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Know Your Data: Exploratory Data Analysis

Shraddha Pai

Analysis Using R

June 13, 2024

bioinformatics.ca

OICR
Ontario Institute for Cancer Research

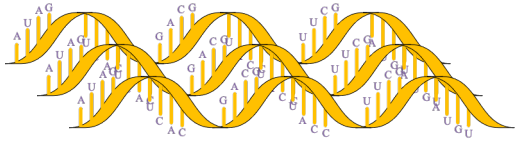
UNIVERSITY OF TORONTO

Learning Objectives

- By the end of this lecture, you will:
  - Know the basic terminology for terms in a statistical model
  - Know how to perform systematic exploratory data analysis to to identify sources of biological and technical variation
  - Appreciate the value of exploring missingness in your data
  - Have a high level understanding of clustering and be able to cluster your data

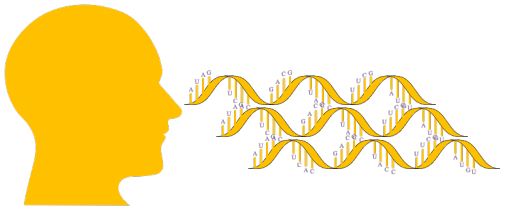
Sources of variation in a study

Goal: “Find transcriptomic biomarkers of disease”



Sources of variation in a study

Basic terminology in statistical model building



$$xpr = \beta_0 + \beta_1(disease) + \epsilon$$

Response variable

Dependent variable

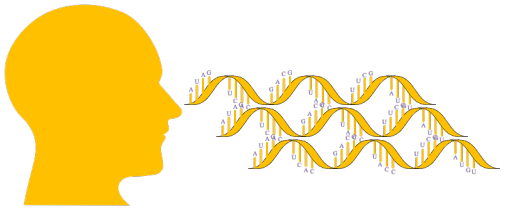
Explanatory variable

Independent variable

Residual; unmodelled variation

Sources of variation in a study

Basic terminology in statistical model building

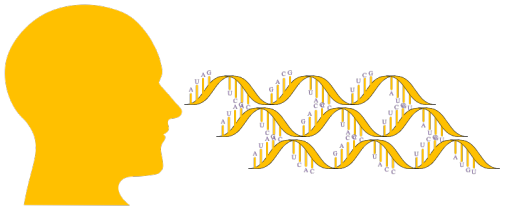


coefficients; weights

$$xpr = \beta_0 + \beta_1(disease) + \epsilon$$

Intercept

Goal: “Find transcriptomic biomarkers of disease”



$$xpr = \beta_0 + \beta_1(disease) + \epsilon$$

Response variable

Explanatory variable

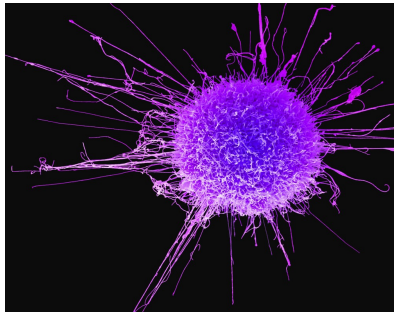
Residual; unmodelled variation

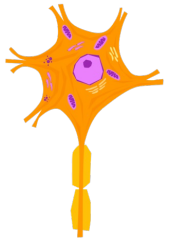
Dependent variable

Independent variable

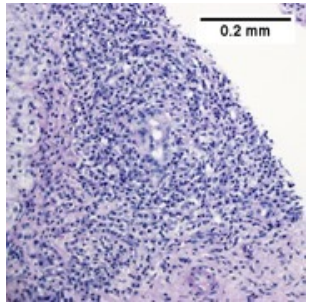
What could be affecting your outcome?

Biological sources of variation





Technical sources of variation



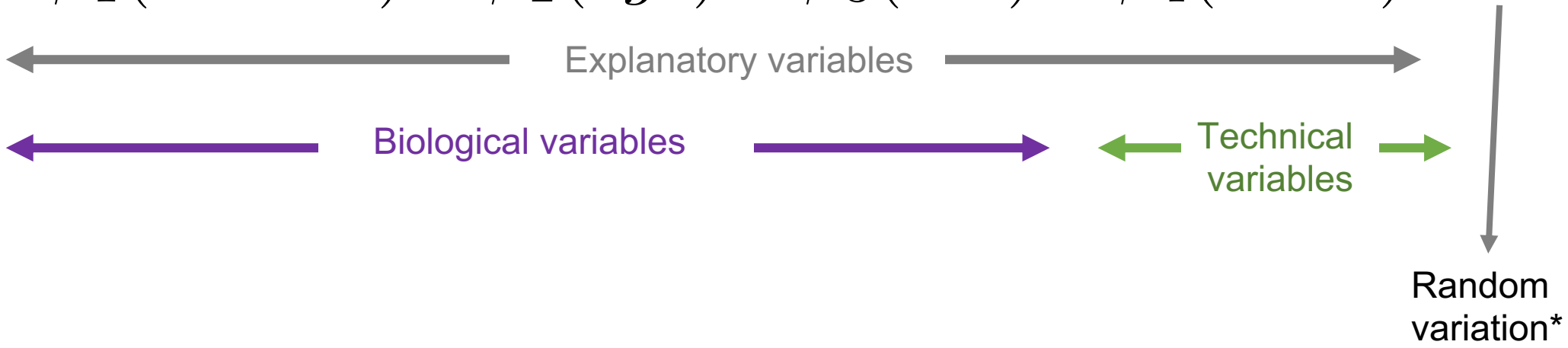
"Which of these is affecting my data?"

Visualize & quantify sources of variation:
* clustering
* dimensionality reduction
  (PCA, UMAP, tSNE)

$$xpr = \beta_0 + \beta_1(disease) + \beta_2(age) + \beta_3(sex) + \beta_4(batch) + \epsilon$$

Final model

$$xpr = \beta_0 + \beta_1(disease) + \beta_2(age) + \beta_3(sex) + \beta_4(batch) + \epsilon$$



- "Random": Values sampled from defined statistical distribution (e.g., Normal distribution, Binomial, Poisson etc.,)

Missingness

Missingness happens!

