

# Exploratory data analysis for microarray data

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# Outline

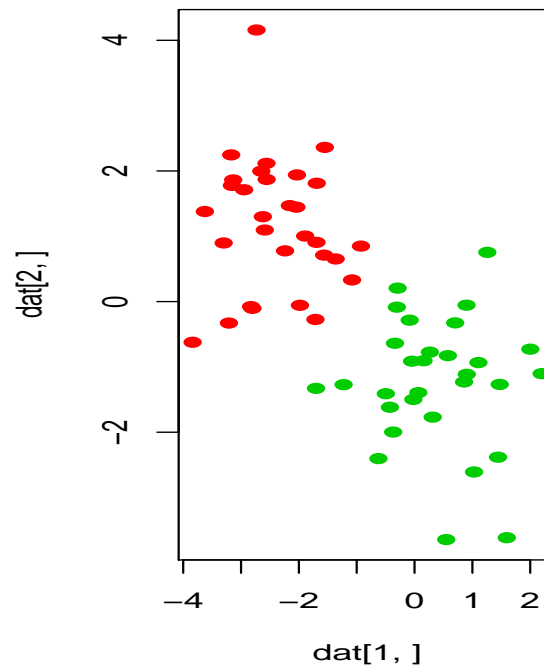
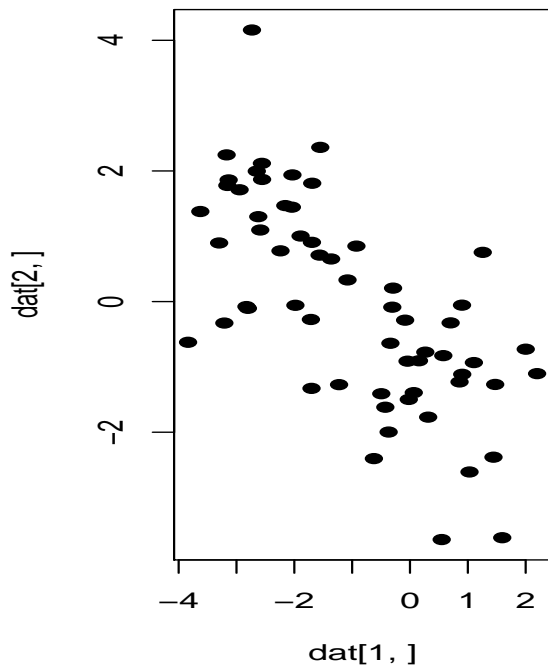
- Goals
- Cluster analysis
  - Distance measures
  - Clustering methods
- Projection methods
- Class discovery

# Exploratory data analysis/unsupervised learning

- “Look at the data”; identify structures in the data and visualize them.
- Can we see biological/experimental parameters; are there outliers?
- Find groups of genes and/or samples sharing similarity.
- Unsupervised learning: The analysis makes no use of gene/sample annotations.

# Clustering

Aim: Group objects according to their similarity.



# Clustering gene expression data

- Clustering can be applied to rows (genes) and/or columns (samples/arrays) of an expression data matrix.
- Clustering may allow for reordering of the rows/columns of an expression data matrix which is appropriate for visualization (heatmap).

# Clustering genes

Aims:

- identify groups of co-regulated genes
- identify typical spatial or temporal expression patterns (e.g. yeast cell cycle data)
- arrange a set of genes in a linear order which is at least not totally meaningless

# Clustering samples

Aims:

- detect experimental artifacts/bad hybridizations (quality control)
- check whether samples are grouped according to known categories (meaning that these are clearly visible in terms of gene expression)
- identify new classes of biological samples (e.g. tumor subtypes)

# Clustering: Distance measures

- Aim: Group objects according to their similarity.
- Clustering requires a definition of distance between the objects, quantifying a notion of (dis)similarity. After this has been specified, a clustering algorithm may be applied.
- The result of a cluster analysis may strongly depend on the chosen distance measure.



# Metrics and distances

A **metric**  $d$  is a function satisfying:

1. non-negativity:  $d(a, b) \geq 0$ ;
2. symmetry:  $d(a, b) = d(b, a)$ ;
3.  $d(a, a) = 0$ .
4. definiteness:  $d(a, b) = 0$  if and only if  $a = b$ ;
5. triangle inequality:  $d(a, b) + d(b, c) \geq d(a, c)$ .

A function only satisfying 1.-3. is called a **distance**.

