Dimension Reduction
PCA, tSNE, UMAP, Integration

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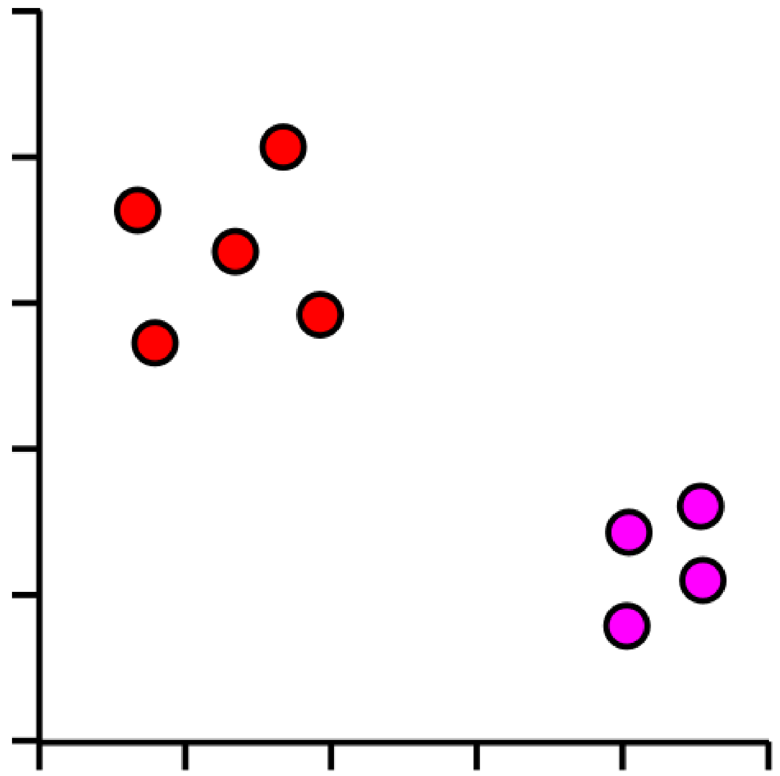
Where are we heading?

Gene	Description	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5
Inpp5d	inositol polyphosphate-5-phosphatase D	7.00	5.45	5.89	6.03	5.75
Aim2	absent in melanoma 2	3.01	4.37	4.59	4.38	4.18
Gldn	gliomedin	3.48	3.63	4.61	4.70	4.74
Frem2	Fras1 related extracellular matrix protein 2	4.75	4.66	3.46	3.74	3.45
Rps3a1	ribosomal protein S3A1	6.10	7.23	7.44	7.36	7.34
Slc38a3	solute carrier family 38, member 3	1.90	3.16	3.52	3.61	3.19
Mt1	metallothionein 1	5.07	6.49	6.46	6.04	6.05
C1s1	complement component 1, s subcomponent 1	2.74	3.02	3.86	4.10	4.10
Cds1	CDP-diacylglycerol synthase 1	4.55	4.22	3.80	3.16	3.12
Ifi44	interferon-induced protein 44	4.82	4.52	3.87	3.42	3.59
Lefty2	left-right determination factor 2	6.95	6.28	5.88	5.60	5.61
Fmr1nb	fragile X mental retardation 1 neighbor	4.28	2.78	3.10	3.25	2.57
Tagln	transgelin	7.93	7.91	7.20	7.02	6.68



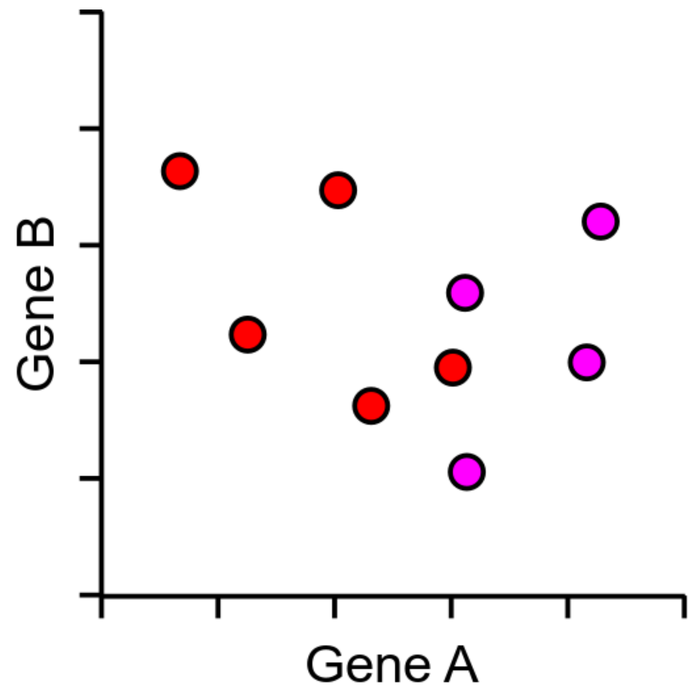
Each dot is a cell

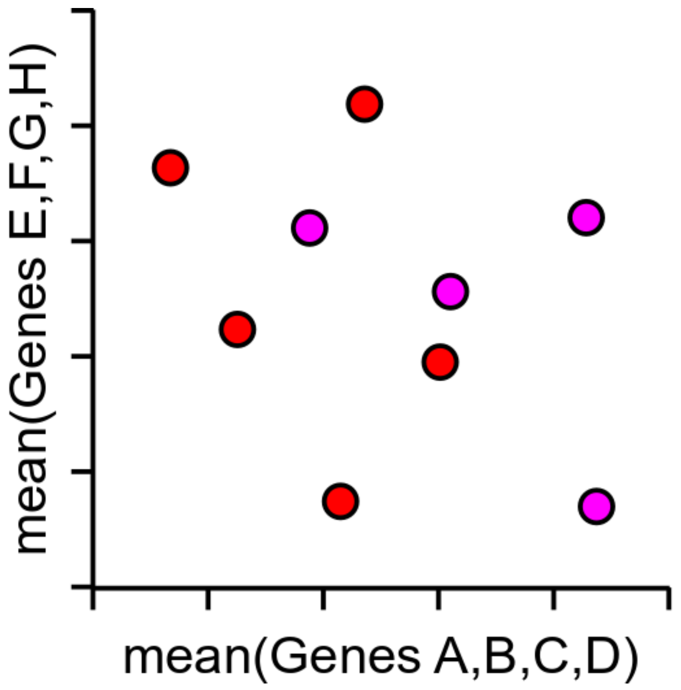
Groups of dots are similar cells

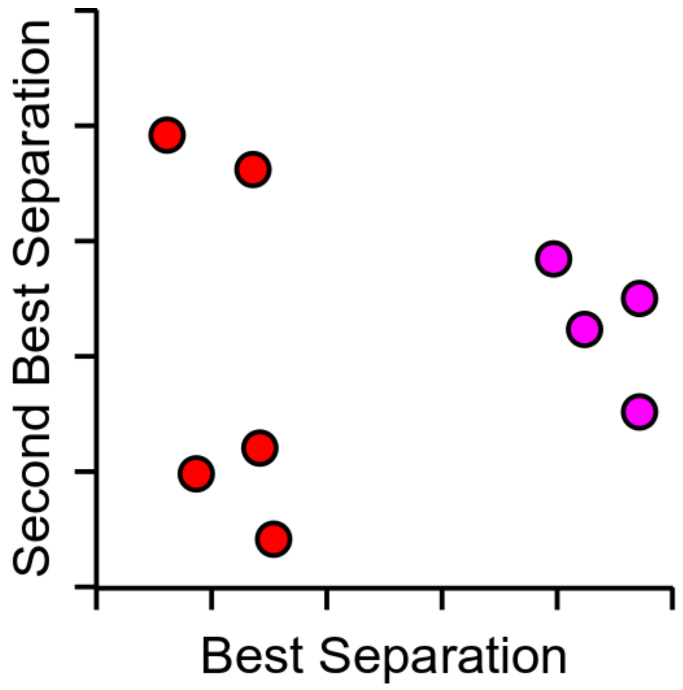
Separation of groups could be interesting biology

Too much data!

- 5000 cells and 2500 measured genes
- Realistically only 2 dimensions we can plot (x,y)







Principle Components Analysis

- Method to optimally summarise large multi-dimensional datasets
- Can find a smaller number of dimensions (ideally 2) which retain most of the useful information in the data

- Builds a recipe for converting large amounts of data into a single value, called a Principle Component (PC), eg:

$$PC = (GeneA*10)+(GeneB*3)+(GeneC*-4)+(GeneD*-20)\ldots$$

Principle Components Analysis

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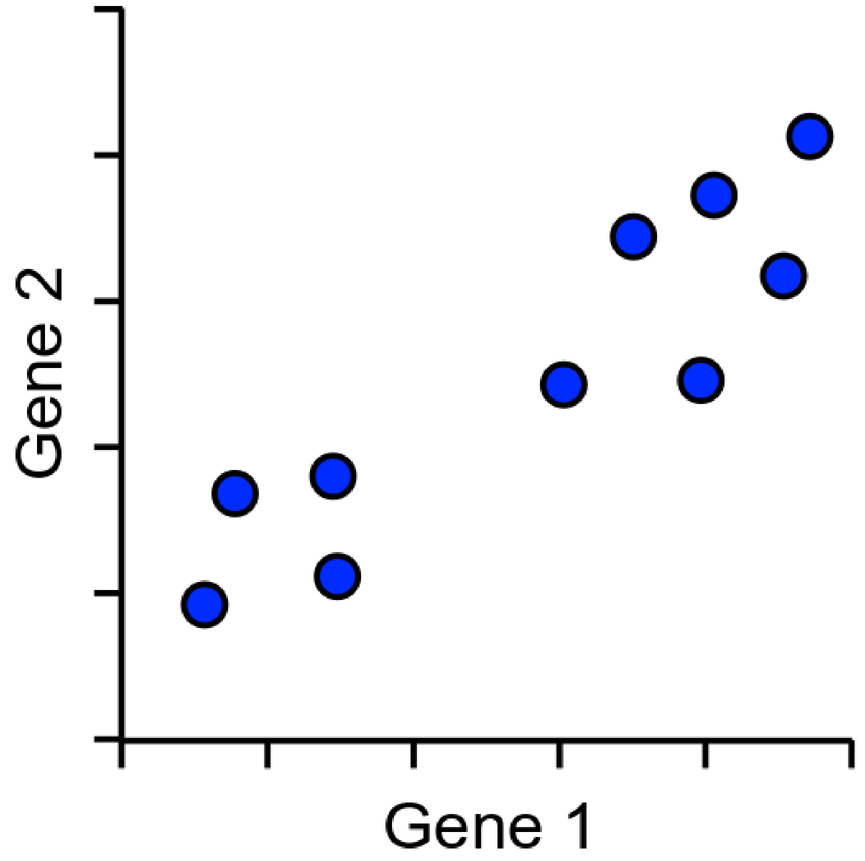
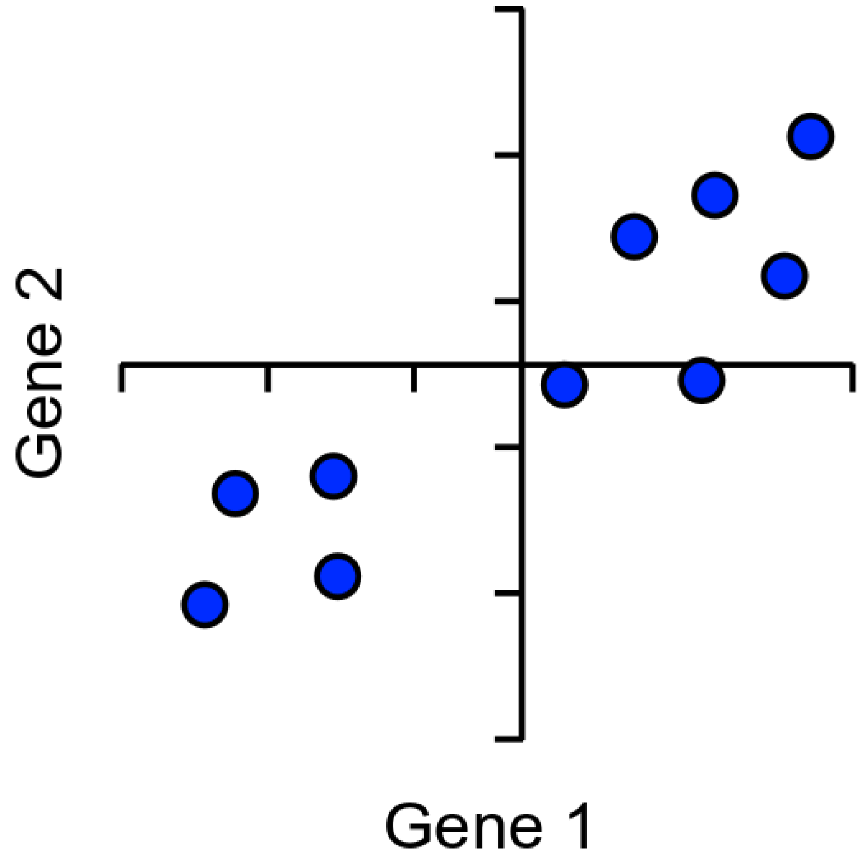
PC = (GeneA*10)+(GeneB*3)+(GeneC*-4)+(GeneD*-20)…

How does PCA work?

