Statistical

Microarray Data Analysis

Clustering and Visualization

國立陽明大學生物資訊研究所
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吳漢銘
hmwu@stat.sinica.edu.tw
http://www.sinica.edu.tw/~hmwu

中央研究院 統計科學研究所
Institute of Statistical Science, Academia Sinica

Outlines

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- Distance and Similarity Measure
- K-Means Clustering
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- Clustering Analysis and Visualization
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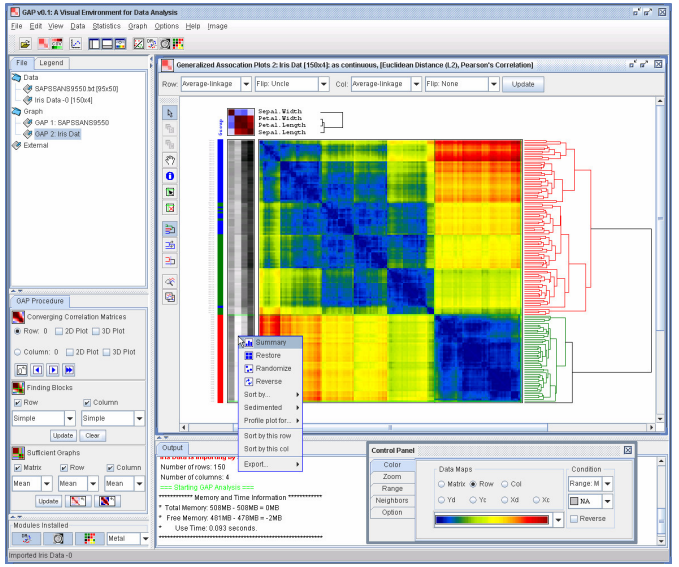
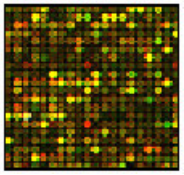
  - Hierarchical Clustering
  - QT (Quality Threshold) Clustering
- Choosing the Number of Clusters
- Generalized Association Plots (GAP)
- Isomap
- Software

GAP: Demo



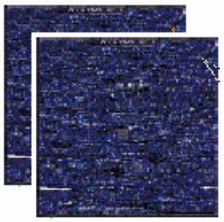
Overview of Microarray Experiment



cDNA Microarray Data

	A	B	C	D
1	UNIQID	Gene Name Description	Array 1	Array 2
2	588029	588029:Hs.79:ACY1	0.645	0.375
3	190929	190929:Hs.247565:RHO	0.615	0.210
4	246550	246550:Hs.293548	0.585	0.665
5	32553	32553:Hs.101248	0.825	0.230
6	446172	446172:	0.570	0.495
7	417978	417978:Hs.268874	0.495	1.835
...				
12000	366879	366879:Hs.169341	1.835	0.300

log2(Cy5/Cy3)

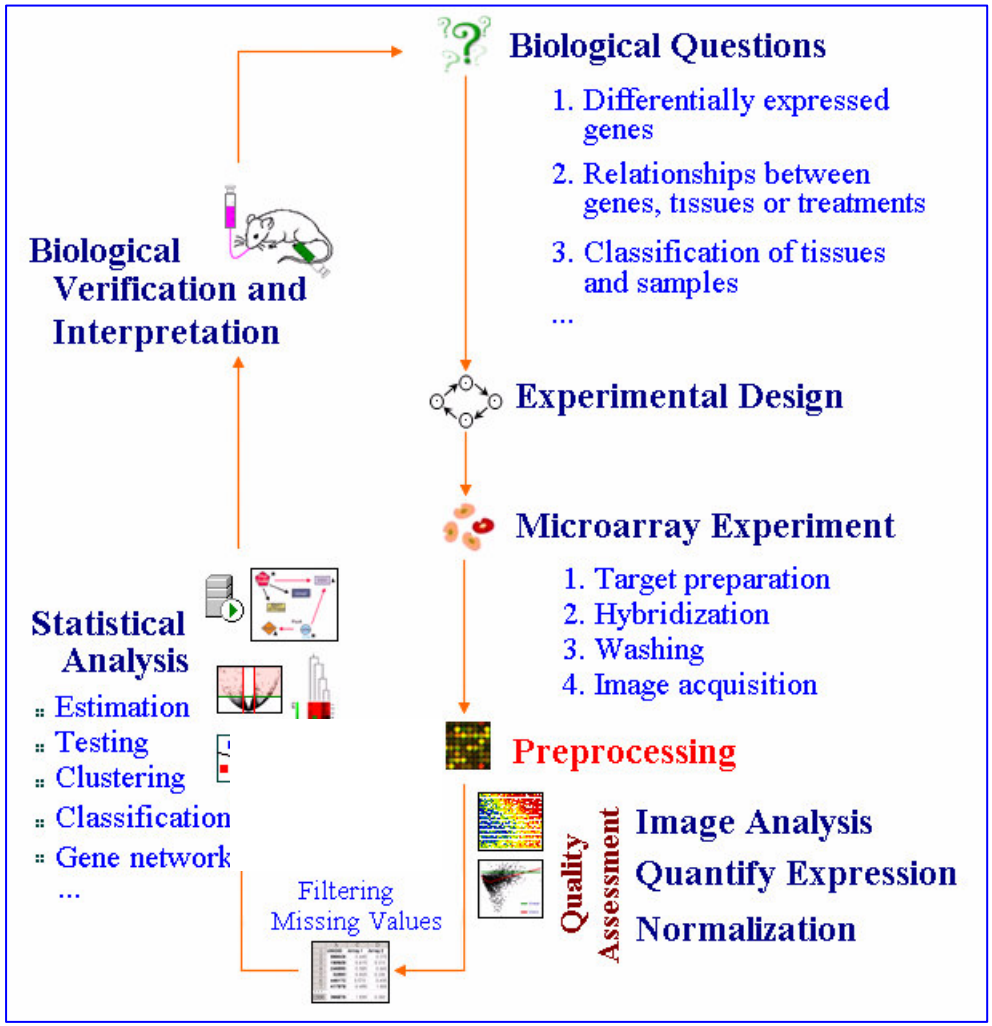


Oligonucleotide Array Data

	A	B	C	D
1	Probeset	Gene Name	Array 1	Array 2
2	103941_at	alpha-spectin 1, erythroid	33.7625	29.2333
3	104432_at	aplysia ras-related homolog N (Rh	127.736	99.6895
4	104137_at	ATP-binding cassette, sub-family A	109.522	65.2727
5	98458_at	baculoviral IAP repeal-containing 5	128.96	123.371
6	93243_at	bone morphogenetic protein 7	174.85	174.019
7	95061_at	breast carcinoma amplified sequer	34.8	43.6696
...				
12600	102632_at	calmodulin binding protein 1	69.888	54.7391

Expression index

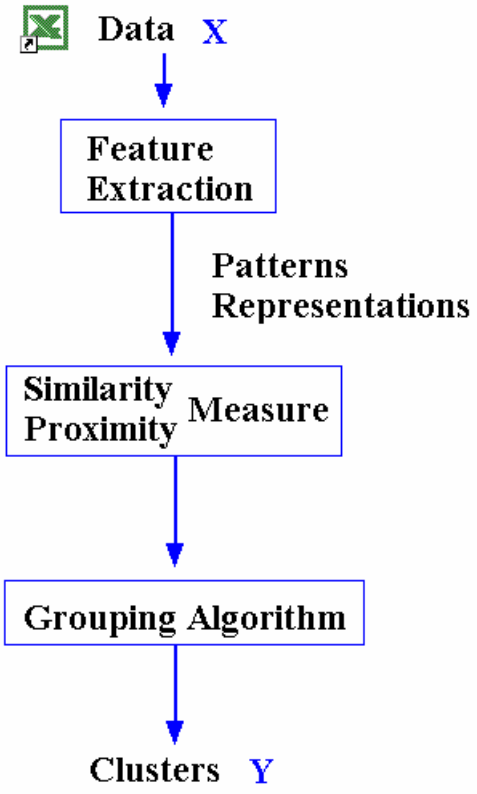
Microarray Life Cycle



Clustering Analysis (Unsupervised Learning)

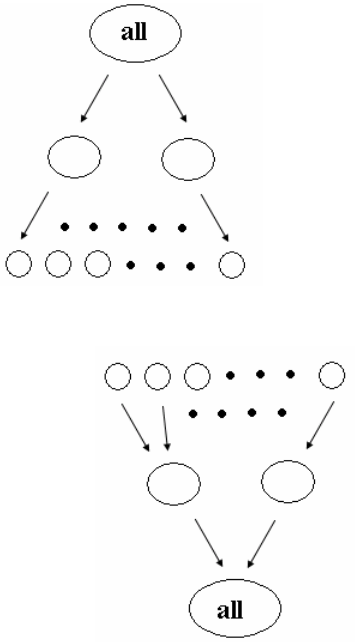
What is Clustering?

Cluster analysis is the organization of a collection of patterns into clusters based on similarity. The problem is to group a given collection of unlabeled patterns into meaningful clusters.



Hierarchical clustering

The result is a tree that depicts the relationships between the objects.



- **Divisive clustering:**
  begin at step 1 with all the data in one cluster.
- **Agglomerative clustering:**
  all the objects start apart., there are n clusters at step 0.

Non-Hierarchical clustering

- k-means, The EM algorithm, K Nearest Neighbor,…

Two important properties of a clustering definition:
1. Most of data has been organized into non-overlapping clusters.
2. Each cluster has a within variance and one between variance for each of the other clusters. A good cluster should have a small within variance and large between variance.

Data/Information Visualization

What is Visualization?

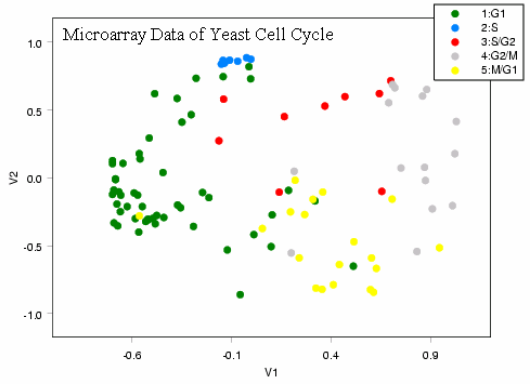
- To visualize = to make visible, to transform into pictures.
- Making things/processes visible that are not directly accessible by the human eye.
- Transformation of an abstraction to a picture.
- Computer aided extraction and display of information from data.

Data/Information Visualization

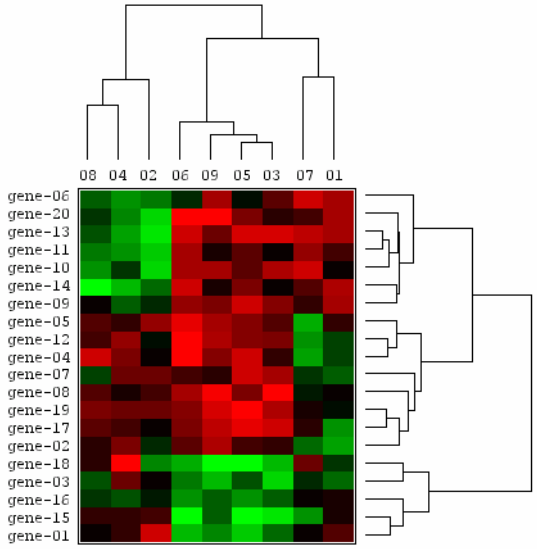
- Exploiting the human visual system to extract information from data.
- Provides an overview of complex data sets.
- Identifies structure, patterns, trends, anomalies, and relationships in data.
- Assists in identifying the areas of interest.

Visualization = Graphing for Data + Fitting + Graphing for Model

Tegarden, D. P. (1999). Business Information Visualization. Communicat



Dimension Reduction
vs.
Dimension Free



Clustering Analysis in Microarray Experiments

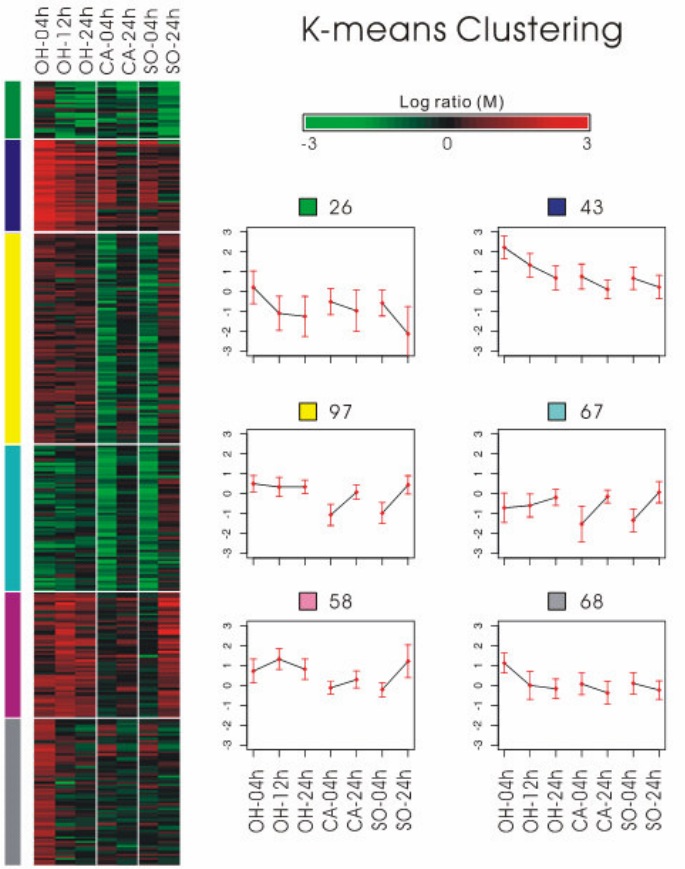
Goals

- Find natural classes in the data
- Identify new classes/gene correlations
- Refine existing taxonomies
- Support biological analysis/discovery

- cluster genes based on samples profiles
- cluster samples based on genes profiles

Hypothesis:

- genes with similar function have similar expression profiles.



Distance and Similarity Measure

Cov	x1	x2	x3	x4		x p
x1	1.00	0.48	0.10	-0.10		-0.28
x2	0.48	1.00	0.41	0.22		-0.23
x3	0.10	0.41	1.00	0.36		-0.05
x4	-0.10	0.22	0.36	1.00		0.10
x p	-0.28	-0.23	-0.05	0.10		1.00

Proximity Matrix

Pearson Correlation Coefficient

$$r_{xy} = \frac{\sum_{i=1}^{n}(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^{n}(x_i - \bar{x})^2}\sqrt{\sum_{i=1}^{n}(y_i - \bar{y})^2}}$$

Data Matrix x y

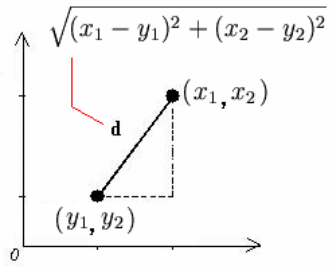
Data	x1	x2	x3	x4	...	x p
subject01	-0.48	-0.42	0.87	0.92		-0.18
subject02	-0.39	-0.58	1.08	1.21		-0.33
subject03	0.87	0.25	-0.17	0.18		-0.44
subject04	1.57	1.03	1.22	0.31		-0.49
subject05	-1.15	-0.86	1.21	1.62		0.16
subject06	0.04	-0.12	0.31	0.16		-0.06
subject07	2.95	0.45	-0.40	-0.66		-0.38
subject08	-1.22	-0.74	1.34	1.50		0.29
subject09	-0.73	-1.06	-0.79	-0.02		0.44
subject10	-0.58	-0.40	0.13	0.58		0.02
subject11	-0.50	-0.42	0.66	1.05		0.06
subject12	-0.86	-0.29	0.42	0.46		0.10
subject13	-0.16	0.29	0.17	-0.28		-0.55
subject14	-0.36	-0.03	-0.03	-0.08		-0.25
subject15	-0.72	-0.85	0.54	1.04		0.24
subject16	-0.78	-0.52	0.26	0.20		0.48
subject17	0.60	-0.55	0.41	0.45		-0.66
⋮						
subject n	-2.29	-0.64	0.77	1.60		0.55
mean	0.07	-0.04	0.44	0.31	...	-0.21

$$x = (x_1, x_2, \cdots, x_n)$$

$$y = (y_1, y_2, \cdots, y_n)$$

Euclidean Distance

$$d_{xy} = \sqrt{\sum_{i=1}^{n}(x_i - y_i)^2}$$



- The standard transformation from a similarity matrix C to a distance matrix D is given by $d_{rs} = (c_{rr} - 2c_{rs} + c_{ss})^{1/2}$.
- (Eisen *et al.* 1998) $d_{rs} = 1 - c_{rs}$
- Other transformations (Chatfield and Collins 1980, Section 10.2)

More Similarity Measures

Dissimilarity/Similarity Measure for Quantitative Data

Kendall's tau

Two pairs of observation (x_i, y_i) and (x_j, y_j)

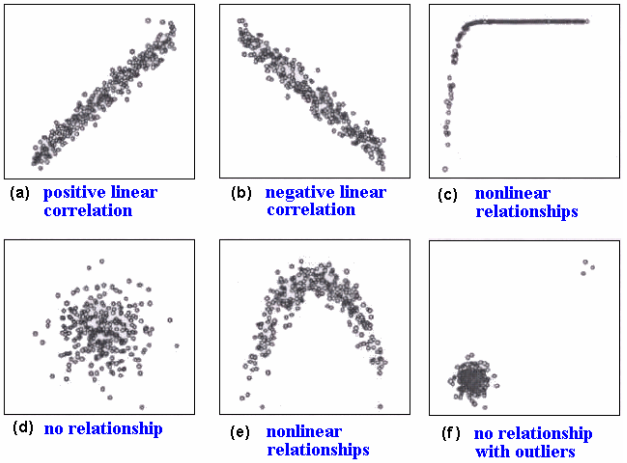
- C: concordant pair: $(x_j - x_i)(y_j - y_i) > 0$
- D: discordant pair: $(x_j - x_i)(y_j - y_i) < 0$
- tie:

E_y: extra y pair in x's: $(x_j - x_i) = 0$

E_x : extra x pair in y's: $(y_j - y_i) = 0$

$$\tau = \frac{C - D}{\sqrt{C + D - E_y}\sqrt{C + D - E_x}}$$

Similarity	Formula
Pearson correlation	$s(i,j) = \frac{\text{cov}(x_i, x_j)}{\sqrt{\text{var}(x_i)\,\text{var}(x_j)}}$
Spearman correlation (r_i is ranked x_i)	$s(i,j) = \frac{\text{cov}(r_i, r_j)}{\sqrt{\text{var}(r_i)\,\text{var}(r_j)}}$
Kendall's Tau	$s(i,j) = \frac{1}{\binom{p}{2}} \sum_{k \neq k'} sign\ [(x_{ik} - x_{ik'})(x_{jk} - x_{jk'})]$



(a) positive linear correlation (b) negative linear correlation (c) nonlinear relationships

(d) no relationship (e) nonlinear relationships (f) no relationship with outliers

- Pearson's rho measures the strength of a linear relationship [(a), (b)].
- Spearman's rho and Kendall's tau measure any monotonic relationship between two variables [(a), (b) ,(c)].
- If the relationship between the two variables is non-monotonic, all three correlation coefficients fail to detect the existence of a relationship [(e)].
- Both Spearman's rho and Kendall's tau are rank-based non-parametric measures of association between variable X and Y.
- The rank-based correlation coefficients are more robust against outliers.