- Clinical data may be incomplete (e.g., participant didn't answer questionnaire)
- Multi-'omic data, some participants missing an assay
- Some measures didn't pass QC

Solutions:

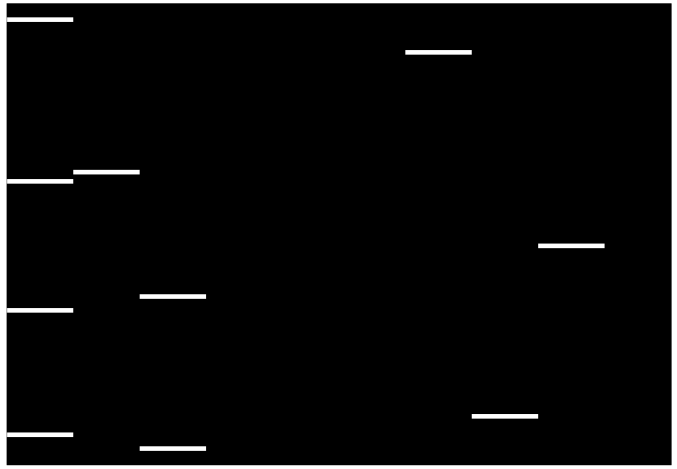
- Remove rows/cols with "excessive" missingness – use field convention where possible
- Use imputation to "guess" at missing values

Ensure your approach is defensible! Careful what you "guess".

What are trade-offs in each solution?

Checkered view of data table. White shows missing (NA).

Unstructured missingness



Structured missingness

Biased, structured missingness

Extreme situation: What if only one group is missing data, and we blindly impute?

Lesson: Where possible, look at your data.

Goals of exploratory data analysis are to:

1. Identify magnitude of KNOWN biological and technical variation

2. Identify sources of UNKNOWN variation

3. Detect OUTLIER samples

4. Characterize MISSINGNESS

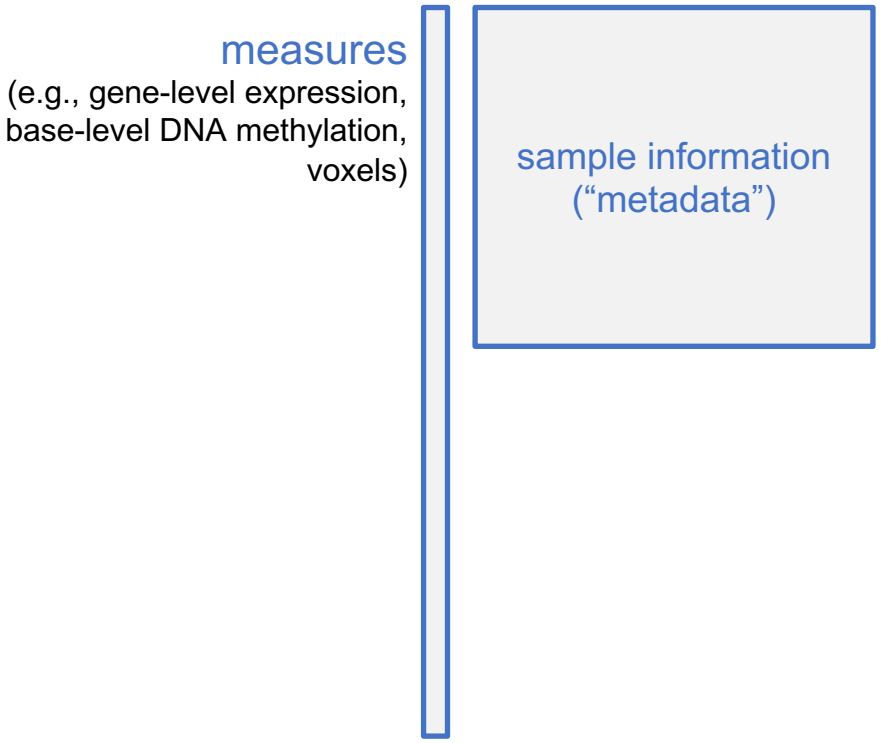
Goals of exploratory data analysis are to:

What you learn	How you learn it	What you do about it
1. Identify magnitude of KNOWN biological and technical variation	PCA*, clustering, prior knowledge	Add terms to model
2. Identify sources of UNKNOWN variation	PCA*, clustering, surrogate value analysis	Add terms to model
3. Detect OUTLIER samples	PCA*, clustering	 Exclude samples from analysis
4. Characterize MISSINGNESS	Plot missingness	• Exclude measures / samples • Define imputation strategy

** Or other dimensionality reduction technique*

Exploratory data analysis: Best practices checklist

Input: 2 tables: DATA and METADATA



Initial exploration

✅ ***How many*** *measures in metadata?*
✅ ***How many*** *measures in data?*
✅ *What are the* ***distribution*** *of the measures?*

✅ Is there **missingness**?

Decide solution

✅ Examine data **correlation** structure
* Does the data have natural groupings?

✅ How many major **sources of variation** are there?
✅ Do they map to biological or technical variables?

Expand model

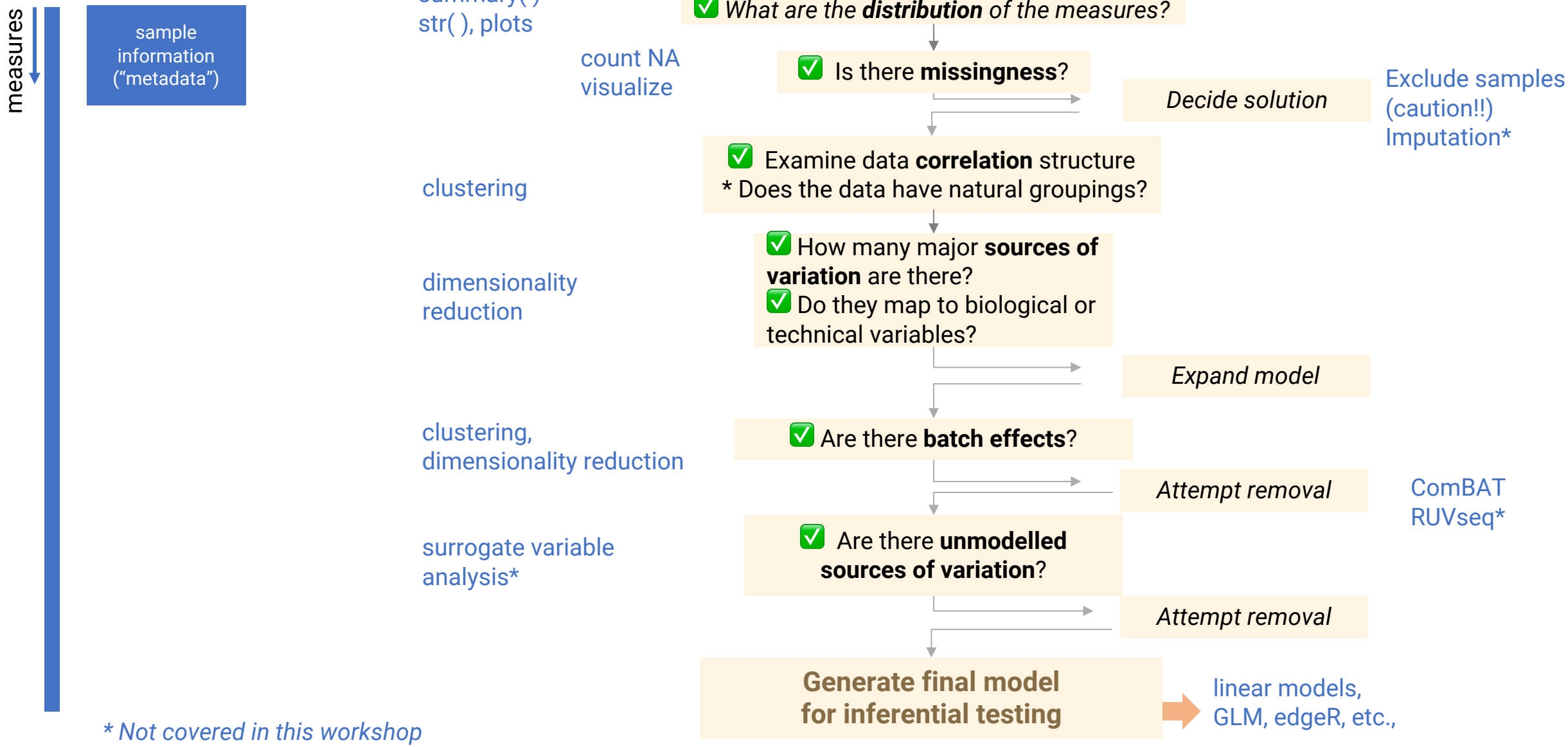
✅ Are there **batch effects**?

