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# Know Your Data: Exploratory Data Analysis

Shraddha Pai

Analysis Using R

June 28-29, 2023

bioinformatics.ca

OICR
Ontario Institute for Cancer Research

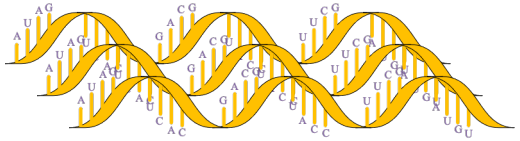
UNIVERSITY OF TORONTO

# Learning Objectives

- By the end of this lecture, you will:
  - Be able to define response variables, explanatory variables, and name broad sources of variation in your data
  - Know how to structure data exploration to systematically identify (un)wanted sources of variation
  - Appreciate the value of exploring missingness in your data
  - Have a high level understanding of clustering and be able to cluster your data

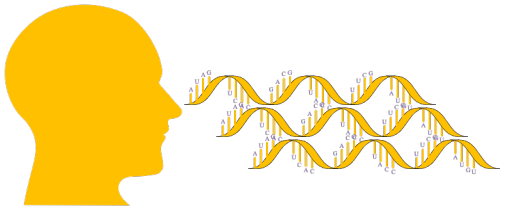
# Studies and sources of variation

Goal: “Find transcriptomic biomarkers of disease”



# Studies and sources of variation

Goal: “Find transcriptomic biomarkers of disease”



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

Response variable

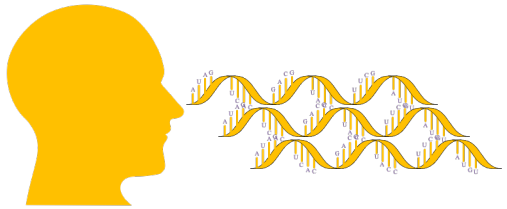
Dependent variable

Explanatory variable

Independent variable

Residual; unmodelled variation

# Studies and sources of variation



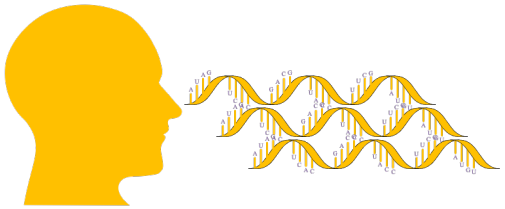
coefficients; weights

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

Intercept

# Studies and sources of variation

Goal: “Find transcriptomic biomarkers of disease”