Data	*Pearson's rho*	*Spearman's rho*	*Kendall's tau*
(a)	0.98	0.98	0.87
(b)	-0.98	-0.98	-0.87
(c)	0.50	0.99	0.98
(d)	-0.02	-0.03	-0.02
(e)	-0.06	-0.02	-0.02
(f)	0.68	0.00	0.00

Algorithm they use different logic for computing the correlation coefficient, they seldom lead to markedly different conclusions (Siegel and Castellan, 1988).

K-Means Clustering

- K-means is a partition methods for clustering.
- Data are classified into k groups as specified by the user.
- Two different clusters cannot have any objects in common, and the k groups together constitute the full data set.

Optimization problem:

Minimize the sum of squared within-cluster distances

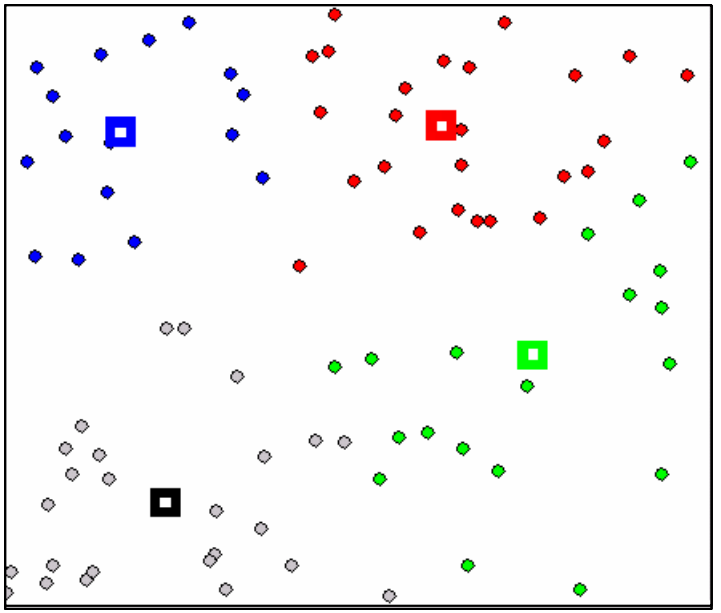
$$W(C) = \frac{1}{2}\sum_{k=1}^{k} \sum_{C(i)=C(j)=k} d_E(x_i, x_j)^2$$

The K-Means Algorithm

1. The data points are randomly assigned to one of the K clusters.
2. The position of the K centroids are determined (initial group centroids).
3. For each data point:
   - Calculate the distance from the data point to each cluster.
   - Assign data point to the cluster that has the closest centroid.
4. Repeat the above step until the centroids no longer move.

The choice of initial partition can greatly affect the final clusters that result.

Converged



Visualizing Clustering Results:

Dimension Reduction Techniques

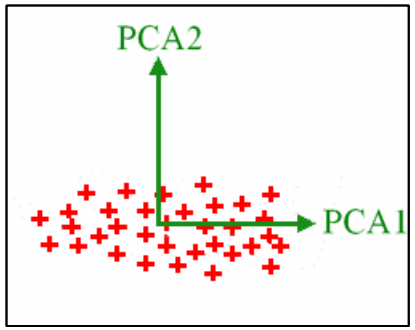
- **Principal Component Analysis (PCA)**
- **Multidimensional Scaling (MDS)**

Dimension reduction visualization is often adopted for presenting grouping structure for methods such as K-means.

Principal Component Analysis (PCA)

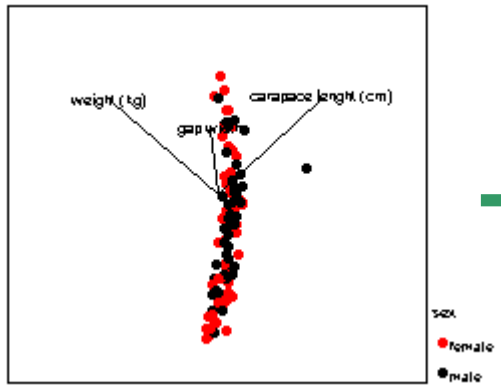
(Pearson 1901; Hotelling 1933; Jolliffe 2002)

PCA is a method that reduces data dimensionality by finding the new variables (major axes, principal components).



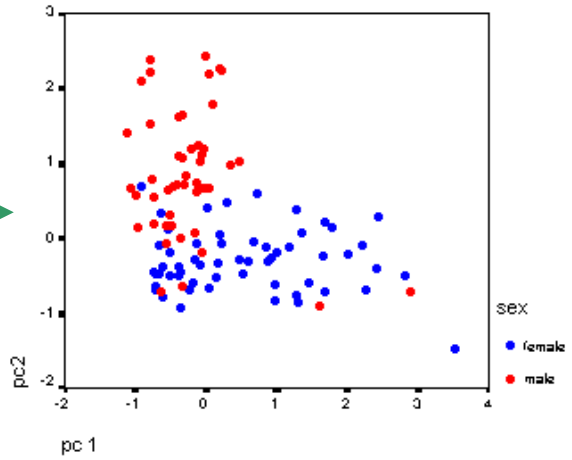
$$\text{PCA}_1 = a_1\mathbf{X} + b_1\mathbf{Y}$$

$$\text{PCA}_2 = a_2\mathbf{X} + b_2\mathbf{Y}$$



$$\text{PCA}_1 = a_1\mathbf{X} + b_1\mathbf{Y} + c_1\mathbf{Z}$$

$$\text{PCA}_2 = a_2\mathbf{X} + b_2\mathbf{Y} + c_2\mathbf{Z}$$



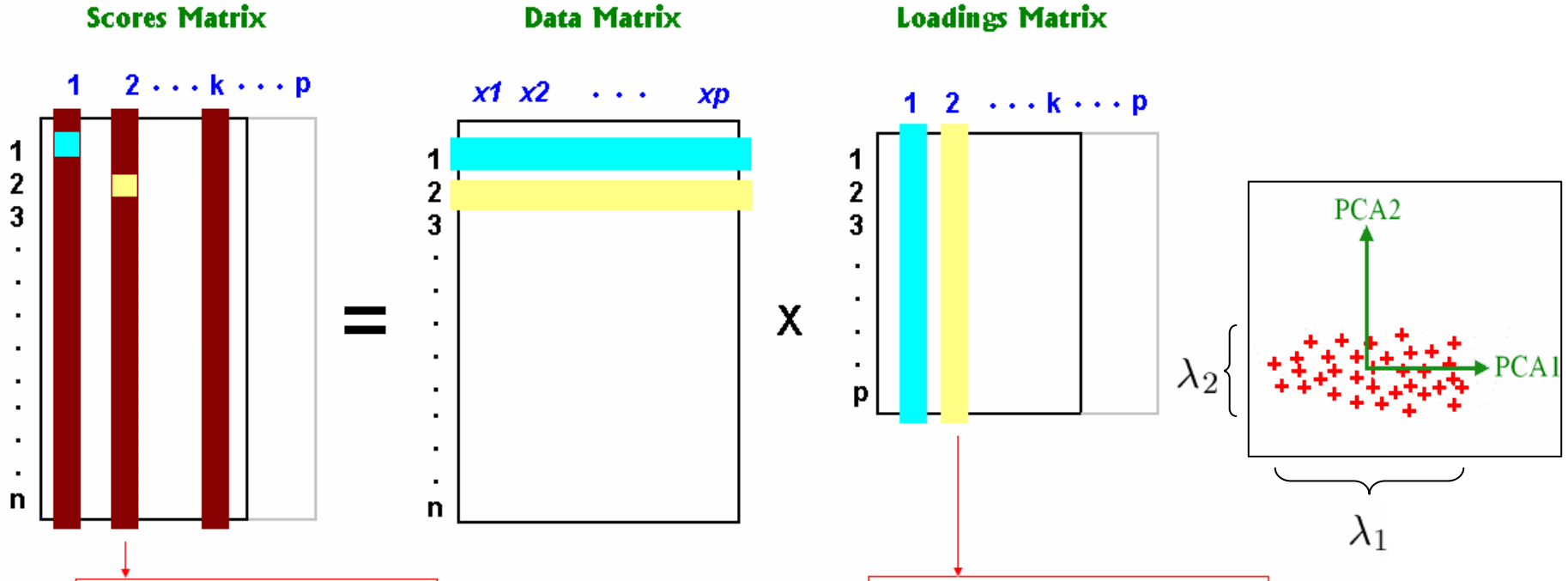
Amongst all possible projections, PCA finds the projections so that the maximum amount of information, measured in terms of variability, is retained in the smallest number of dimensions.

$$\text{PCA}_1 = a_{11}\mathbf{X}_1 + a_{12}\mathbf{X}_2 + \cdots + a_{1p}\mathbf{X}_p$$

$$\text{PCA}_2 = a_{21}\mathbf{X}_1 + a_{22}\mathbf{X}_2 + \cdots + a_{2p}\mathbf{X}_p$$

PCA: Loadings and Scores

$$\mathbf{Z} = \mathbf{X}\,\mathbf{W}$$



The *i*th principal component of $\mathbf{X}$ is $\mathbf{X}\mathbf{w}_i$, where $\mathbf{w}_i$ is the *i*th normalized eigenvector of $\Sigma_{\mathbf{x}}$ corresponding to the *i*th largest eigenvaules.

Eigenvalues $\lambda_1 \geq \lambda_2 \geq \cdots \geq \lambda_p$

$$\text{proportion} = \frac{\sum_{i=1}^{k} \lambda_i}{\sum_{i=1}^{p} \lambda_i}$$

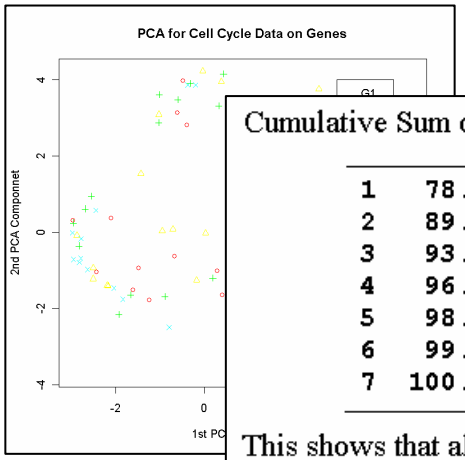
PCA (conti.)

Microarray Data Matrix

MA Table	exp01	exp02	exp03	exp04	exp05	exp•••	exp p
gene001	-0.48	-0.42	0.87	0.92	0.67		-0.35
gene002	-0.39	-0.58	1.08	1.21	0.52		-0.58
gene003	0.87	0.25	-0.17	0.18	-0.13		-0.13
gene004	1.57	1.03	1.22	0.31	0.16		-1.02
gene005	-1.15	-0.86	1.21	1.62	1.12		-0.44
gene006	0.04	-0.12	0.31	0.16	0.17		0.08
gene007	2.95	0.45	-0.40	-0.66	-0.59		-0.76
gene008	-1.22	-0.74	1.34	1.50	0.63		-0.55
gene009	-0.73	-1.06	-0.79	-0.02	0.16		0.03
gene010	-0.58	-0.40	0.13	0.58	-0.09		-0.45
gene011	-0.50	-0.42	0.66	1.05	0.68		0.01
gene012	-0.86	-0.29	0.42	0.46	0.30		-0.63
gene013	-0.16	0.29	0.17	-0.28	-0.02		-0.04
gene014	-0.36	-0.03	-0.03	-0.08	-0.23		-0.21
gene015	-0.72	-0.85	0.54	1.04	0.84		-0.64
gene016	-0.78	-0.52	0.26	0.20	0.48		0.27
gene017	0.60	-0.55	0.41	0.45	0.18		-1.02
gene018	-0.20	-0.67	0.13	0.10	0.38		0.05
gene019	-2.29	-0.64	0.77	1.60	0.53		-0.38
gene020	-1.46	-0.76	1.08	1.50	0.74		-0.70
gene021	-0.57	0.42	1.03	1.35	0.64		-0.40
gene022	-0.11	0.13	0.41	0.60	0.23		0.19
gene•••							
gene n	-1.79	0.94	2.13	1.75	0.23		-0.66

PCA on Conditions

MA Table	PCA-1	PCA-2	PCA-3
gene001	-0.18	-0.11	-0.03
gene002	0.51	-0.53	0.54
gene003	-0.35	-0.39	0.26
gene004	-0.18	-1.08	0.41
gene005	-0.62	-0.8	0.13
gene006	-0.09	-0.23	0.77
gene007	-0.38	-0.32	1.08
gene008	-0.88	-0.55	1.03
gene009	-1.26	0.45	0.41
gene010	0.12	-0.36	-0.16
gene011	-0.28	-0.44	2.13
gene012	-0.45	-0.23	0.82
gene013	-0.2	-0.43	0.44
gene014	0.03	-0.26	-0.68
gene015	-0.7	-0.76	0.5
gene016	-0.61	0.07	-0.04
gene017	-0.23	-0.71	0.01
gene018	0.1	0.1	0.11
gene019	-0.94	-0.97	0.24
gene020	-0.55	-0.53	0.86
gene021	-0.47	-0.87	-0.02
gene022	-0.34	-1.1	0.51
gene•••	-0.49	-0.2	0.91
gene n	-0.15	-1.04	-0.01

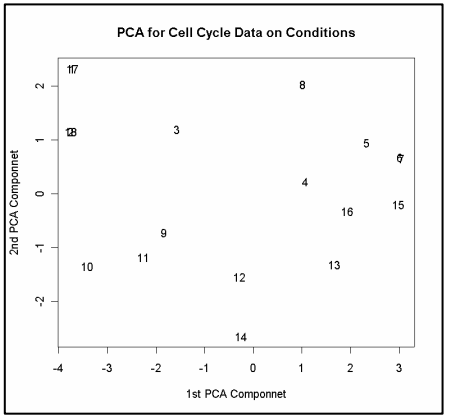


Cumulative Sum of the Variances:

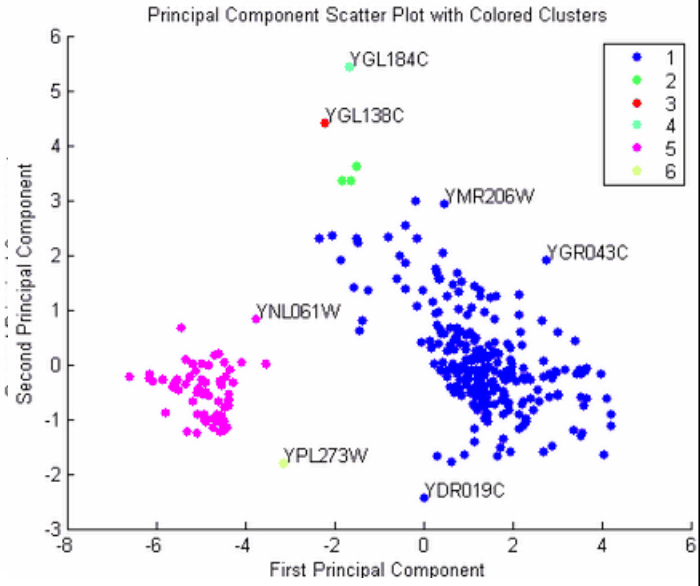
1	78.3719
2	89.2140
3	93.4357
4	96.0831
5	98.3283
6	99.3203
7	100.0000

This shows that almost 90% of the variance is accounted for by the first two principal components.