Attempt removal

✅ Are there **unmodelled sources of variation**?

Attempt removal

Generate final model for inferential testing



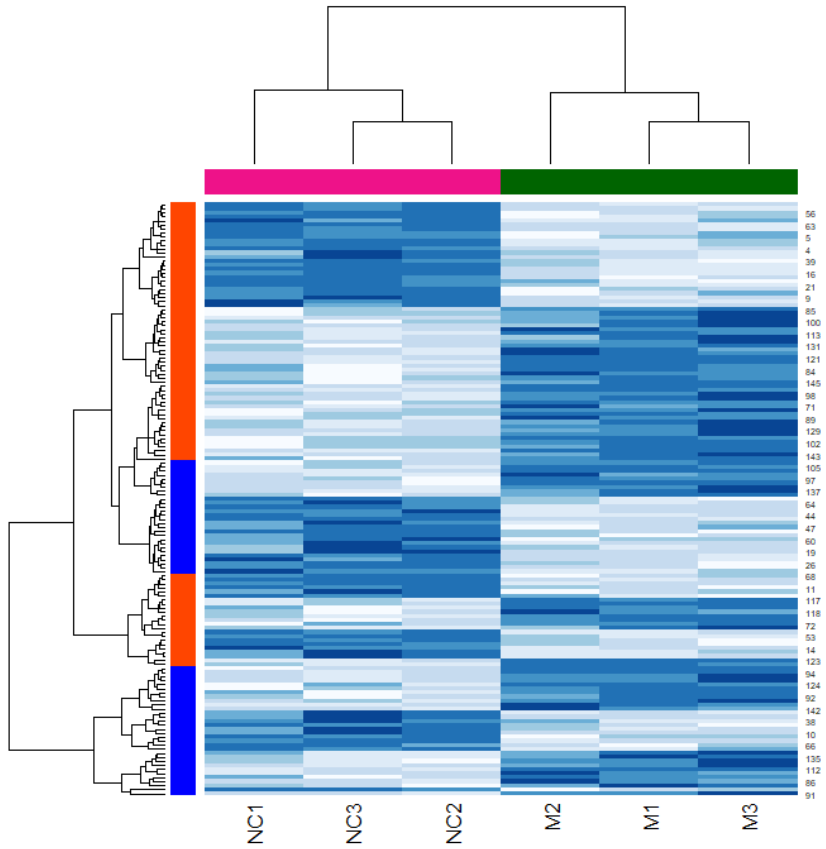
** Not covered in this workshop*

Clustering

High-level purpose: find groups in your data

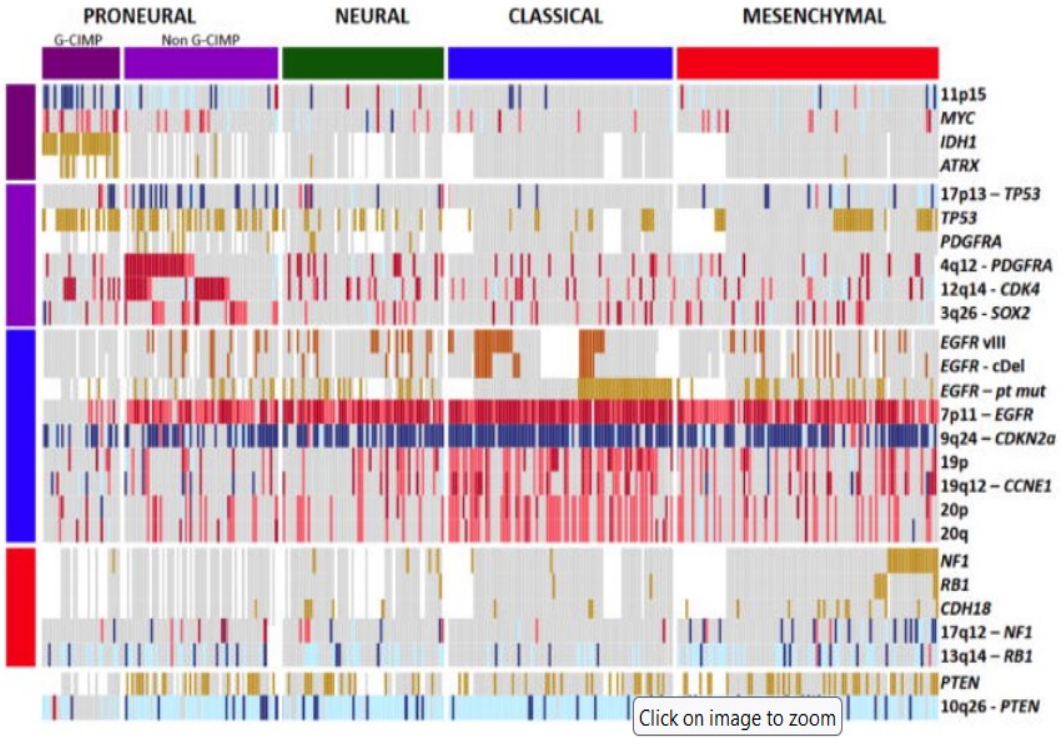
Your particular purpose (may be):

- Identify batches in your data
- Identify patient subtypes
- Identify groups of coexpressed genes