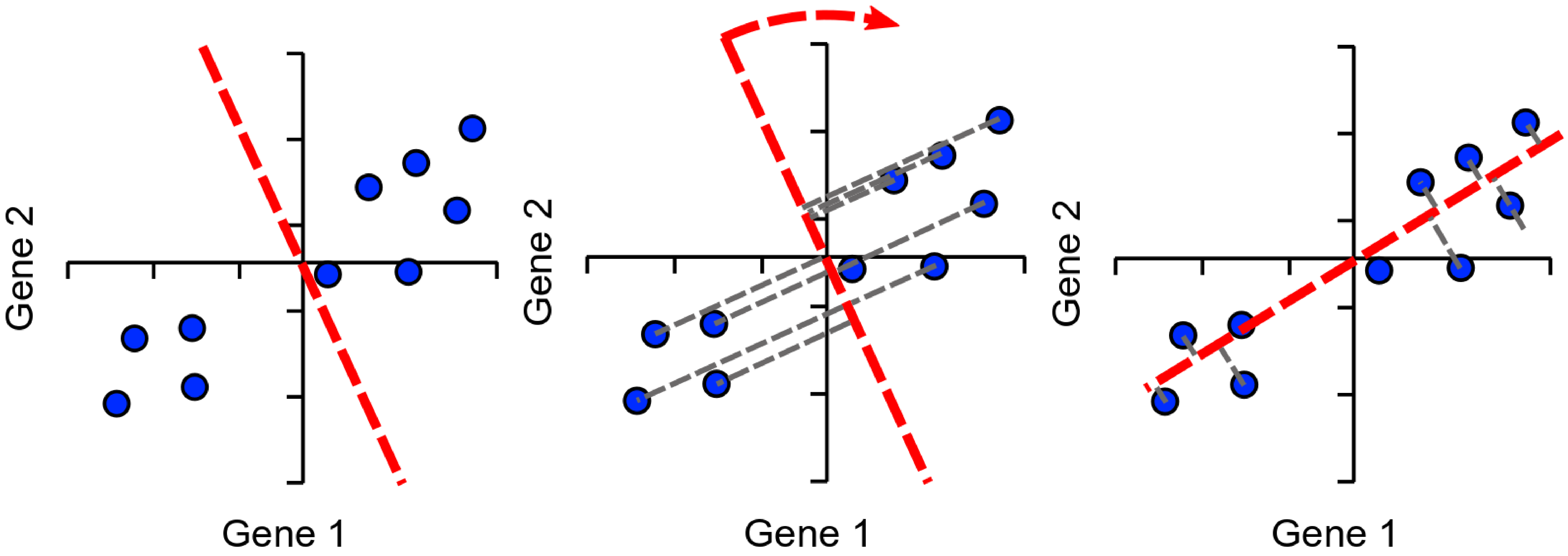
Simple example using 2 genes and 10 cells



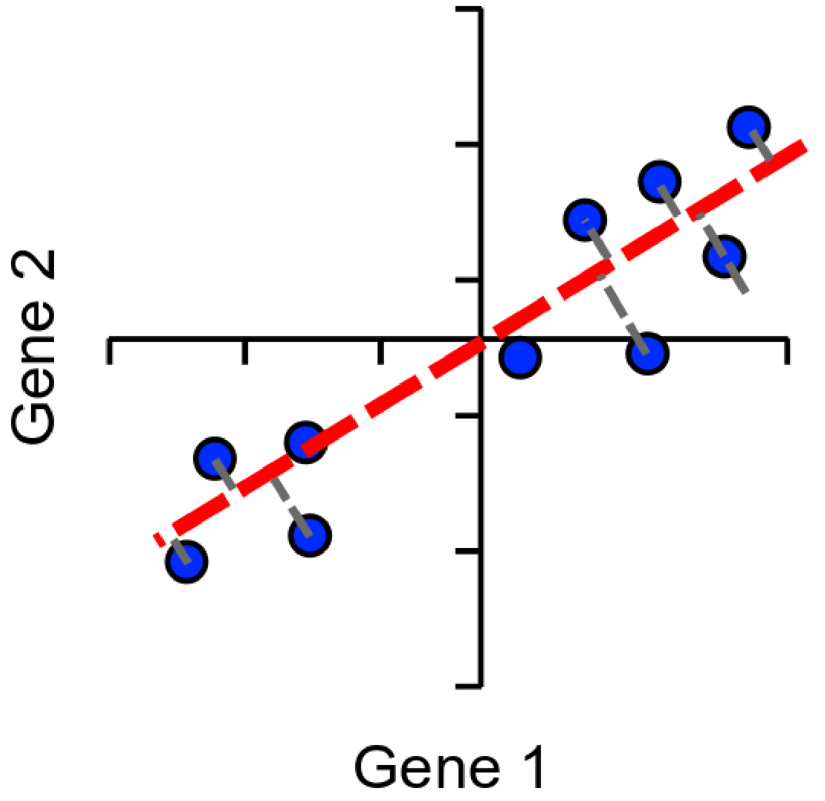


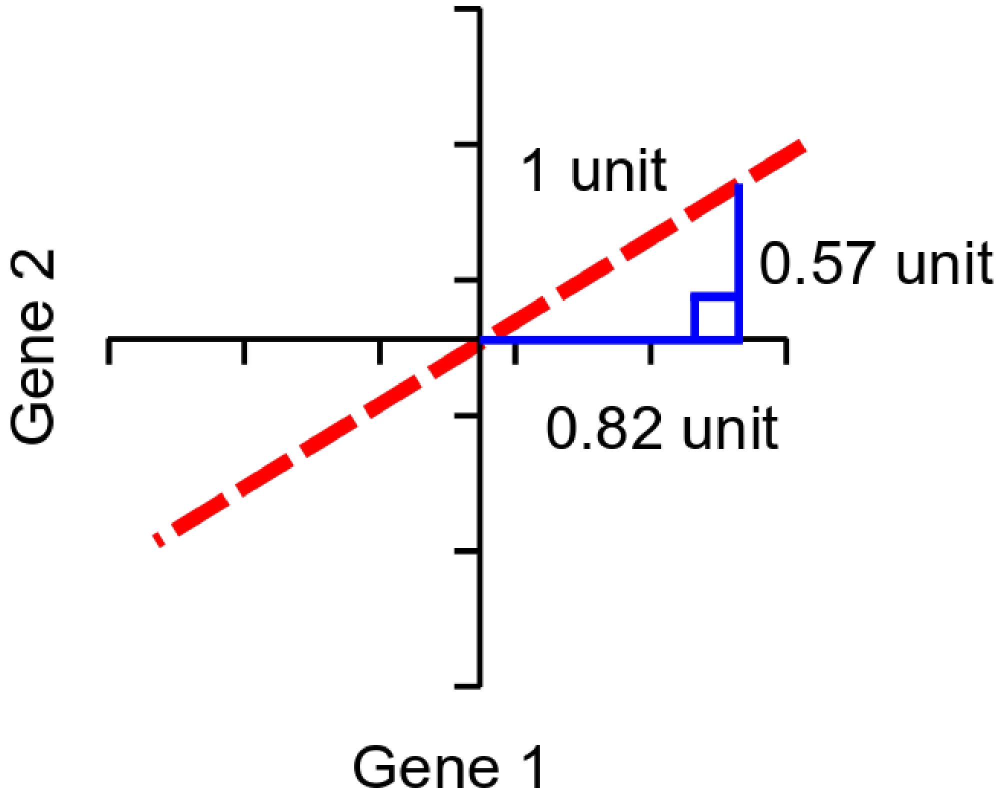
How does PCA work?

Find line of best fit, passing through the origin



Assigning Loadings to Genes





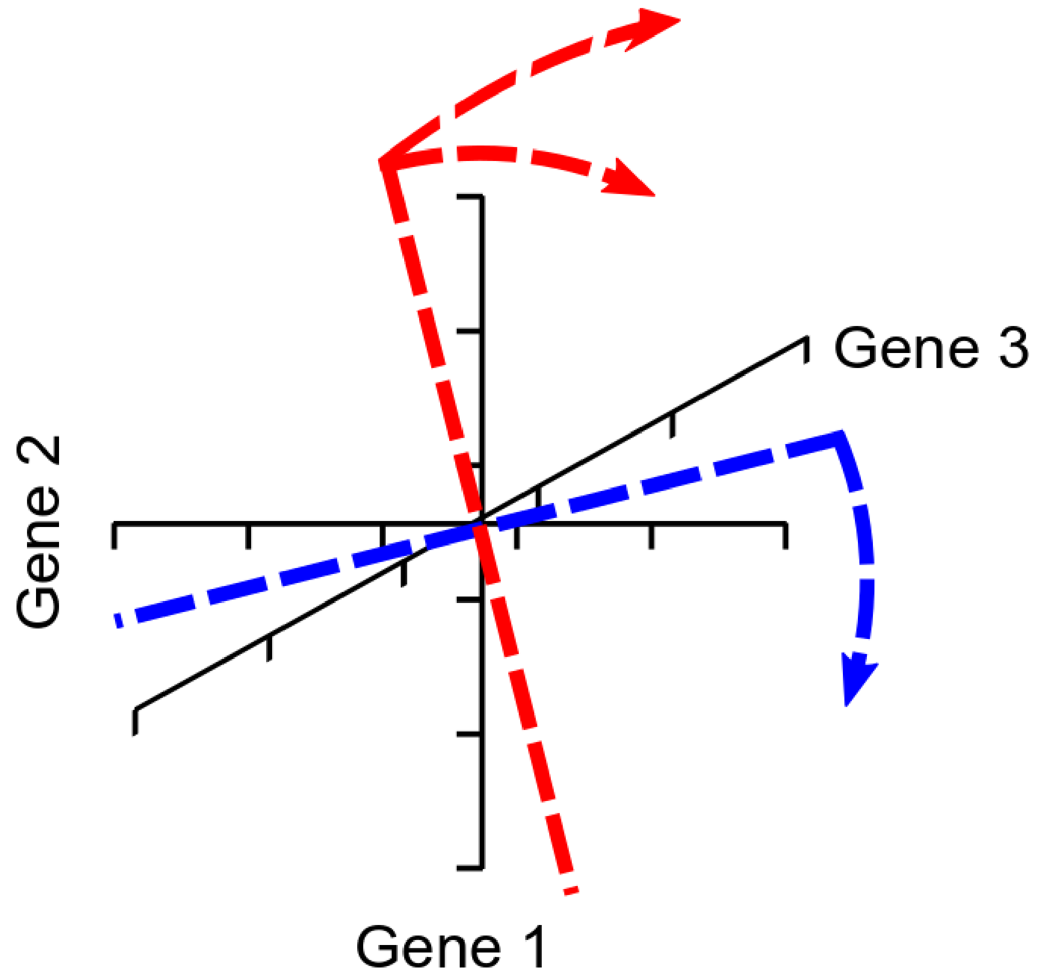
Single Vector or ‘**eigenvector**’

Loadings:

- Gene1 = 0.82
- Gene2 = 0.57

Higher loading equals more influence on PC

More Dimensions



- The same idea extends to larger numbers of dimensions (n)
- First PC rotates in (n-1) dimensions
  - Next PC is perpendicular to PC2, but rotated similarly (n-2)
  - Last PC is remaining perpendicular (no choice)
  - Same number of PCs as genes

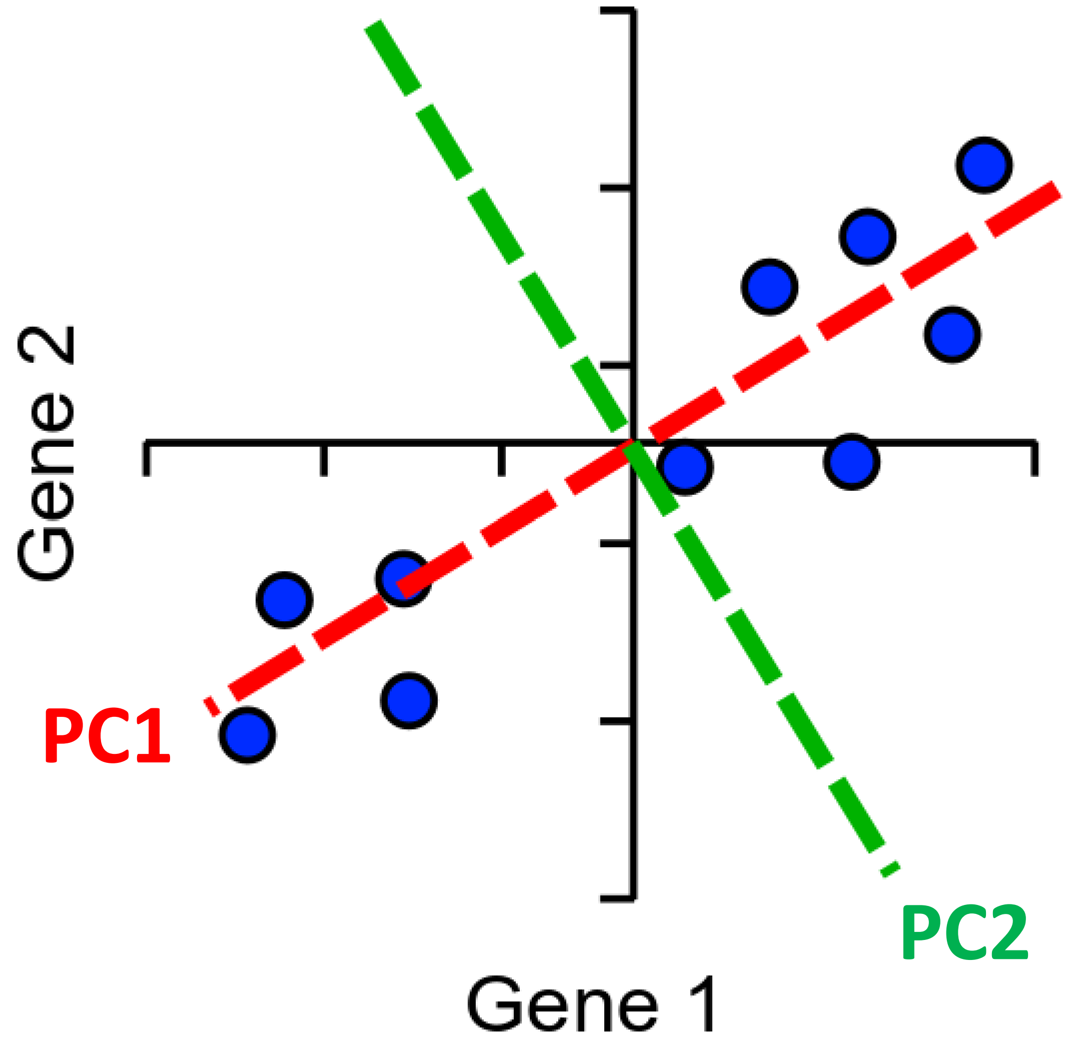
Explaining Variance

- Each PC always explains some proportion of the total variance in the data. Between them they explain everything
  - PC1 always explains the most
  - PC2 is the next highest etc. etc.