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

Response variable

Dependent variable

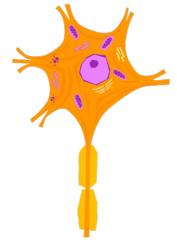
Explanatory variable

Independent variable

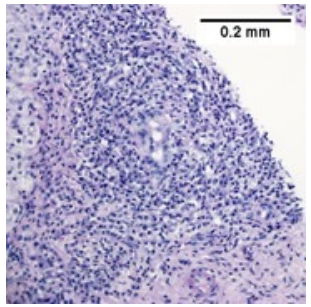
Residual; unmodelled variation

# Studies and sources of variation

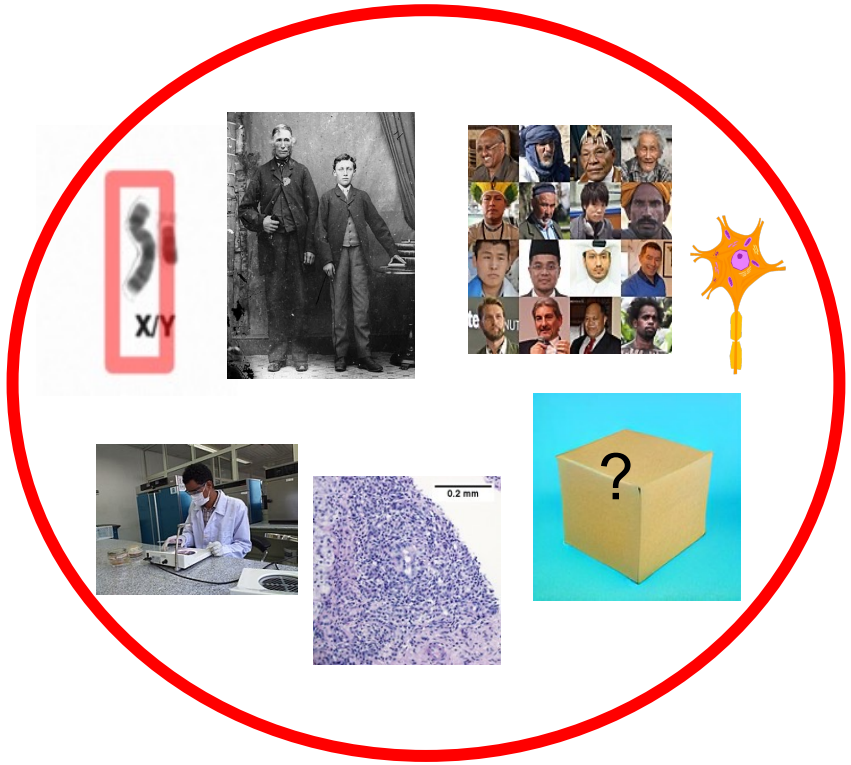
## **Biological** sources of variation

## **Technical** sources of variation




*"Which of these is affecting my data?"*



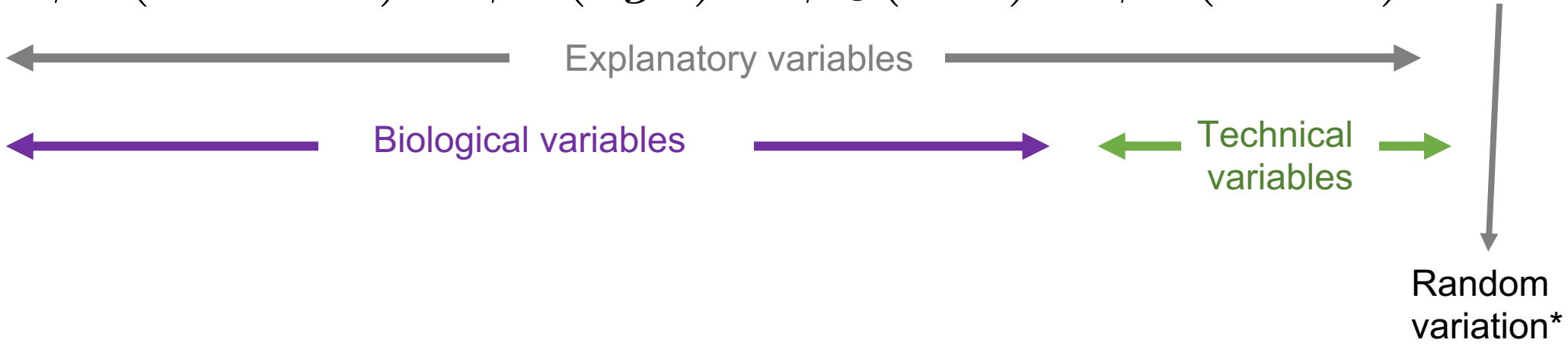

Visualize & quantify sources of variation:

- clustering
- dimensionality reduction (PCA, UMAP, tSNE)

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

# Final model

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




- Values drawn 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)
- Data pooled from multiple sources, not all collected a particular set of measures
- Multi-'omic data, some participants missing an assay

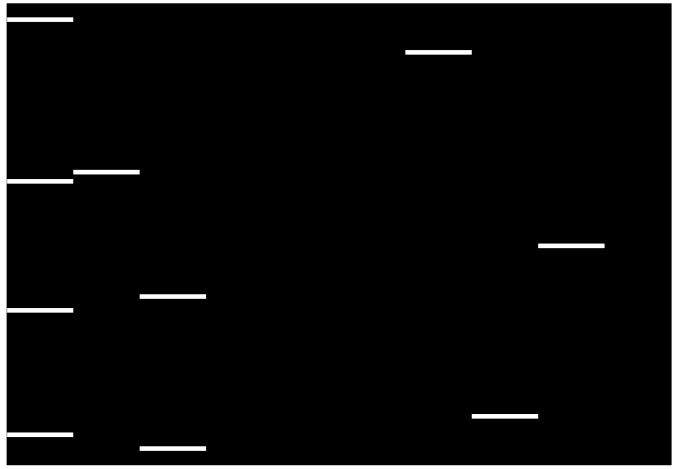
Solutions:

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

What are trade-offs in each solution?

Checkered view of data table. White is 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