PCA on Genes



MA Table	exp01	exp02	exp03	exp04	exp05	exp•••	exp p
PCA-1	0.18	0.3	-0.12	-0.44	0.19	-0.39	-0.61
PCA-2	-0.16	-0.58	-0.43	-0.22	0.53	0.69	0.08
PCA-3	0.16	-0.44	-0.93	-1.23	-0.62	0.62	1.3



Yeast Microarray Data is from
DeRisi, JL, Iyer, VR, and Brown, PO.(1997). "Exploring the metabolic and genetic control of gene expression on a genomic scale"; Science, Oct 24;278(5338):680-6.

Multidimensional Scaling (MDS)

(Torgerson 1952; Cox and Cox 2001)

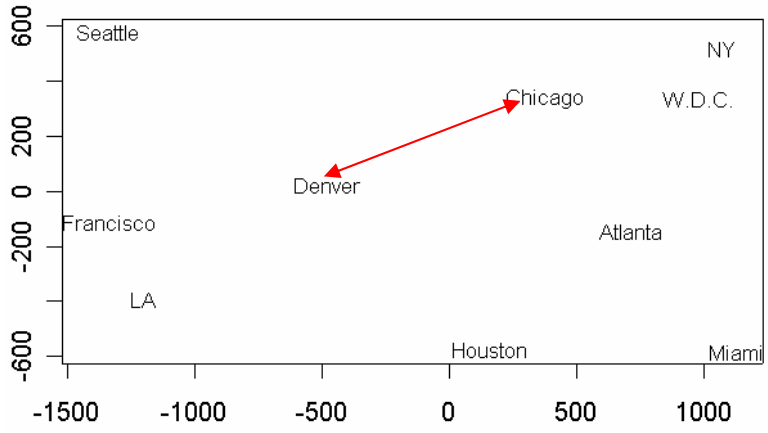
http://www.lib.utexas.edu/maps/united_states.html

- Classical MDS takes a set of dissimilarities and returns a set of points such that the distances between the points are approximately equal to the dissimilarities.
- projection from some unknown dimensional space to 2-d dimension.

Flying Mileages Between Ten U.S. Cities

0										Atlanta
587	0									Chicago
1212	920	0								Denver
701	940	879	0							Houston
1936	1745	831	1374	0						Los Angeles
604	1188	1726	968	2339	0					Miami
748	713	1631	1420	2451	1092	0				New York
2139	1858	949	1645	347	2594	2571	0			San Francisco
2182	1737	1021	1891	959	2734	2408	678	0		Seattle
543	597	1494	1220	2300	923	205	2442	2329	0	Washington D.C.

MDS



MDS: Metric and Non-Metric Scaling

Question

Given a *dissimilarity matrix* D of certain objects, can we construct points in k-dimensional (often 2-dimensional) space such that

Goal of metric scaling

the Euclidean distances between these points approximate the entries in the dissimilarity matrix?

Goal of non-metric scaling

the order in distances coincides with the order in the entries of the dissimilarity matrix approximately?

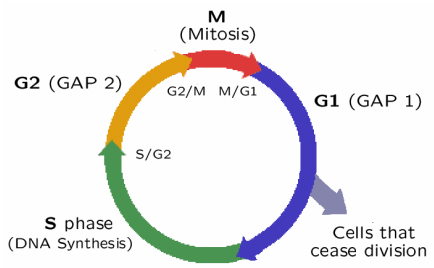
$$S = \sum_{i,j} (\hat{d}_{ij} - d_{ij})^2$$

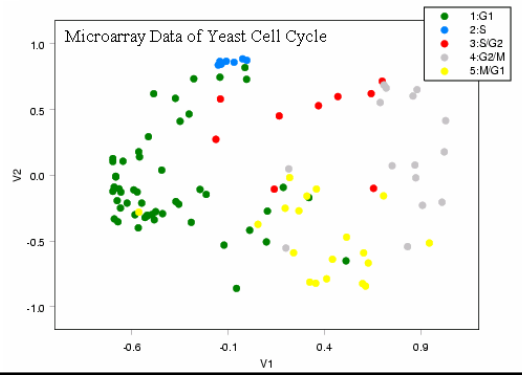
Mathematically: for given k, compute points $x_1,\ldots,x_n$ in k-dimensional space such that the object function is minimized.

$$Stress = \sqrt{\frac{\sum_{i,j} (\hat{d}_{ij} - d_{ij})^2}{\sum_{i,j} d_{ij}^2}}$$

Microarray Data of Yeast Cell Cycle

- **Synchronized by alpha factor arrest method (Spellman et al. 1998; Chu et al. 1998)**
- **103 known genes: every 7 minutes and totally 18 time points.**
- **2D MDS Configuration Plot for 103 known genes.**



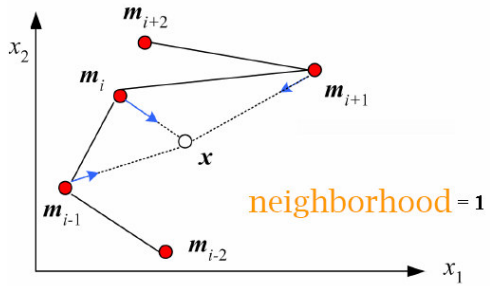


Clustering Analysis and Visualization

- Self-Organizing Maps (SOM)
- Heat Map
- Hierarchical Clustering
- QT (Quality Threshold) Clustering

Self-Organizing Maps (SOM)

- SOMs were developed by **Kohonen** in the early **1980's**, original area was in the area of speech recognition.
- ***Idea:*** Organise data on the basis of **similarity** by putting entities **geometrically** close to each other.



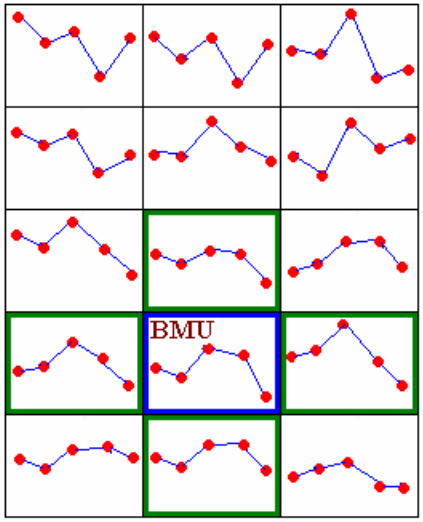
- SOM is unique in the sense that it combines both aspects. It can be used at the same time both to reduce the amount of data by clustering, and to construct a nonlinear projection of the data onto a low-dimensional display.

Step 0:
Initialize weights $\mathbf{w}_i(t)$.
Set $\alpha(t)$ and $h_{ci}(t)$.

Learning process:

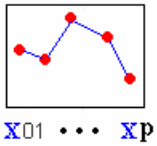
$$\mathbf{w}_i(t+1) = \begin{cases} \mathbf{w}_i(t) + h_{ci}(t)[\,\mathbf{x}(t) - \mathbf{w}_i(t)\,] & i \in N_c(t) \\ \mathbf{w}_i(t), \quad \text{o.w.} \end{cases}$$

5 x 3 output node



Data Matrix

Table	X01	X02	X03	•••	Xp
obs 001	-0.48	-0.42	0.87		-0.35
obs 002	-0.39	-0.58	1.08		-0.58
obs 003	0.87	0.25	-0.17		-0.13
obs 004	1.57	1.03	1.22		-1.02
obs 005	-1.15	-0.86	1.21		-0.44
obs 006	0.04	-0.12	0.31		0.08
obs 007	2.95	0.45	-0.40		-0.76
obs 008	-1.22	-0.74	1.34		-0.55
obs 009	-0.73	-1.06	-0.79		0.03
obs 010	-0.58	-0.40	0.13		-0.45
obs 011	-0.50	-0.42	0.66		0.01
obs 012	-0.86	-0.29	0.42		-0.63
obs 013	-0.16	0.29	0.17		-0.04
obs •••					
obs n	-1.79	0.94	2.13		-0.66



input node

Incrementally decrease the learning rate and the neighborhood size, and repeat

Algorithm of SOM

Step 0: Initialize weights $\mathbf{w}_i(t)$.
Set topological neighborhood parameters $N_c(t)$.
Set learning rate parameters $\alpha(t)$ and $h_{ci}(t)$.

Step 1: For each input vector $\mathbf{x}(t)$, do

a. Finding a BMU: $\|\mathbf{x}(t) - \mathbf{w}_c(t)\| = \min_i \|\mathbf{x}(t) - \mathbf{w}_i(t)\|$

b. Learning process:

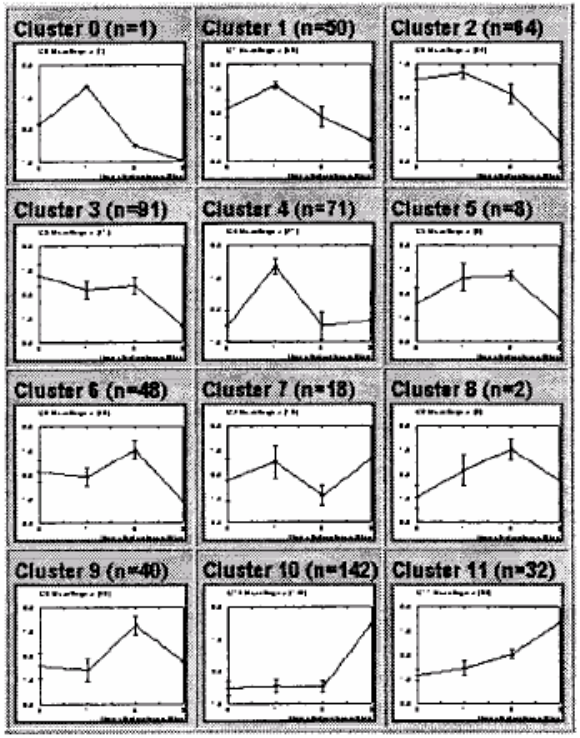
$$\mathbf{w}_i(t+1) = \begin{cases} \mathbf{w}_i(t) + h_{ci}(t)[\,\mathbf{x}(t) - \mathbf{w}_i(t)\,], & i \in N_c(t) \\ \mathbf{w}_i(t), & \text{o.w.} \end{cases}$$

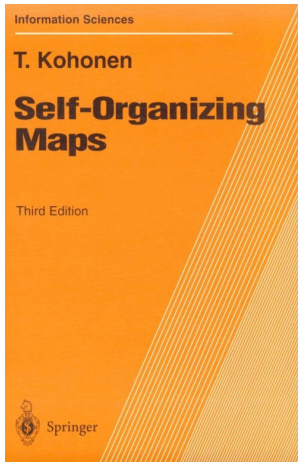
c. Go to the next unvisited input vector. If there are no unvisited input vector left then go back to the very first one and go to Step 2.

Step 2: Incrementally decrease the learning rate and the neighborhood size, and repeat Step 1.

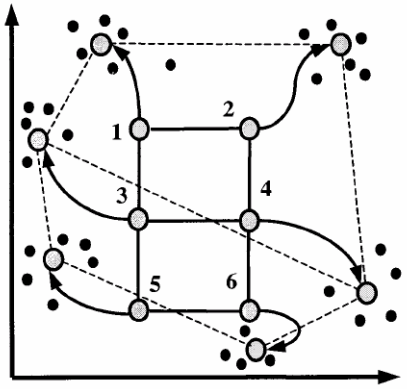
Step 3: Keep doing Steps 1 and 2 for a sufficient number of iterations.

HL-60 4×3 SOM 567 genes





1995, 1997, 2001



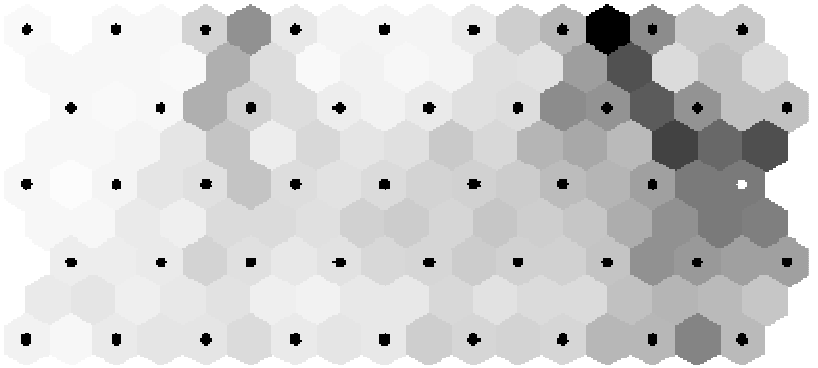
Macrophage Differentiation in HL-60 cells

Tamayo, P. et al. (1999). Interpreting patterns of gene expression with self-organizing maps: Methods and application to hematopoietic differentiation. *Proc Natl Acad Sci* 96:2907-2912.

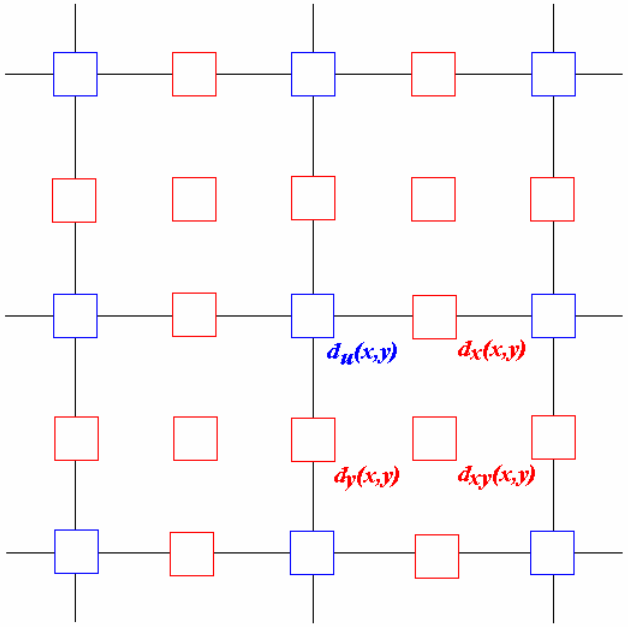
U-matrix: Unified Matrix Method

(Ultsch and Siemon 1989, Ultsch 1993)

U-matrix representation of SOM visualizes the distance between the neurons. The distance between the adjacent neurons is calculated and presented with different colorings between the adjacent nodes.



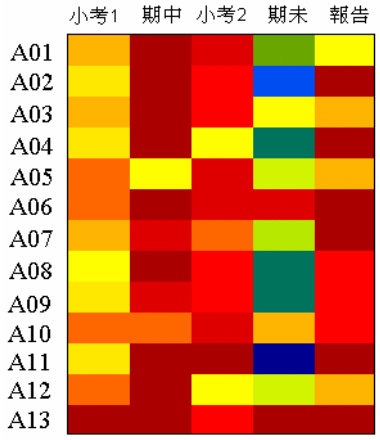
U-matrix representation of the SOM

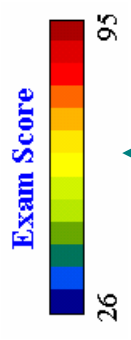


$b(x,y)$: matrix of neurons, of size $n_x \times n_y$.
$w_i(x,y)$: matrix of weights.
$u(x,y)$: U-matrix of size $(2n_x - 1) \times (2n_y - 1)$.

$d_x(x,y)$: $\|b(x,y) - b(x+1,y)\| = \sqrt{\sum_i [w_i(x,y) - w_i(x+1,y)]^2}$
$d_y(x,y)$: $\|b(x,y) - b(x,y+1)\| = \sqrt{\sum_i [w_i(x,y) - w_i(x,y+1)]^2}$
$d_{xy}(x,y)$: $\frac{1}{2}[\frac{\|b(x,y)-b(x+1,y+1)\|}{\sqrt{2}} + \frac{\|b(x,y+1)-b(x+1,y)\|}{\sqrt{2}}]$
$d_u(x,y)$: the median of the surrounding elements.