# Distance measures: Examples

Vectors  $\mathbf{x} = (x_1, \dots, x_n)$ ,  $\mathbf{y} = (y_1, \dots, y_n)$

○ Euclidean distance:  $d_M(\mathbf{x}, \mathbf{y}) = \sqrt{\sum_{i=1}^n (x_i - y_i)^2}$

○ Manhattan distance:  $d_E(\mathbf{x}, \mathbf{y}) = \sum_{i=1}^n |x_i - y_i|$

○ One minus Pearson correlation:

$$d_C(\mathbf{x}, \mathbf{y}) = 1 - \frac{\sum_{i=1}^n (x_i - \bar{x})(y_i - \bar{y})}{(\sum_{i=1}^n (x_i - \bar{x})^2)^{1/2} (\sum_{i=1}^n (y_i - \bar{y})^2)^{1/2}}$$

## Distance measures/standardization

- The **correlation distance** is invariant wrt shifting and scaling of its arguments:

$$d_C(\mathbf{x}, \mathbf{y}) = d_C(\mathbf{x}, a\mathbf{y} + b), a > 0.$$

- One may apply **standardization** to observations or variables:

$$\mathbf{x} \mapsto \frac{\mathbf{x} - \bar{\mathbf{x}}}{\sigma(\mathbf{x})},$$

- The correlation distance and the Euclidean distance between standardized vectors are closely related:

$$d_E(\mathbf{x}, \mathbf{y}) = \sqrt{2nd_C(\mathbf{x}, \mathbf{y})}.$$

## Distances between clusters

Extend a distance measure  $d$  to a measure of distance between clusters.

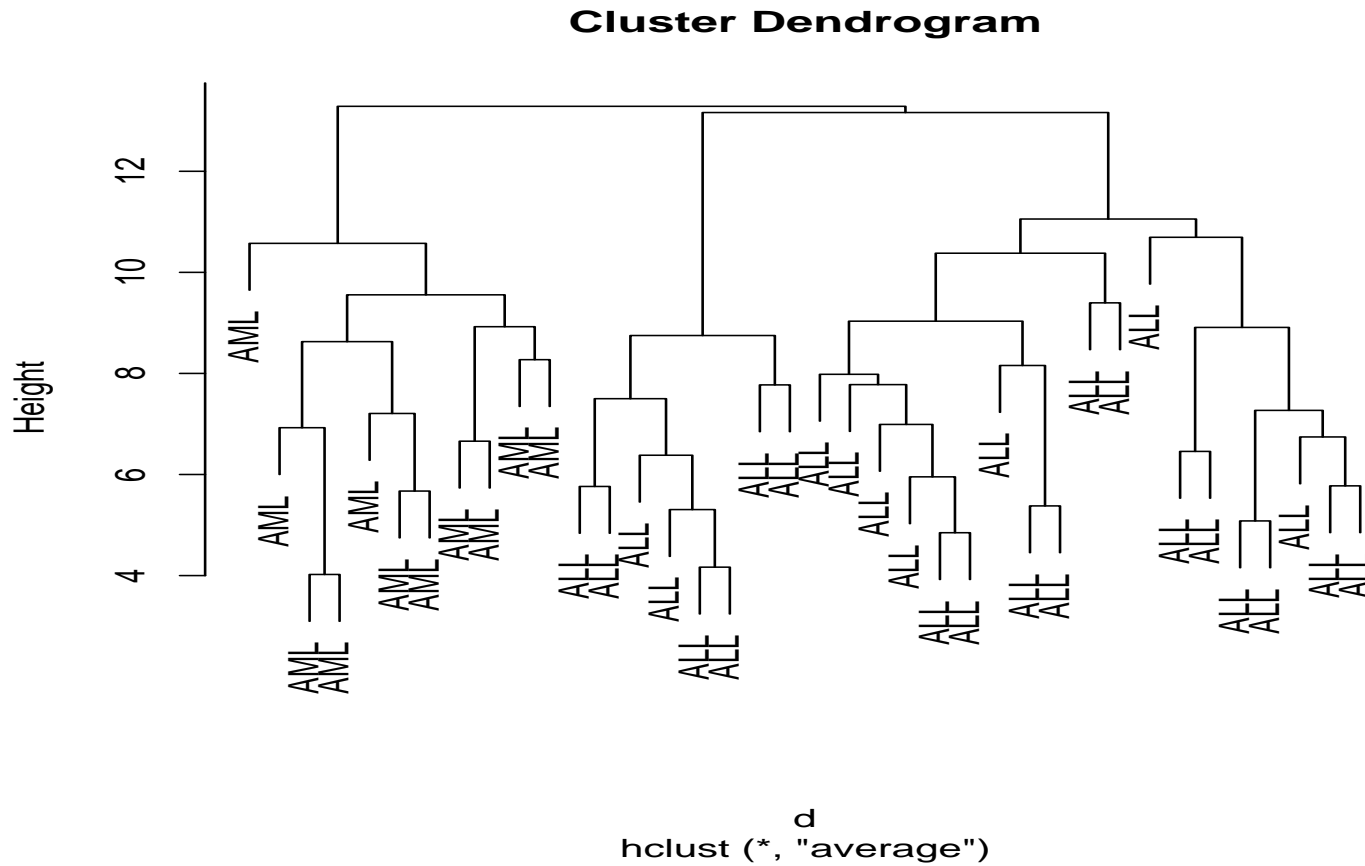
- **Single linkage** The distance between two clusters is the minimal distance between two objects, one from each cluster.
- **Average linkage** The distance between two clusters is the average of the pairwise distance between members of the two clusters.
- **Complete linkage** The distance between two clusters is the maximum of the distances between two objects, one from each cluster.
- **Centroid linkage** The distance between two clusters is the distance between their *centroids*.