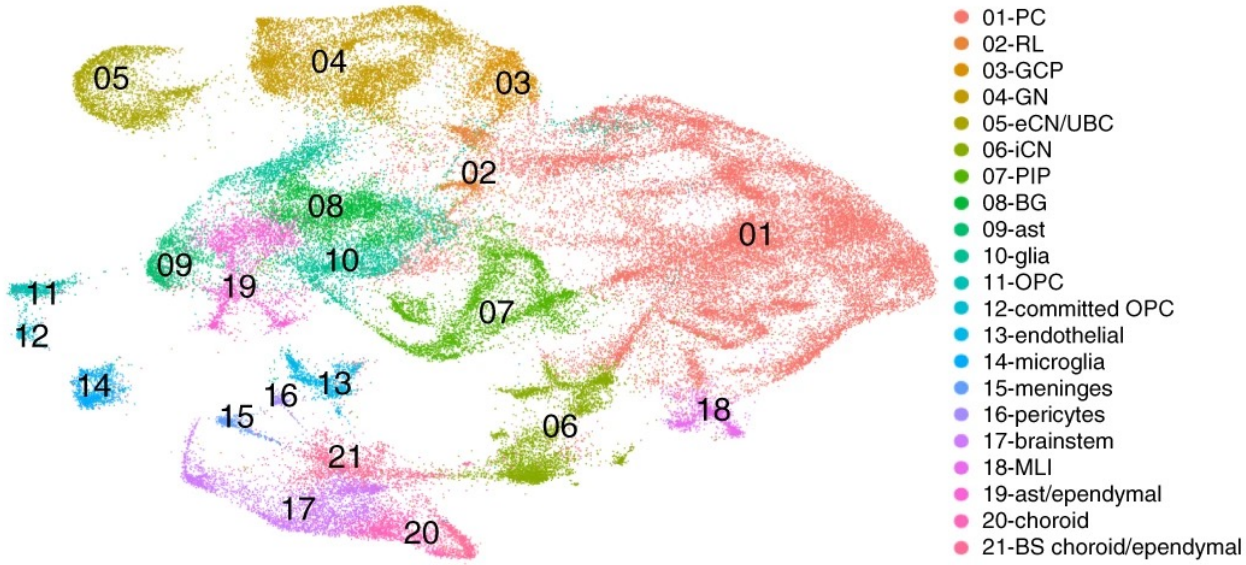


Applications of clustering

Molecular subgroups of glioblastoma



Cell clusters in single-cell genomics



Brennan et al (2013). *Cell*.
Aldinger et al. (2021). *Nat Neurosci*.

The heart of clustering: Defining distance between samples

When clustering, you need to find a way to quantify how similar/dissimilar observations are from one another.

This quantity is your **"distance metric"**

Different data types require different distance metrics

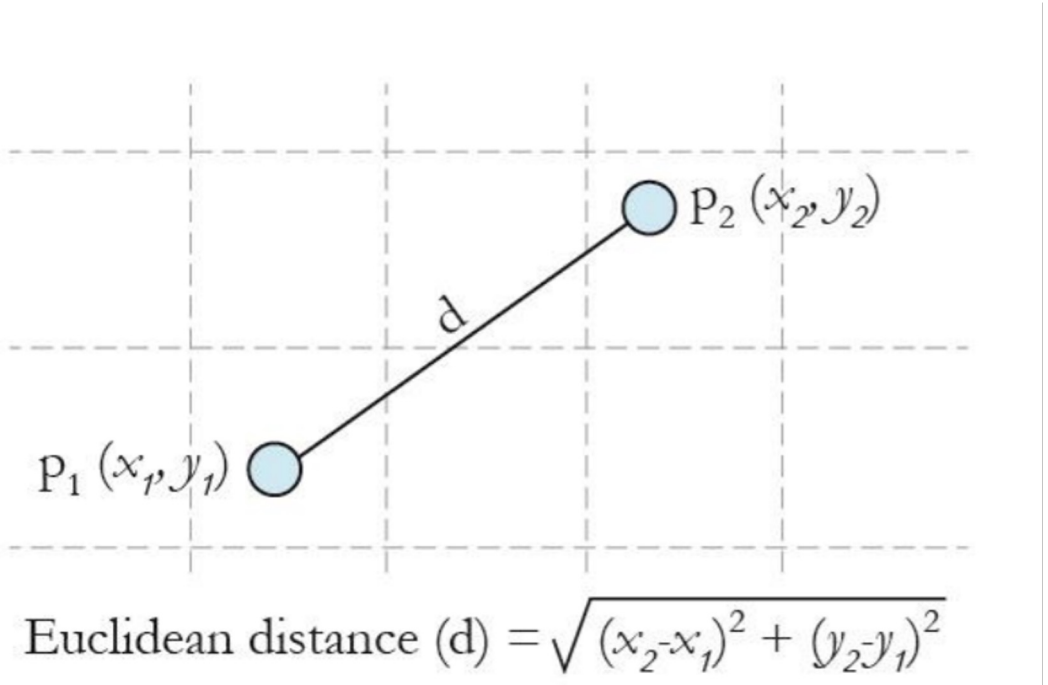
Some data types have many distance metrics, all which come with their own properties.

Distance metrics

Continuous variables:

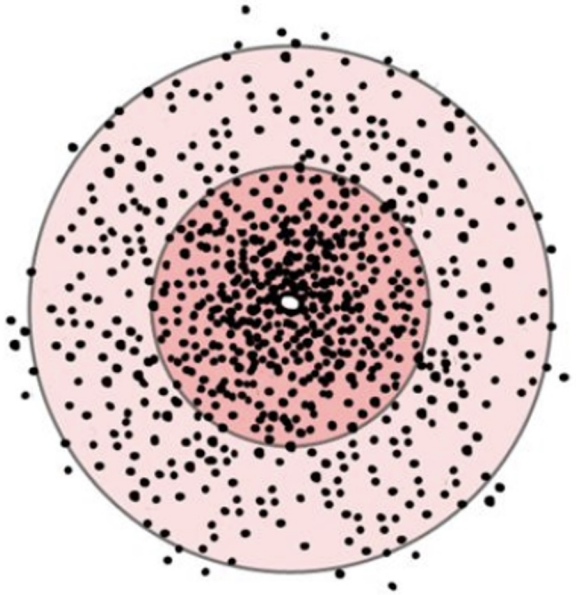
- Euclidean distance: Root squared error

$$\sqrt{\sum_{i=1}^{n}(q_i - p_i)^2}$$



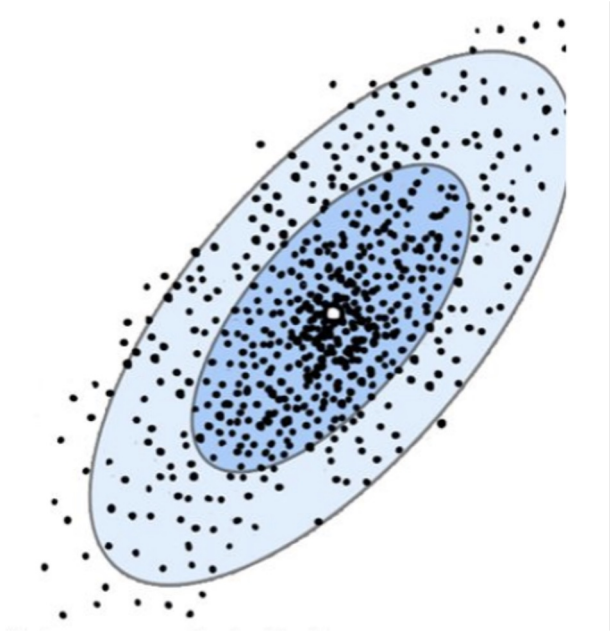
Distance metrics

Euclidean Distance
Ignores correlation between the variables; best if you know data are uncorrelated
(*i.e.,* knowing x does not tell us anything about y).