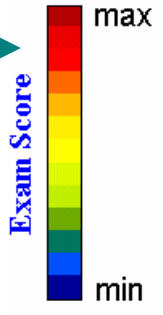
Heat Map: Data Image, Matrix Visualization

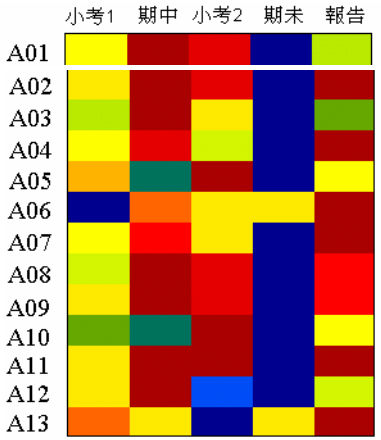




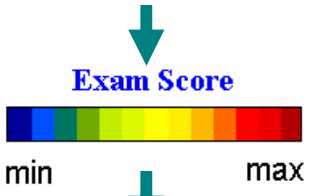
Range Matrix Condition

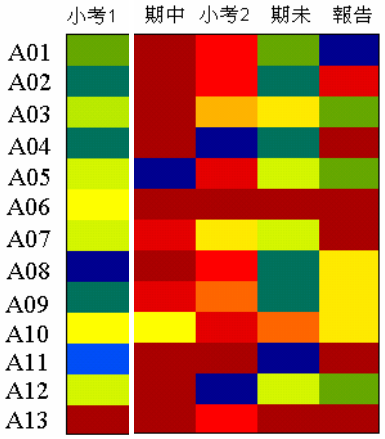
	A	B	C	D	E	F
1	學號	小考1	期中考	小考2	期末考	報告
2	A01	69	92	85	45	62
3	A02	66	90	83	36	90
4	A03	72	92	80	62	70
5	A04	68	90	60	37	95
6	A05	74	60	86	54	70
7	A06	77	90	88	88	95
8	A07	73	88	77	51	95
9	A08	61	90	84	40	82
10	A09	66	88	82	39	80
11	A10	76	75	87	72	80
12	A11	64	90	90	26	95
13	A12	75	90	60	55	70
14	A13	92	90	83	90	95





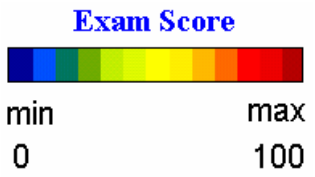
Range Raw Condition





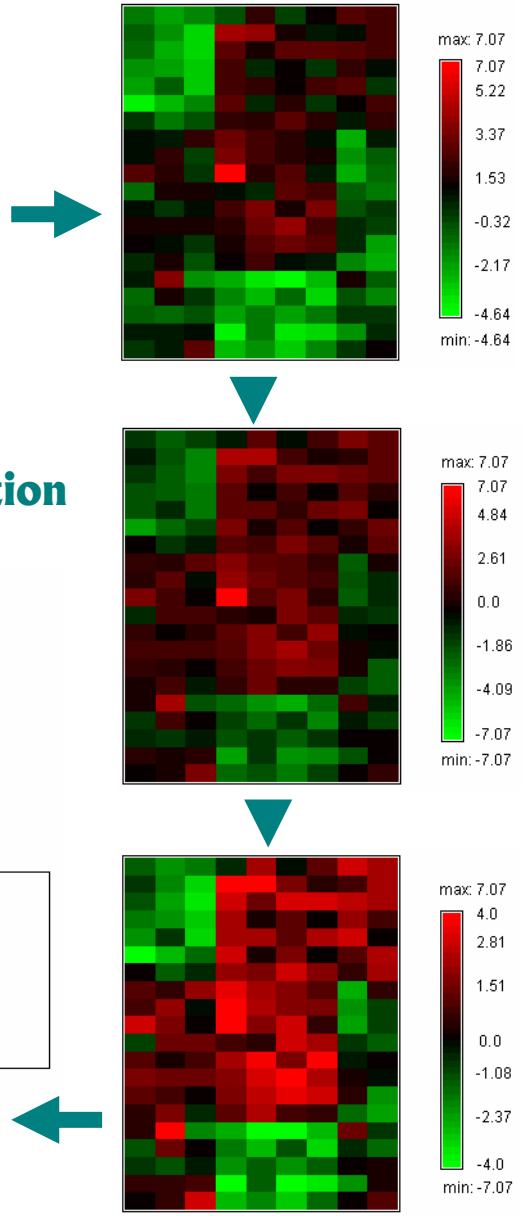
Range Column Condition

What about this one?

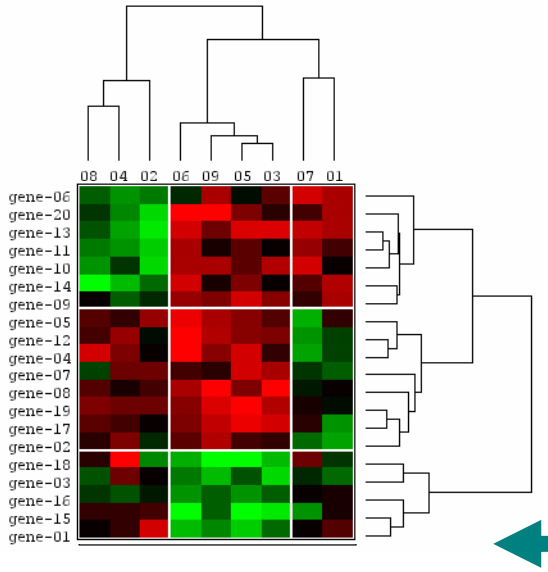


Heat Map (conti.)

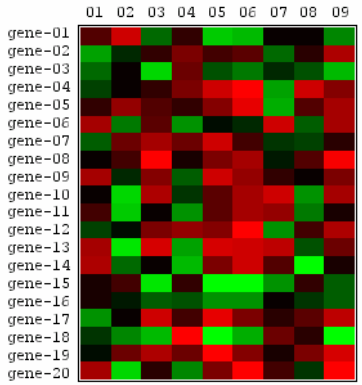
	A	B	C	D	E	F	G	H	I
1	-1.37	-2.30	-1.80	-0.55	2.45	-0.13	1.49	3.03	2.48
2	-0.68	-2.11	-3.42	4.67	4.57	1.75	0.61	0.92	2.52
3	-1.19	-2.49	-3.66	3.14	1.70	3.29	3.33	2.92	2.48
4	-1.93	-2.28	-3.16	2.51	0.32	1.49	0.21	2.20	1.03
5	-2.21	-0.79	-3.29	2.55	2.44	1.45	2.68	3.03	0.19
6	-4.14	-2.91	-1.64	3.21	0.37	1.93	0.14	1.27	2.67
7	0.21	-1.36	-0.44	2.22	1.85	3.11	2.03	0.67	2.40
8	1.13	0.79	2.25	3.65	2.52	2.09	1.13	-2.59	0.67
9	0.95	2.33	-0.07	3.89	2.72	2.13	1.75	-2.17	-0.90
10	3.04	1.85	0.21	7.07	2.01	3.05	0.76	-2.58	-1.04
11	-1.02	1.65	1.53	0.95	0.60	3.12	2.52	-0.77	-1.40
12	1.21	0.24	1.04	2.50	3.69	1.81	3.98	-0.33	0.11
13	1.74	1.60	1.70	2.02	3.45	4.46	2.69	0.41	-0.09
14	1.34	1.06	0.06	1.81	2.90	3.64	3.04	0.49	-2.33
15	0.57	1.81	-0.47	1.40	2.70	0.99	0.82	-1.61	-2.56
16	0.61	4.22	-2.03	-2.61	-4.00	-4.64	-2.92	1.55	-0.71
17	-1.13	1.64	0.01	-1.77	-2.85	-1.24	-3.41	-0.59	-1.64
18	-0.86	-1.17	-0.41	-2.20	-1.30	-2.37	-1.41	0.08	0.25
19	0.75	0.66	1.04	-4.26	-1.41	-3.99	-3.53	-2.17	0.34
20	0.15	0.68	3.18	-2.86	-2.01	-3.18	-1.58	0.10	1.28



Center Matrix Condition



Without ordering



Microarray Data of Yeast Cell Cycle

- **Synchronized by alpha factor arrest method (Spellman et al. 1998; Chu et al. 1998)**
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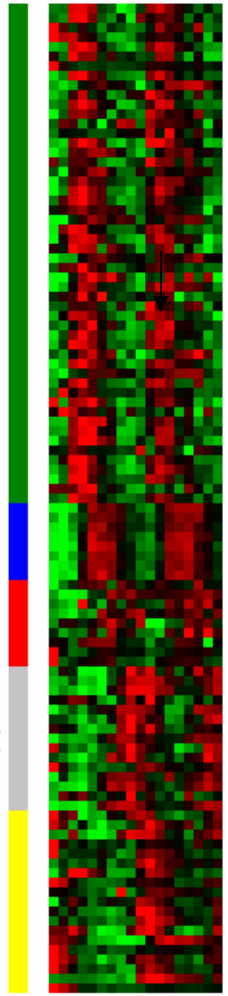


K-Means Clustering

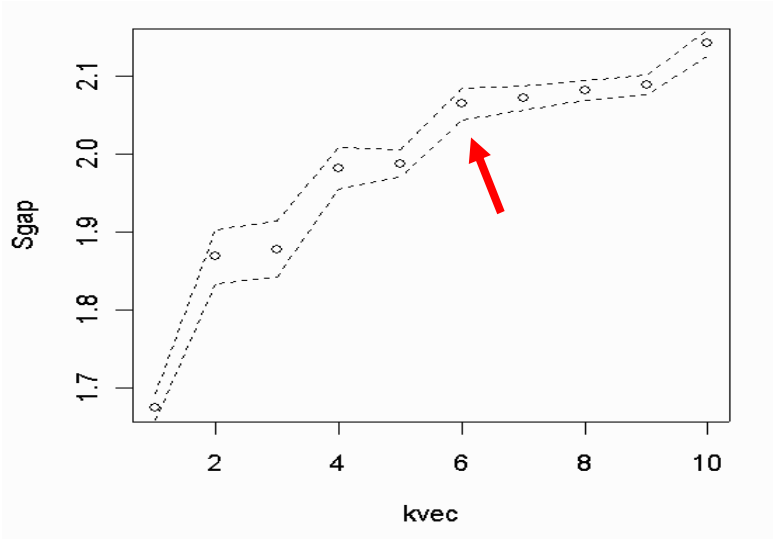
- Data

Baseline: Culture Medium (CM-00h)

OH-04h, OH-12h, OH-24h

CA-04h, CA-24h

SO-04h, SO-24h

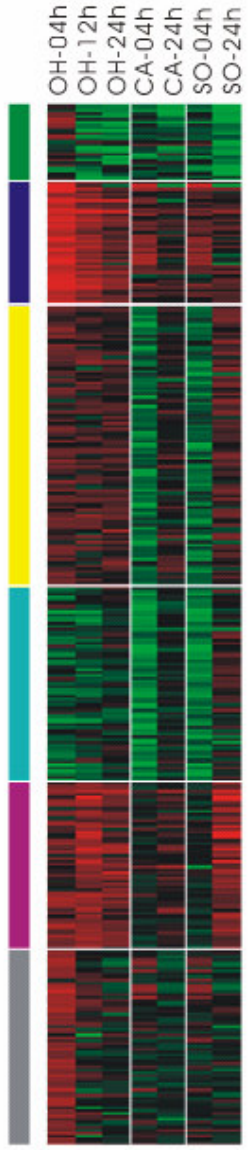


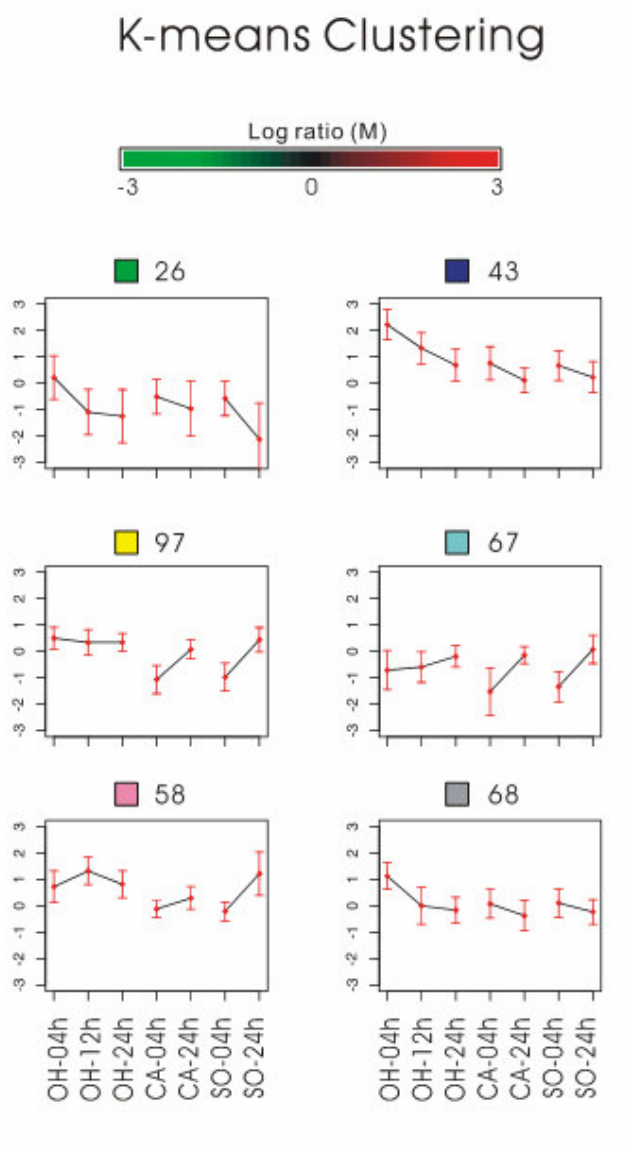
J. R. Statist. Soc. B (2001)
63, *Part* 2, *pp.* 411–423

Estimating the number of clusters in a data set via the gap statistic

Robert Tibshirani, Guenther Walther and Trevor Hastie

Stanford University, USA





Hierarchical Clustering and Dendrogram

(Kaufman and Rousseeuw, 1990)

Example:

Average-Linkage

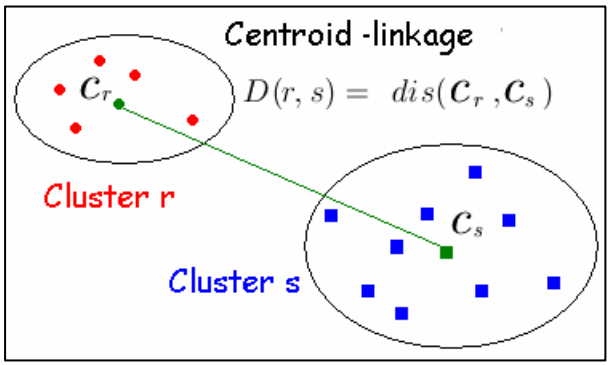
	a	b	c	d	e
a	0	2	6	10	9
b		0	5	9	8
c			0	4	5
d				0	3
e					0

	{a, b}	c	d	e
{a, b}	0	5.5	9.5	8.5
c		0	4	5
d			0	3
e				0

	{a,b}	c	{d, e}
{a, b}	0	5.5	9.0
c		0	4.5
{d, e}			0

	{a, b}	{c, d, e}
{a, b}	0	7.83
{c, d, e}		0

UPGMC (Unweighted Pair-Groups Method Centroid)



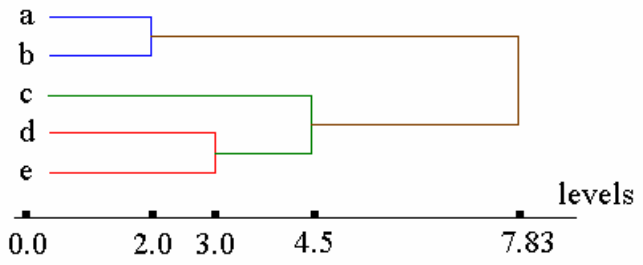
$$D(\{a,b\},\{c\}) = \frac{1}{2}[D(a,c) + D(b,c)]$$

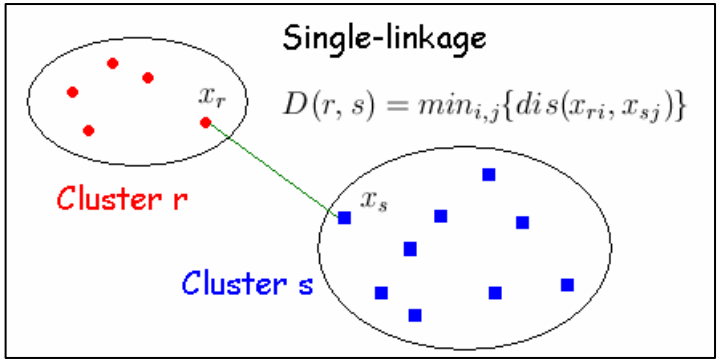
$$= \frac{1}{2}(6+5) = 5.5$$

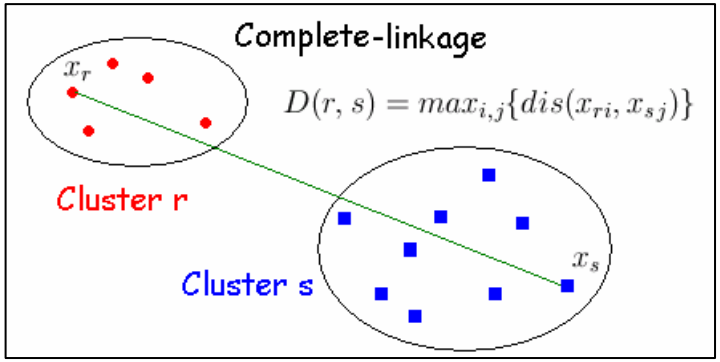
$$D(\{a,b\},\{d,e\})$$

$$= \frac{1}{4}[D(a,d)+D(a,e)+D(b,d)+D(b,e)]$$

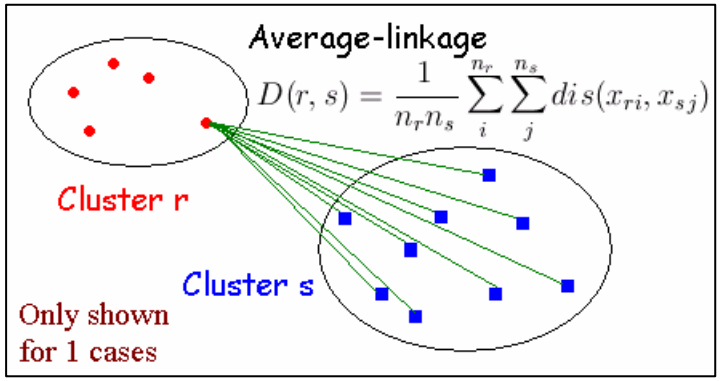
$$= \frac{1}{4}(10+9+9+8) = 9$$







UPGMA (Unweighted Pair-Groups Method Average)



Ward's Method

- The Ward's method does not compute distances between clusters.
- It forms clusters by maximizing within-clusters homogeneity.
- The within-group (i.e., within-cluster) sum of squares is used as the measure of homogeneity.
- The Ward's method tries to minimize the total within-group or within-cluster sum of squares.
- Clusters are formed at each step such that the resulting cluster solution has the fewest within-cluster sums of squares.
- The within-cluster sums of squares that is minimized is also known as the error sums of squares (ESS).