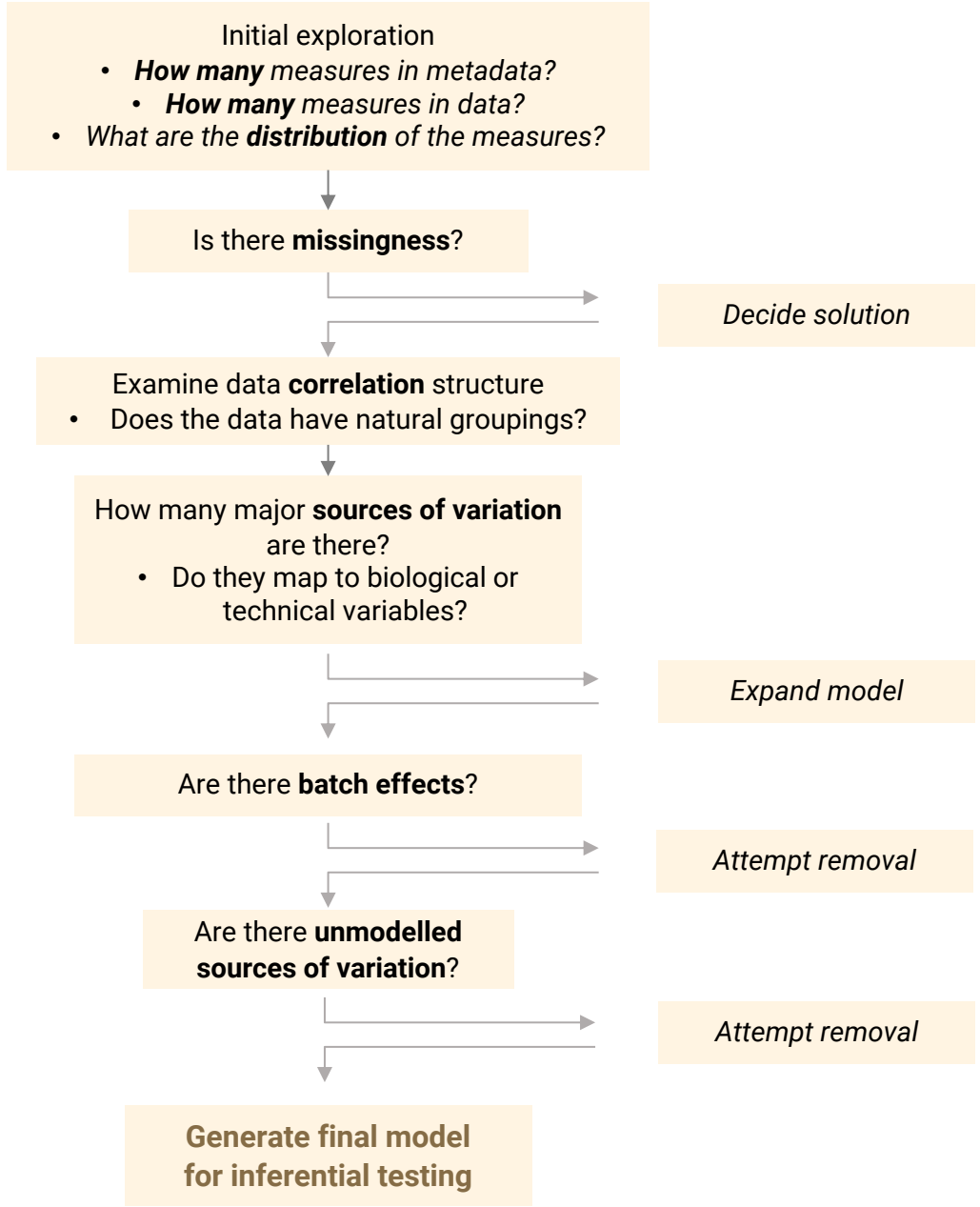
# Goals of exploratory data analysis are to:

| Goal | Tool | Action |
| --- | --- | --- |
| 1. Identify magnitude of KNOWN biological and technical variation | PCA, clustering, prior knowledge | Add terms to model |

# EDA workflow

measures
(e.g., gene-level expression, base-level DNA methylation, voxels)

sample information
("metadata")



# EDA workflow

measures

sample information ("metadata")

dim( )
head( )
summary( )
str( ), plots

Initial exploration
- ***How many*** *measures in metadata?*
- ***How many*** *measures in data?*
- *What are the* ***distribution*** *of the measures?*

count NA
visualize

Is there **missingness**?

*Decide solution*

clustering

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

dimensionality reduction

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

*Expand model*

clustering, dimensionality reduction

Are there **batch effects**?

*Attempt removal*

ComBAT
RUVseq*

surrogate variable analysis*

Are there **unmodelled sources of variation**?