Mahalanobis Distance
Metric takes into account correlation between variables. Best used if you suspect variables are correlated.

$$D_M(\vec{x}) = \sqrt{(\vec{x} - \vec{\mu})^T S^{-1} (\vec{x} - \vec{\mu})}$$



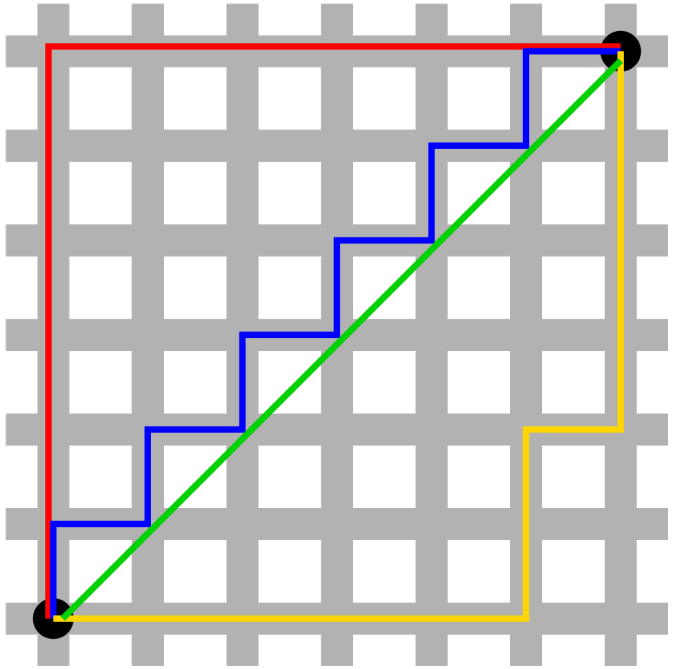
Related term: “similarity”

Distance metrics

Continuous variables:

- Euclidean distance
- Mahalanobis distance
- Manhattan or “block” distance:

$$d_M(x, y) = \sum_{i=1}^{n} |x_i - y_i|$$



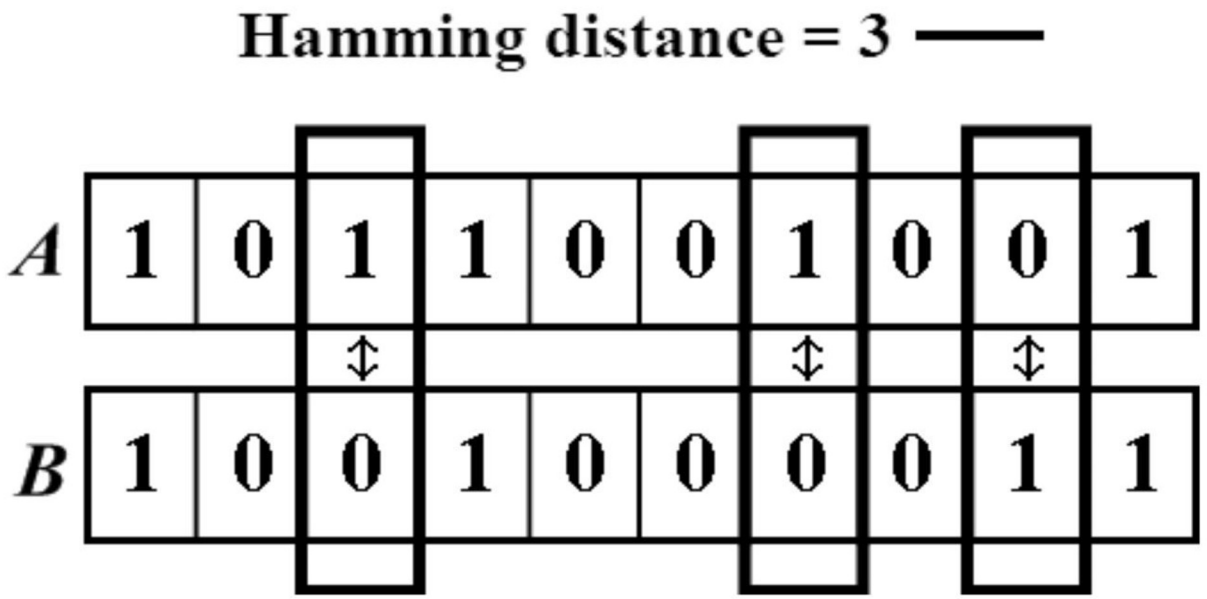
Distance metrics

Continuous variables:

- Euclidean distance: Root squared error
- Mahalanobis distance (Normalized Euclidean Distance)
- Manhattan distance

Categorical variable:

- Hamming distance (number of mismatches)



