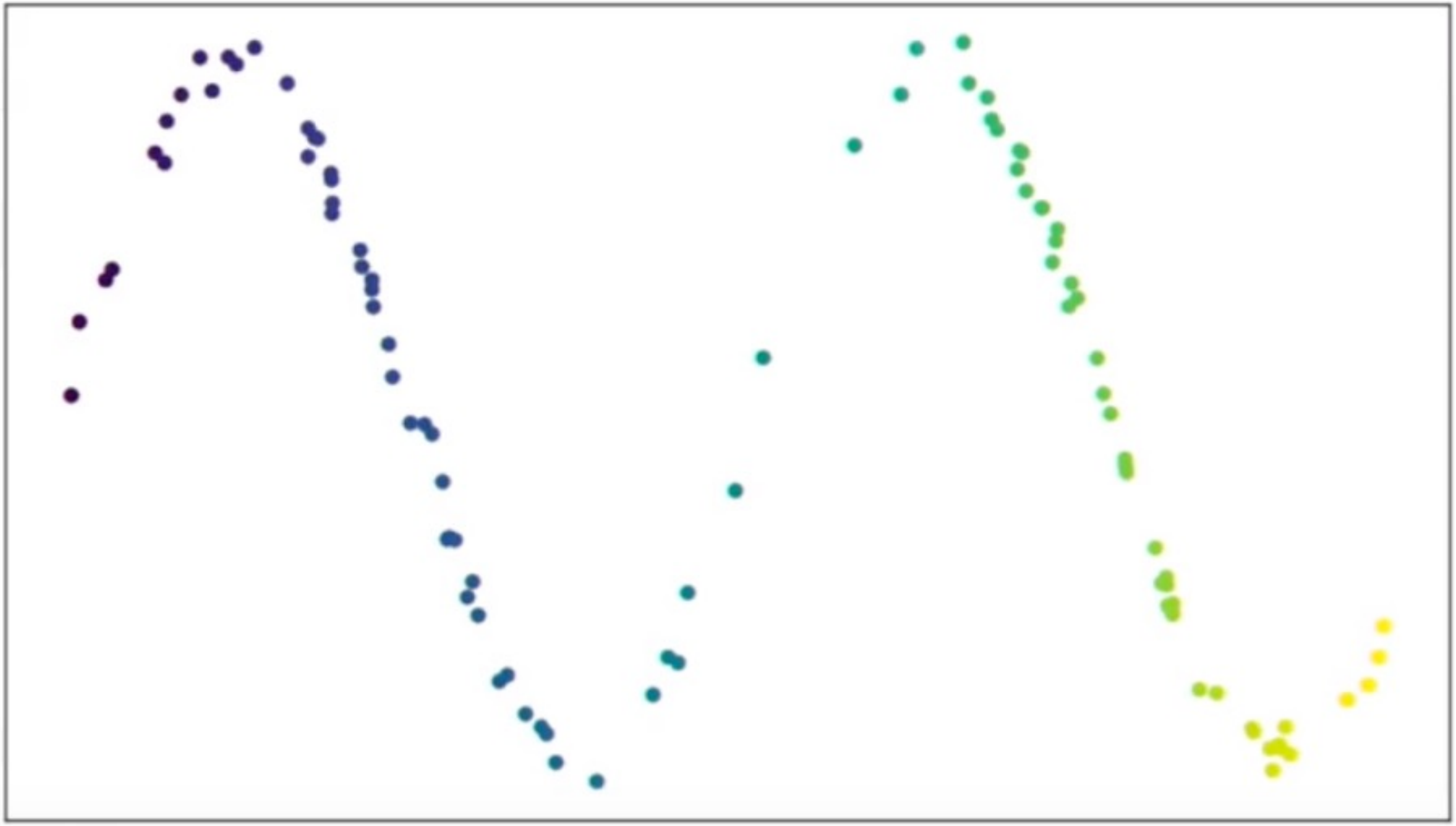


1. Combinatorial
2. Simple to implement
3. Keeps the information of the global structure
4.Nice theorems exist on those (Nerve theorem)

From L.McInnes, SciPy 2018

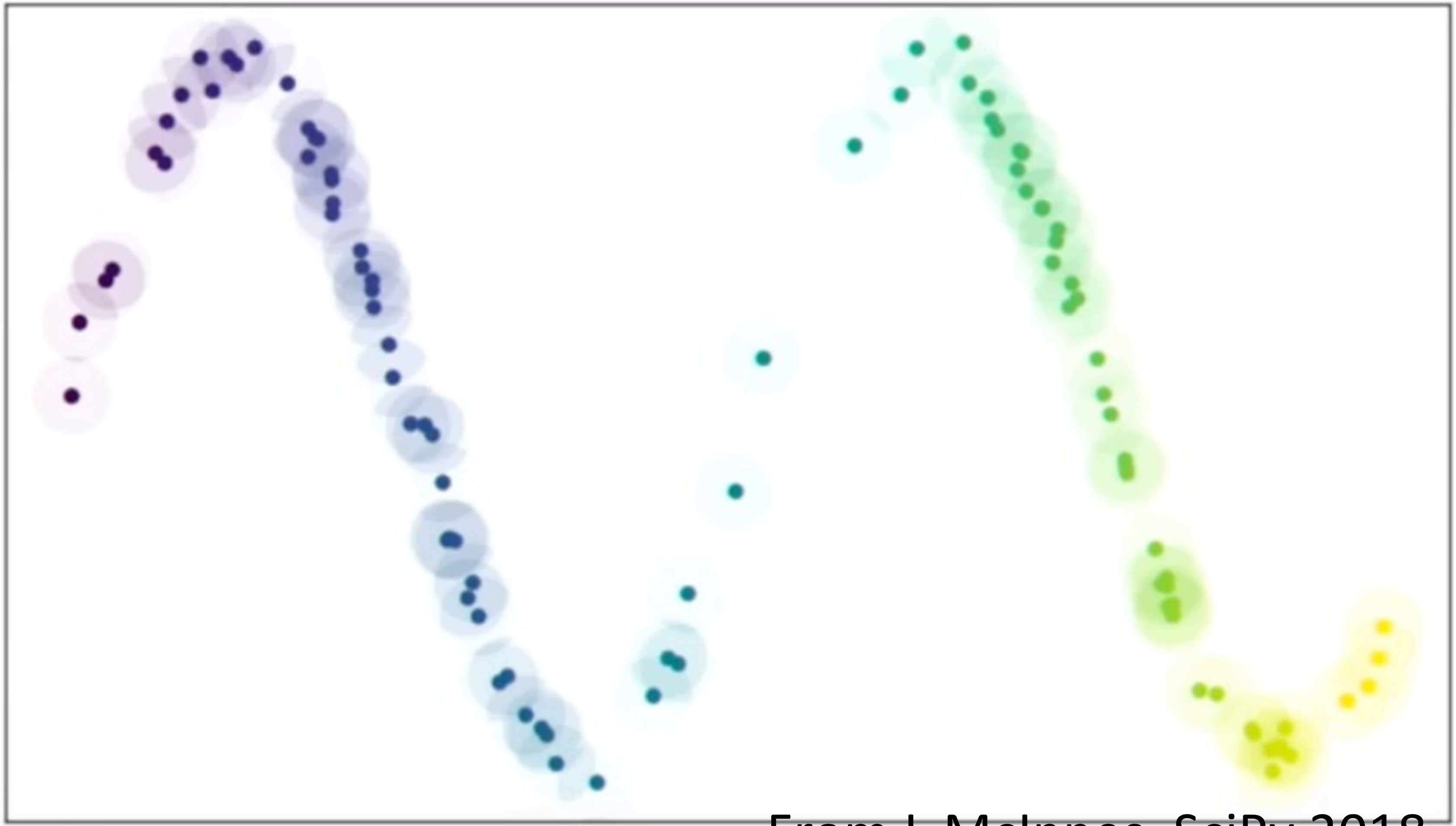


How do we build a simplicial complex on top of a data set?