# Hierarchical clustering

- Build a cluster tree/dendrogram, starting from the individual objects as clusters.
- In each step, merge the two clusters with the minimum distance between them - using one of the above linkage principles.
- Continue until everything is in one cluster.
- If you want a partition of the set of objects, cut the tree at a certain height.
- R function `hclust` in package `mva`.

# Hierarchical clustering, example

Golub data, 150 genes with highest variance



# k-means clustering

- User specifies the number  $k$  of desired clusters. Input: Objects given as vectors in  $n$ -dimensional space (Euclidean distance is used).
- For an initial choice of  $k$  cluster centers, each object is assigned to the closest of the centers.
- The centroids of the obtained clusters are taken as new cluster centers.
- This procedure is iterated until convergence.

## How many clusters?

- Many methods require the user to specify the number of clusters. Generally it is not clear which number is appropriate for the data at hand.
- Several authors have proposed criteria for determining the number of clusters, see Dudoit and Fridlyand 2002.
- Sometimes there may not be a clear answer to this question - there may be a hierarchy of clusters.



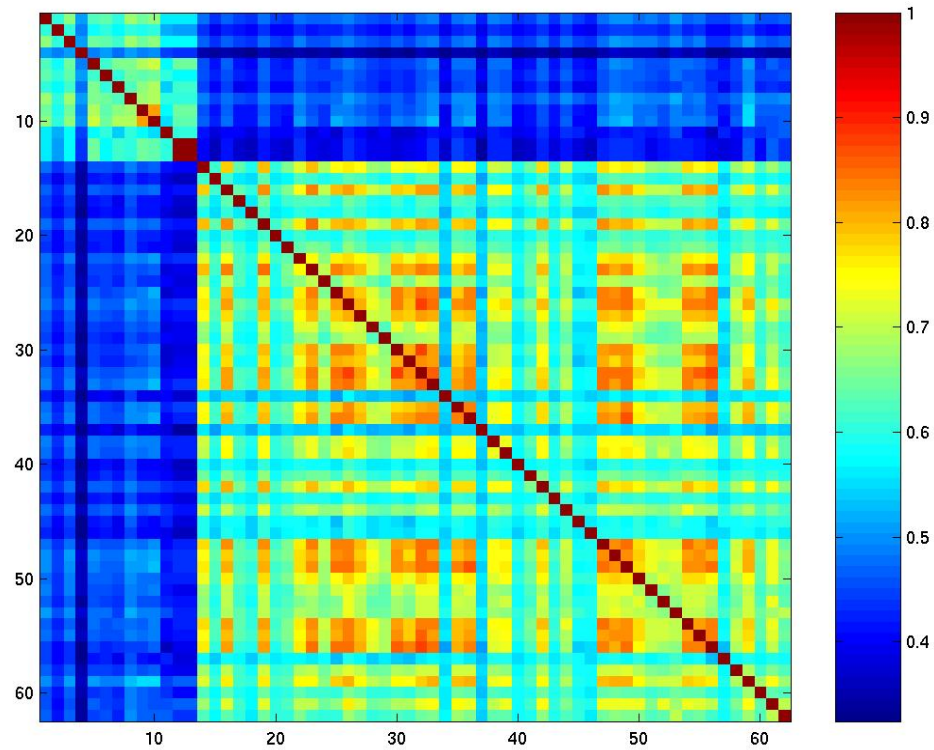
## Which scale, which distance measure to use for clustering?

- Data should be normalized and transformed to appropriate scale before clustering (log or generalized log (R package `vsN`)).
- Clustering genes: Standardization of gene vectors or the use of the correlation distance is useful when looking for patterns of relative changes - independent of their magnitude.
- Clustering samples: Standardizing genes gives relatively smaller weight for genes with high variance across the samples - not generally clear whether this is desirable.
- Gene filtering (based on intensity/variability) may be reasonable - also for computational reasons.

## Some remarks on clustering

- A clustering algorithm will always yield clusters, whether the data are organized in clusters or not.
- The bootstrap may be used to assess the variability of a clustering (Kerr/Churchill 2001, Pollard/van der Laan 2002).
- If a class distinction is not visible in cluster analysis, it may still be accessible for supervised methods (e.g. classification).