Example:

Charles H. Romesburg (1984)

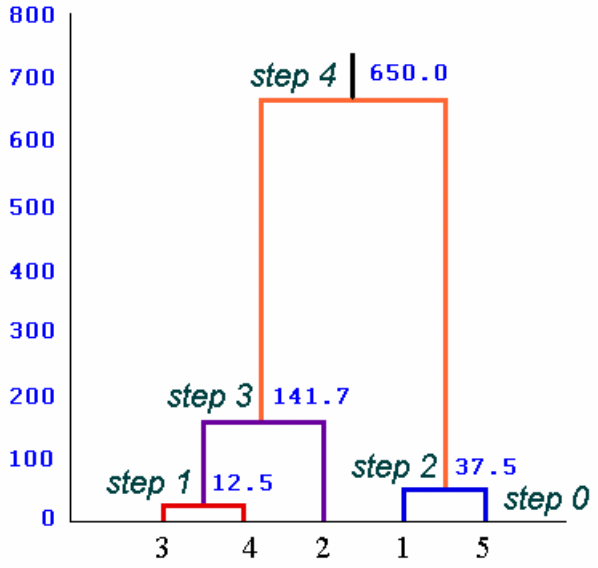
Toy Data

data	x1	x2
1	10	5
2	20	20
3	30	10
4	30	15
5	5	10

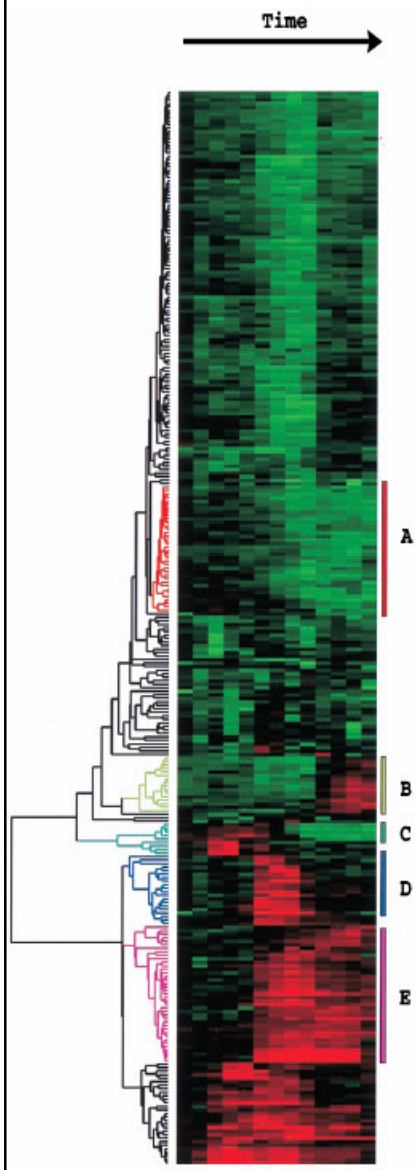
step	Possible Partitions				ESS
1	(12)	3	4	5	?

$$\{\overline{12}\} = [\ (10+20)/2, (5+20)/2\ ]$$
$$= [\ 15, 12.5]$$
$$ESS = wss\{12\} + wss\{3\} + wss\{4\} + wss\{5\}$$
$$= ss(1, \{\overline{12}\}) + ss(2, \{\overline{12}\})$$
$$= (10-15)^2 + (5-12.5)^2 + (20-15)^2 + (2-12.5)^2$$
$$= 162.5$$

step	Possible Partit			ESS
1	(12)	3	4 5	162.5
	(13)	2	4 5	212.5
	(14)	2	3 5	250.0
	(15)	2	3 4	25.0
	(23)	1	4 5	100.0
	(24)	1	3 5	62.5
	(25)	1	3 4	162.5
	(34)	1	2 5	12.5*
	(35)	1	2 4	312.5
	(45)	1	2 3	325.0
2	(34)	(12)	5	175.0
	(34)	(15)	2	37.5*
	(34)	(25)	1	175.0
	(134)	2	5	316.7
	(234)	1	5	116.7
	(345)	1	2	433.3
3	(234)	(15)		141.7*
	(125)	(34)		245.9
	(1345)	2		568.8
4	(12345)			650.0



Display of Genome-Wide Expression Patterns



Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 14863–14868, December 1998
Genetics

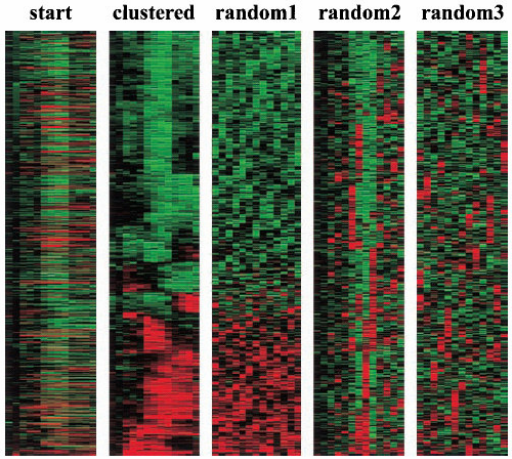
Cluster analysis and display of genome-wide expression patterns

MICHAEL B. EISEN*, PAUL T. SPELLMAN*, PATRICK O. BROWN†, AND DAVID BOTSTEIN*‡

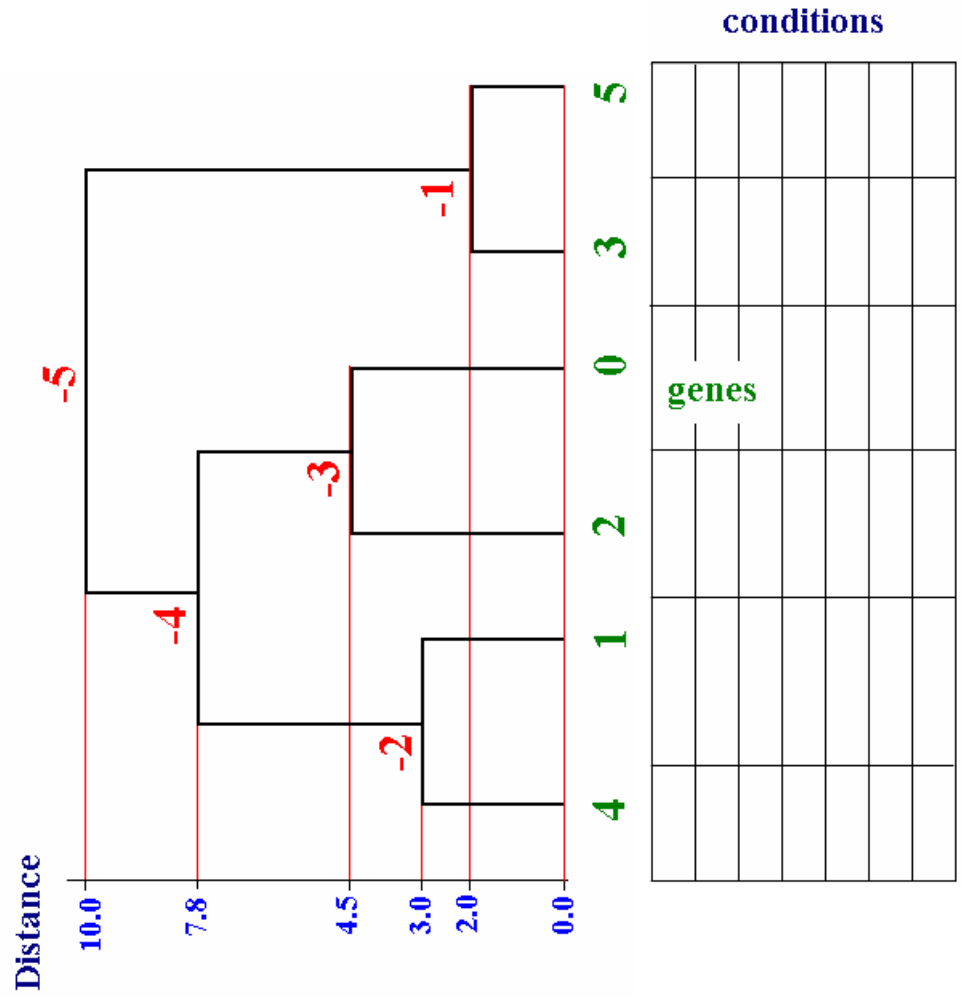
FIG. 1. Clustered display of data from time course of serum stimulation of primary human fibroblasts. Experimental details are described elsewhere (11). Briefly, foreskin fibroblasts were grown in culture and were deprived of serum for 48 hr. Serum was added back and samples taken at time 0, 15 min, 30 min, 1 hr, 2 hr, 3 hr, 4 hr, 8 hr, 12 hr, 16 hr, 20 hr, 24 hr. The final datapoint was from a separate unsynchronized sample. Data were measured by using a cDNA microarray with elements representing approximately 8,600 distinct human genes. All measurements are relative to time 0. Genes were selected for this analysis if their expression level deviated from time 0 by at least a factor of 3.0 in at least 2 time points. The dendrogram and colored image were produced as described in the text; the color scale ranges from saturated green for log ratios −3.0 and below to saturated red for log ratios 3.0 and above. Each gene is represented by a single row of colored boxes; each time point is represented by a single column. Five separate clusters are indicated by colored bars and by identical coloring of the corresponding region of the dendrogram. As described in detail in ref. 11, the sequence-verified named genes in these clusters contain multiple genes involved in (*A*) cholesterol biosynthesis, (*B*) the cell cycle, (*C*) the immediate–early response, (*D*) signaling and angiogenesis, and (*E*) wound healing and tissue remodeling. These clusters also contain named genes not involved in these processes and numerous uncharacterized genes. A larger version of this image, with gene names, is available at http://rana.stanford.edu/clustering/serum.html.

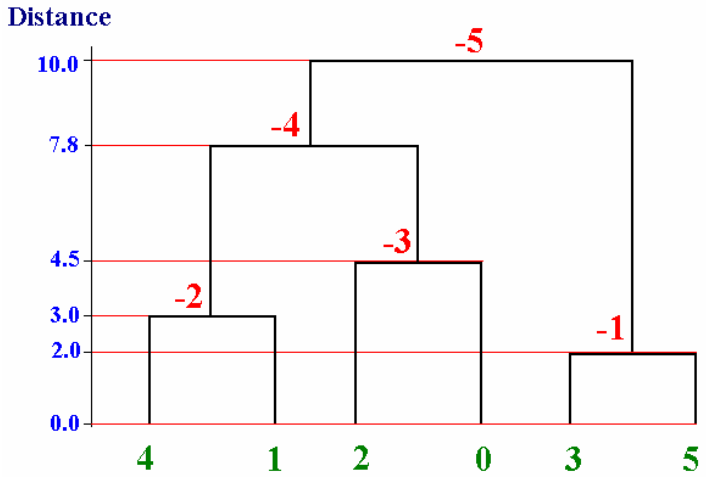
Software:
Cluster and TreeView

FIG. 3. To demonstrate the biological origins of patterns seen in Figs. 1 and 2, data from Fig. 1 were clustered by using methods described here before and after random permutation within rows (random 1), within columns (random 2), and both (random 3).



Dendrogram and Tree Storage

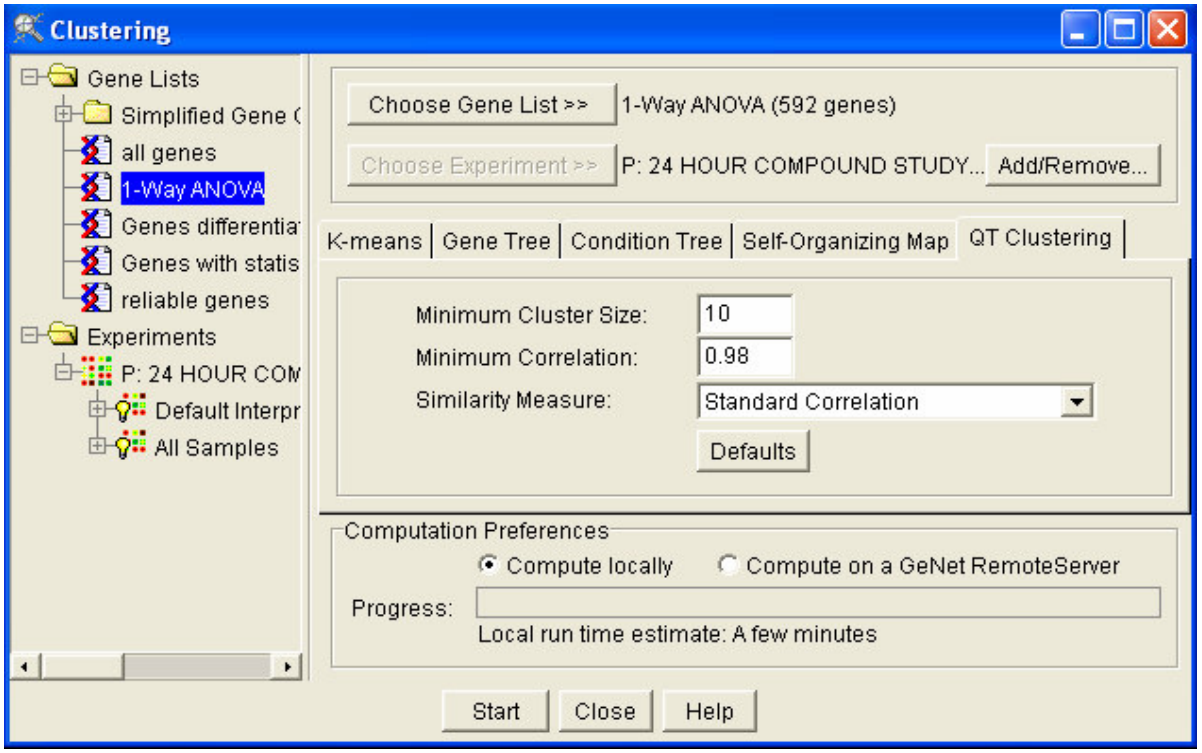




no	NodeID	Left	Right	Distance
0	-1	3	5	2
1	-2	4	1	3
2	-3	2	0	4.5
3	-4	-2	-3	7.8
4	-5	-4	-1	10

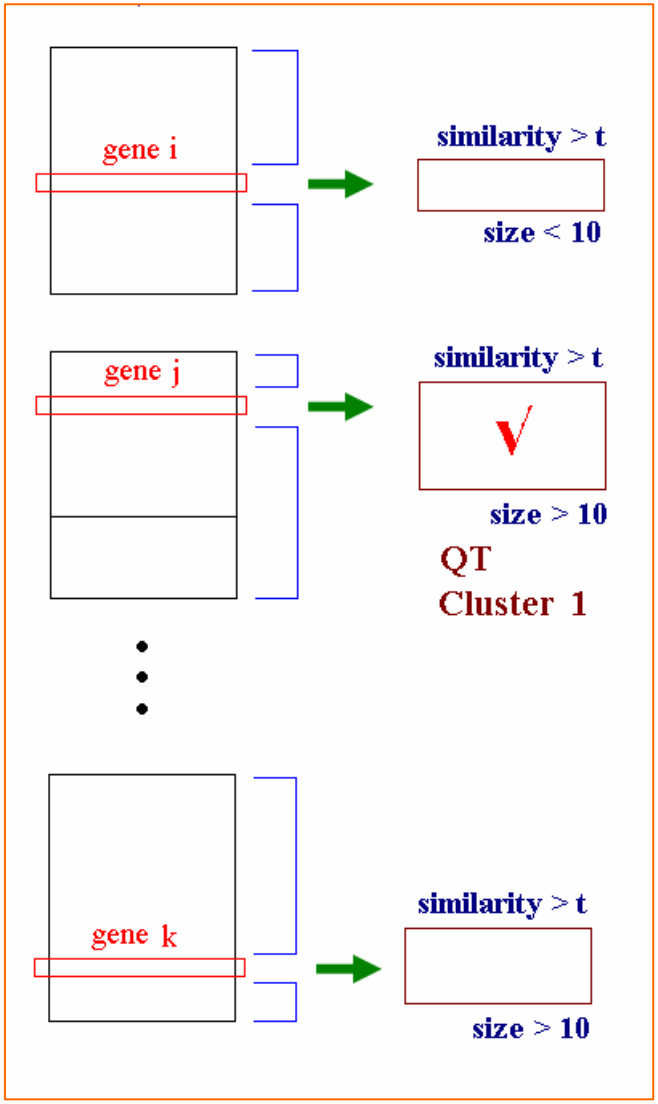
QT (Quality Threshold) Clustering

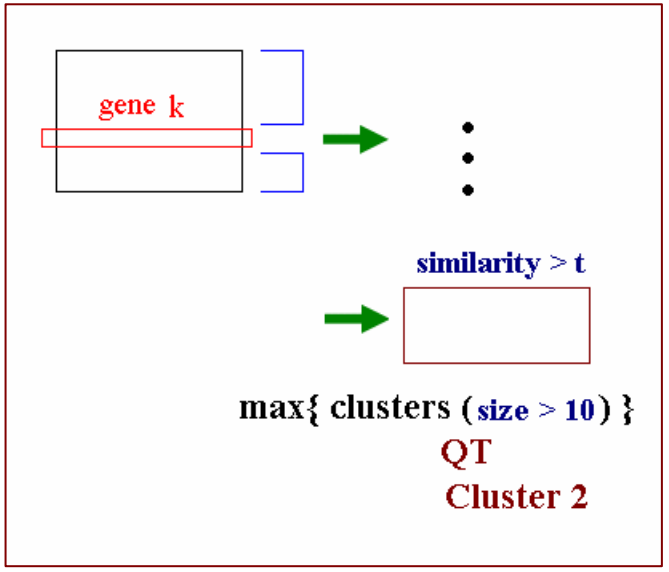
GeneSpring GX v7.3



- **Minimum Cluster Size:** Minimum number of genes that you would like to have in each cluster.
- **Minimum Correlation:** Minimum correlation that genes within each cluster must have to one another.
- The diameter is the equivalent of 1 minus the minimum correlation.