- Since we only plot 2 dimensions we'd like to know that these are a good explanation

- How do we calculate this?

Explaining variance

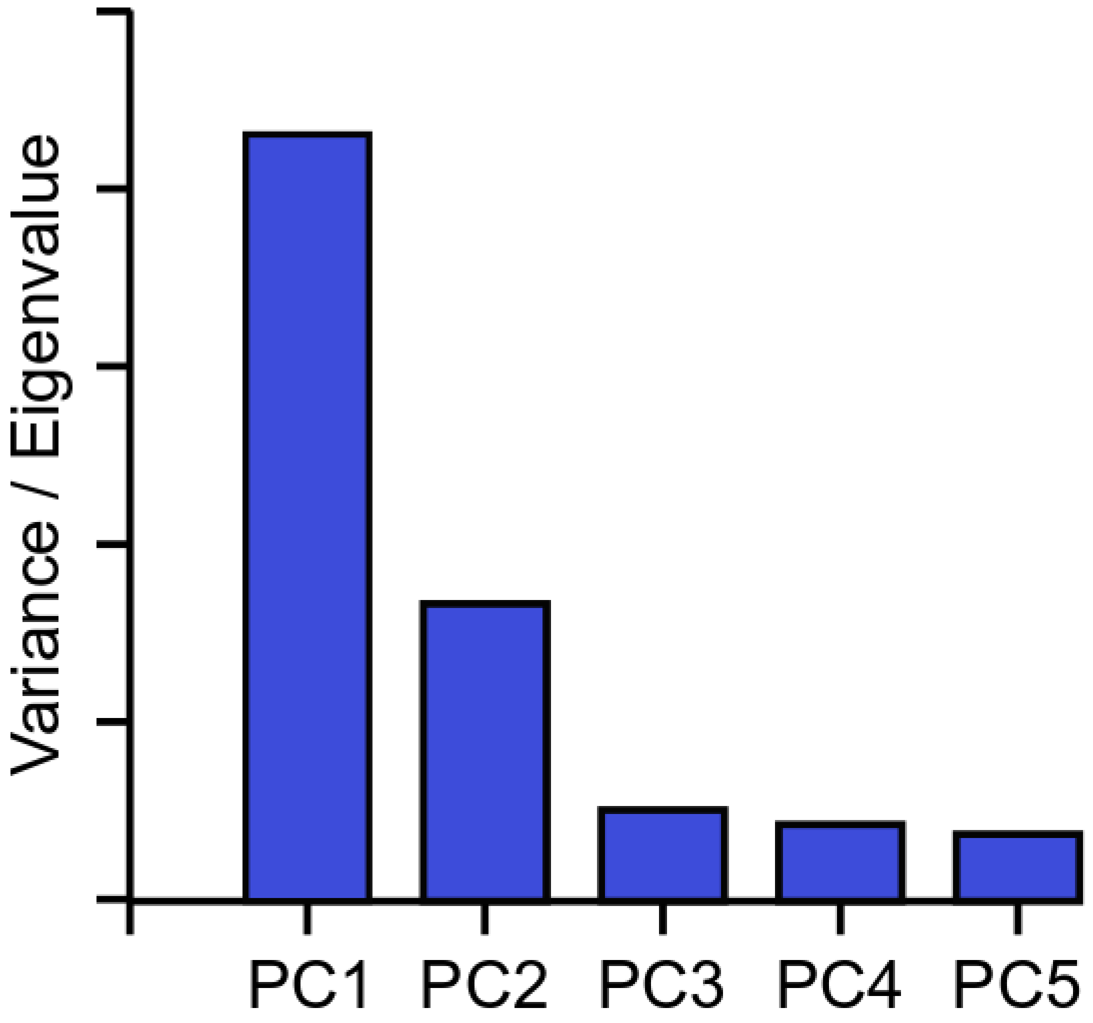


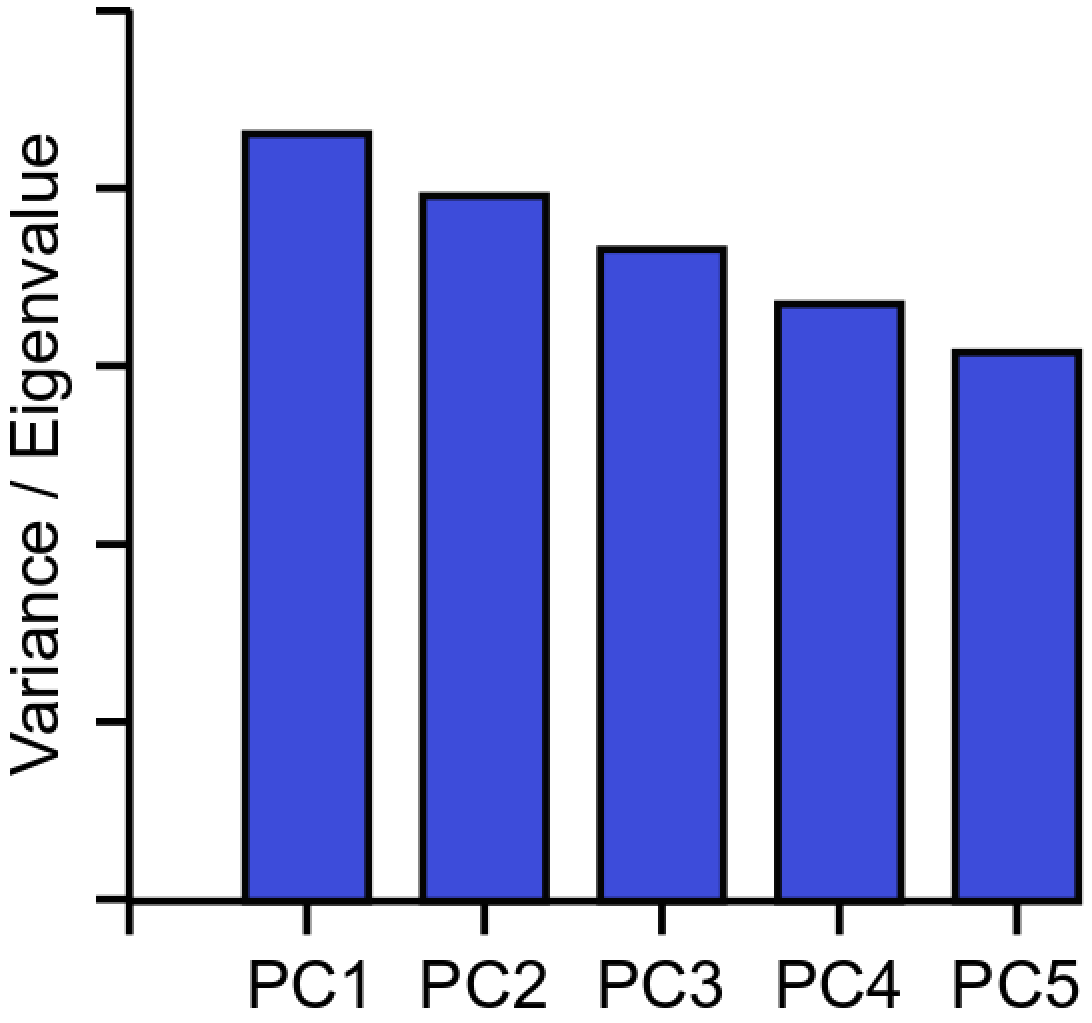
- Project onto PC
- Calculate distance to the origin

- Calculate sum of squared differences (SSD)
  - This is a measure of variance called the 'eigenvalue'

  - Divide by (points-1) to get actual variance

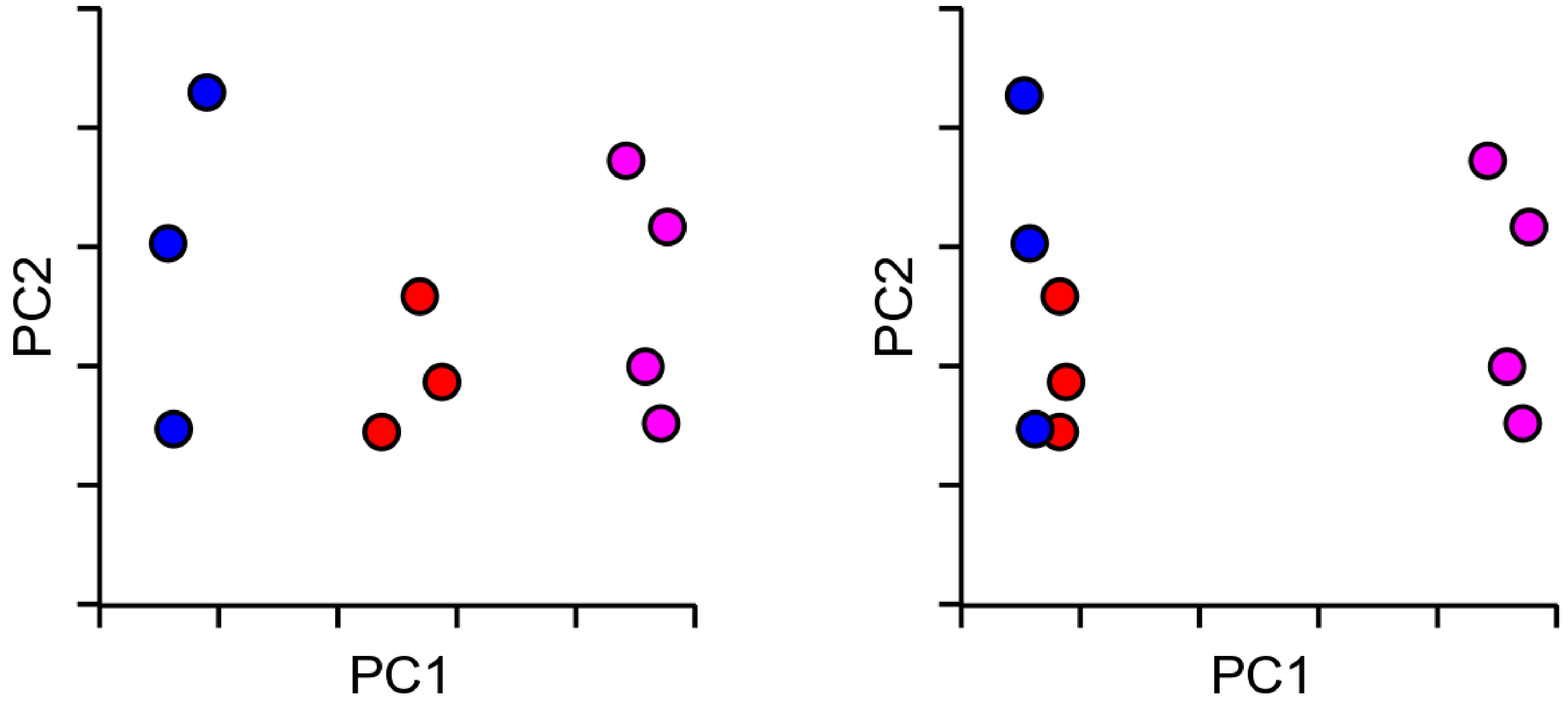
Explaining Variance – Scree Plots





So PCA is great then?