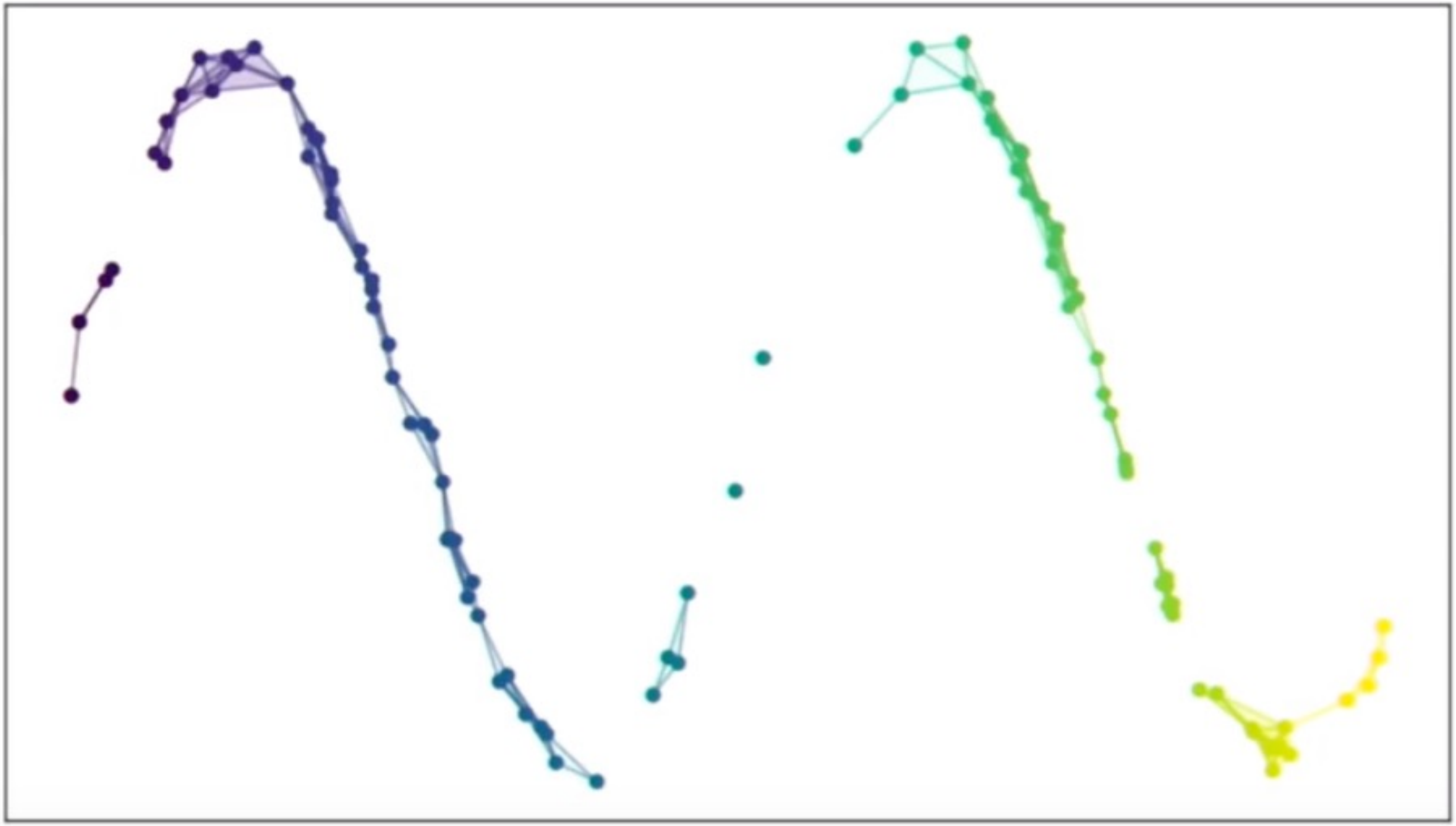
From L.McInnes, SciPy 2018

Step 1: draw unit-balls with a



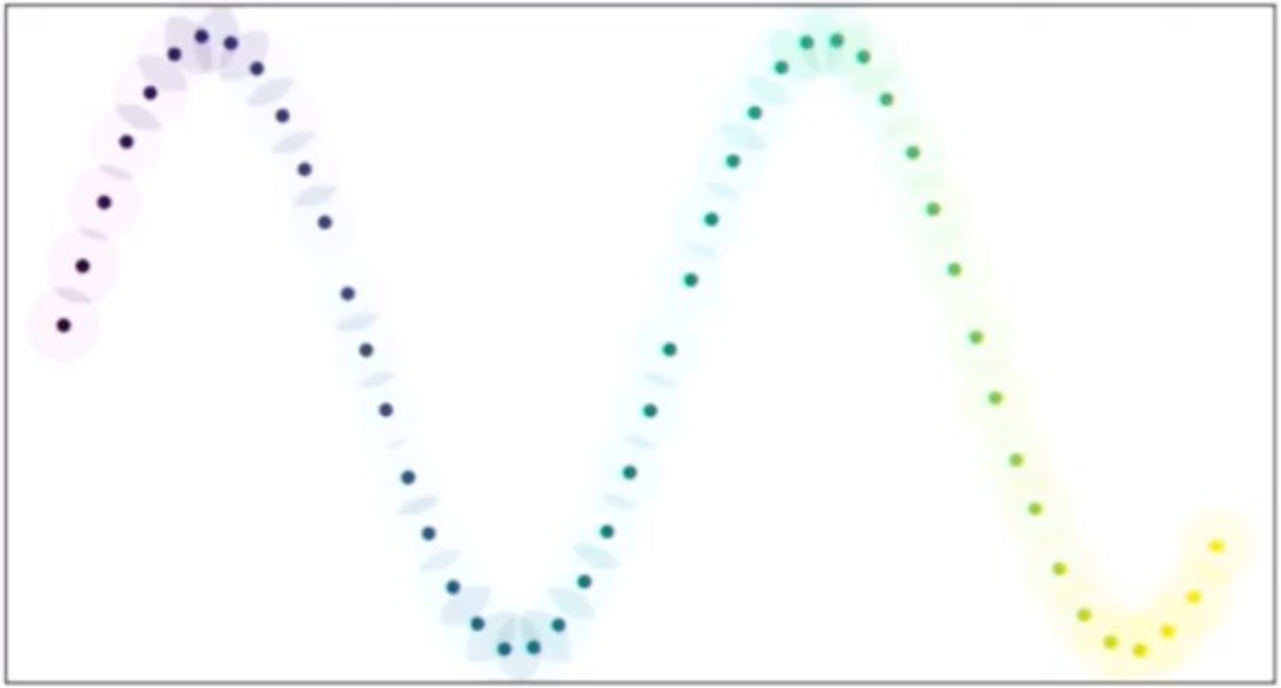
From L.McInnes, SciPy 2018

Step 2: Draw the Nerve of that



From L.McInnes, SciPy 2018

The data is not uniformly distributed on the underlying manifold

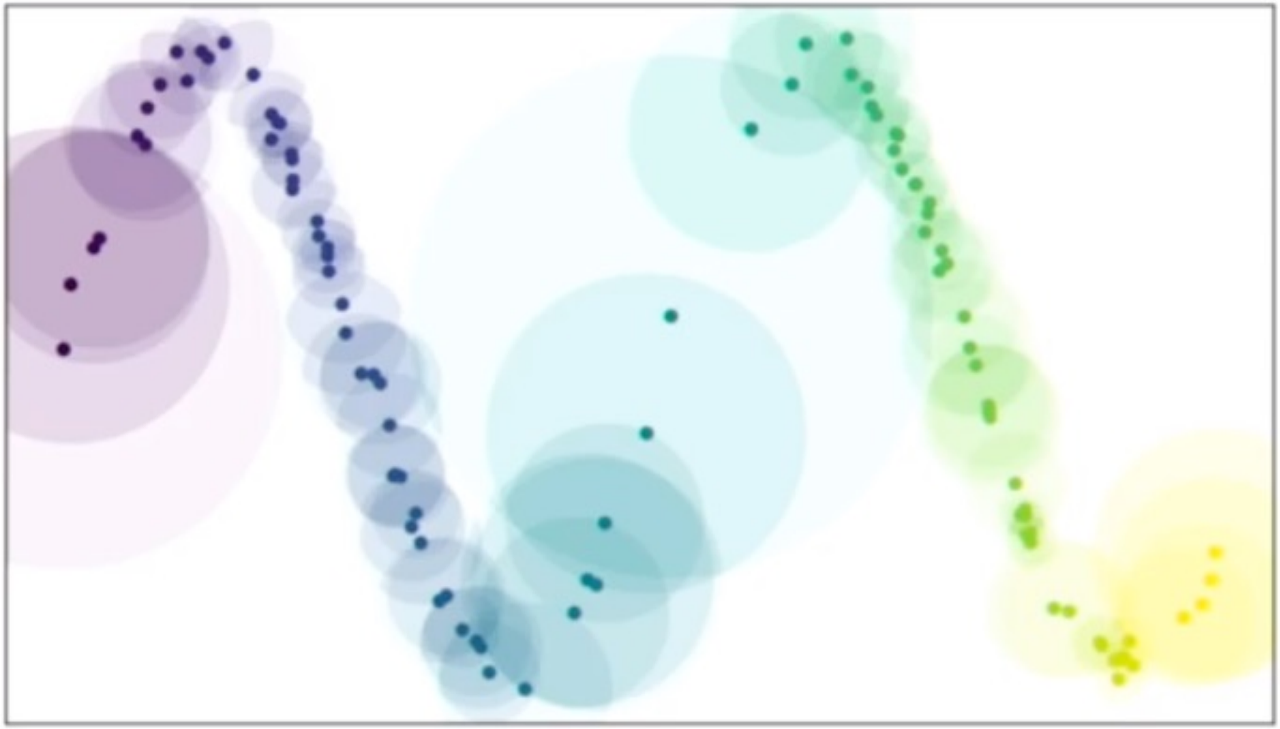


From L.McInnes, SciPy 2018

However… Data is not so nicely distributed