*Attempt removal*

**Generate final model for inferential testing**

linear models, GLM, edgeR, etc.,

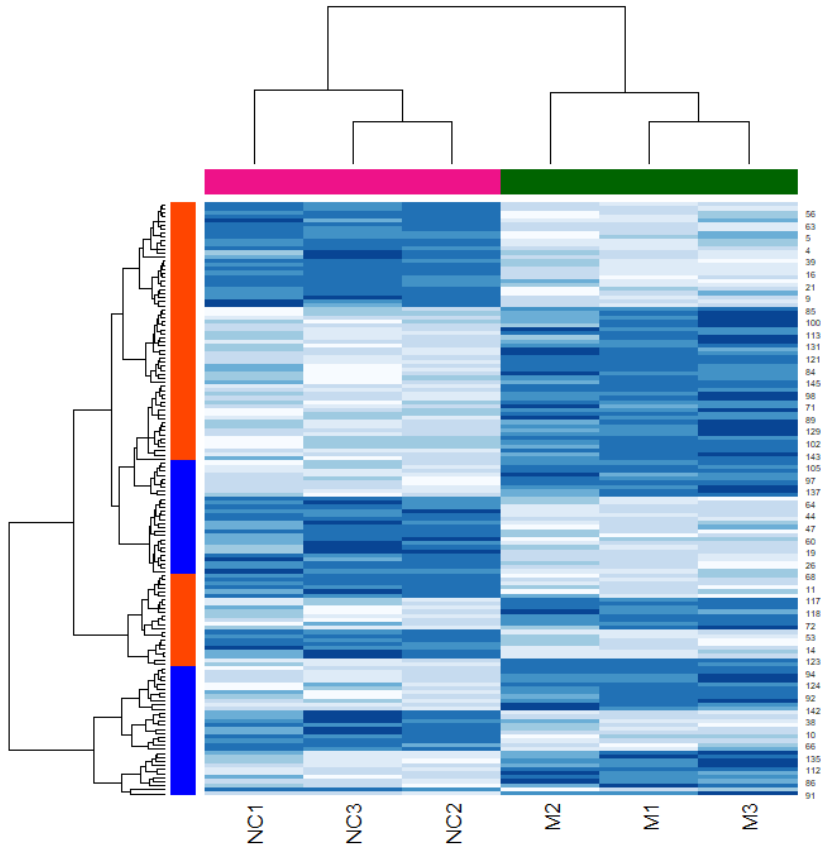
** Not covered in this workshop*

# 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



# Distance metric

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.

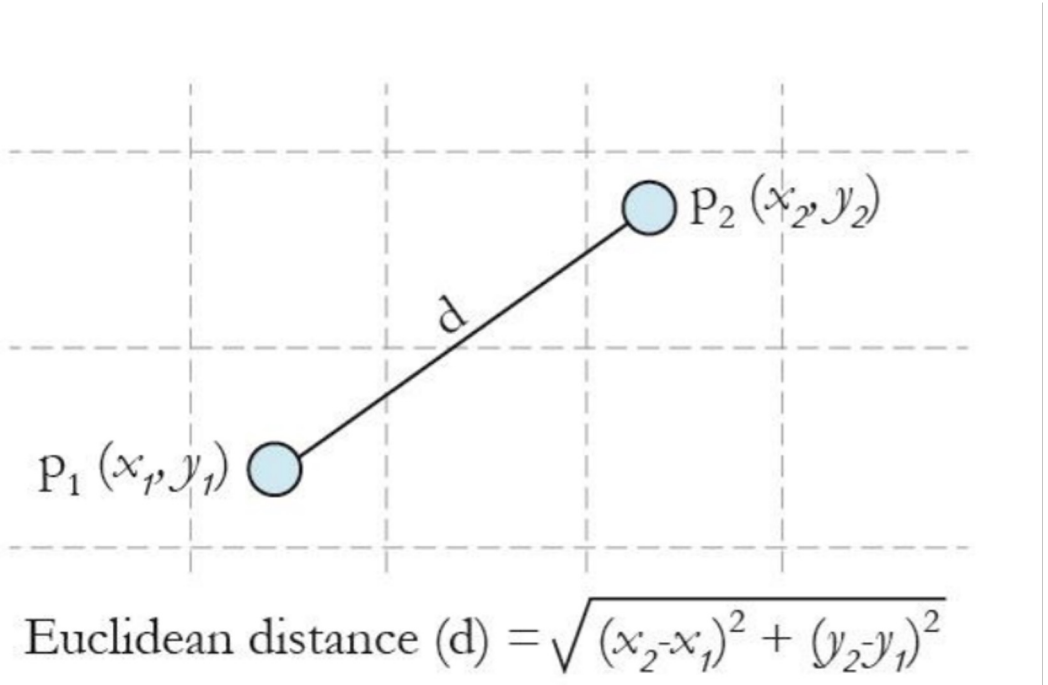
Related term: "similarity"

# Distance metrics

Continuous variables:

- Euclidean distance: Root squared error

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



# Distance metrics

Continuous variables:

- Euclidean distance: Root squared error

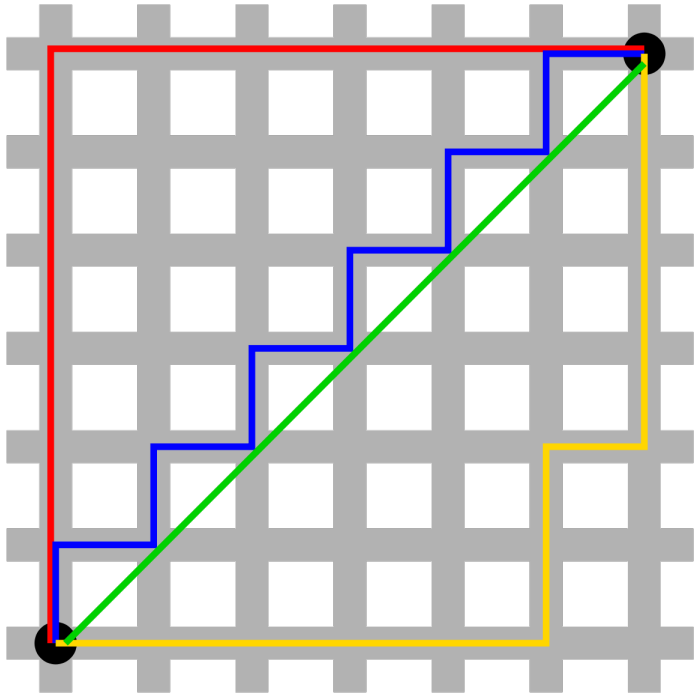
- Mahalanobis distance
  - Euclidean distance that builds in the covariance in the data

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

# Distance metrics

Continuous variables:

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



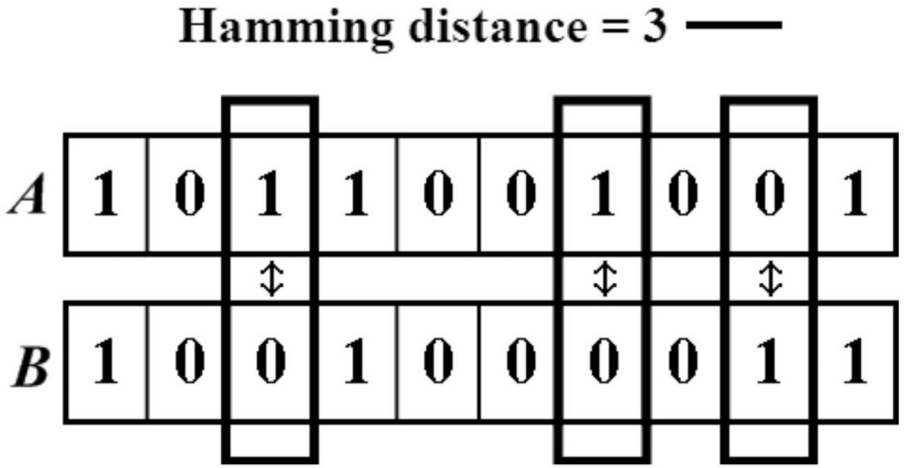
# Distance metrics

Continuous variables:

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

Categorical variable:

- Hamming distance (number of mismatches)