Algorithm of QT Clustering

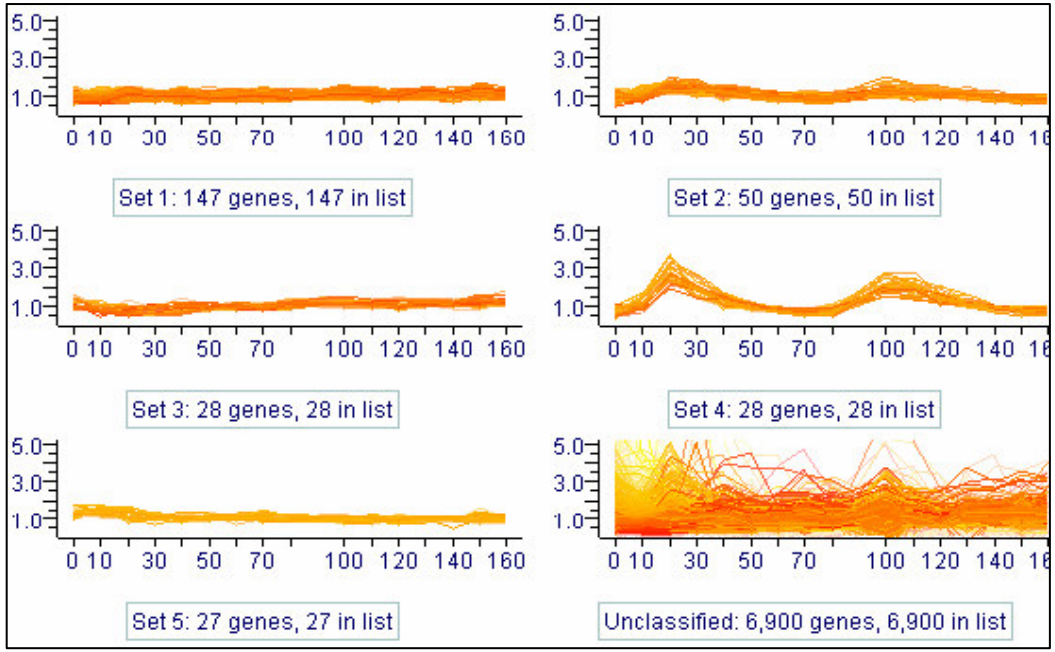




- The result is a set of non-overlapping QT clusters that meet quality threshold for both size, with respect to number of genes, and similarity, with respect to maximum allowable diameter.
- Genes that do not belong in any clusters will be grouped under the “unclassified” group.

Interpreting the Results

- QT Clusters are displayed according to the cluster size, from the largest to the smallest.
- Set 1 is the largest cluster, followed by set 2, etc…
- All sets will have **at least** the user-defined minimum cluster size and the minimum correlation (diameter).
- For example, all 147 genes in Set 1 below are at least 0.98 correlated to each other.
- Genes that did not meet the minimum quality are grouped under the "unclassified" category.



QT Clustering (conti.)

Advantages

- Quality Guarantee
- Number of clusters is not specified a priori
- All possible clusters are considered

Disadvantages

- Computationally Intensive/Time Consuming

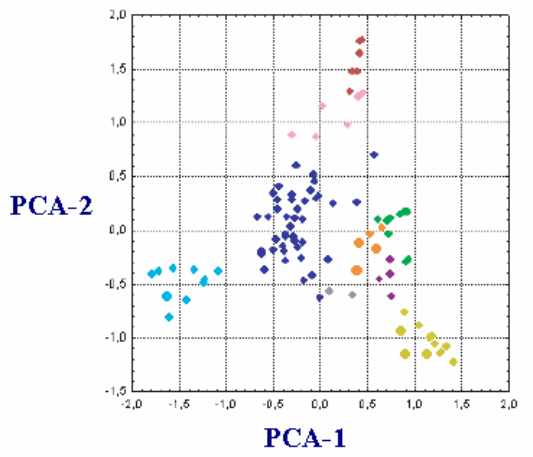
Main differences between QT clustering and K-means clustering?

	K-means	QT clustering	Consequence
Need to specify cluster number?	Yes	No	K-means: if users specify too few clusters, genes that are not similar will be forced to group together.
Very computationally intensive?	No	Yes	QT clustering: may be too computationally intensive, depending on available RAM and number of genes in starting gene list, for some desktop computer.
Every gene must be clustered?	Yes	No	K-means: every gene on the selected gene list must belong to a cluster. This could potentially group genes that are not very similar into the same cluster. QT clustering: only cluster with user-specified quality will be formed.

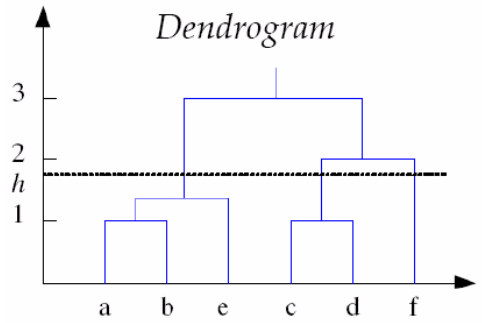
Choosing the Number of Clusters

(1) K is defined by the application.

(2) Plot the data in two PAC dimensions.

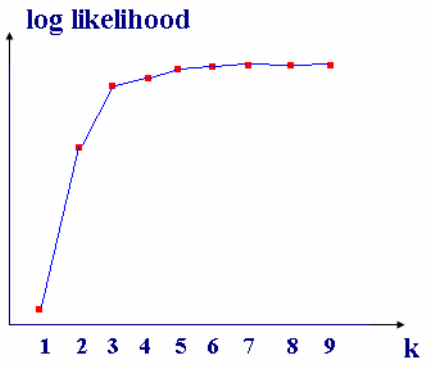


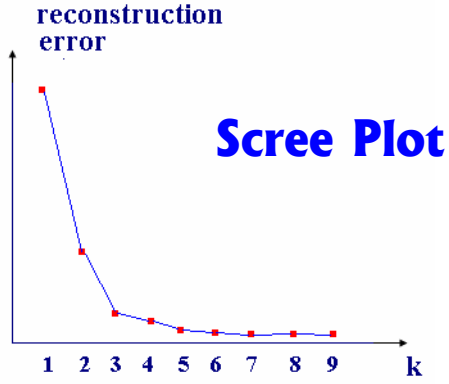
(4) Hierarchical clustering: look at the difference between levels in the tree.



(e.g., k-means: within-cluster sum of squares)

(3) Plot the **reconstruction error** or log likelihood as a function of k, and look for the elbow.





Scree Plot

Calinski and Harabasz (1974): CH(k)
Hartigan (1975): $H(k)$
Krzanowski and Lai (1985): KL(k)
Kaufman and Rousseeuw (1990): $s(i)$

J. R. Statist. Soc. B (2001)
63, *Part* 2, *pp.* 411–423

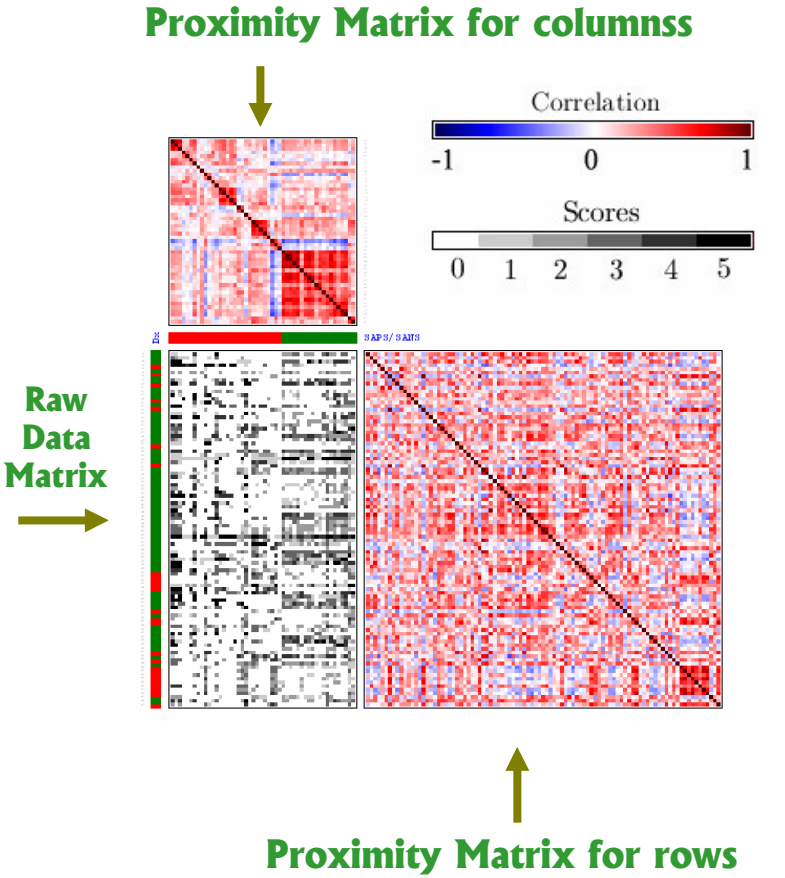
Estimating the number of clusters in a data set via the gap statistic

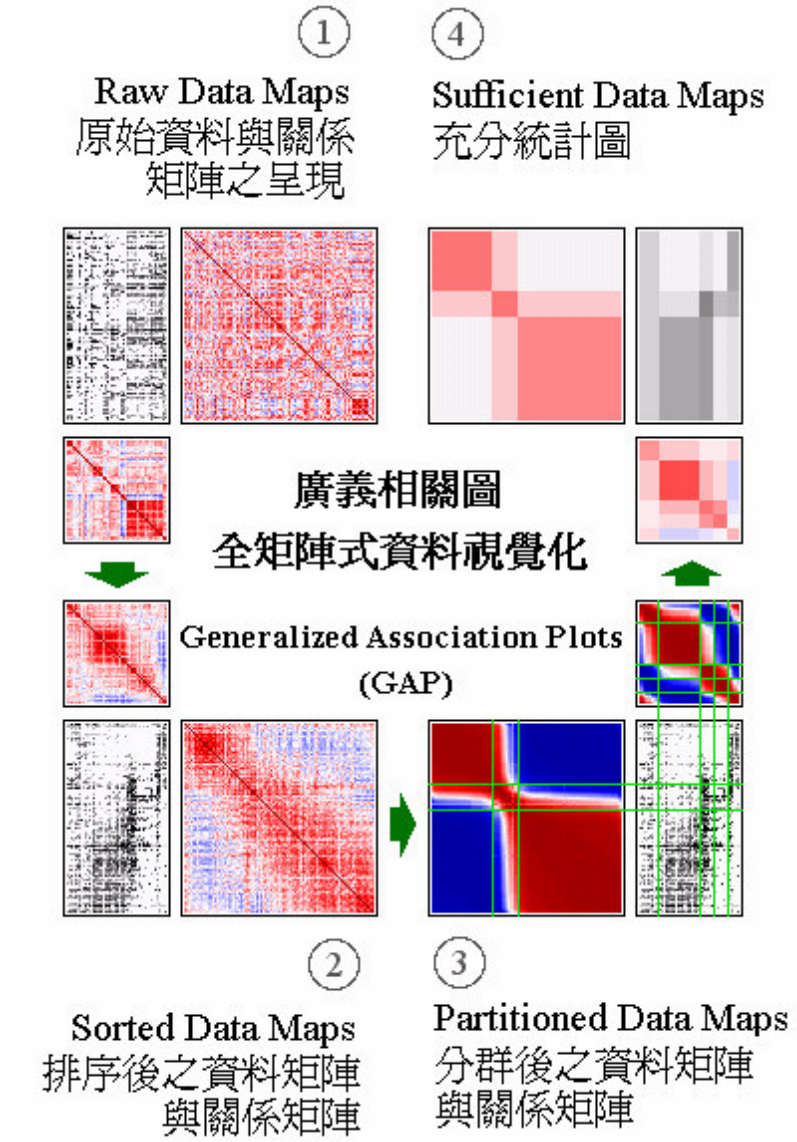
Robert Tibshirani, Guenther Walther and Trevor Hastie
Stanford University, USA

Generalized Association Plots (GAP)

(Chen, 2002)

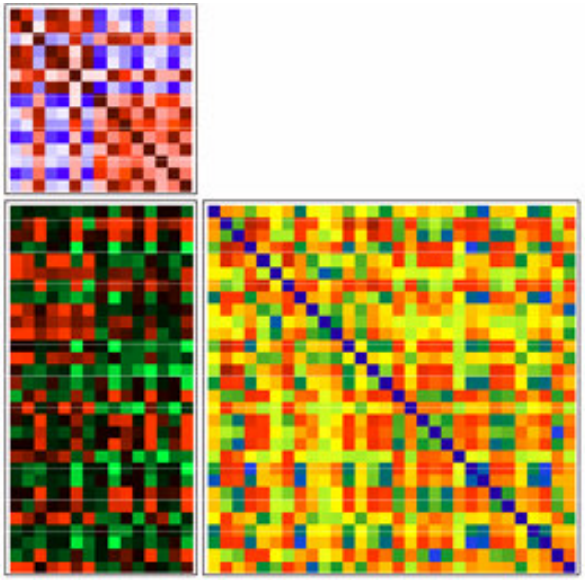
- 95 patients: 69 schizophrenic and 26 bipolar disorders
- SAPS: 30 items, SANS: 20 items
- Six point scale (0-5).





Presentation of Raw Data Matrix

1. Color spectrum
2. Variable transformation
3. Selection of proximity



"Resolution" of a Statistical Graph

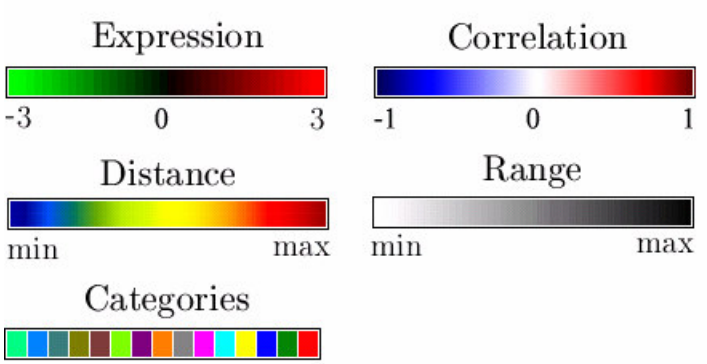
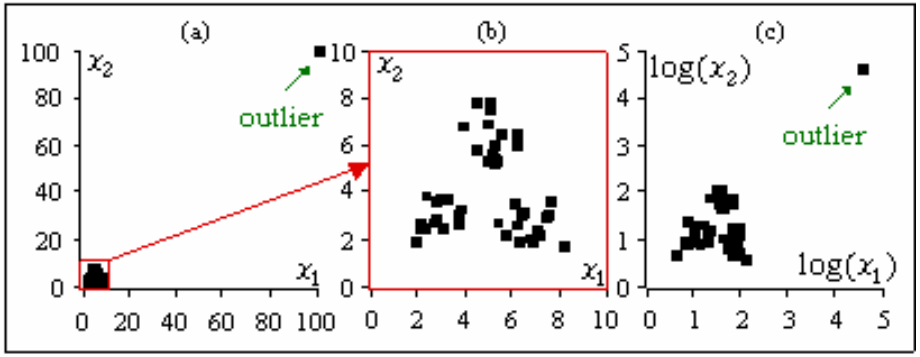
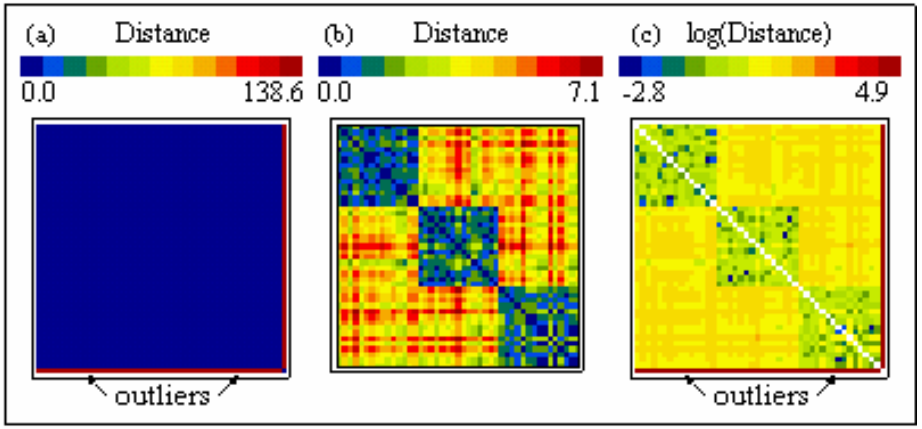
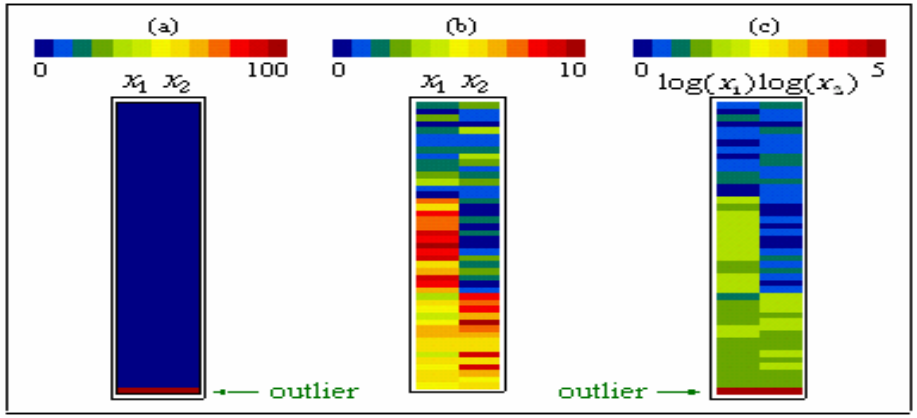