Solution: We vary the notion of metric and effectively the data will be with that metric uniformly distributed on the underlying manifold

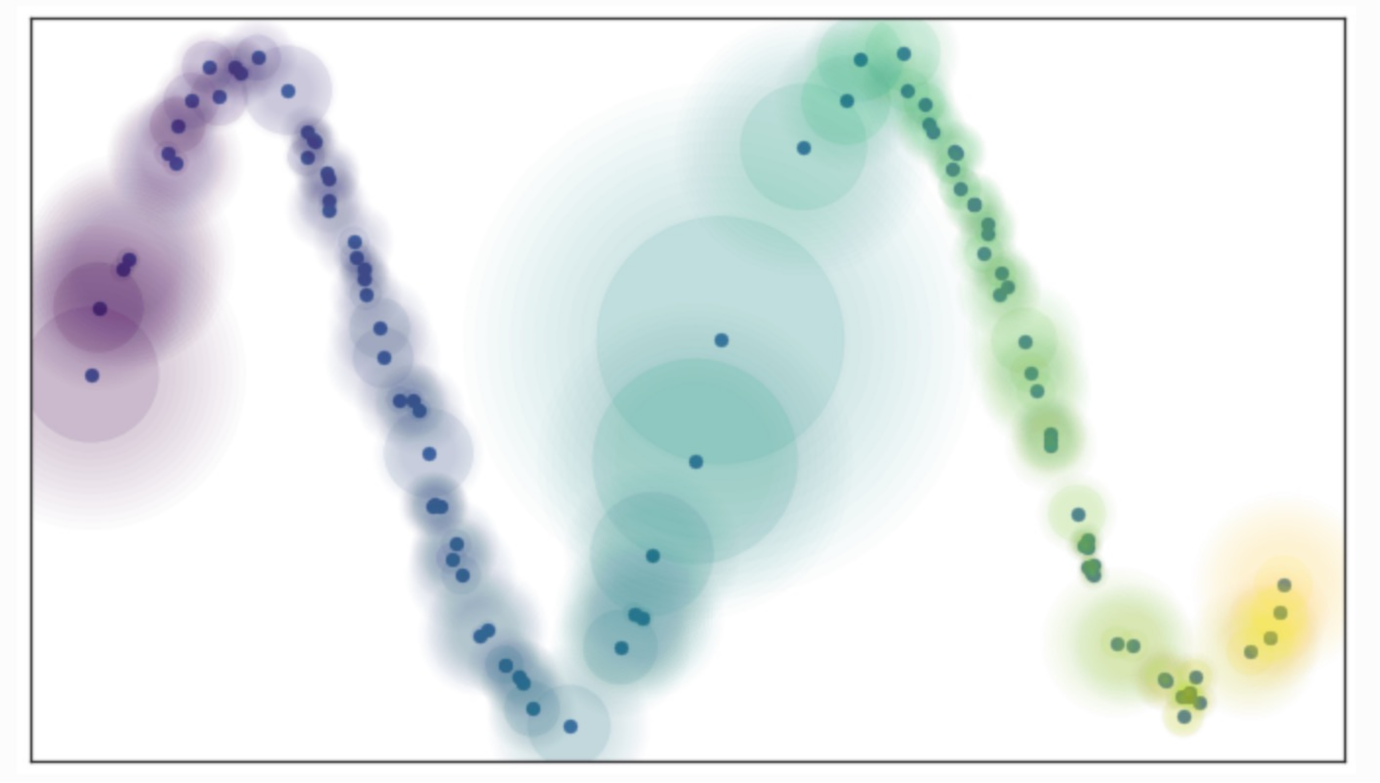
How it looks like on the example



The radius of each ball is equal to one.

From L.McInnes, SciPy 2018

How it looks like on the example

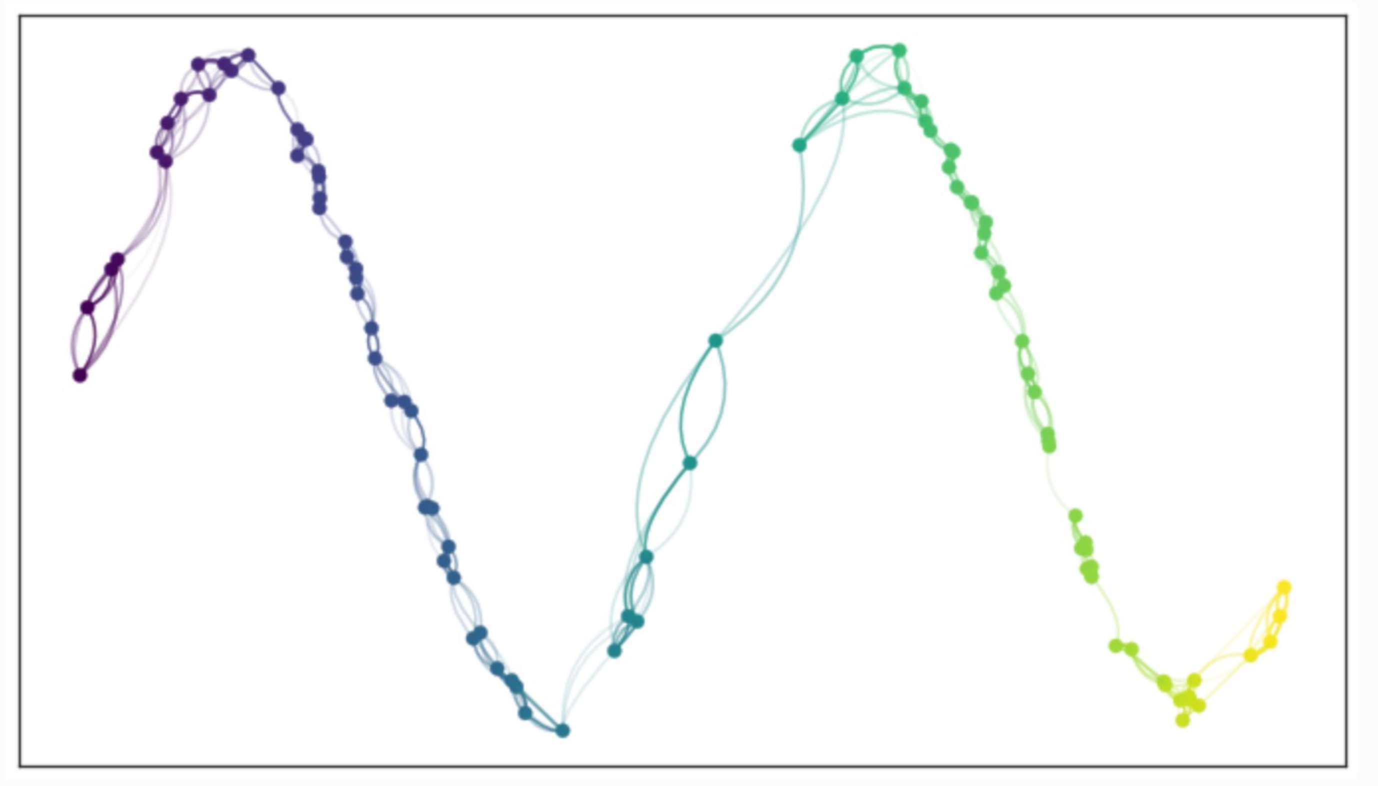


Equivalent to choosing a cover of balls with varying radia. This is what Fuzzy covers try to do.