# Visualization of similarity/distance matrices

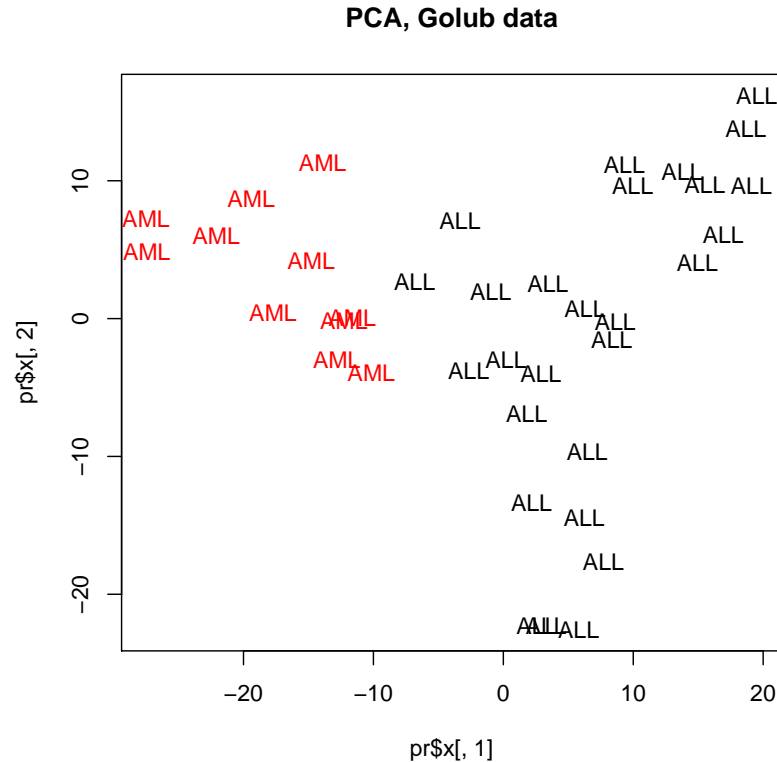


Matrix of correlation coefficients, rows/columns ordered by **array batch**.

# Projection methods

○ Map the rows and/or columns of the data matrix to a plane such that similar rows/columns are located close to each other.

○ Different methods (principal component analysis, multidimensional scaling, correspondence analysis) use different notions of similarity.



## Principal component analysis

- Imagine  $k$  observations (e.g. tissue samples) as points in  $n$ -dimensional space (here:  $n$  is the number of genes).
- Aim: Dimension reduction while retaining as much of the variation in the data as possible.
- Principal component analysis identifies the direction in this space with maximal variance (of the observations projected onto it).
- This gives the first principal component (PC). The  $i + 1$ st PC is the direction with maximal variance among those orthogonal to the first  $i$  PCs.
- The data projected onto the first PCs may then be visualized in scatterplots.

# Principal component analysis

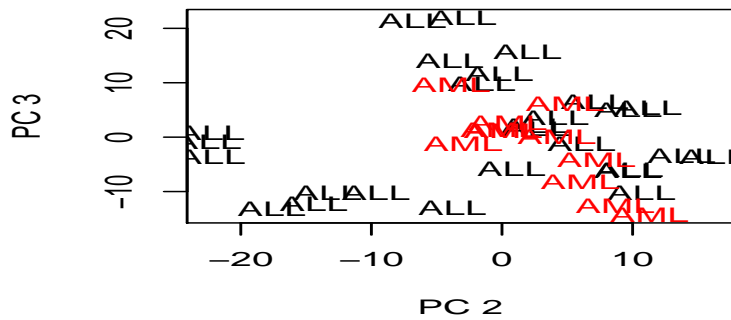
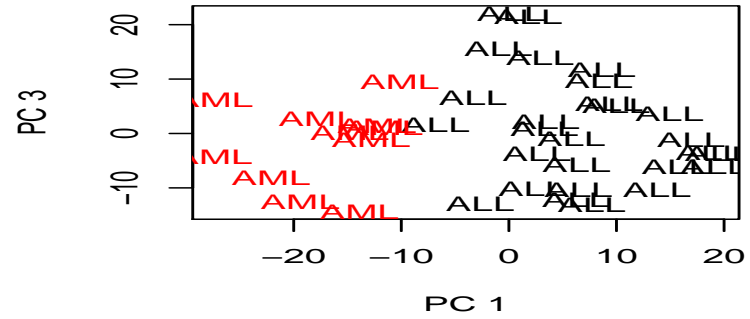
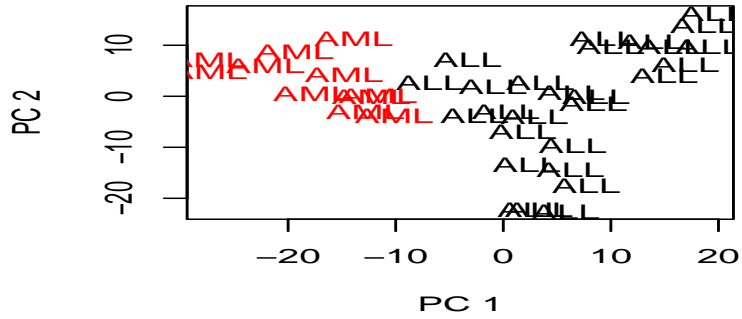
- PCA can be explained in terms of the eigenvalue decomposition of the covariance/correlation matrix  $\Sigma$ :

$$\Sigma = S\Lambda S^t,$$

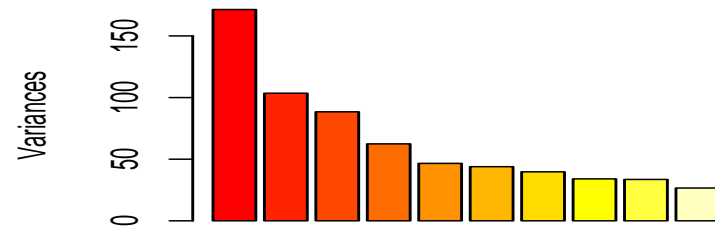
where the columns of  $S$  are the eigenvectors of  $\Sigma$  (the principal components), and  $\Lambda$  is the diagonal matrix with the eigenvalues (the variances of the principal components).