Image source: Dr. Chen Chun-houh's Silde

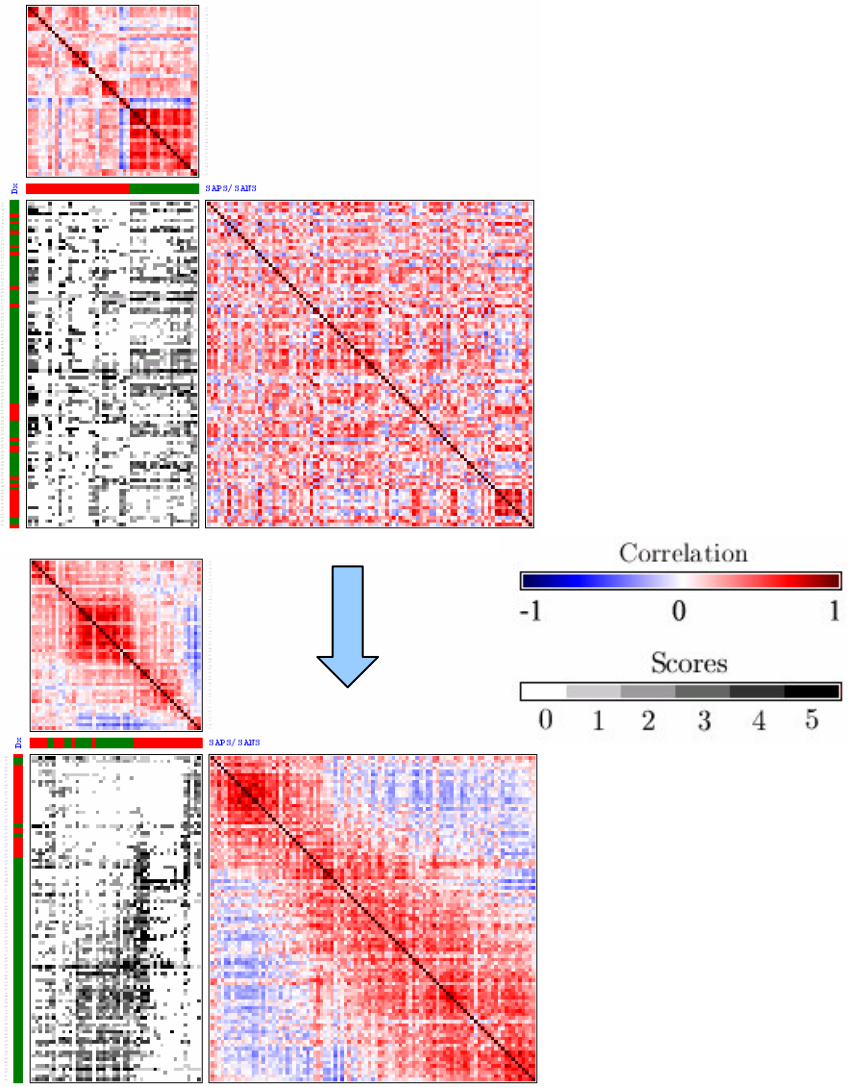


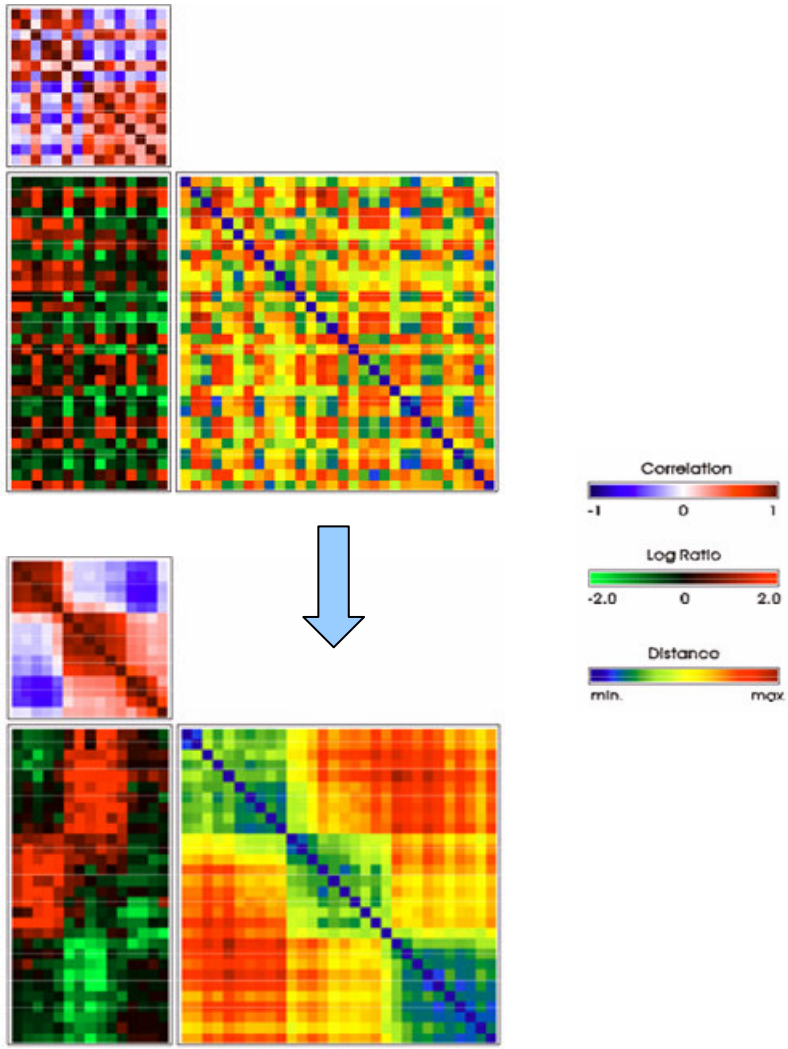




Concept of Relativity of a Statistical Graph

Placing similar (different) objects at closer (distant) positions

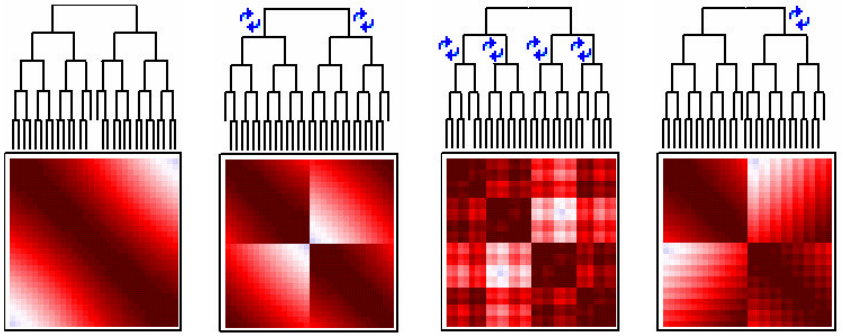




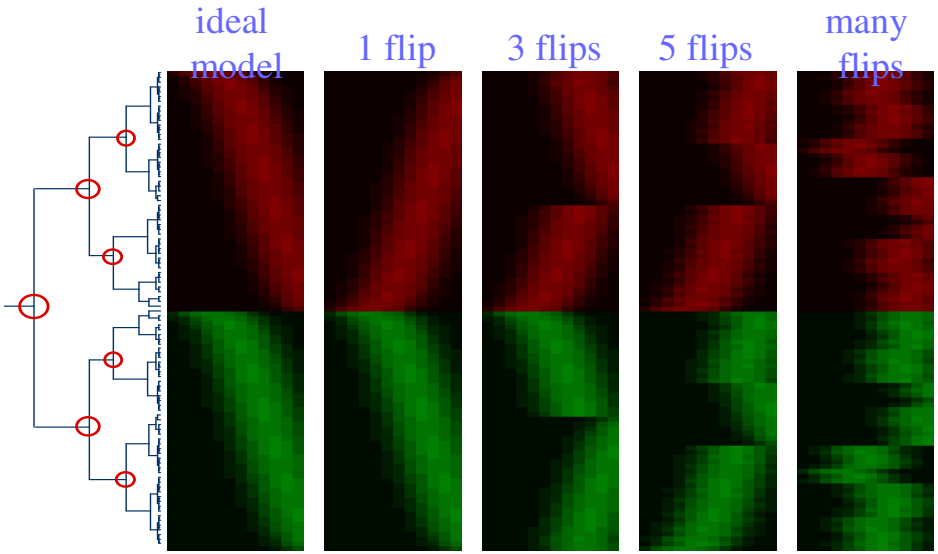
Seriation Problem for Hierarchical Clustering

Different Seriations
Generated from
Identical Tree Structure

Tree seriation for proximity matrices

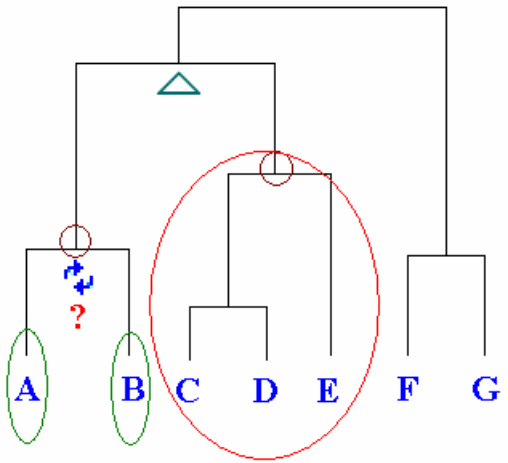


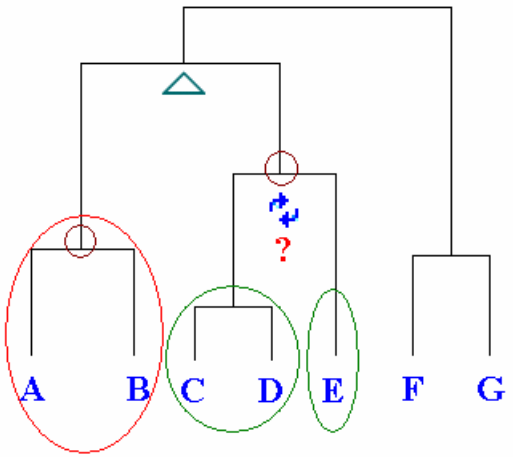
Tree seriation for raw data matrices



Internal Tree Flips

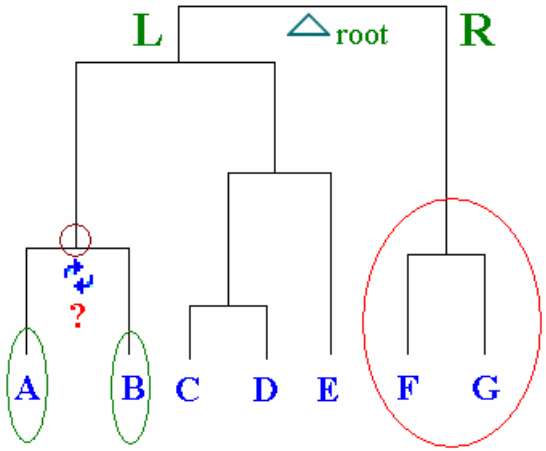
Uncle Approach

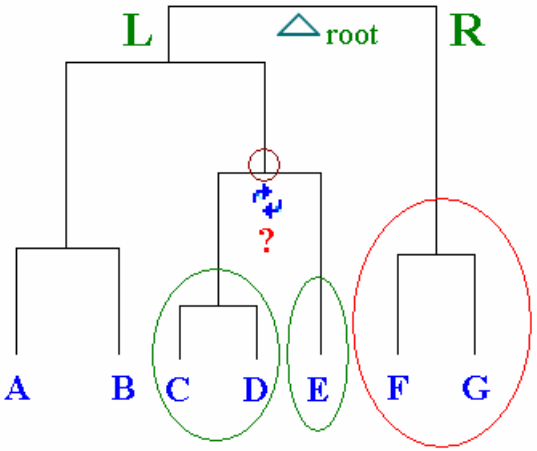


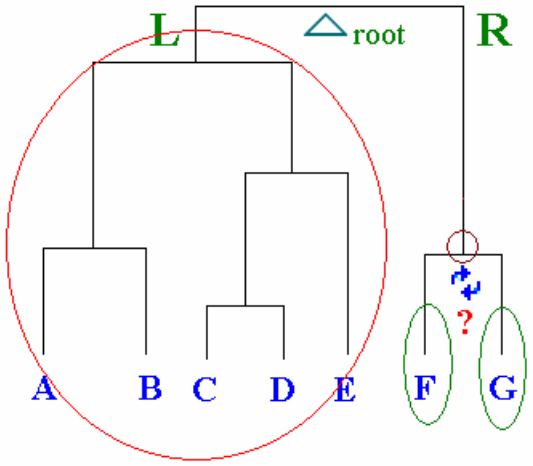


if d(A, {C,D, E}) < d(B, {C,D, E}) then flip

GrandPa Approach

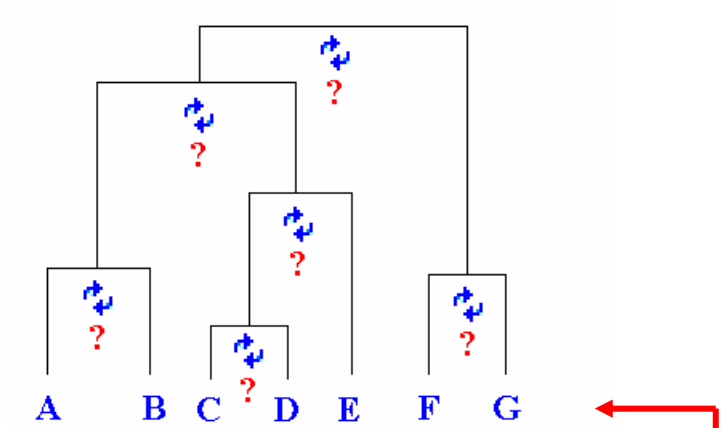






Further reading: Ziv Bar-Joseph, David K. Gifford, and Tommi S. Jaakkola, (2001), **Fast Optimal Leaf Ordering** for Hierarchical Clustering. Bioinformatics 17(Suppl. 1):S22–S29.

External Tree Flips



External Ordering D E A F B C G

As match as possible

How to build an external ordering?

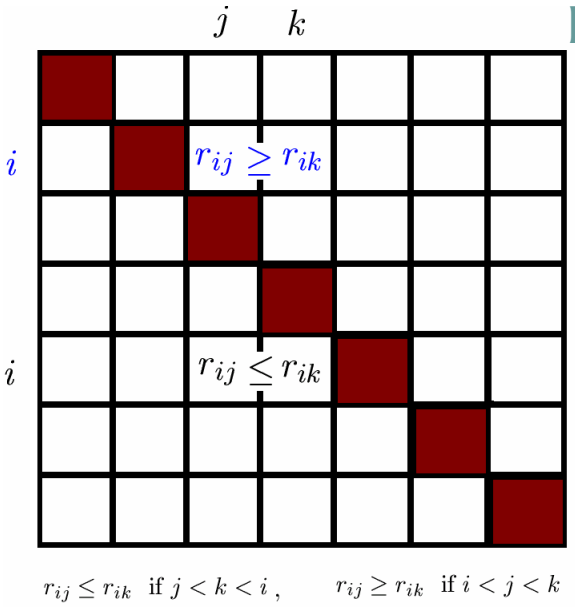
(1) Based on average expression level (Cluster Software, Eisen et al 1998)
(2) Using the results of a one-dimensional SOM
(3) …

Further reading: Tien, Y. J., Lee, Y. S, Wu, H. M. and Chen, C. H. (2006) Integration of clustering and visualization methods for simultaneously identifying coherent local clusters with smooth global patterns in gene expression profiles.