There are nice theorems again justifying that all of this is valid.

From L.McInnes, SciPy 2018

New directed graph



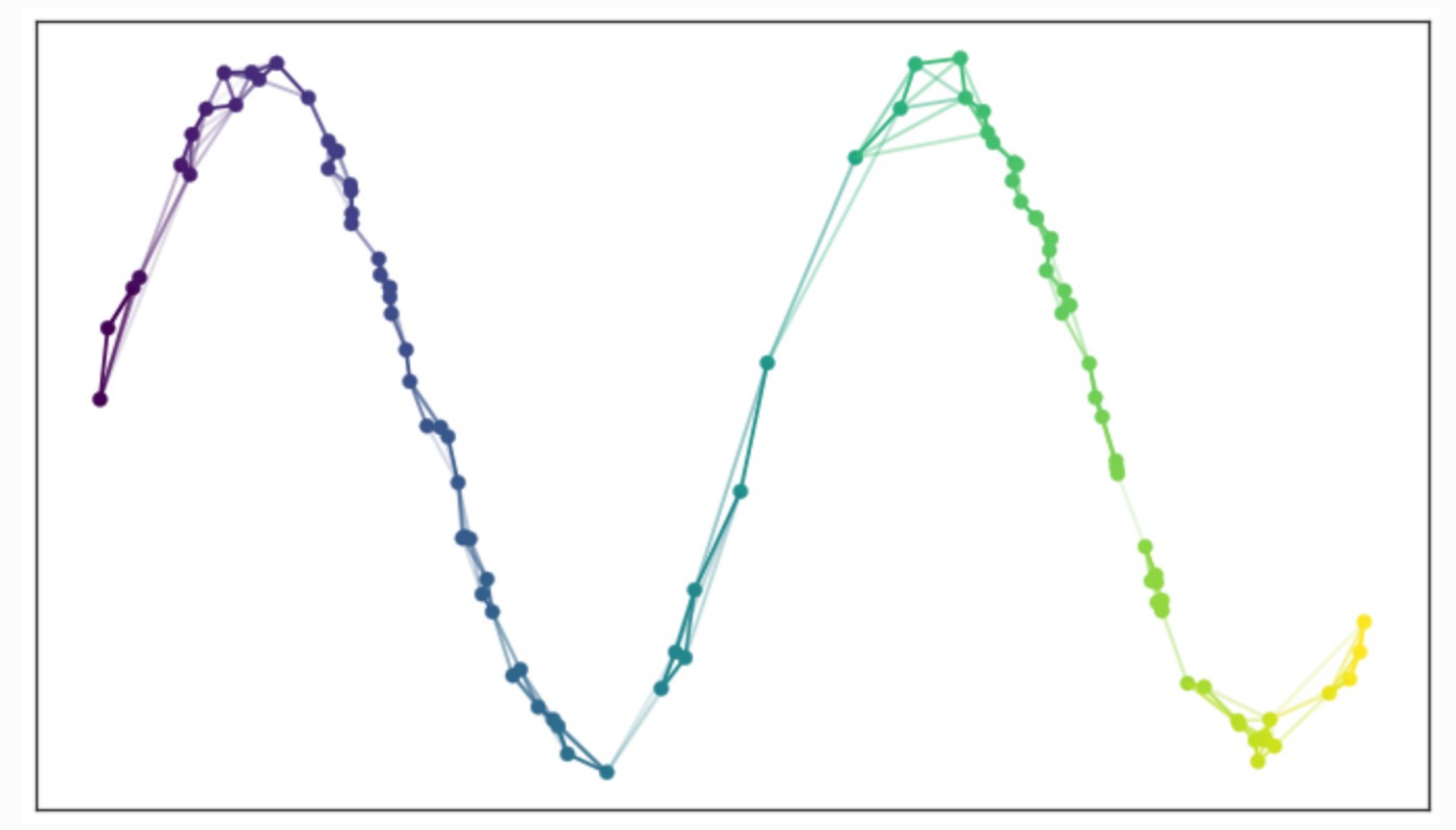
From L.McInnes, SciPy 2018

But we needed a (weighted) simplicial complex…

$$f(a,b) = a+b - a*b$$

Solving the problem…

New simplicial complex



From L.McInnes, SciPy 2018

2nd assumption

The second assumption : the manifold is locally connected.
They use that for mathematics to work but has as an implication that in practice you will not find isolated points in your dataset.

Dimension reduction

Now, UMAP is a dimension reduction method. Let us say you would like to project the data onto IR^2

It will therefore take Y ={y1,…,yN} in IR^2

Compute the fuzzy topological considering IR^2 to be the underlying manifold.

Optimizing this dimension reduction

Given fuzzy simplicial set representations : X and Y , a means of comparison is required.

For the purpose of calculations only the 1-skeleton of the fuzzy simplicial sets is considered (the l-skeletons are calculated using the 1-skeleton and can therefore be shown to be negligible)

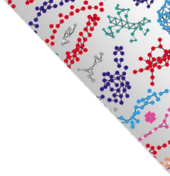
To compare two fuzzy sets we will make use of fuzzy set ***cross entropy***.

$$\sum_{a\in A} \overbrace{\mu(a)\log\left(\frac{\mu(a)}{\nu(a)}\right)}^{\text{Get the clumps right}} + \underbrace{(1-\mu(a))\log\left(\frac{1-\mu(a)}{1-\nu(a)}\right)}_{\text{Get the gaps right}}$$

Summary

The first phase consists of constructing a fuzzy topological representation (edges and weights).

The second phase is optimizing the low dimensional representation to have as close as possible a fuzzy topological representation as measured by cross entropy.