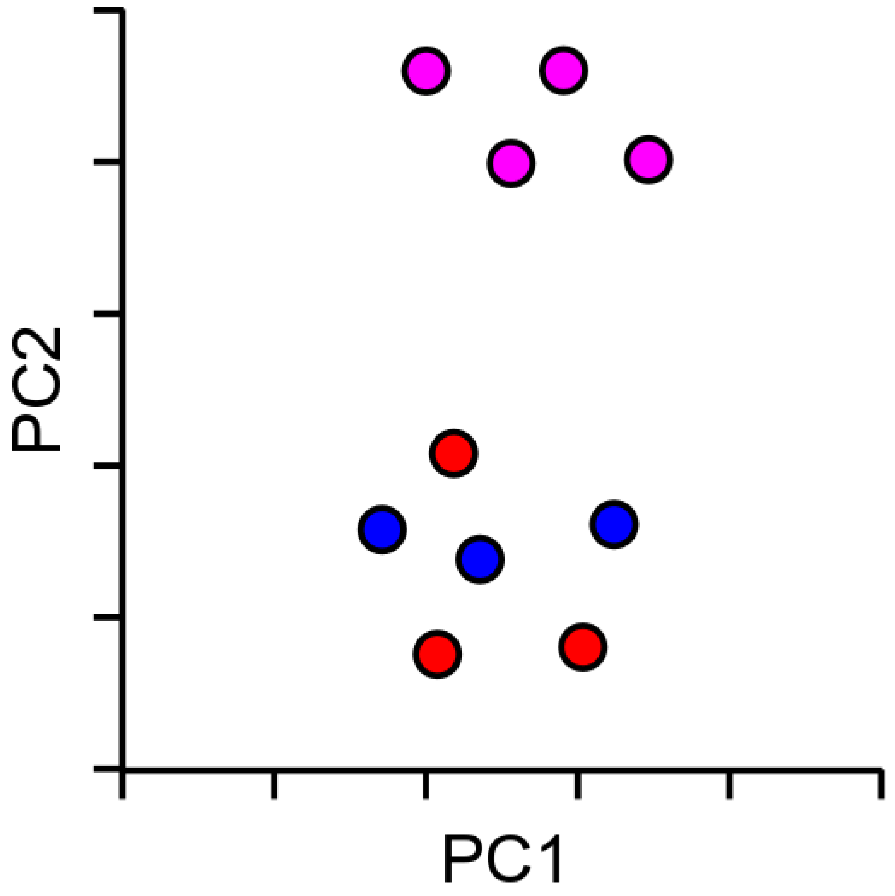
Kind of…

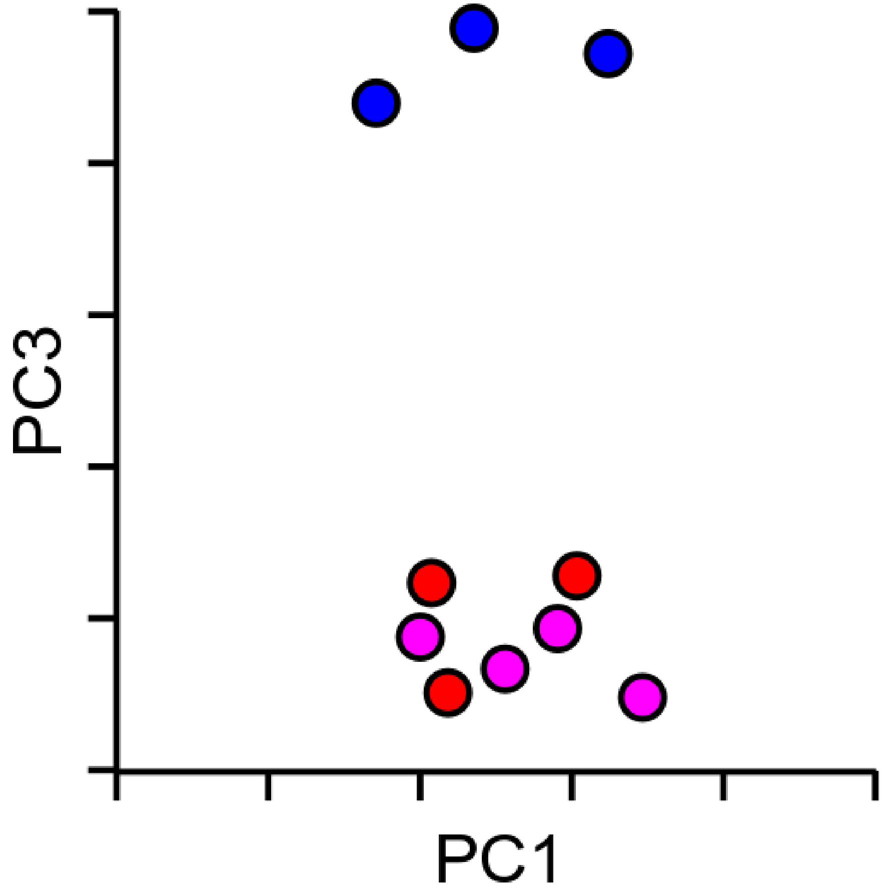


Non-linear separation of values

So PCA is great then?

Kind of…





Not optimised for 2-dimensions

tSNE to the rescue…

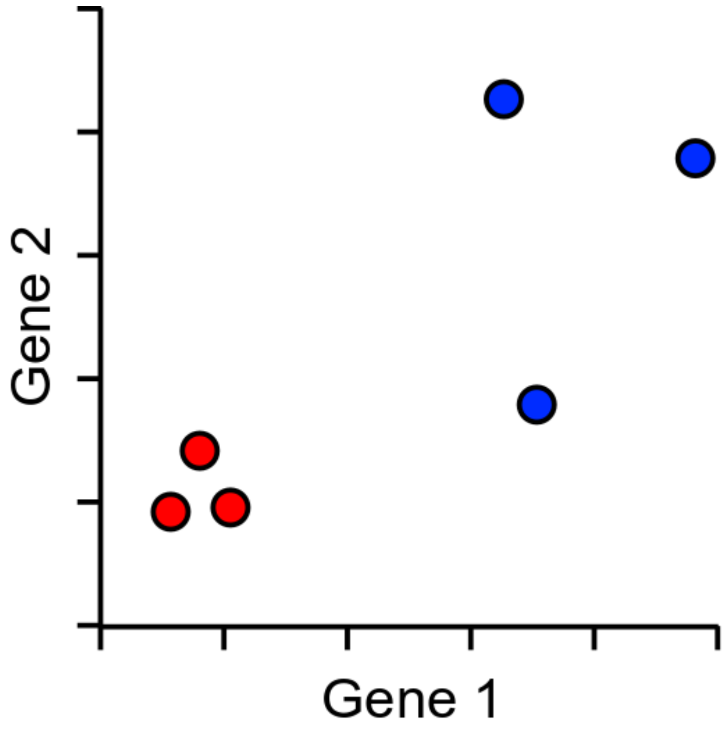
- T-Distributed Stochastic Neighbour Embedding

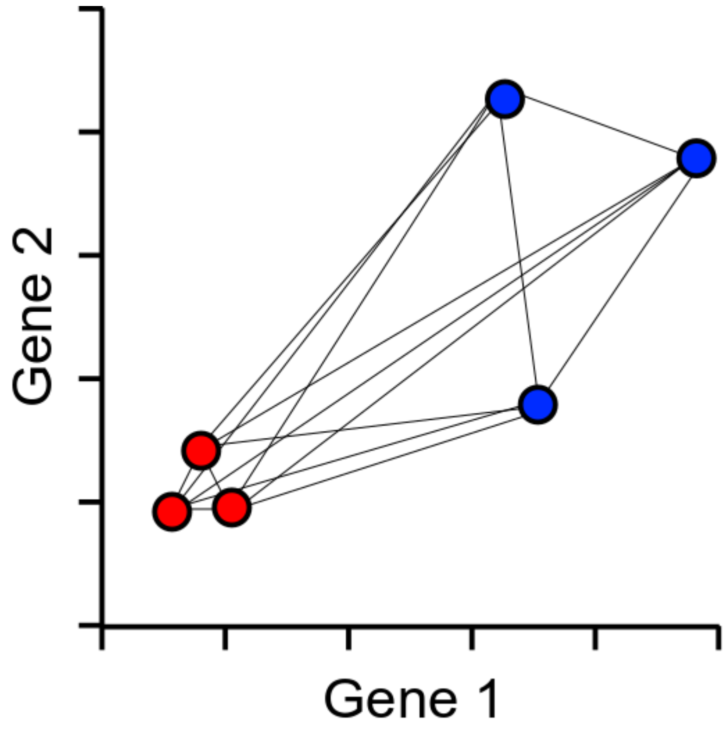
- Aims to solve the problems of PCA
  - Non-linear scaling to represent changes at different levels

  - Optimal separation in 2-dimensions

How does tSNE work?

- Based around all-vs-all table of pairwise cell to cell distances

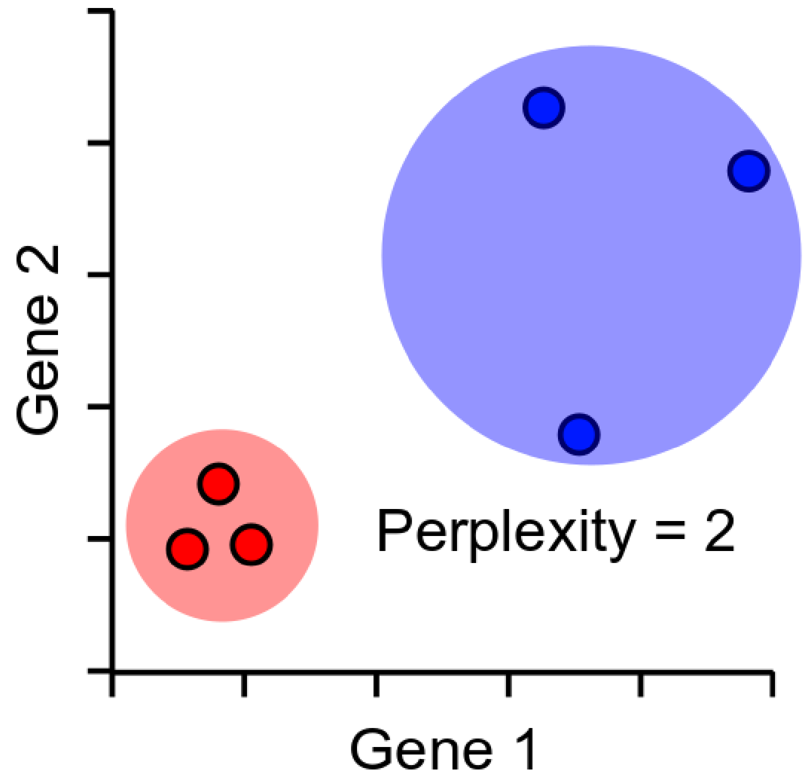




	🟥	🟥	🟥	🟦	🟦	🟦
🟥	0	10	10	295	158	153
🟥	9	0	1	217	227	213
🟥	1	8	0	154	225	238
🟦	205	189	260	0	23	45
🟦	248	227	246	44	0	54
🟦	233	176	184	41	36	0

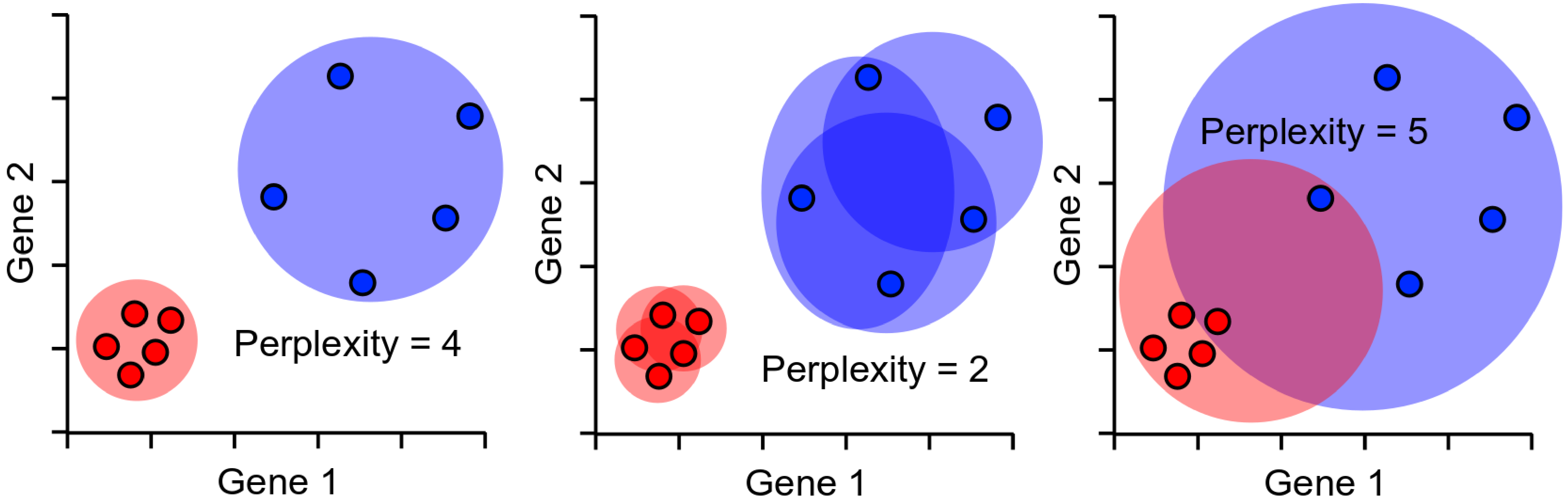
Distance scaling and perplexity

- Perplexity = expected number of neighbours within a cluster
- Distances scaled relative to perplexity neighbours



	🟥	🟥	🟥	🟦	🟦	🟦
🟥	0	4	6	586	657	836
🟥	4	0	4	815	527	776
🟥	9	3	0	752	656	732
🟦	31	28	29	0	4	7
🟦	31	24	25	4	0	7
🟦	40	37	32	8	8	0

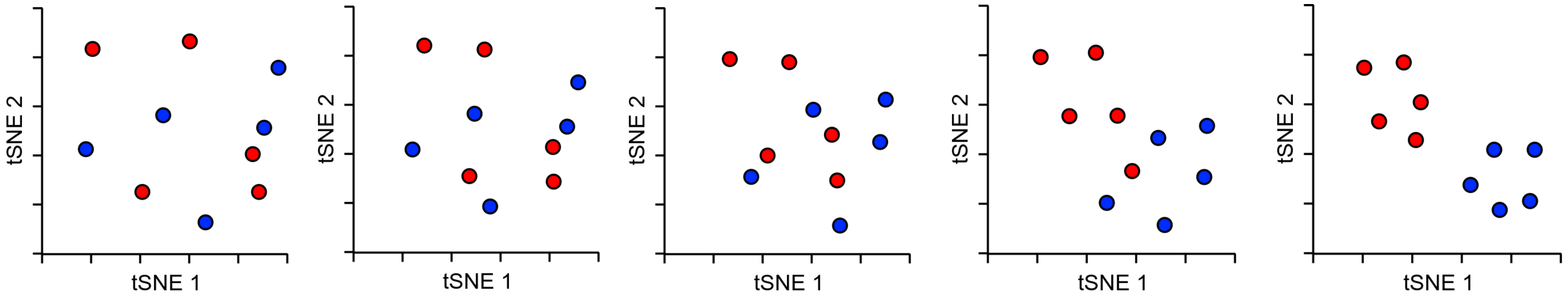
Perplexity Robustness



tSNE Projection

- Randomly scatter all points within the space (normally 2D)
- Start a simulation
  - Aim is to make the point distances match the distance matrix
  - Shuffle points based on how well they match
  - Stop after fixed number of iterations, or
  - Stop after distances have converged