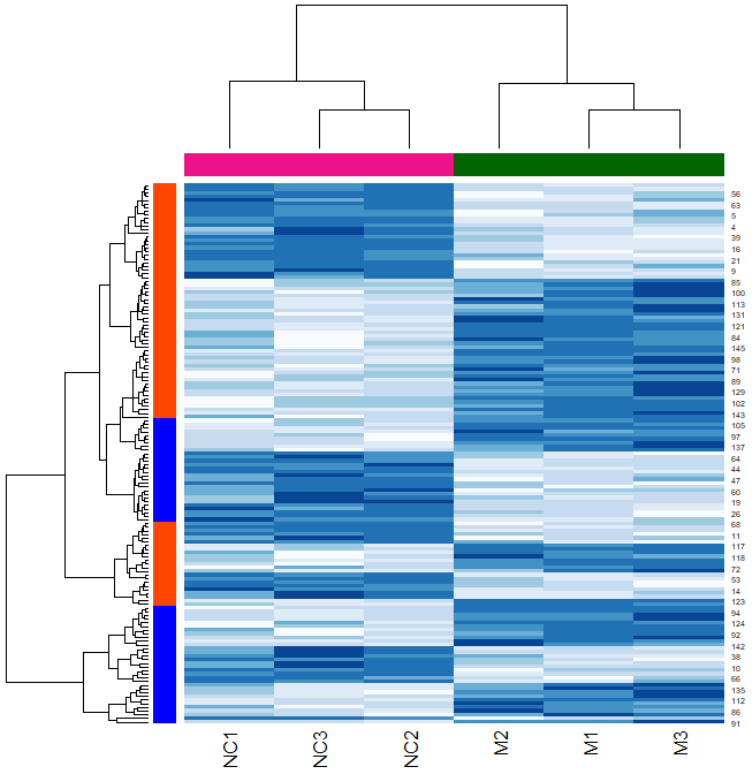
# Common clustering approaches

- 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
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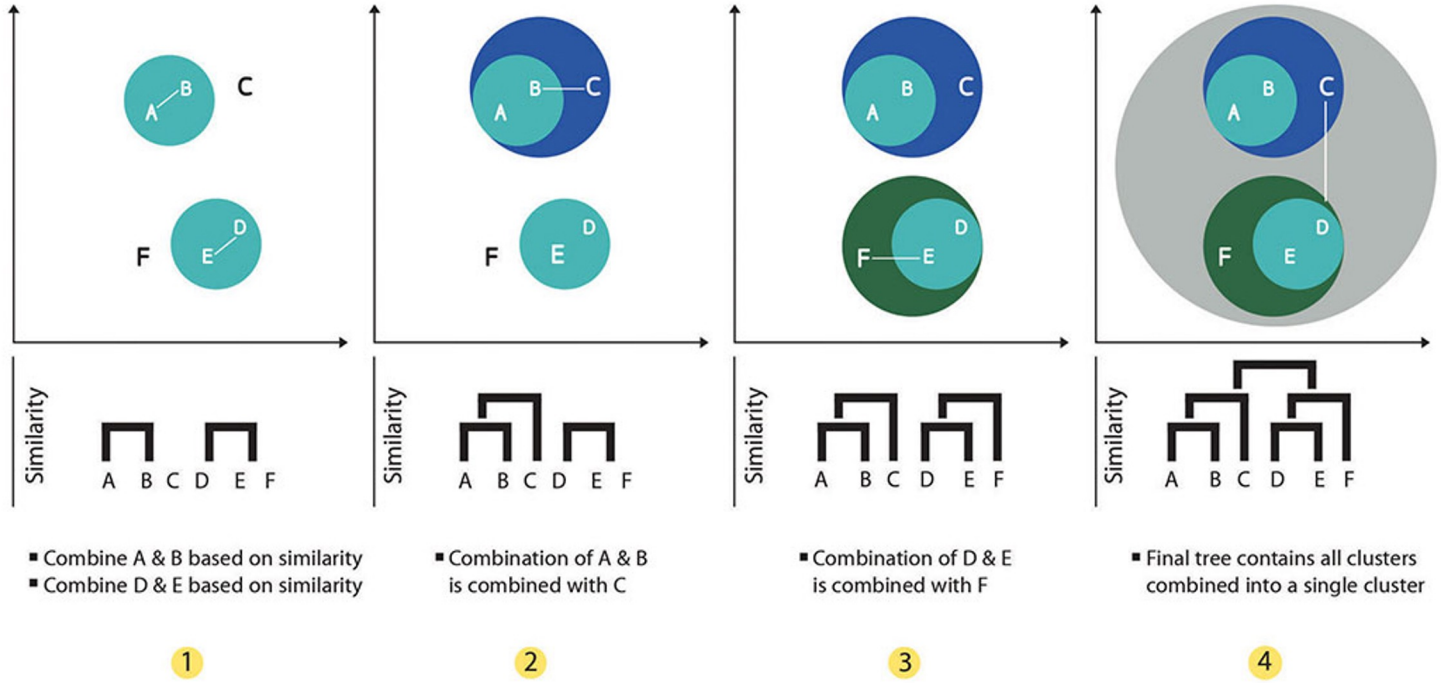
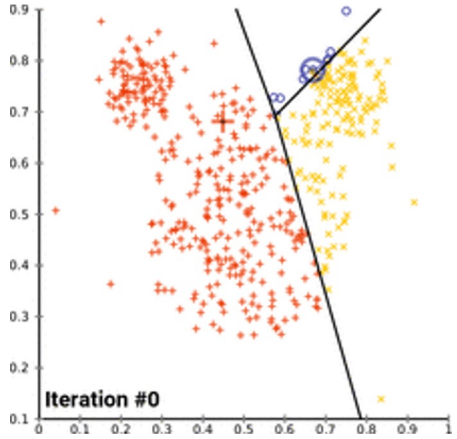