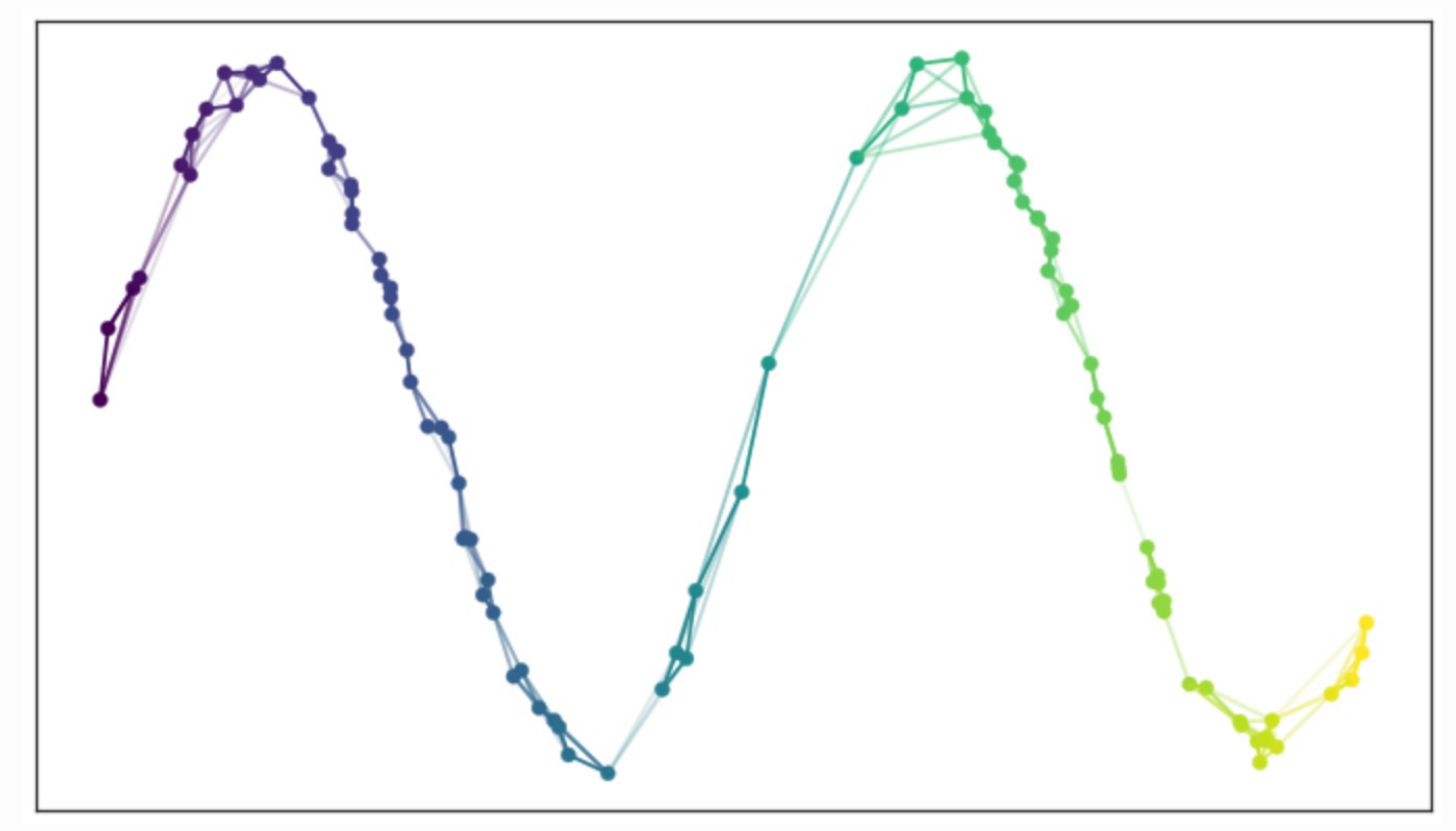


Two shortcuts are needed for the computations

NNdescent: kNN approximation algorithm

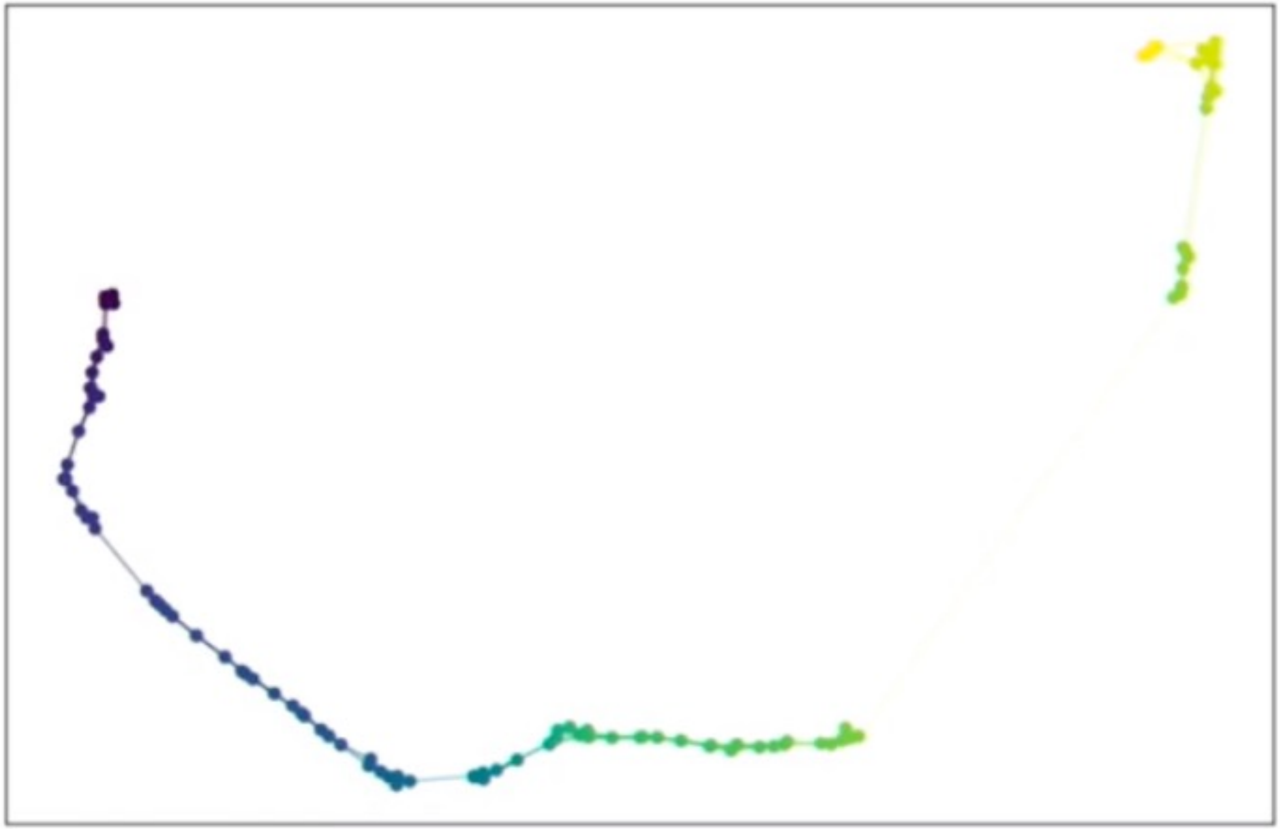
stochastic gradient descent + negative sampling trick: Algorithms for optimizing the cross entropy.

New simplicial complex



From L.McInnes, SciPy 2018

How the UMAP embedding looks



From L.McInnes, SciPy 2018

Input parameters

X: the data

n: the neighborhood parameter: number of neighbors to consider when approximating the local metric

d: the target embedding dimension (2 usually)

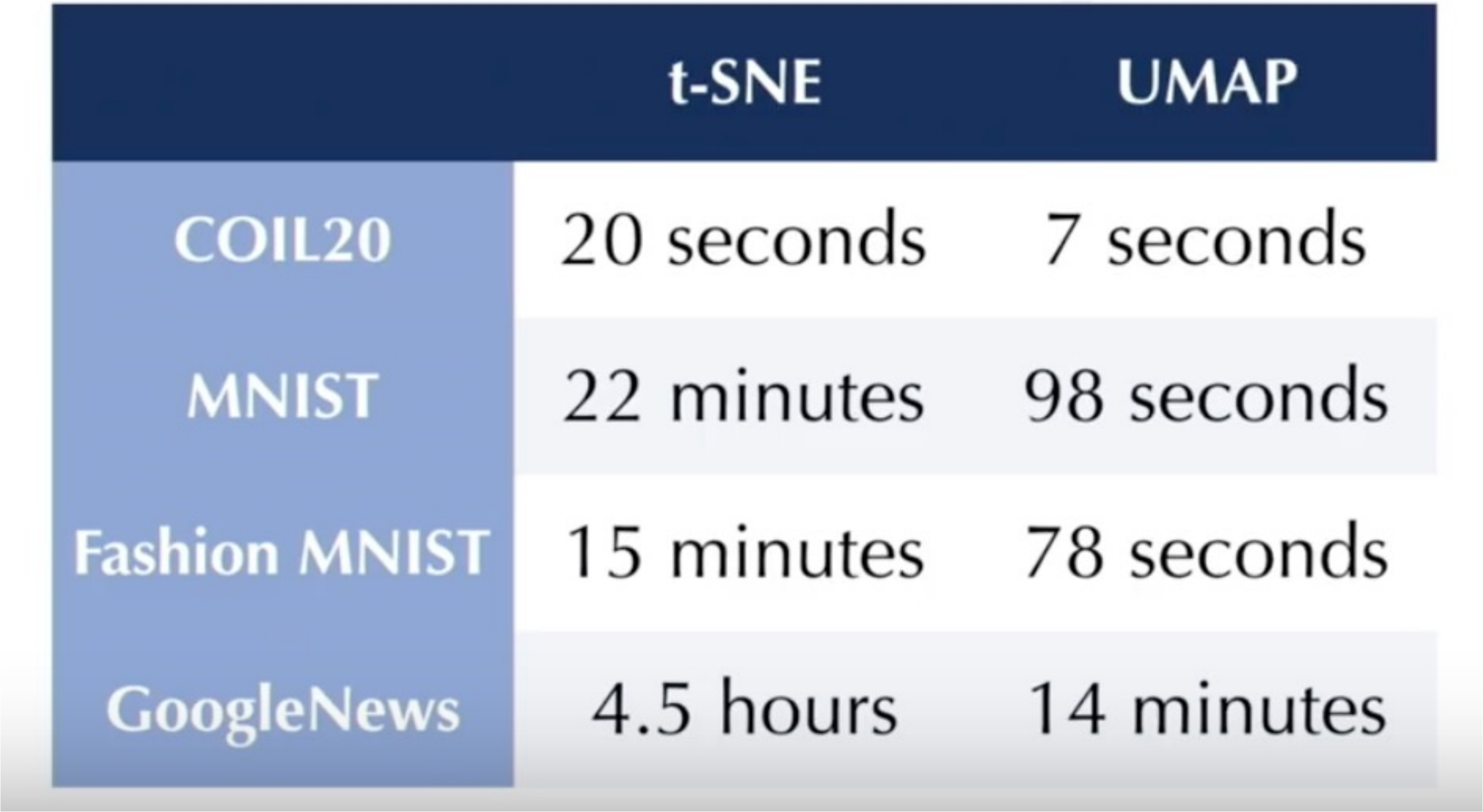
min-dist: »beauty» parameter for the local embedding in 2D: the desired separation between close points in the embedding space: this determines how closely points can be packed together in the low dimensional representation

n-epochs: optimization parameter for the local embedding in 2D the number of training *epochs (batches)* to use when optimizing the low dimensional representation.