Common clustering approaches

- Hierarchical
- K-means
- Many more…
  - e.g., Spectral clustering for networks

Hierarchical Clustering

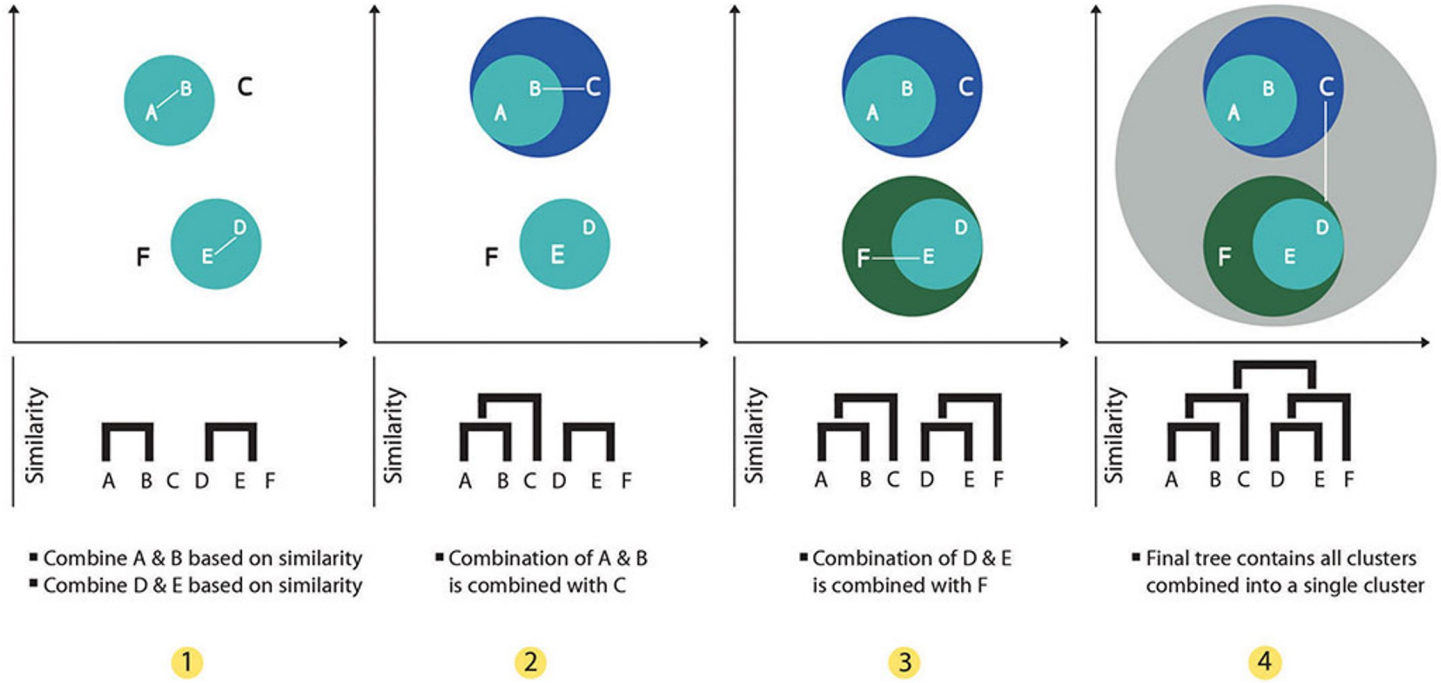
Steps:

- Build dendrogram
- Choose cut point (based on dendrogram or K)

Hierarchical Clustering

Steps:

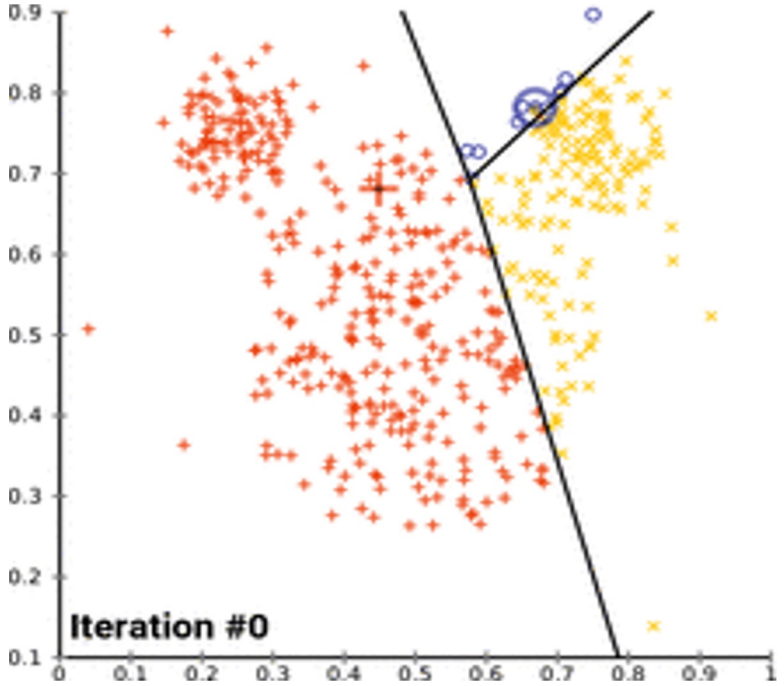
- Build dendrogram
- Choose cut point (based on dendrogram or K)



K-Means Clustering

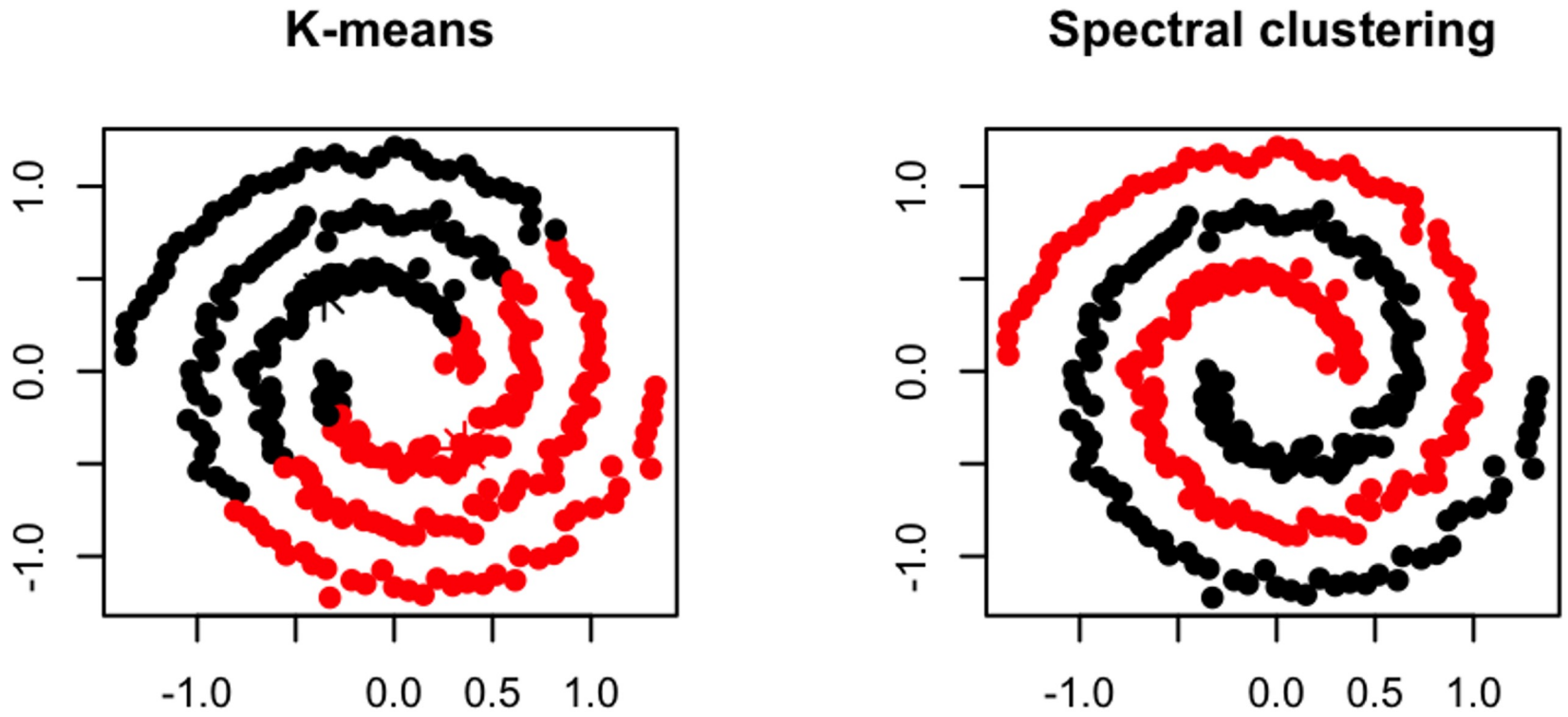
How it works:

- Choose number of clusters: k
- Set random cluster centers (“centroid”)
- For each point:
  - find closest centroid
  - assign it to that cluster
- Recompute the new centroid for each cluster
- Repeat until centroids stop moving (convergence)



Spectral Clustering

- Commonly used for networks/graphs
- Operates on pairwise sample similarity ("adjacency")



Deciding on the number of clusters

- Arbitrarily cutting the dendrogram (by eye)
- Silhouette statistic
- Dunn Index
- Connectivity

Measured in the ***clValid*** package

We will do this with ***hclust( )*** in the lab

And others…

Silhouette width

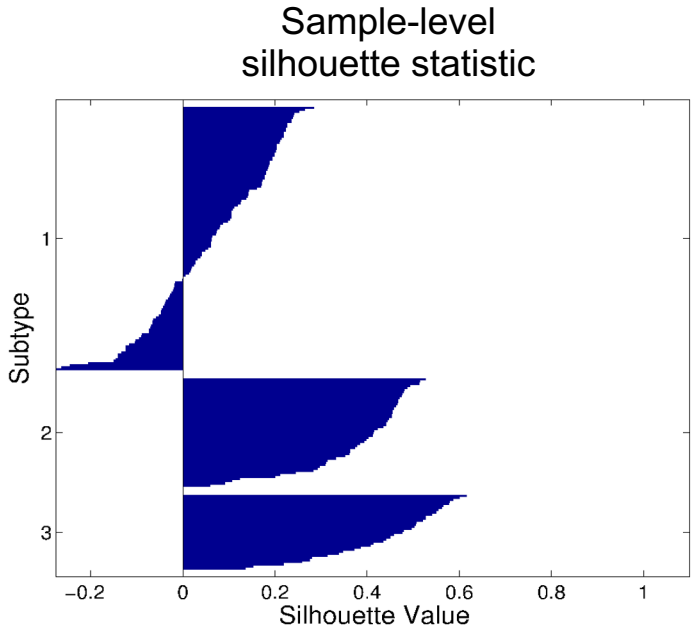
On average, how similar is a sample to its assigned cluster, compared to other clusters.

Requires identified clusters.

average distance to samples in nearest neighbouring cluster

average distance to within-cluster samples

$$S(i) = \frac{b_i - a_i}{max(b_i, a_i)}$$



Worst cluster separation

Best cluster separation

-1 0 +1

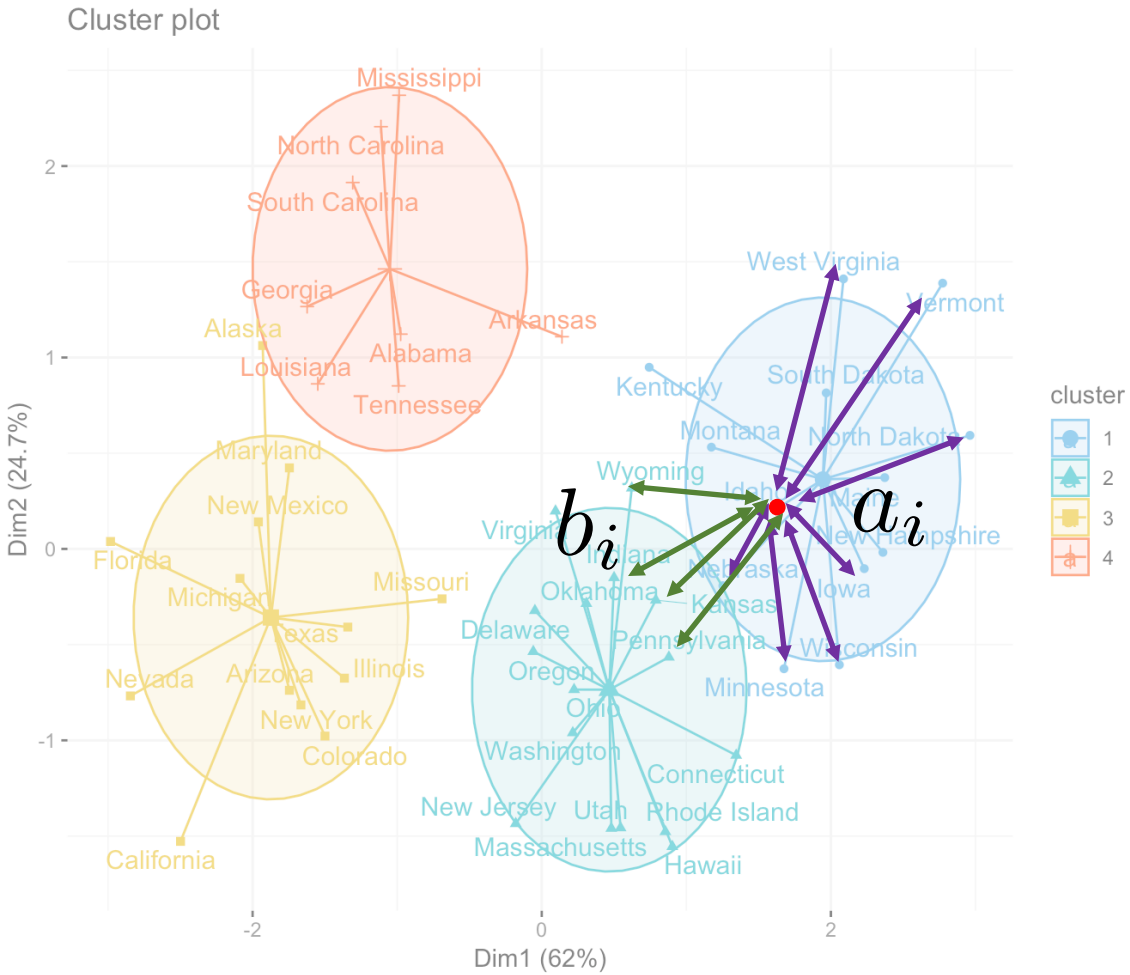


Image source: https://www.datanovia.com/en/lessons/k-means-clustering-in-r-algorith-and-practical-examples/

Dunn Index

Dunn Index

The Dunn Index is the ratio of the smallest distance between observations not in the same cluster to the largest intra-cluster distance. It is computed as

$$D(\mathcal{C}) = \frac{\min\limits_{C_k, C_l \in \mathcal{C}, C_k \neq C_l} \left(\min\limits_{i \in C_k, j \in C_l} dist(i,j) \right)}{\max\limits_{C_m \in \mathcal{C}} diam(C_m)},$$

where $diam(C_m)$ is the maximum distance between observations in cluster C_m. The Dunn Index has a value between zero and ∞, and should be maximized.