- Use of the correlation matrix instead of the covariance matrix amounts to standardizing variables (genes).
- R function `prcomp` in package `mva`

# PCA, Golub data



variances of PCs



# Multidimensional scaling

○ Given an  $n \times n$  dissimilarity matrix  $D = (d_{ij})$  for  $n$  objects (e.g. genes or samples), multidimensional scaling (MDS) tries to find  $n$  points in Euclidean space (e.g. plane) with a similar distance structure  $D' = (d'_{ij})$  - more general than PCA.

○ The similarity between  $D$  and  $D'$  is scored by a **stress function**.

○ Least-squares scaling:  $S(D, D') = (\sum (d_{ij} - d'_{ij})^2)^{1/2}$ .  
Corresponds to PCA if the distances are Euclidean.

In R: **cmdscale** in package `mva`.

○ Sammon mapping:  $S(D, D') = \sum (d_{ij} - d'_{ij})^2 / d_{ij}$ . Puts more emphasis on the smaller distances being preserved.

In R: **sammon** in package `MASS`.

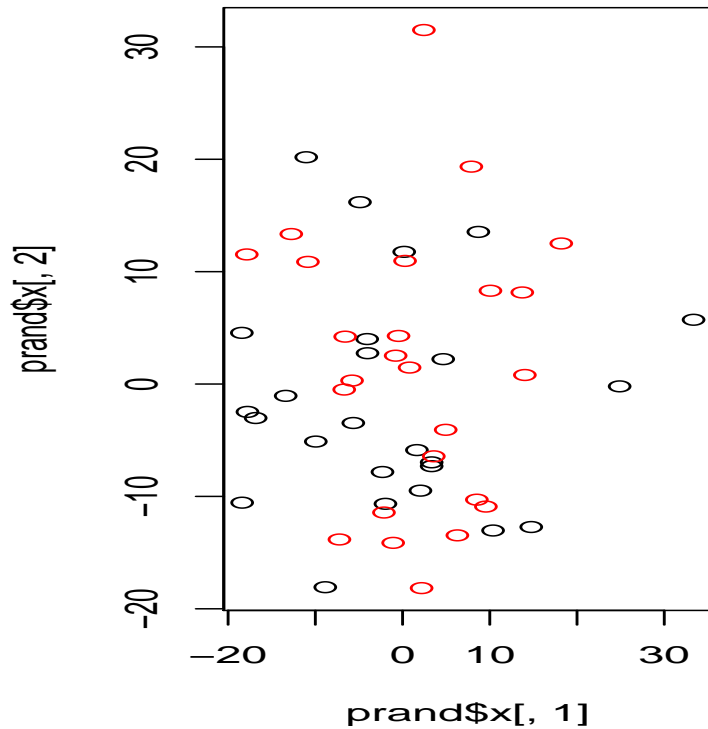


## Projection methods: feature selection

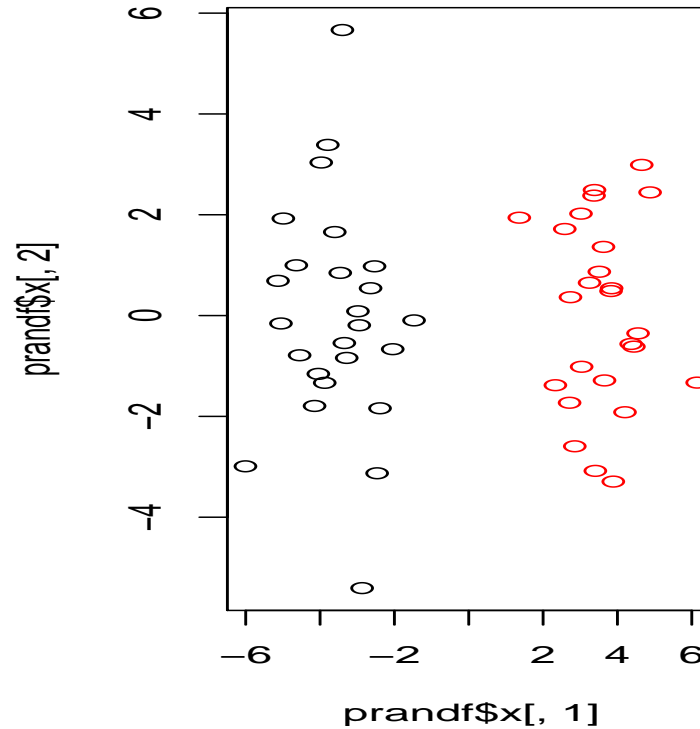
- The results of a projection method also depend on the features (genes) selected.
- If those genes are selected that discriminate best between two groups, it is no wonder if they appear separated.
- This may also happen if there is no real difference between the groups.