# K-Means

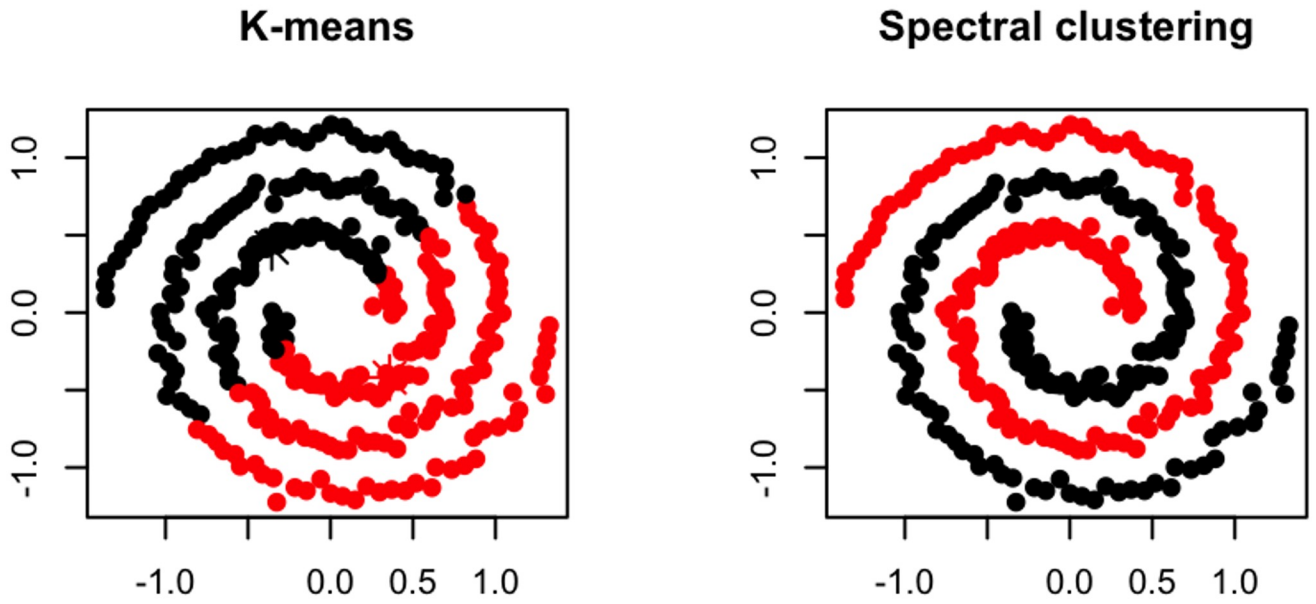
How it works:

- 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

We will do this with hclust in the lab

Measured in the clValid package

And others…

# Silhouette width

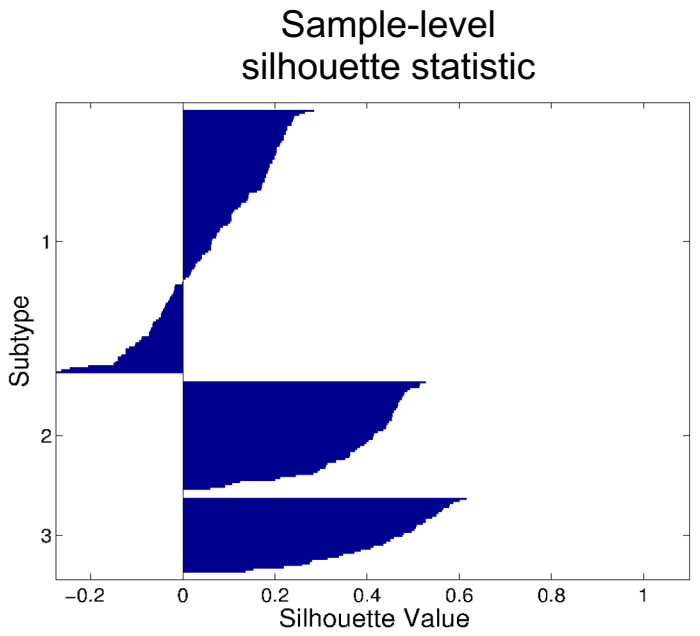
On average, how similar is a sample to its assigned cluster, compared to other clusters.

Requires identified clusters.