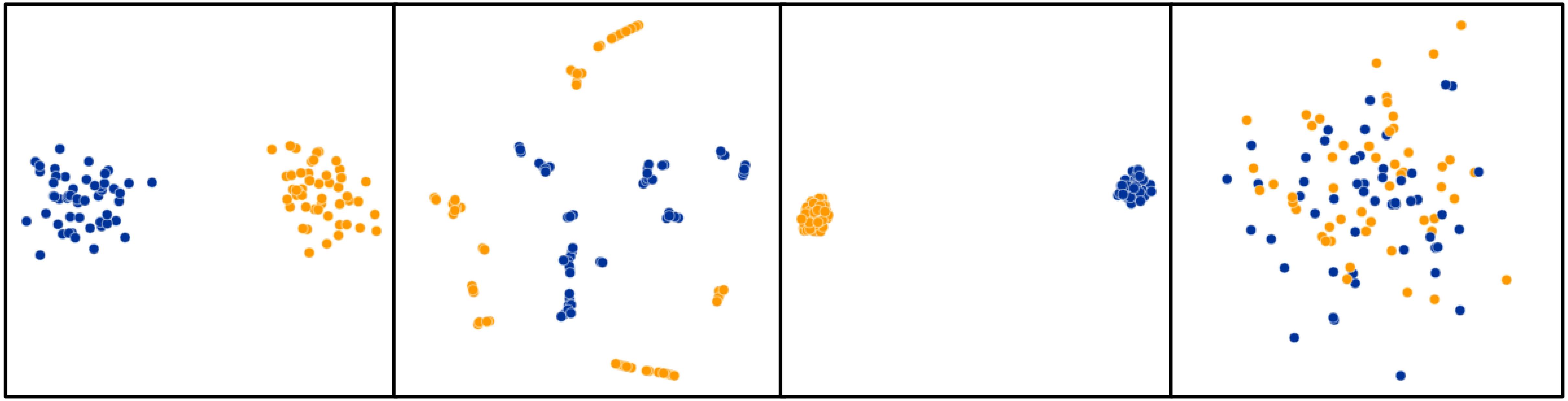
tSNE Projection



- X and Y don’t mean anything (unlike PCA)
- Distance doesn’t mean anything (unlike PCA)
- Close proximity is highly informative
- Distant proximity isn’t very interesting
- Can’t rationalise distances, or add in more data

tSNE Practical Examples

Perplexity Settings Matter

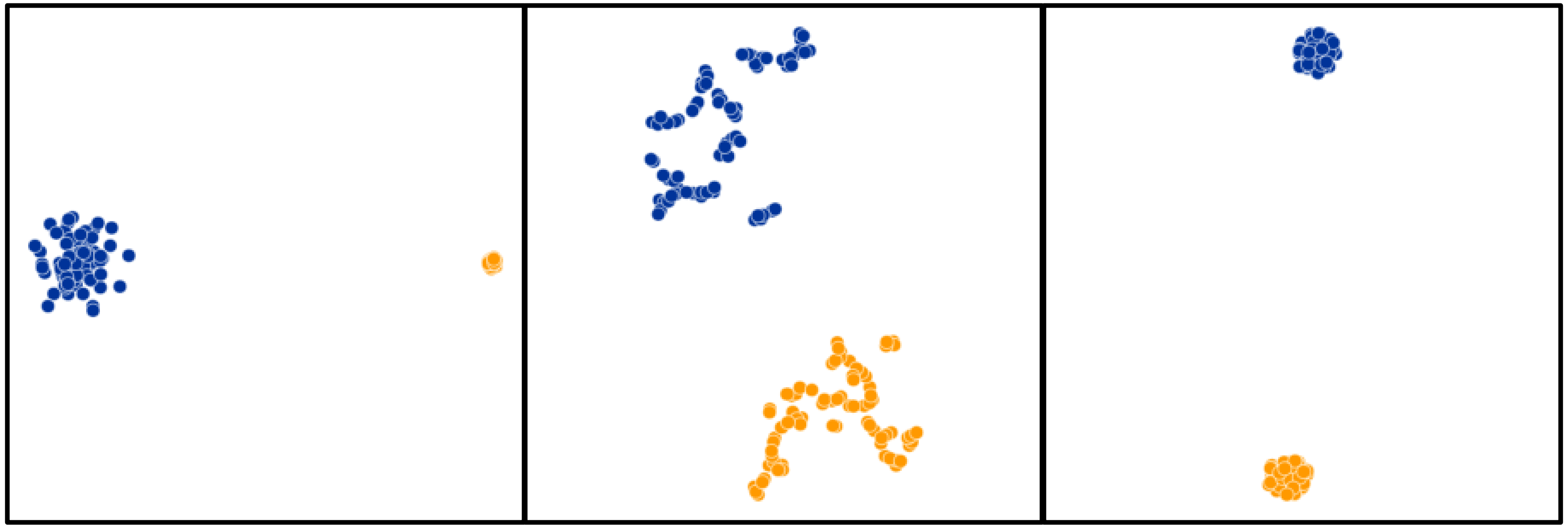


Original Perplexity = 2 Perplexity = 30 Perplexity = 100

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

tSNE Practical Examples

Cluster Sizes are Meaningless



Original

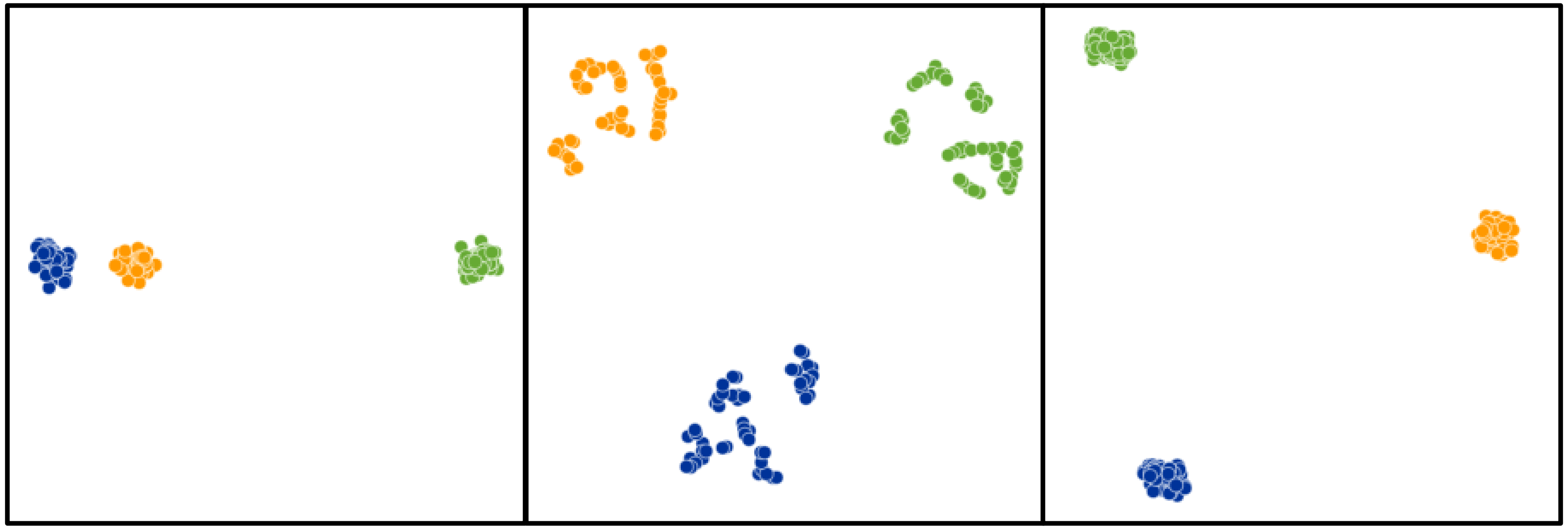
Perplexity = 5

Perplexity = 50

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

tSNE Practical Examples

Distances between clusters can't be trusted



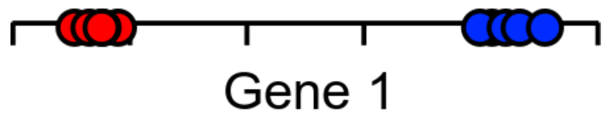
Original Perplexity = 5 Perplexity = 30

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

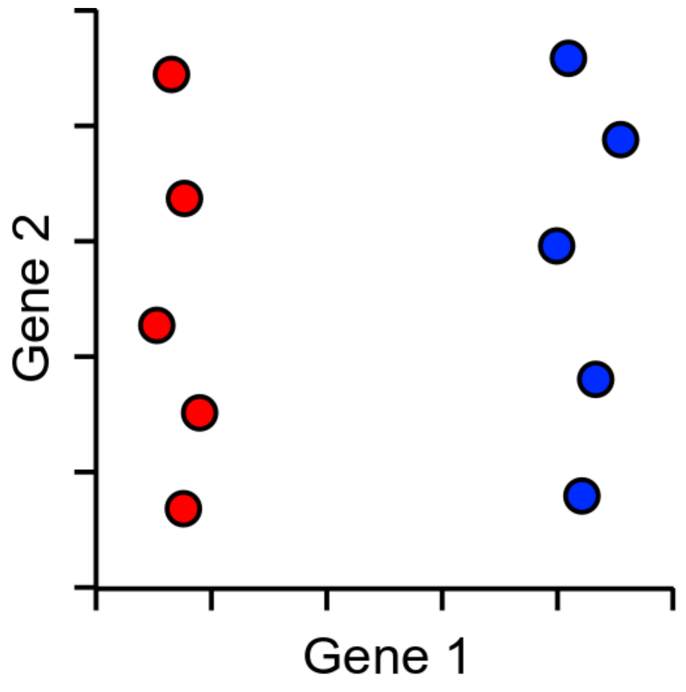
So tSNE is great then?

Kind of…

Imagine a dataset with only one super informative gene



Distance within cluster = low
Distance between clusters = high



Distance within cluster = higher
Distance between clusters = lower

- Now 3 genes
- Now 3,000 genes

- Everything is the same distance from everything

So everything sucks?

- PCA
  - Requires more than 2 dimensions
  - Thrown off by quantised data
  - Expects linear relationships
- tSNE
  - Can’t cope with noisy data
  - Loses the ability to cluster

Answer: Combine the two methods, get the best of both worlds

- PCA
  - Good at extracting signal from noise
  - Extracts informative dimensions
- tSNE
  - Can reduce to 2D well
  - Can cope with non-linear scaling

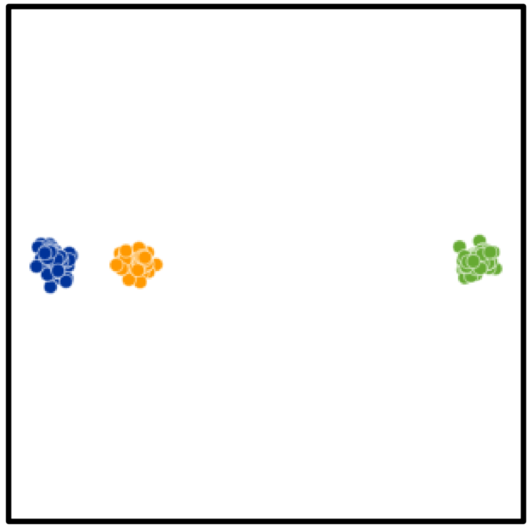
This is what many pipelines do in their default analysis

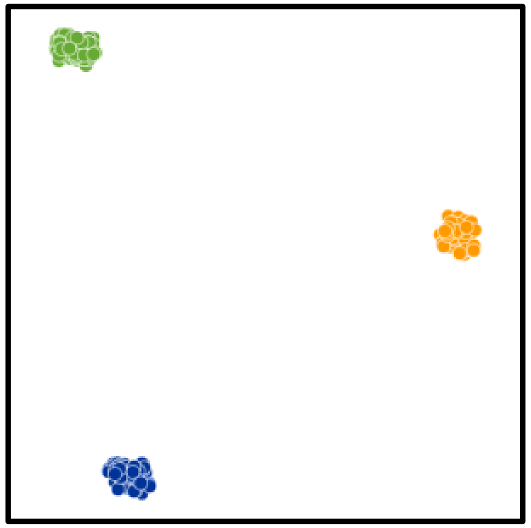
So PCA + tSNE is great then?

Kind of…

- tSNE is slow. This is probably it’s biggest crime
  - tSNE doesn’t scale well to large numbers of cells (10k+)

- tSNE only gives reliable information on the closest neighbours large distance information is almost irrelevant





UMAP to the rescue!

- UMAP is a replacement for tSNE to fulfil the same role
- Conceptually very similar to tSNE, but with a couple of relevant (and somewhat technical) changes
- Practical outcome is:
  - UMAP is quite a bit quicker than tSNE
  - UMAP can preserve more global structure than tSNE*
  - UMAP can run on raw data without PCA preprocessing*
  - UMAP can allow new data to be added to an existing projection

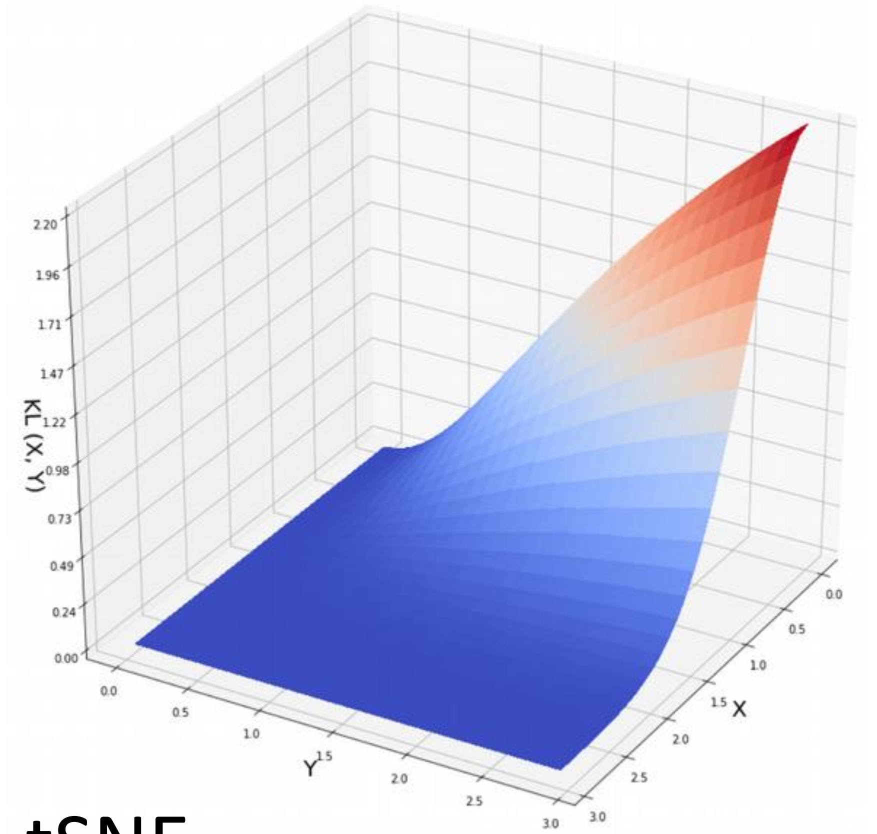
* In theory, but possibly not in practice