# Projection methods: feature selection

PCA, all features

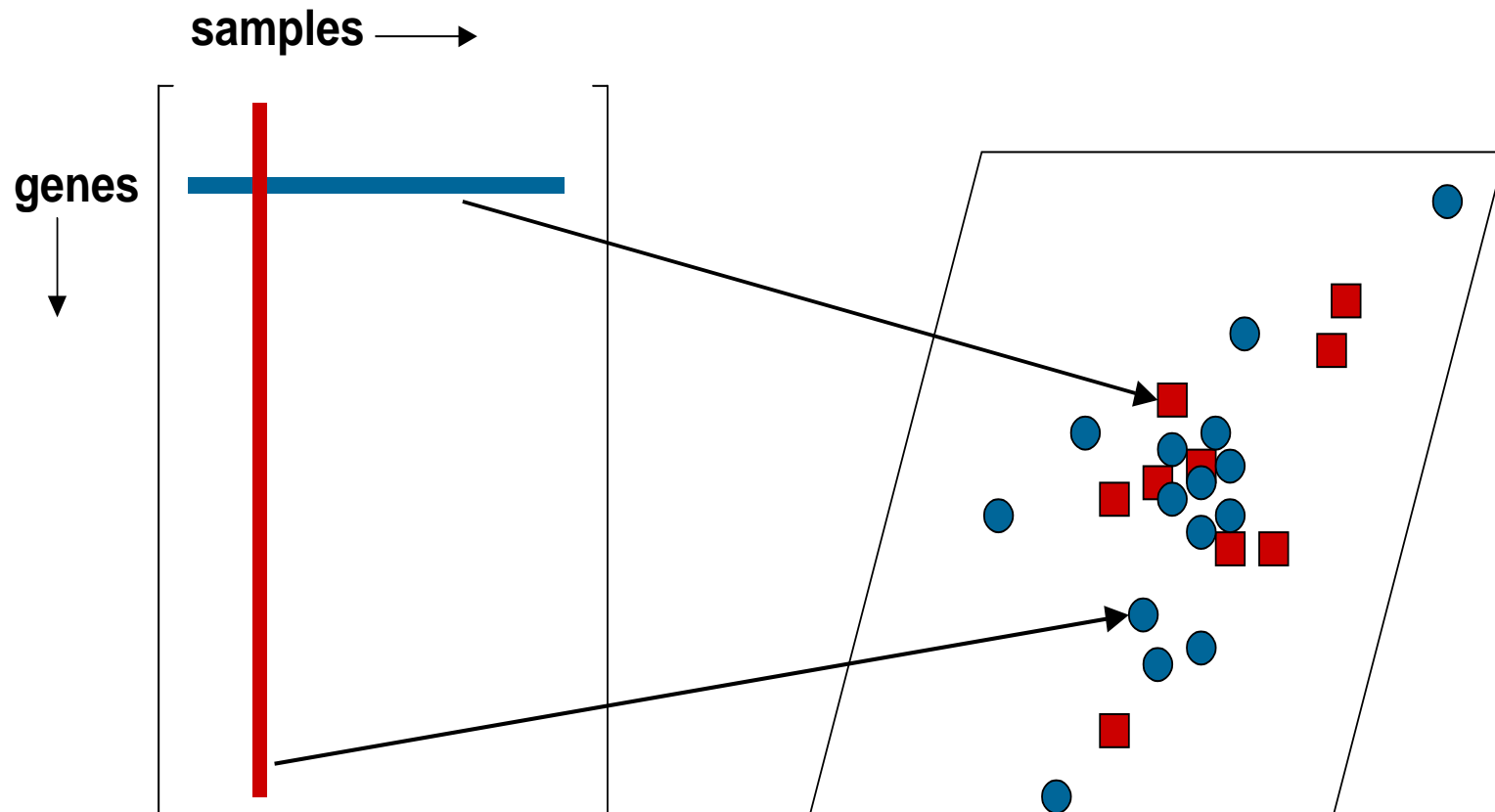


PCA, feature selection



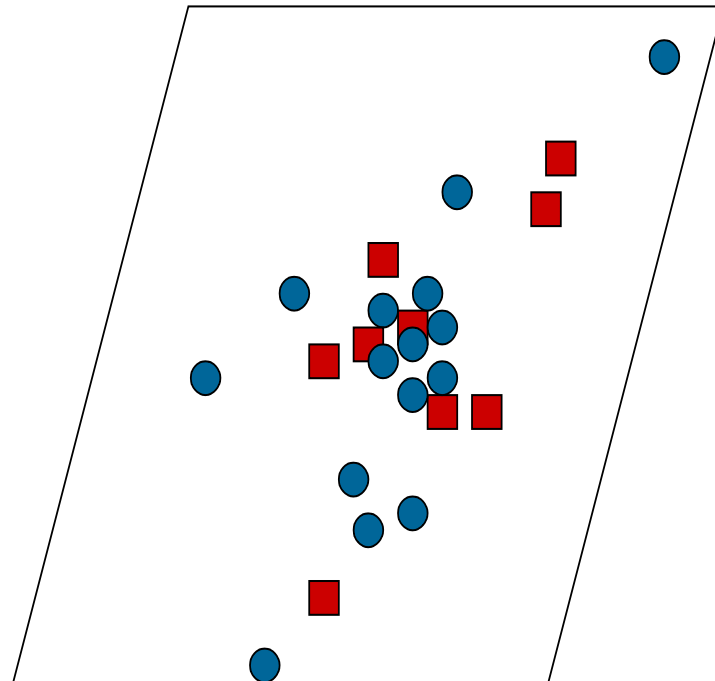
Left: PCA for a 5000 x 50 **random** data matrix. For the right plot, 90 “genes” with best discrimination between red and black (t-statistic).

# Correspondence analysis: Projection onto plane



# Correspondence analysis: Properties of projection

- Similar **row/column** profiles (small  $\chi^2$ -distance) are projected close to each other.