Some parameters in Seurat:

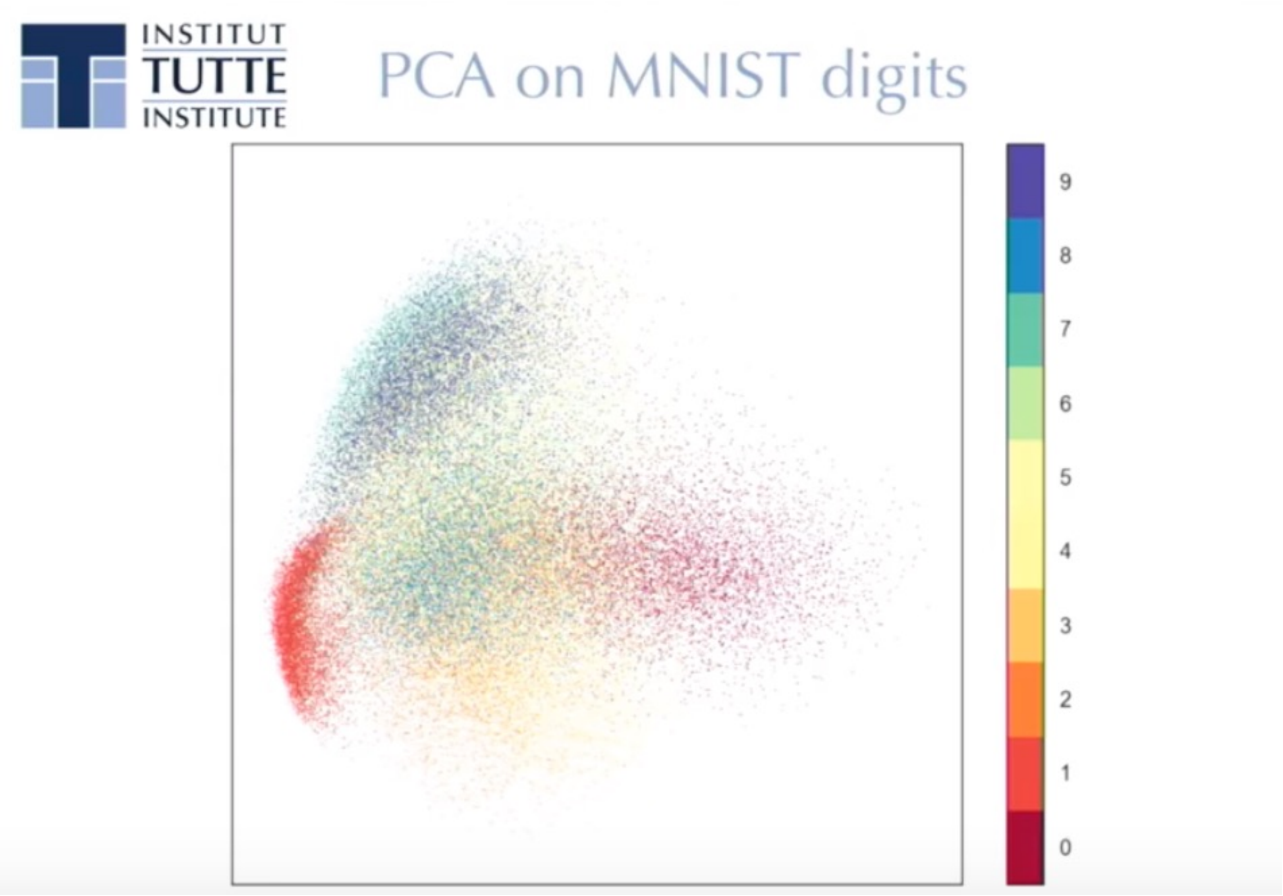
```
n_neighbors = 30L,
min_dist = 0.3,
metric = "correlation",
seed.use = 42,
n_epochs=200
```

Comparing tSNE and UMAP in terms of computation time



	t-SNE	UMAP
COIL20	20 seconds	7 seconds
MNIST	22 minutes	98 seconds
Fashion MNIST	15 minutes	78 seconds
GoogleNews	4.5 hours	14 minutes

PCA is good, but one can do better!



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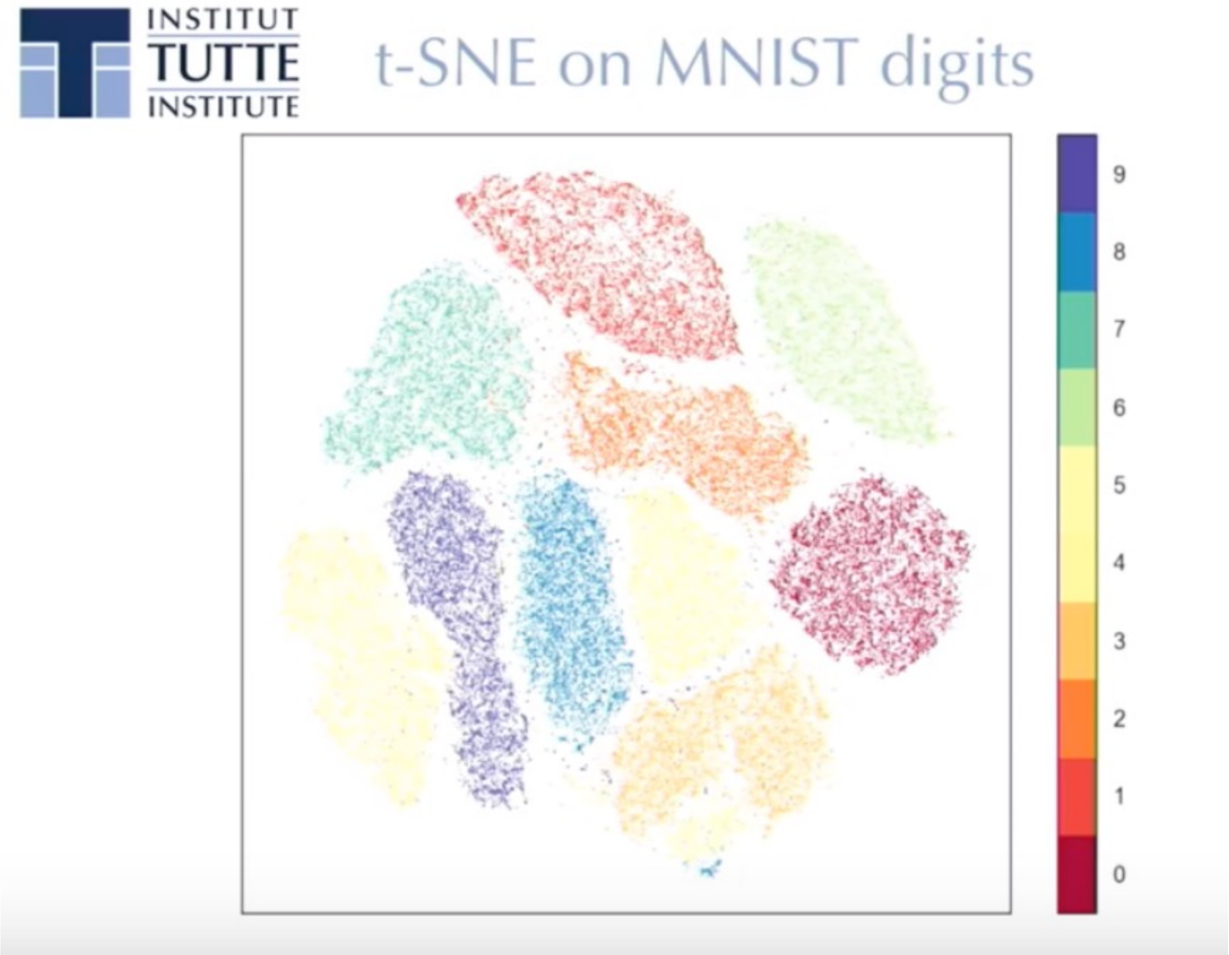