* Similar to silhouette width. Want to maximize

clValid, an R package for cluster validation

Connectivity

Connectivity

Let N denote the total number of observations (rows) in a dataset and M denote the total number of columns, which are assumed to be numeric (e.g., a collection of samples, time points, etc.). Define $nn_{i(j)}$ as the jth nearest neighbor of observation i, and let $x_{i,nn_{i(j)}}$ be zero if i and j are in the same cluster and $1/j$ otherwise. Then, for a particular clustering partition $\mathcal{C} = \{C_1, \ldots, C_K\}$ of the N observations into K disjoint clusters, the connectivity is defined as

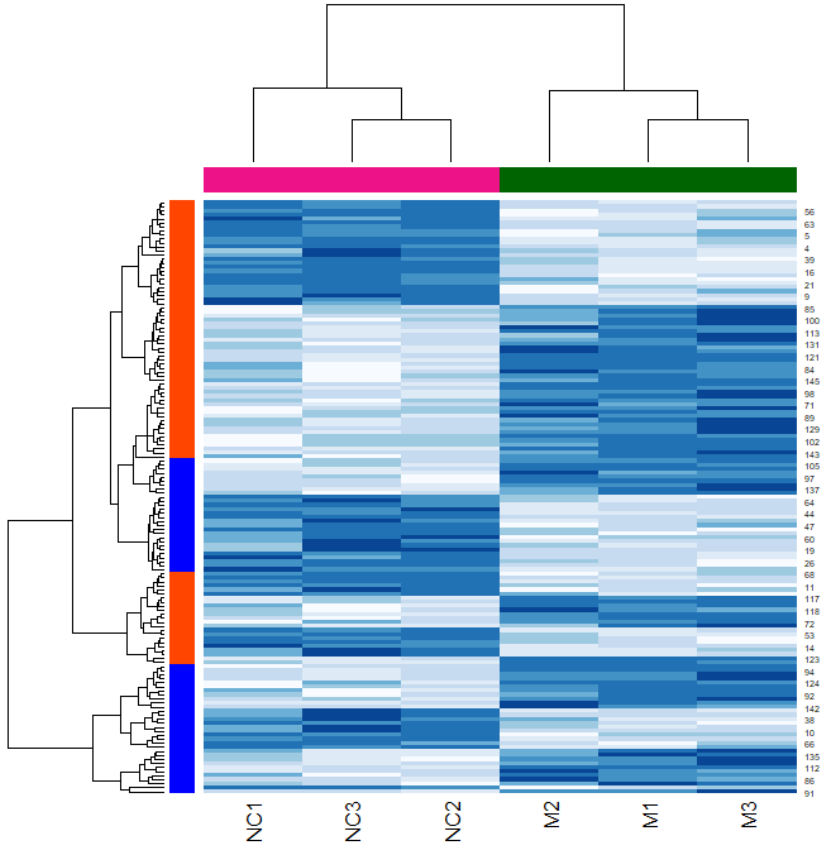
$$Conn(\mathcal{C}) = \sum_{i=1}^{N} \sum_{j=1}^{L} x_{i,nn_{i(j)}} \ ,$$

where L is a parameter giving the number of nearest neighbors to use. The connectivity has a value between zero and ∞ and should be minimized.

* Counts what fraction of nearest neighbours are not in the same cluster. Note: Should be minimized.

clValid, an R package for cluster validation

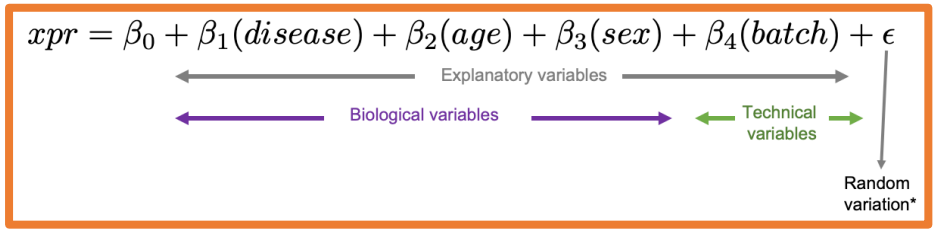
Clustering



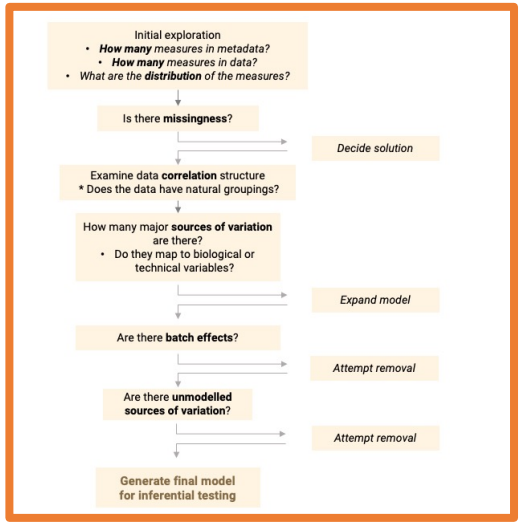
- Find groups in your data
- Requires a distance
- Multiple clustering methods exist, pick one appropriate to your application.
- Measure goodness of clustering with silhouette, Dunn score or connectivity

Let’s recap!

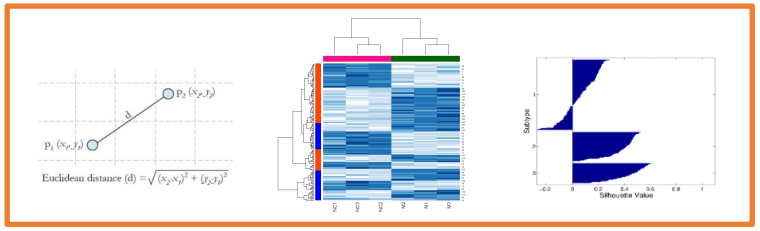
Main points:



The goals of exploratory data analysis are to identify major sources of variation and co-variation, and identify outliers / missing data



Perform exploratory data analysis in a systematic way using an approach like the one on the left.



Clustering can be used to find natural groupings in data. It requires a distance metric. Clustering can be validated with metrics.

Let’s look at how to achieve EDA and clustering using R.

We are on a Coffee Break & Networking Session

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