- A **gene** with positive/negative association with a **sample** will lie in the same/opposite direction from the centroid.

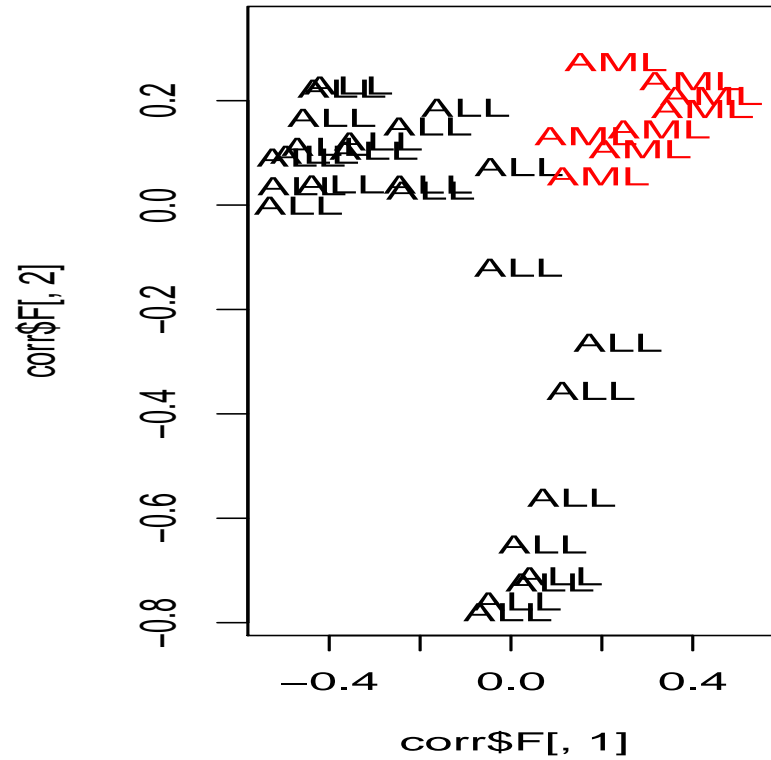
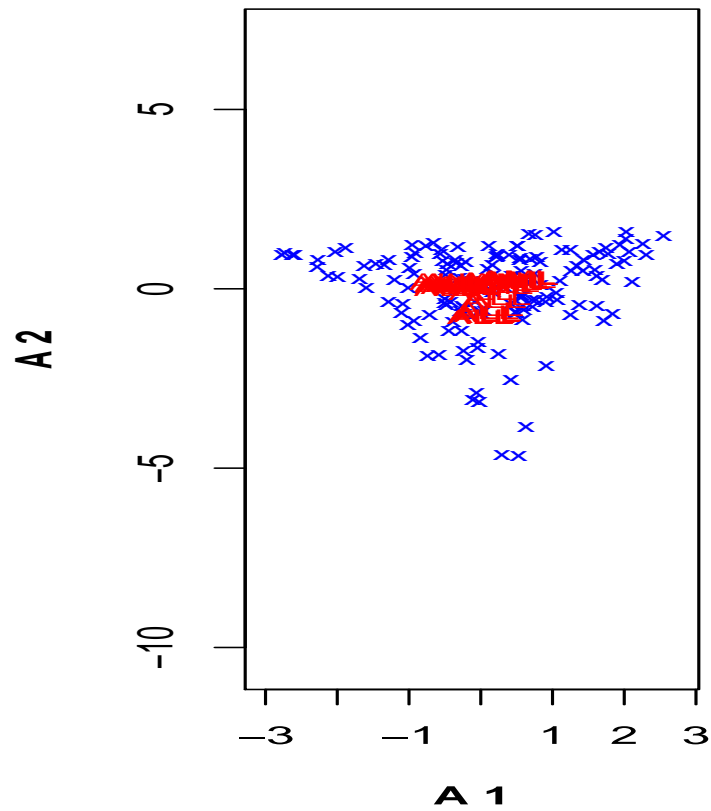


# Projection methods: Correspondence analysis

- Correspondence analysis is usually applied to tables of frequencies (contingency tables) in order to show associations between particular rows and columns – in the sense of deviations from homogeneity, as measured by the  $\chi^2$ -statistic.
- Data matrix is supposed to contain only positive numbers - may apply global shifting to achieve this.
- R packages `CoCoAn`, `multiv`.