UMAP differences

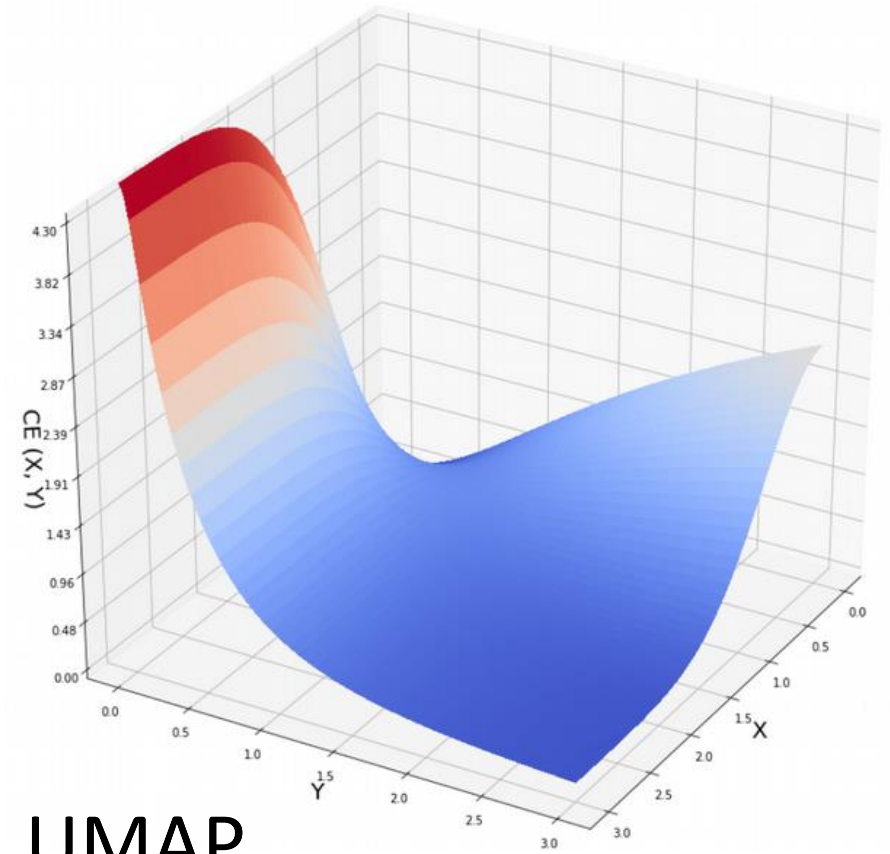
- Instead of the single perplexity value in tSNE, UMAP defines
  - **Nearest neighbours**: the number of expected nearest neighbours – basically the same concept as perplexity
  - **Minimum distance**: how tightly UMAP packs points which are close together
- Nearest neighbours will affect the influence given to global vs local information. Min dist will affect how compactly packed the local parts of the plot are.

UMAP differences

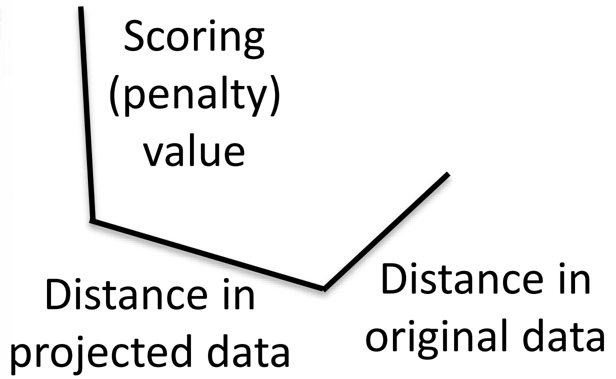
- Structure preservation – mostly in the 2D projection scoring



tSNE



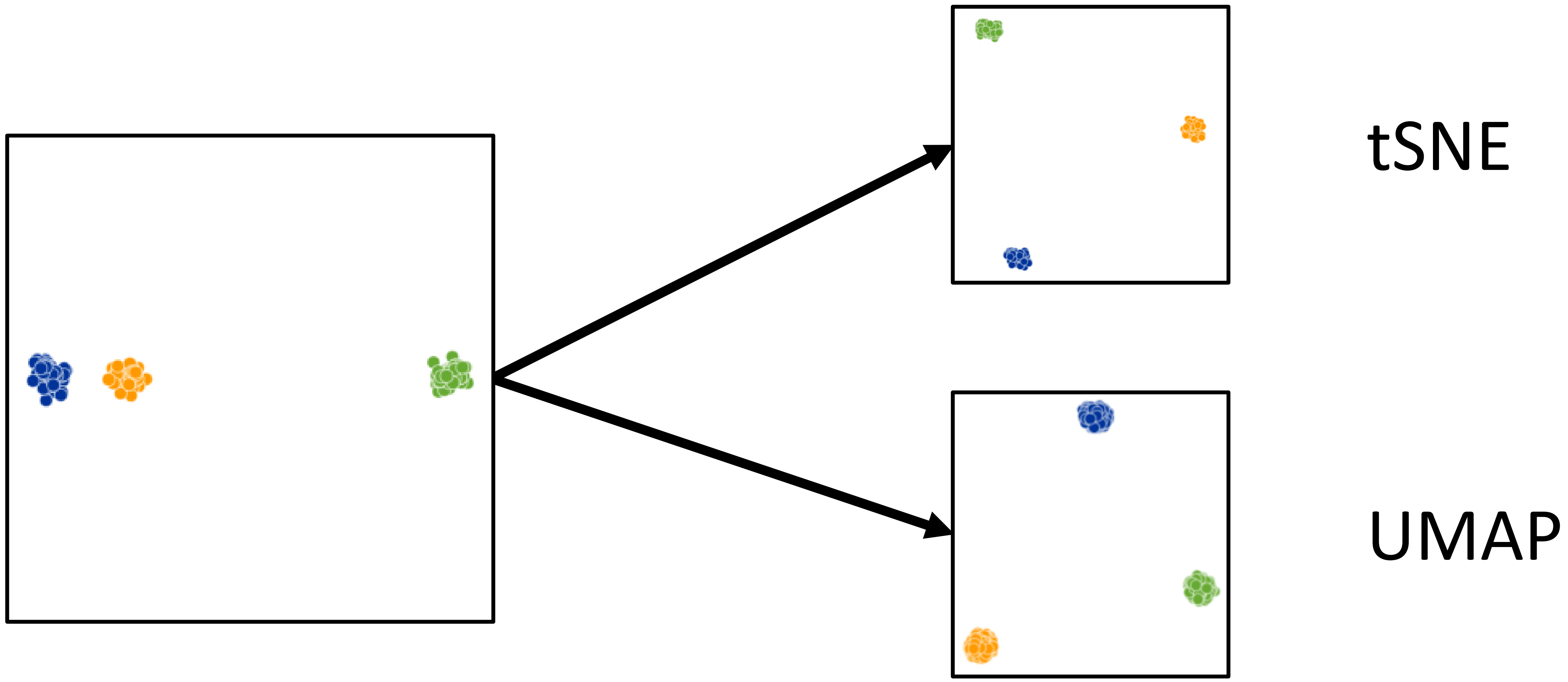
UMAP



https://towardsdatascience.com/how-exactly-umap-works-13e3040e1668

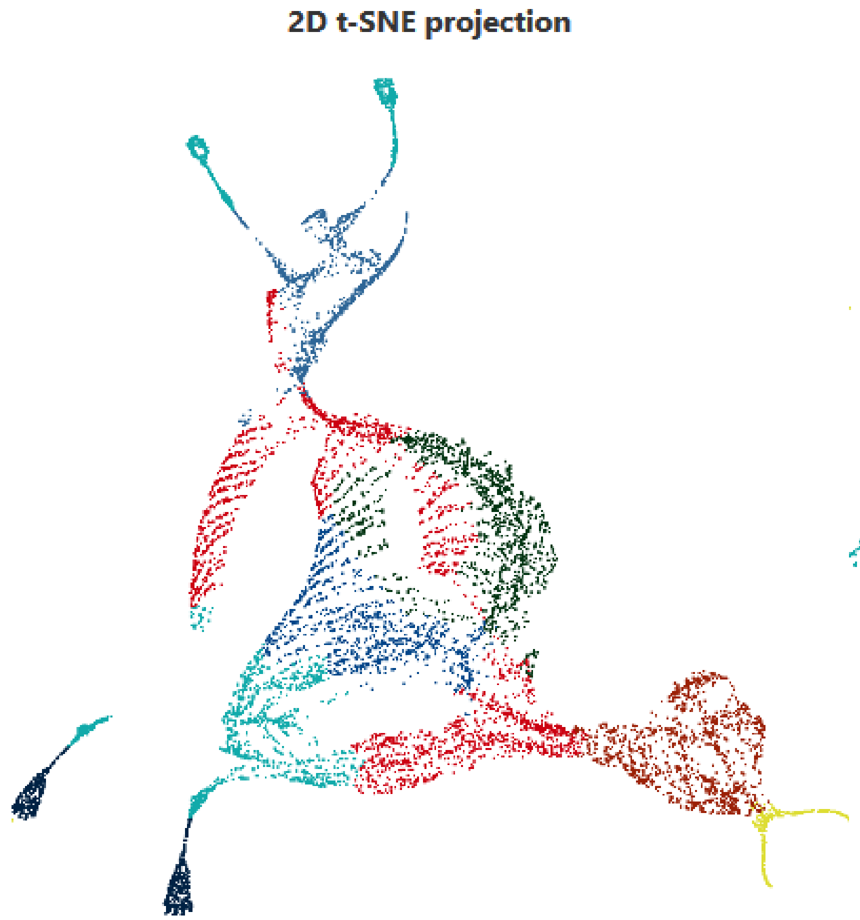
So UMAP is great then?

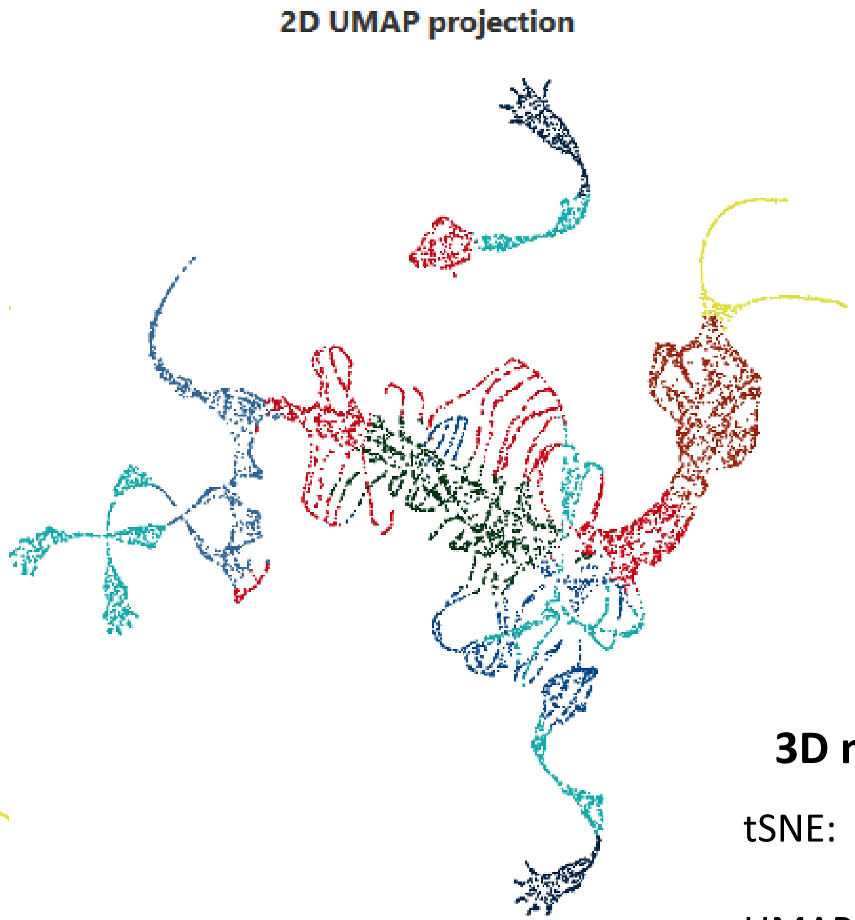
Kind of…



So UMAP is all hype then?