t-SNE on MNIST digits

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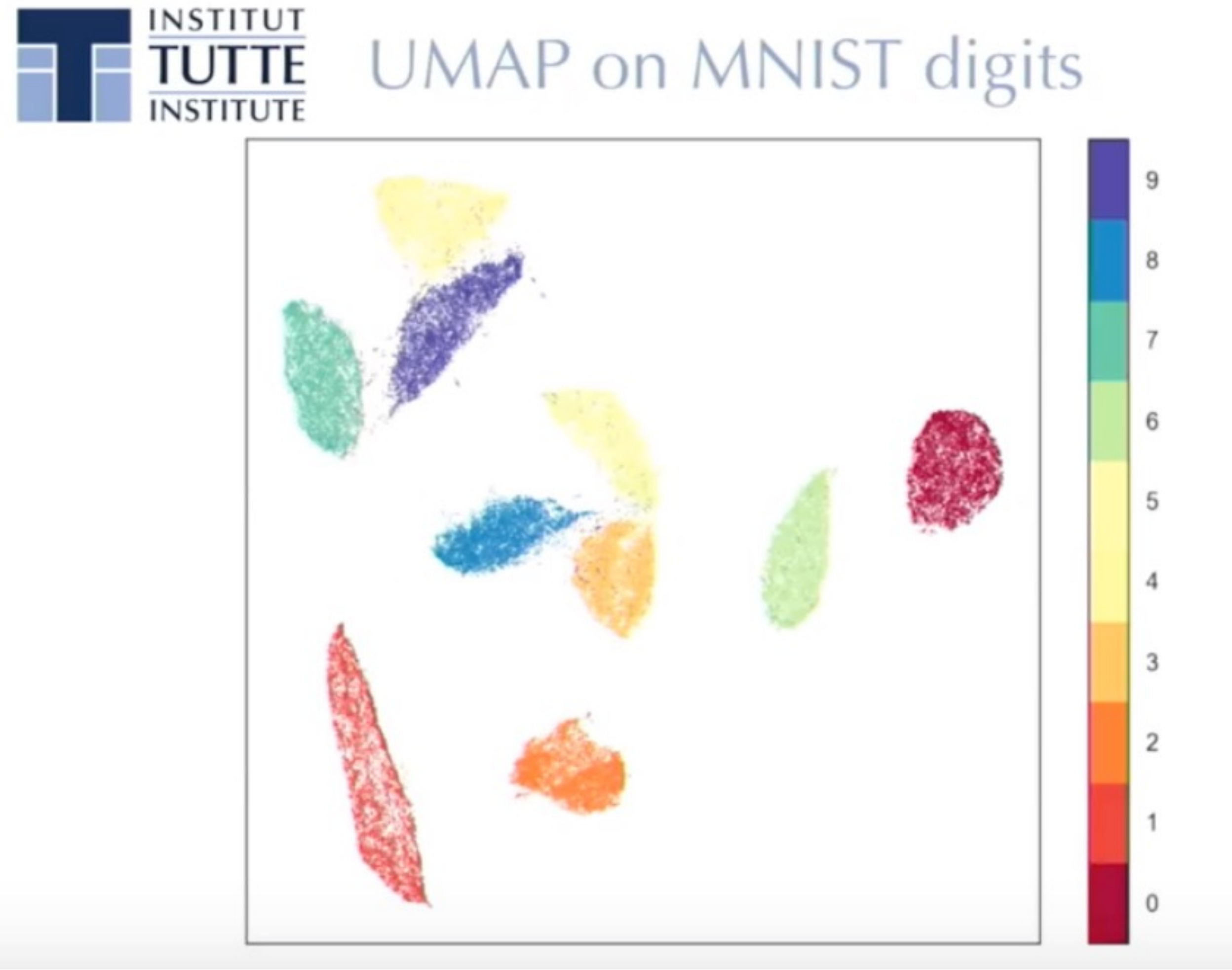
UMAP



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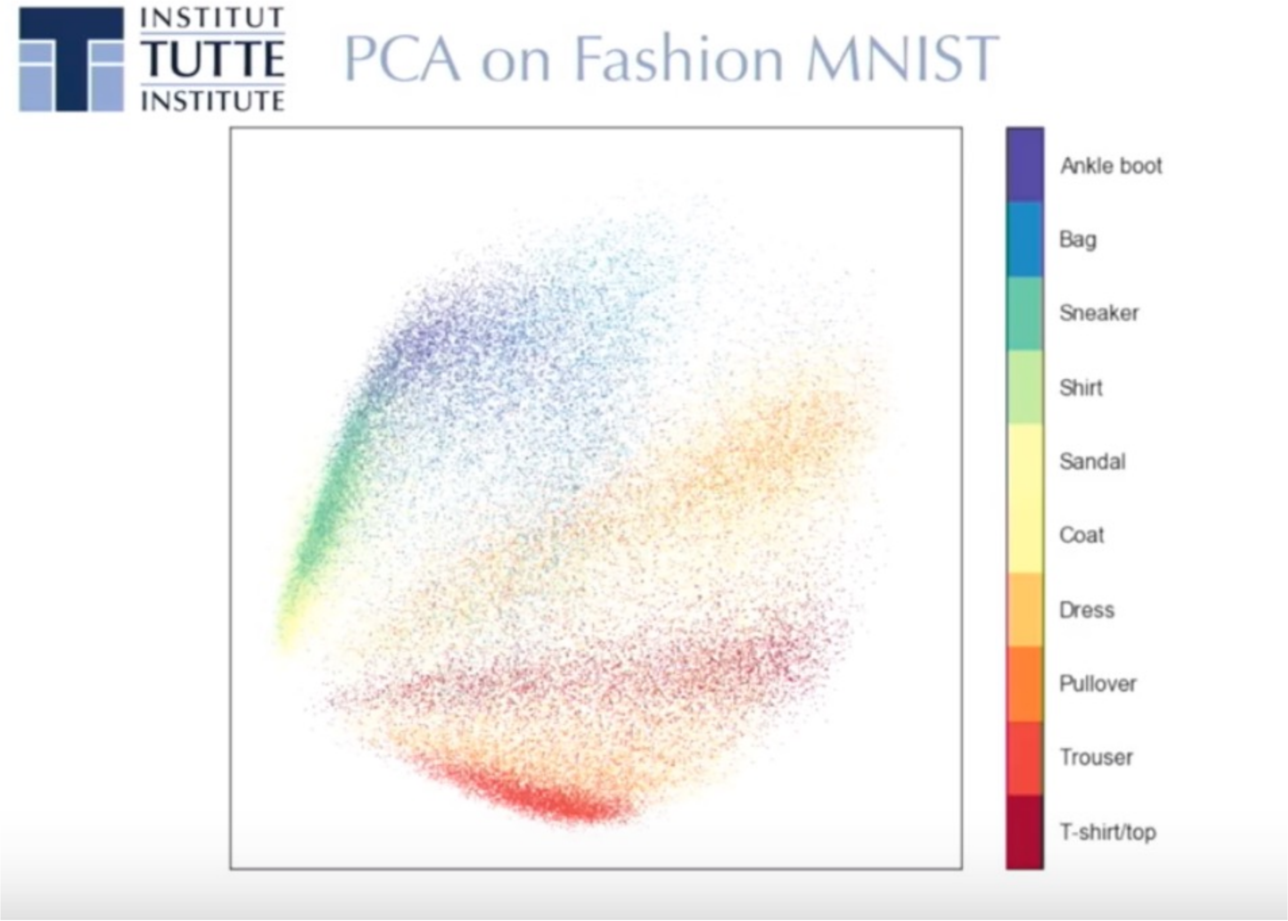