**average distance to nearest-neighbour-cluster samples**

**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

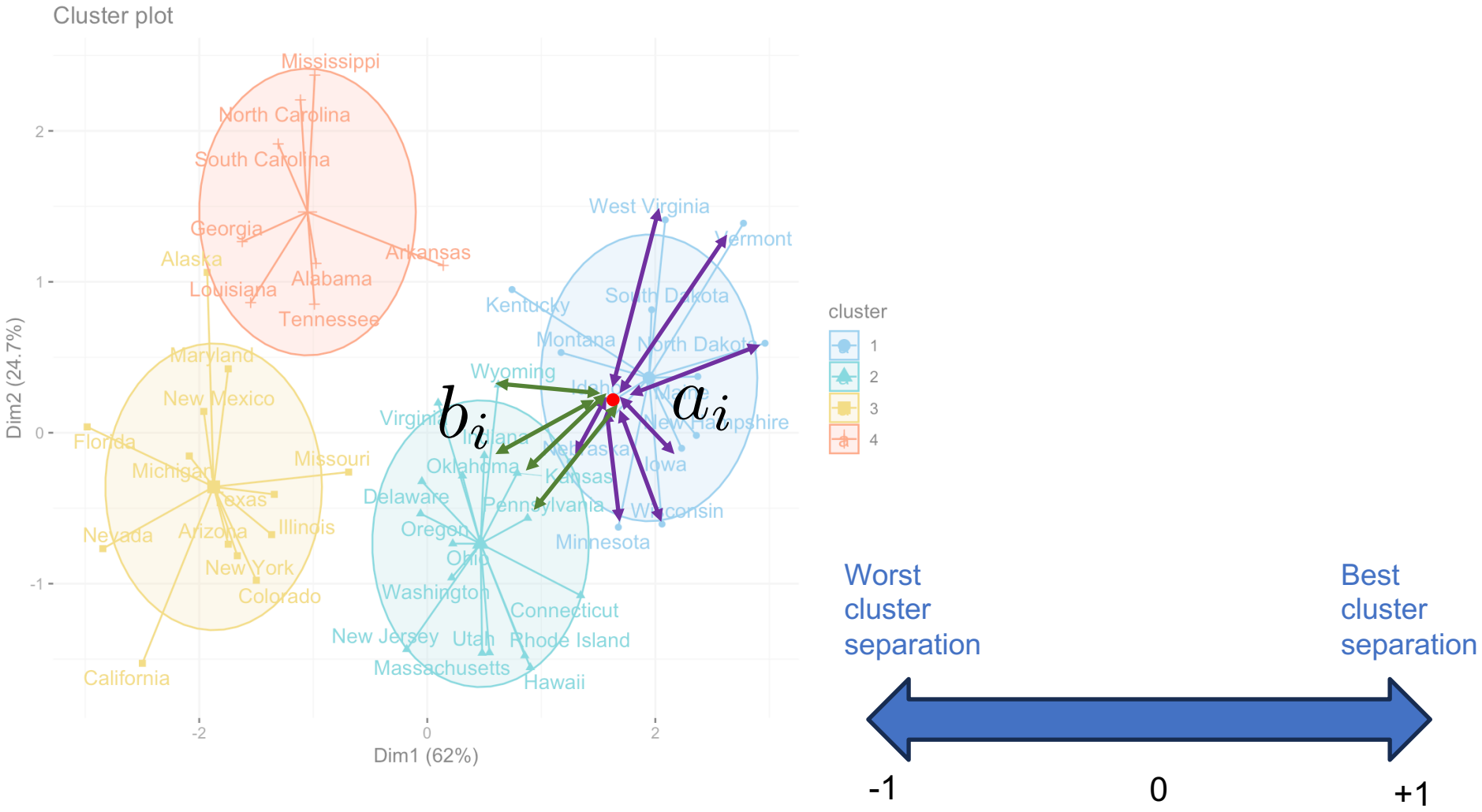



Image source: https://www.datanovia.com/en/lessons/k-means-clustering-in-r-algorith-and-practical-examples/

# 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 $\infty$, 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 $j$th 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 $\infty$ 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

# Let’s recap!

# Take home

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

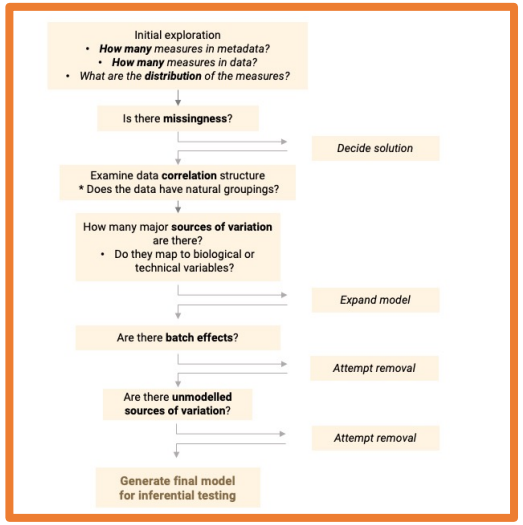
Explanatory variables

Biological variables

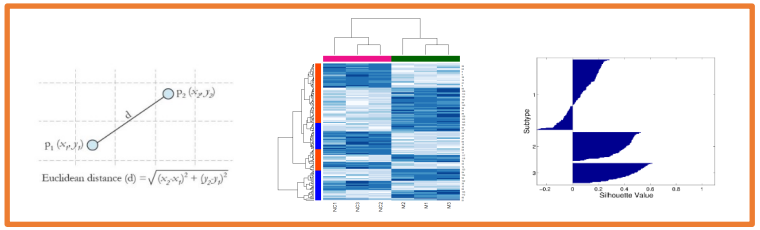
Technical variables

Random variation*

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




EDA can be structured using a systematic 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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