No, it really does better for some datasets…





3D mammoth skeleton projected into 2D

tSNE:	Perplexity 2000	2h 5min
UMAP:	Nneigh 200, mindist 0.25,	3min

https://pair-code.github.io/understanding-umap/

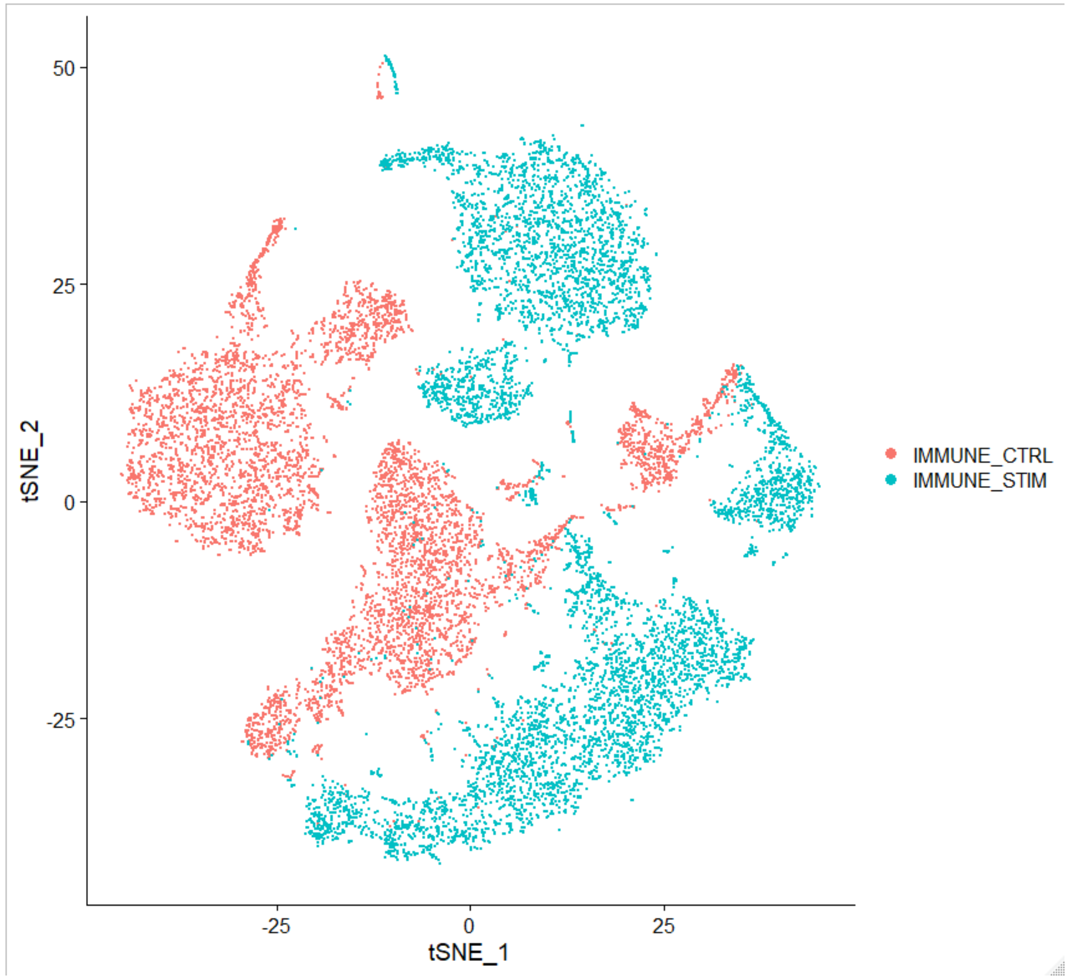
Practical approach PCA + tSNE/UMAP

- Filter heavily before starting
  - Nicely behaving cells
  - Expressed genes
  - Variable genes
- Do PCA
  - Extract most interesting signal
  - Take top PCs. Reduce dimensionality (but not to 2)
- Do tSNE/UMAP
  - Calculate distances from PCA projections
  - Scale distances and project into 2-dimensions

So PCA + UMAP is great then?

Kind of… as long as you only have one dataset

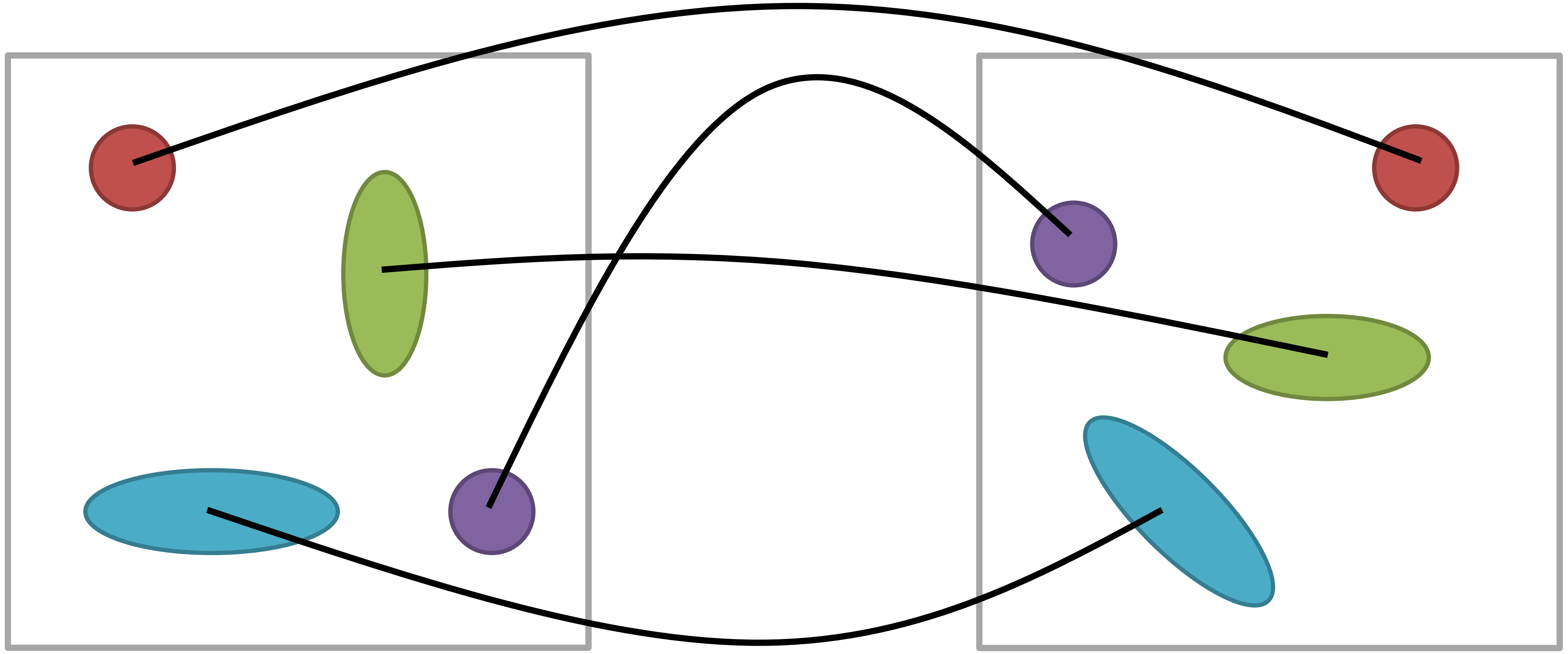
- In 10X every library is a 'batch'
- More biases over time/distance
- Biases prevent comparisons
- Need to align the datasets



Data Integration

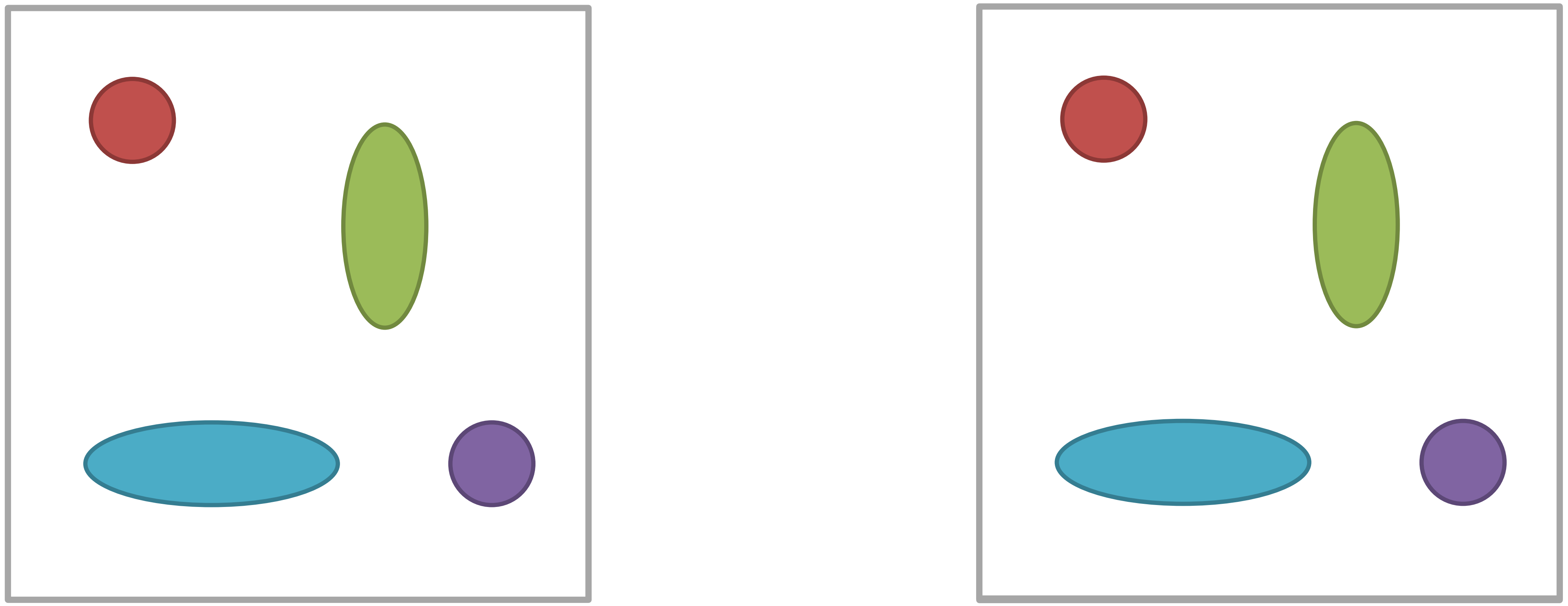
- Works on the basis that there are 'equivalent' collections of cells in two (or more) datasets
- Find 'anchor' points which are equivalent cells which should be aligned
- Quantitatively skew the data to optimally align the anchors

UMAP/tSNE integration



Define key 'anchor' points between equivalent cells

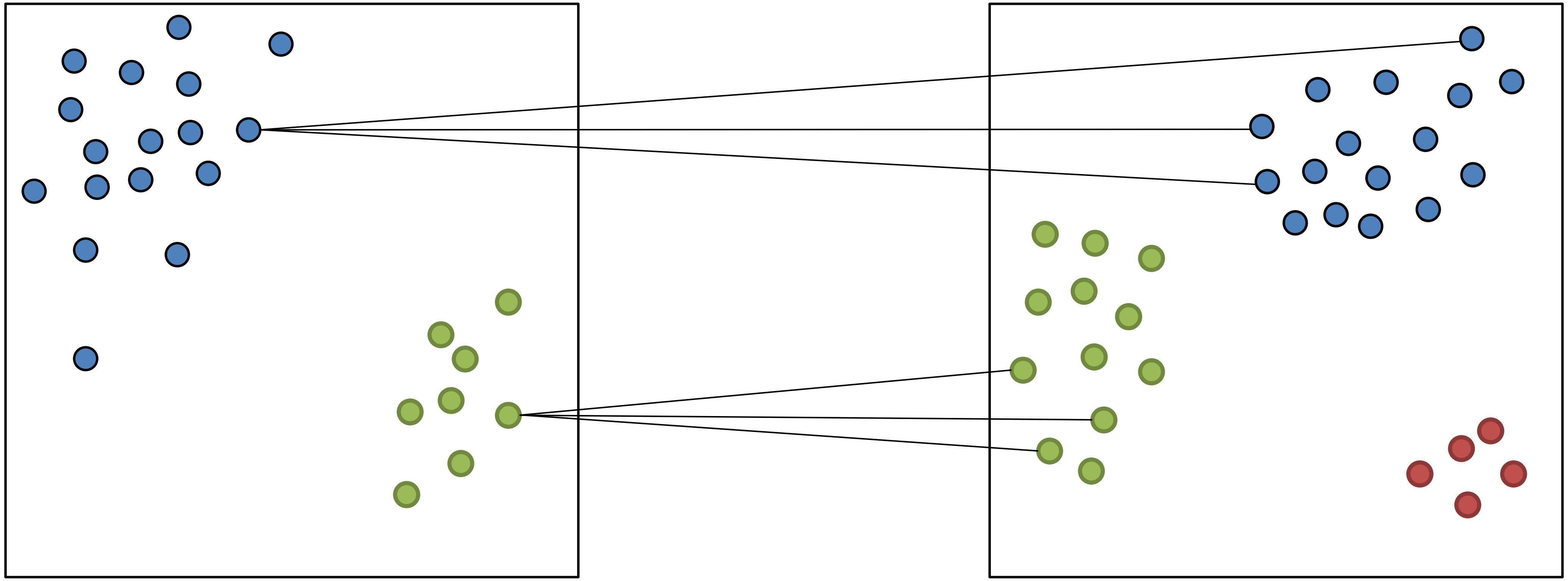
UMAP/tSNE integration



Skew data to align the anchors

Defining Integration Anchors

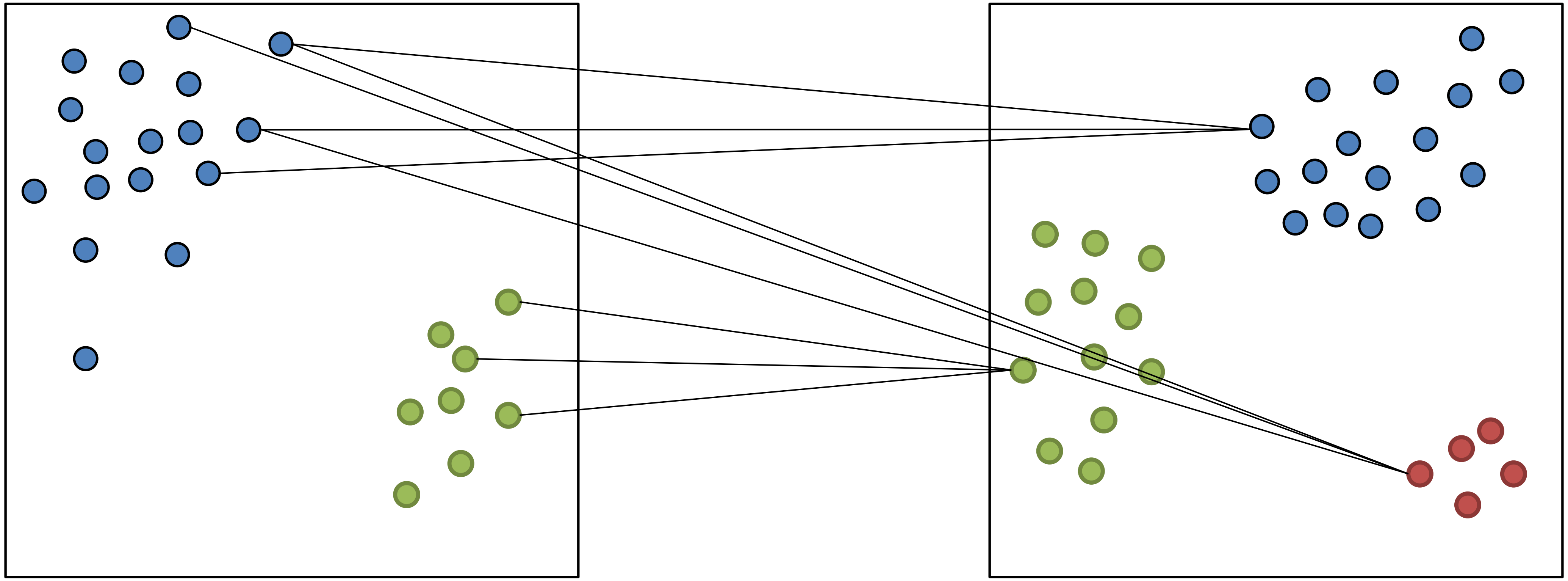
Mutual Nearest Neighbours (MNN)