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PCA on Fashion MNIST

See the
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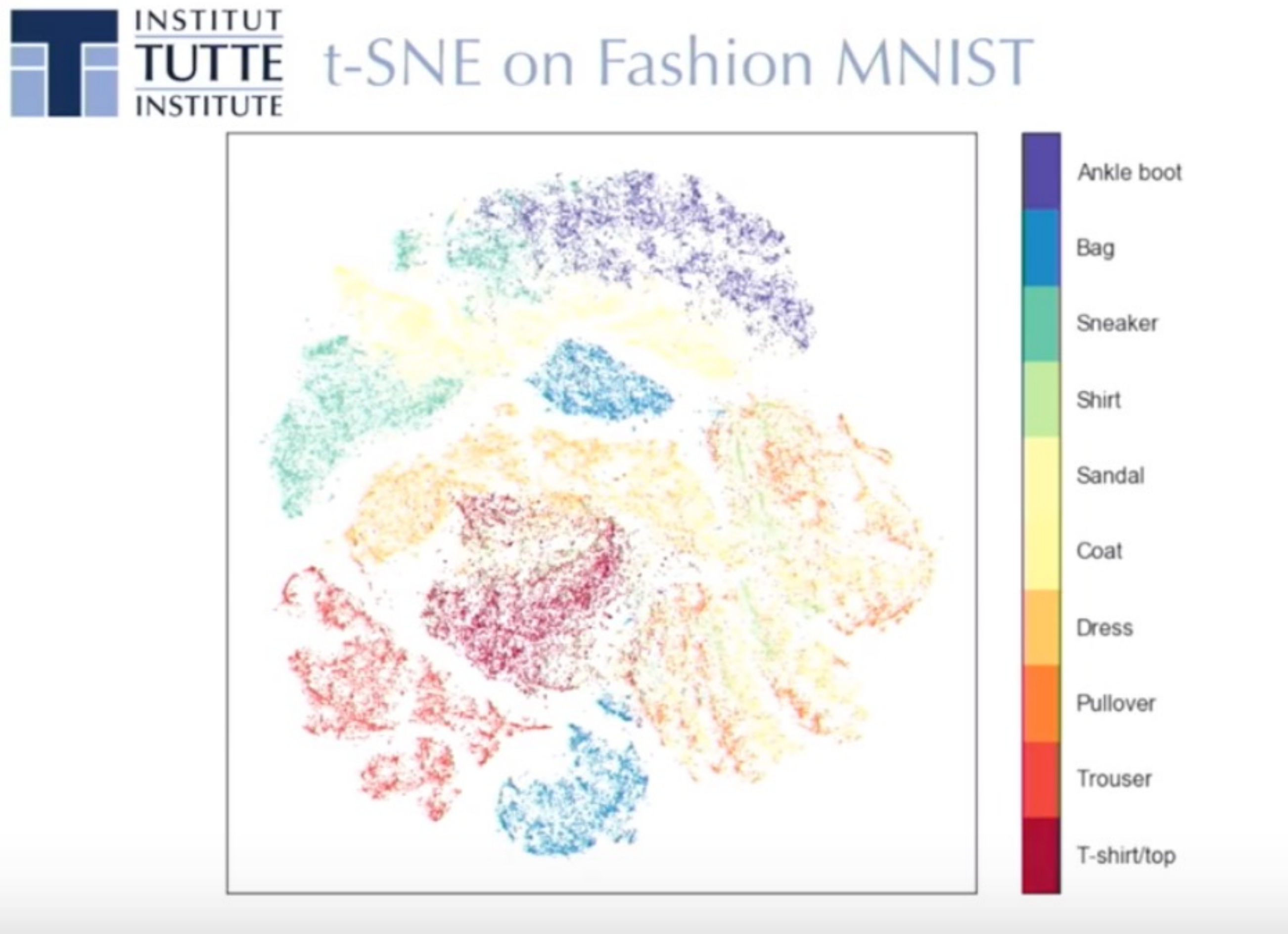
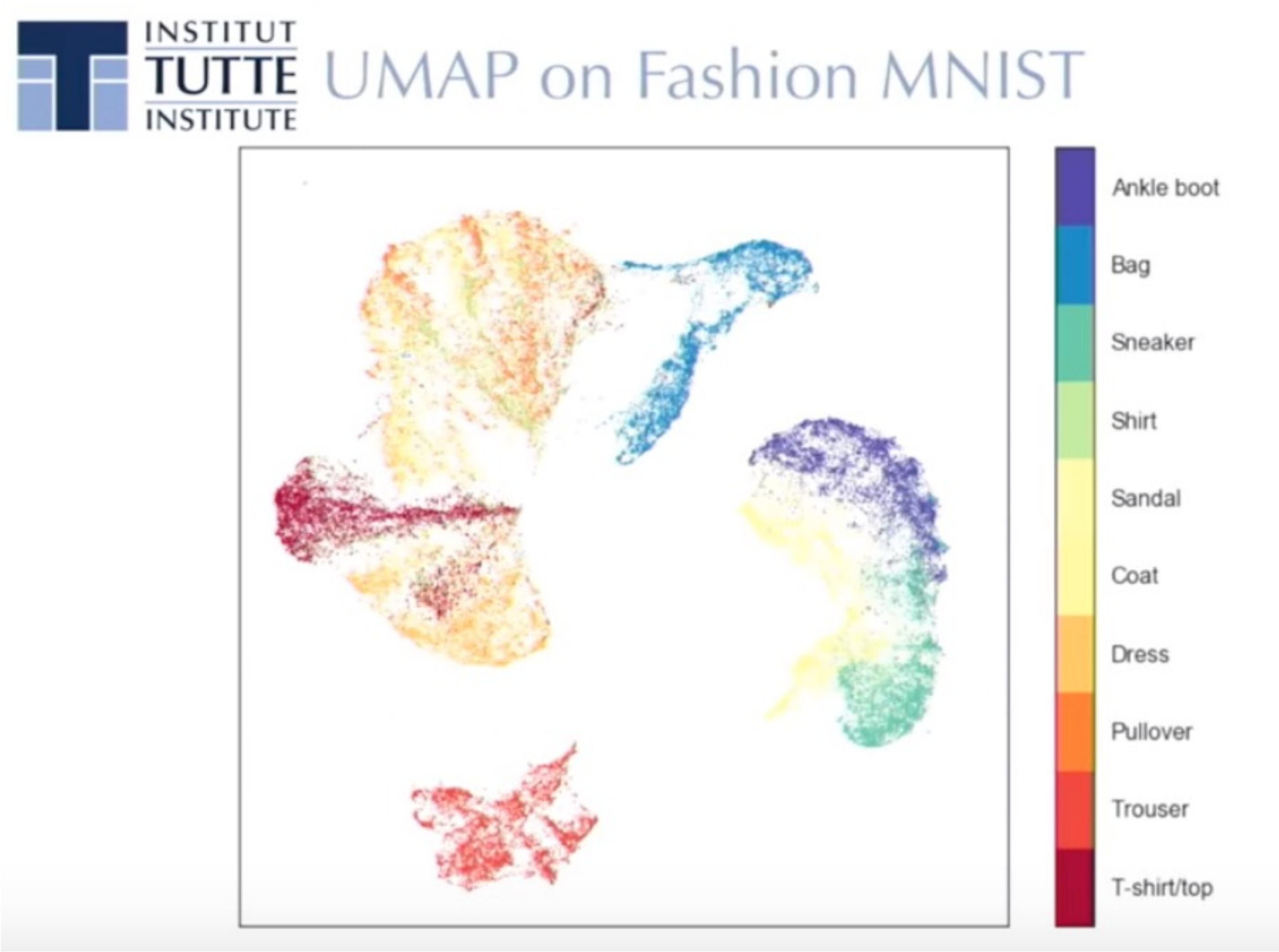


t-SNE on Fashion MNIST

From L.McInnes, SciPy 2018

UMAP



UMAP on Fashion MNIST

	Seurat v3	Scater	Pagoda v2	Monocle v3
➡	PCA	PCA	PCA	PCA
	ICA	-	-	ICA
	-	MDS	-	-
➡	tSNE (BH, FIt)	tSNE (BH)	tSNE (BH)	tSNE (BH)
➡	UMAP	UMAP	-	UMAP
	-	-	LargeVis	-
	Diff. Maps	Diff. Maps	Isomap	-
	-	-	-	DDRTree
	PHATE	-	-	-
	-	-	-	SimplePPT

```
obj <-RunPCA( obj )
obj <-RunTSNE( obj )
obj <-RunUMAP( obj )
```

Paper comparing dimensionality reduction techniques:
https://www.biorxiv.org/content/biorxiv/early/2018/06/28/120378.full.pdf