# Correspondence analysis - Example

Golub data

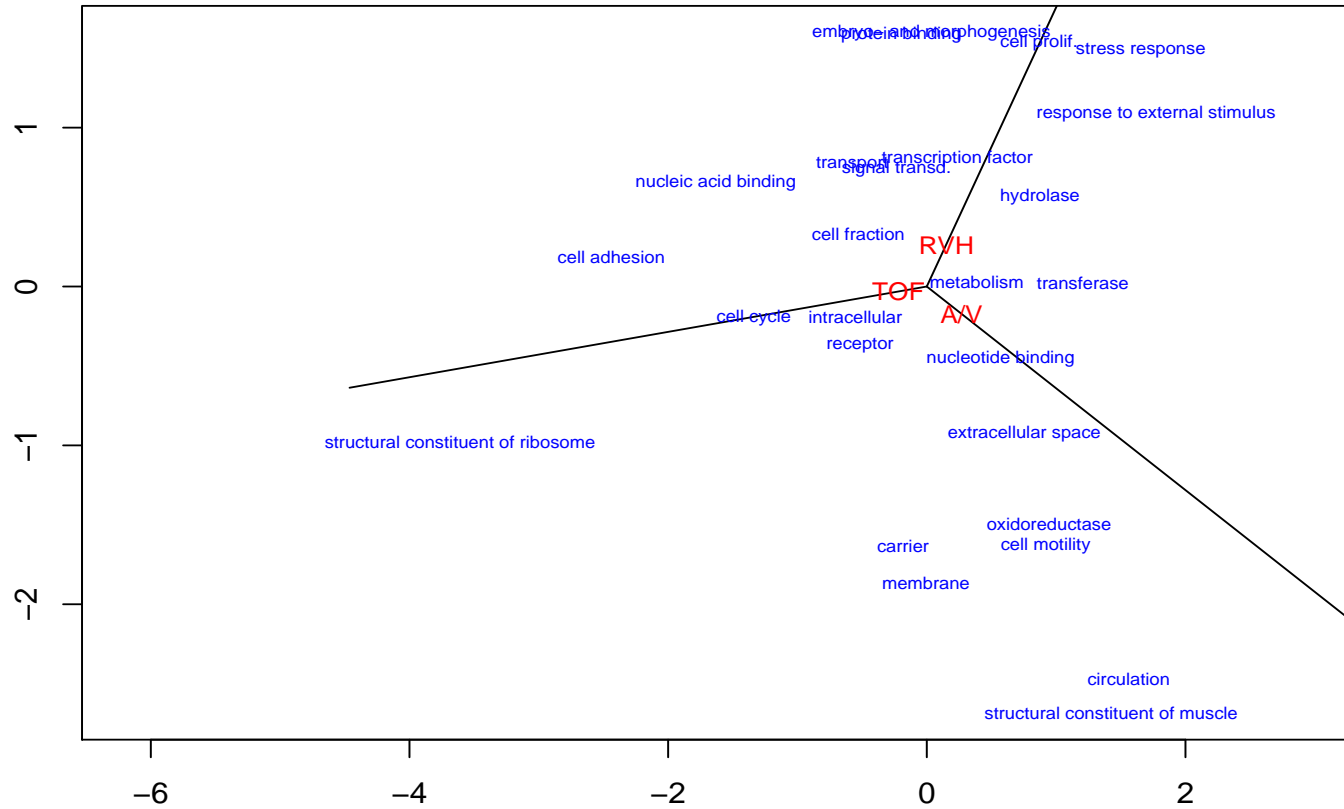


# Contingency table of differentially expressed genes

	right ventricular hypertrophy	tetralogy of Fallot	atrium/ventricle
stress response	11	8	9
constituent of muscle	7	29	20
constituent of ribosome	9	20	8
cell proliferation	7	7	5
signal transduction	14	25	11
metabolism	38	66	44
cell motility	5	12	12
...	...	...	...

# Correspondence analysis

Association between Gene Ontology categories and tissue/disease phenotypes





# ISIS - a class discovery method

- Aim: detect subtle class distinctions among a set of tissue samples/gene expression profiles (application: search for disease subtypes)
- Idea: Such class distinctions may be characterized by differential expression of just a small set of genes, not by global similarity of the gene expression profiles.
- The method quantifies this notion and conducts a search for interesting class distinctions in this sense.
- R package ISIS available at <http://www.molgen.mpg.de/~heydebre>

# References

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