For each cell in data1 find the 3 closest cells in data2

Defining Integration Anchors

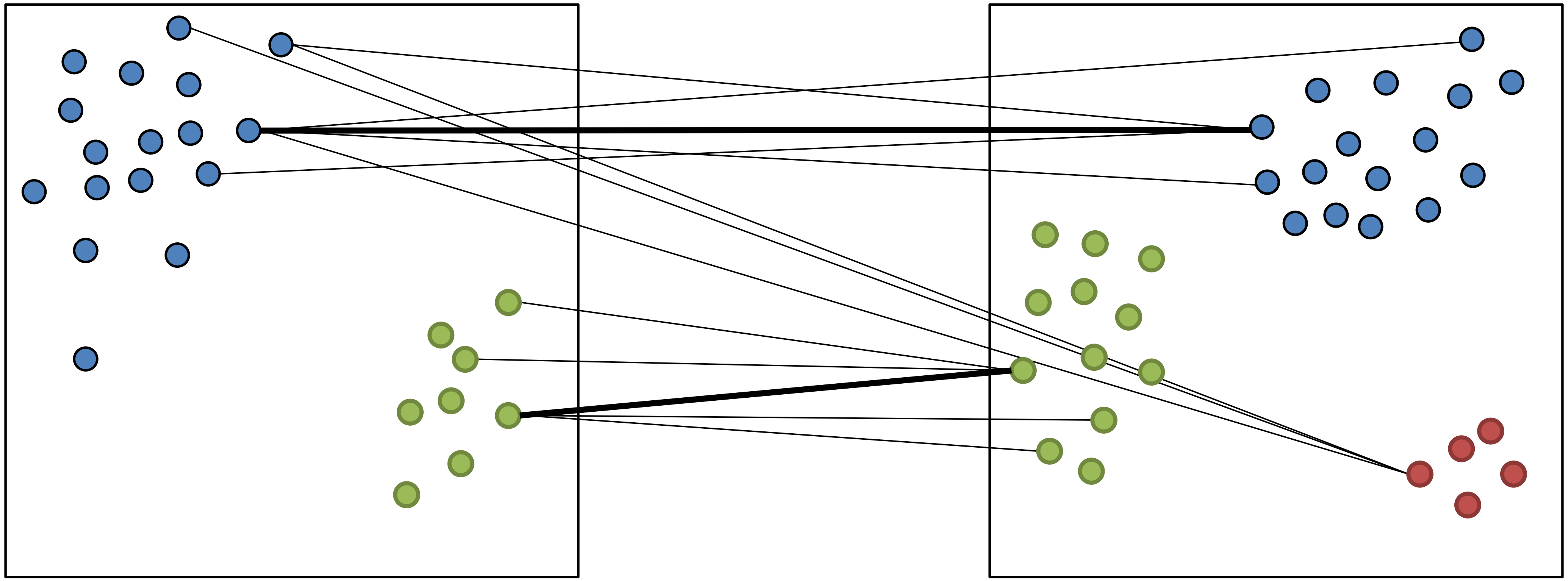
Mutual Nearest Neighbours (MNN)



Do the same thing the other way around

Defining Integration Anchors

Mutual Nearest Neighbours (MNN)



Select pairs of cells which are in others nearest neighbour groups

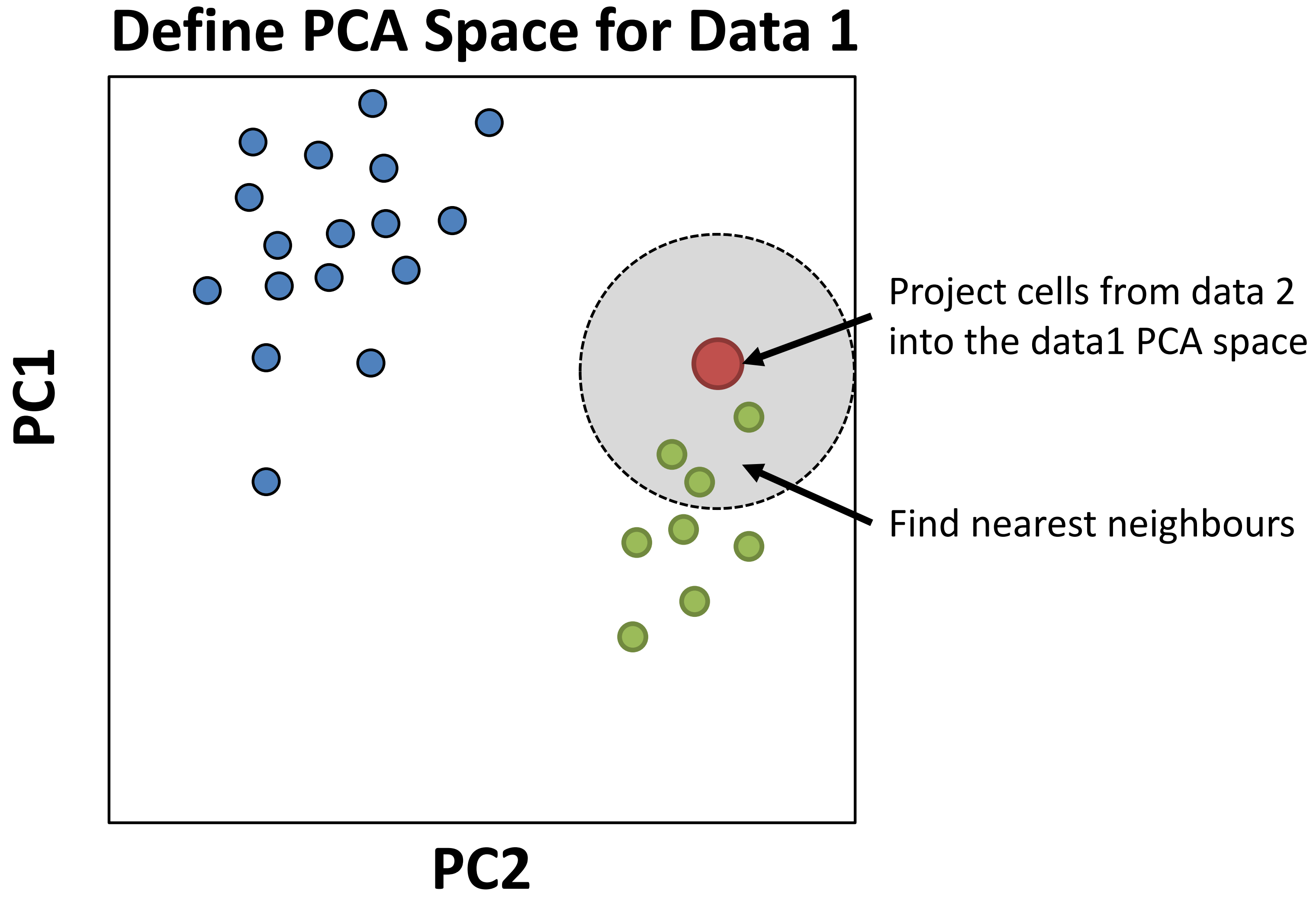
Defining nearest neighbours

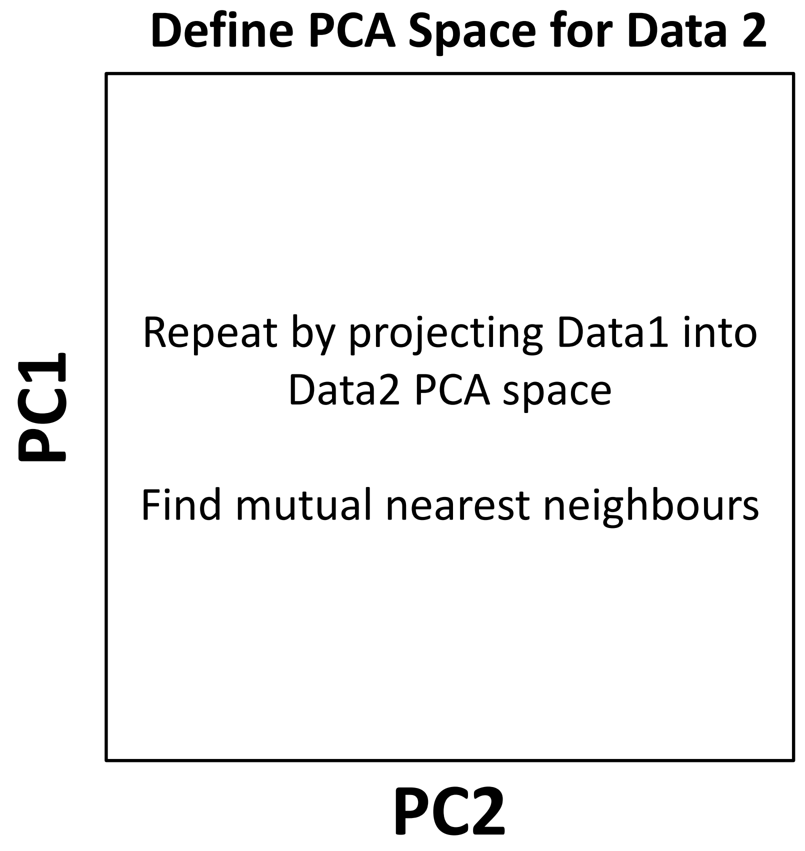
- Distance in original expression quantitation
  - Really noisy (different technology, normalisation, depth)
  - Slow and prone to mis-prediction

- Use a cleaner (less noisy) representation
  - Principal Components (rPCA)

Defining Integration Anchors

Reciprocal PCA

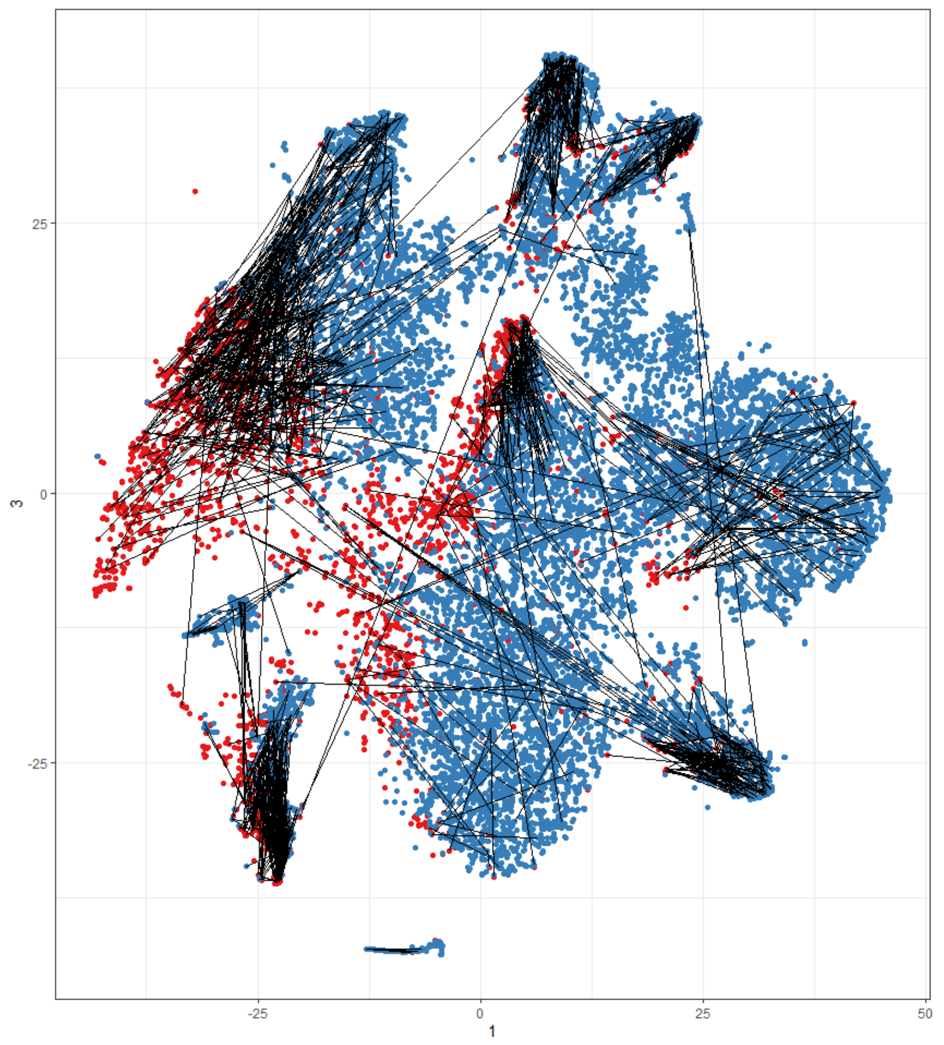

Define PCA Space for Data 1
PC1
PC2
Project cells from data 2 into the data1 PCA space
Find nearest neighbours



Repeat by projecting Data1 into Data2 PCA space

Find mutual nearest neighbours

Integration Anchors



Factors Affecting Integration

- Which genes are submitted to the integration
  - Expressed in all datasets
  - Variable in all datasets
- Which method is used to define nearest neighbours
  - Normalised data, Correlation, Reverse PCA
- How many nearest neighbours you consider
  - Default is around 5, some clusters require more (20ish)
- Other filters to remove artefacts