Criteria for a "good" Permutation

When T is symmetric, we usually want T' to approximate a Robinson form (Robinson (1951)).

Robinson Form



Global/local Criterion:
Anti-Robinson Measurements

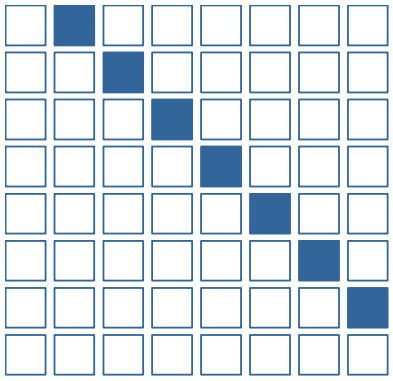
permuted matrix, $D = [d_{ij}]$

$$AR(i) = \sum_{i=1}^{p} \Big[\sum_{j<k<i} I(d_{ij} < d_{ik}) + \sum_{i<j<k} I(d_{ij} > d_{ik}) \Big],$$

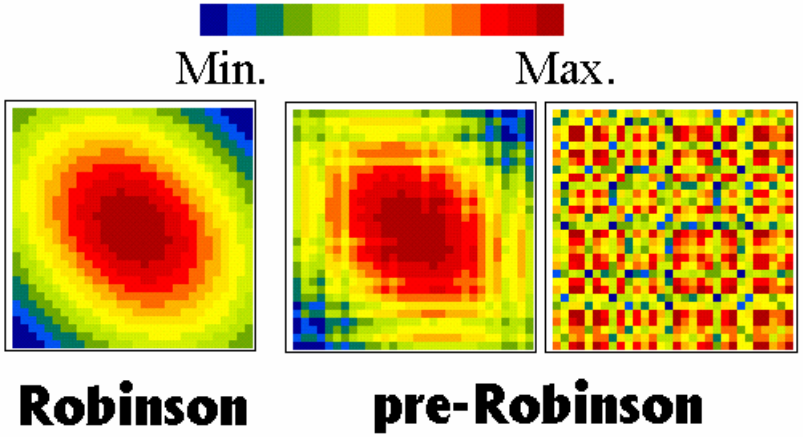
$$AR(s) = \sum_{i=1}^{p} \Big[\sum_{j<k<i} I(d_{ij} < d_{ik}) \cdot |d_{ij} - d_{ik}| + \sum_{i<j<k} I(d_{ij} > d_{ik}) \cdot |d_{ij} - d_{ik}| \Big],$$

$$AR(w) = \sum_{i=1}^{p} \Big[\sum_{j<k<i} I(d_{ij} < d_{ik}) |j-k| |d_{ij} - d_{ik}| + \sum_{i<j<k} I(d_{ij} > d_{ik}) |j-k| |d_{ij} - d_{ik}| \Big].$$

Local criterion: Minimal Span Loss Function



$$MS = \sum_{i=1}^{n-1} d_{i,i+1}$$

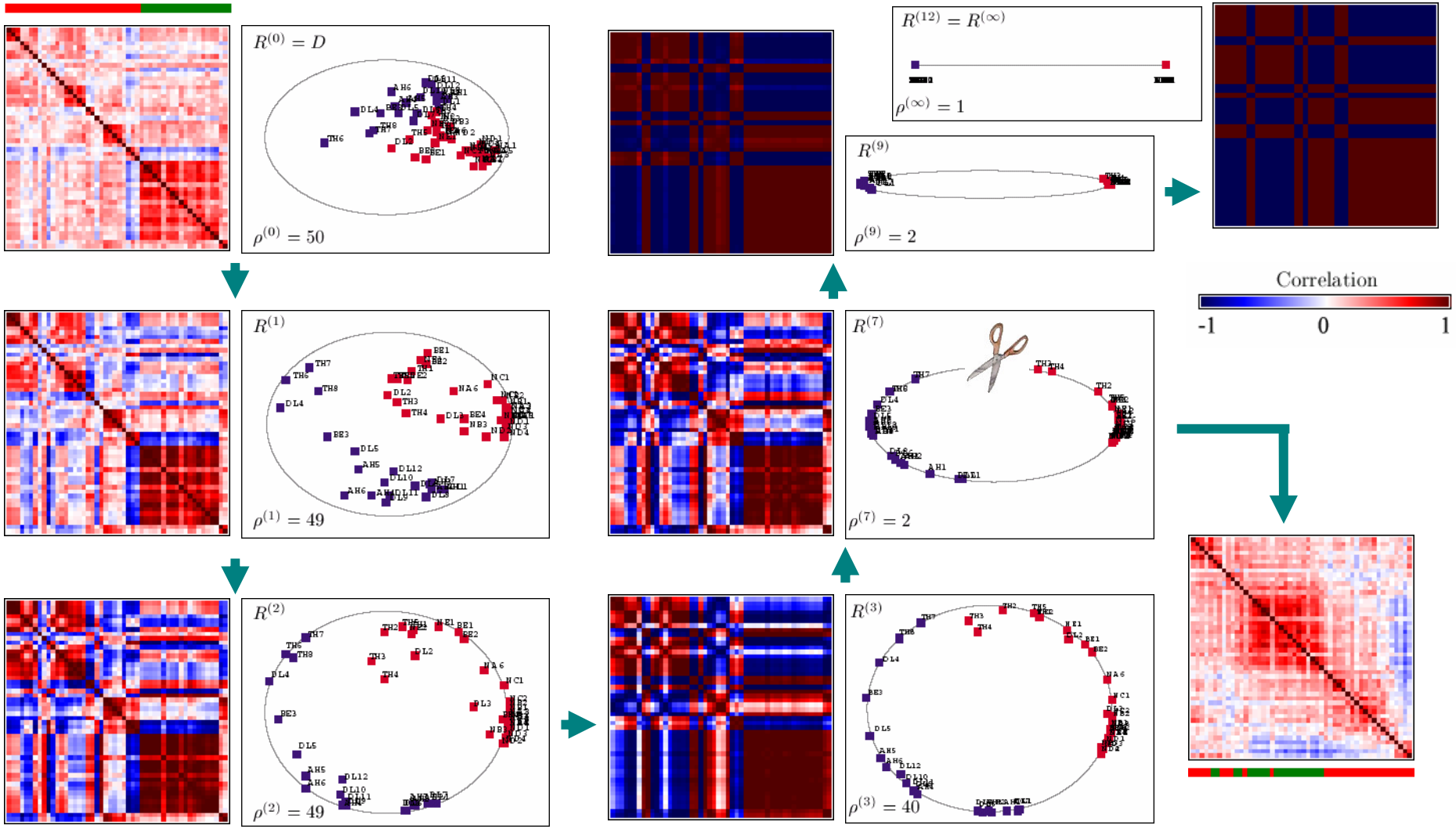


Further Reading

Michael Friendly , Ernest Kwan, (2003) Effect ordering for data displays, Computational Statistics & Data Analysis, v.43 n.4, p.509-539.

GAP Rank-Two Elliptical Seriation

- Seriation Algorithms with Converging Correlation Matrices
- When the sequence reaches an iteration with rank two, the p objects fall on an ellipse and have unique relative position on the ellipse.



Global vs Local Seriation

GAP Elliptical Seriation

An algorithm for identifying global clustering patterns and smoothing temporal expression profiles

GAP Elliptical Seriation Michael Eisen Tree Seriation

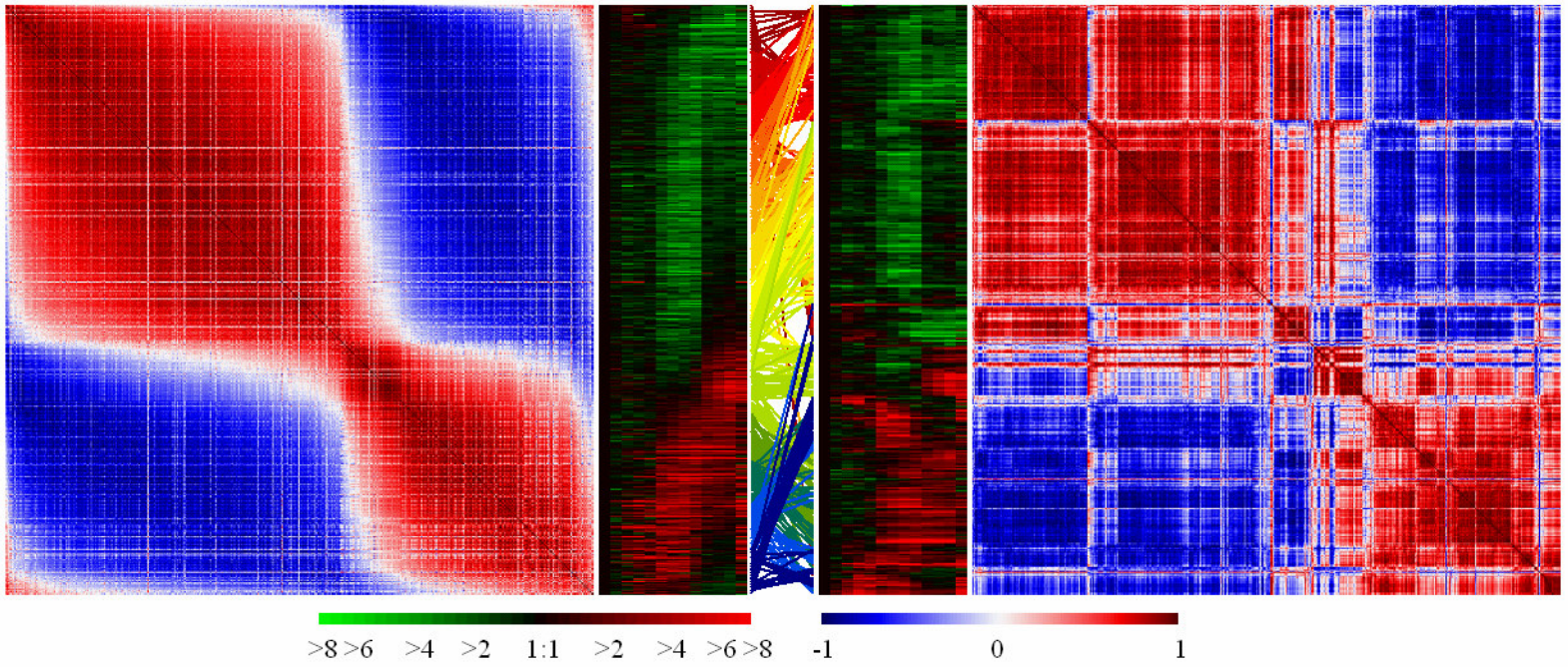
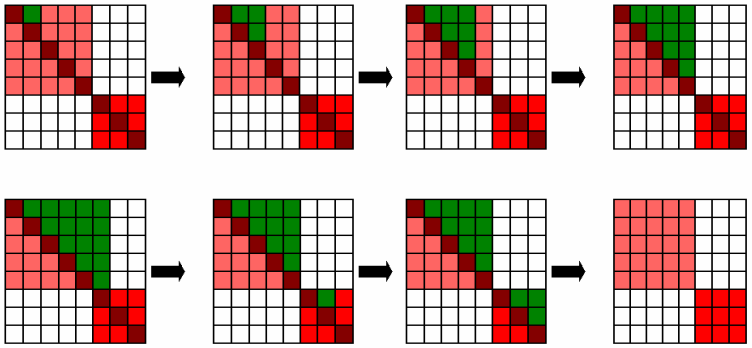


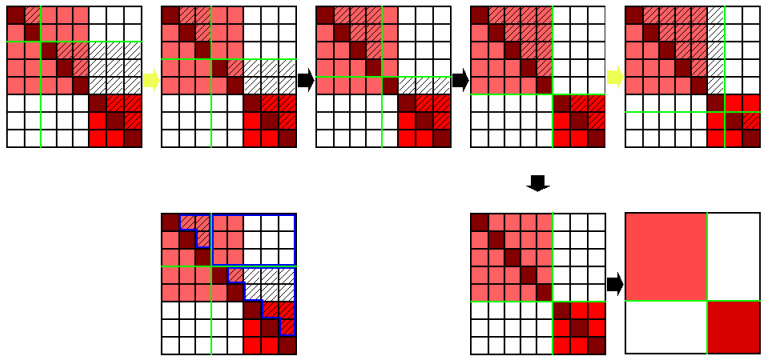
Image source: Dr. Chen Chun-houh's slide

Partitions of Permuted Matrix Maps

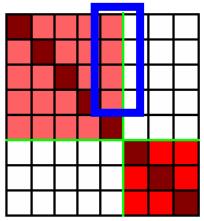
One-Way block Searching

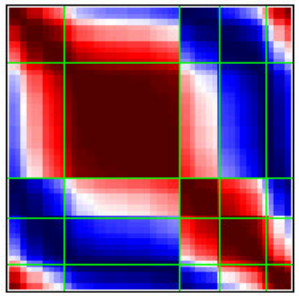


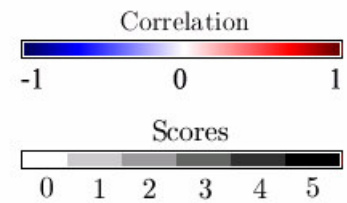
Within-Sum-of-Square Approach



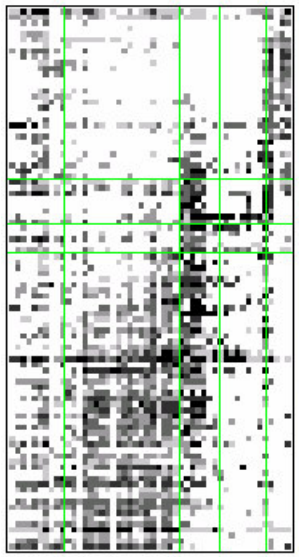
Two-Sample Problem

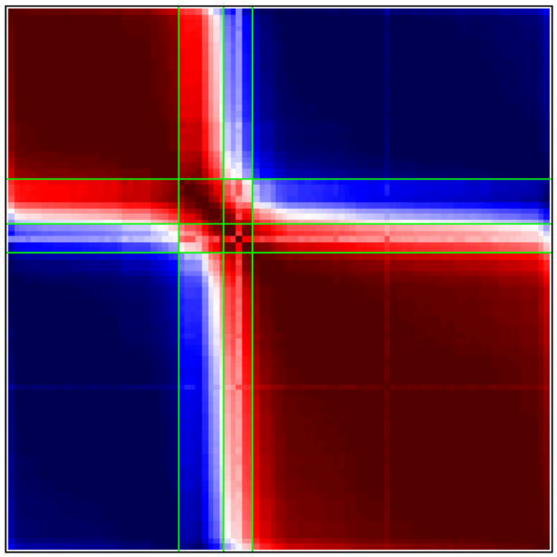




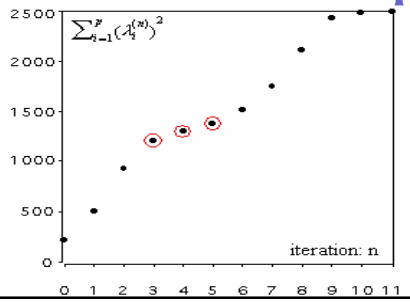


Row: $R^{(3)}$, Column: $R^{(4)}$





Sum squared eigenvalues (sum squared correlations)



Further Reading

J. A. Hartigan. Direct clustering of a data matrix. Journal of the American Statistical Association, 67(337):123-129, March 1972.
Duffy, D. & Quiroz, A. (1991), `A permutation-based algorithm for block clustering', J. of Classification 8, 65--91.

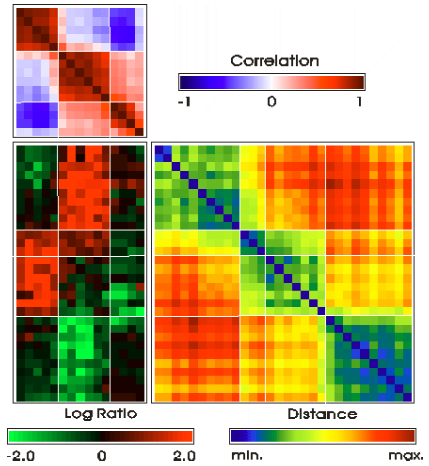
Sufficient Graph

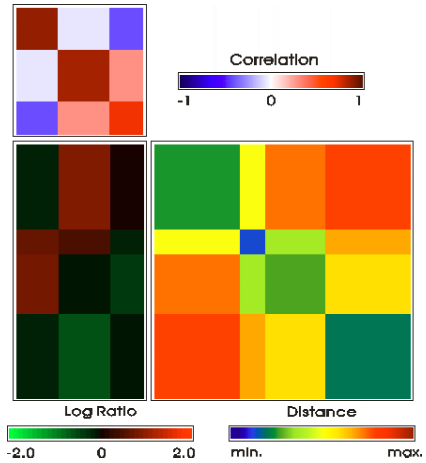
	A	B	C	D	E	F
1	學號	小考1	期中考	小考2	期末考	報告
2	A01	69	92	85	45	62
3	A02	66	90	83	36	90
4	A03	72	92	80	62	70
5	A04	68	90	60	37	95
6	A05	74	60	86	54	70
7	A06	77	90	88	88	95
8	A07	73	88	77	51	95
9	A08	61	90	84	40	82
10	A09	66	88	82	39	80
11	A10	76	75	87	72	80
12	A11	64	90	90	26	95
13	A12	75	90	60	55	70
14	A13	92	90	83	90	95

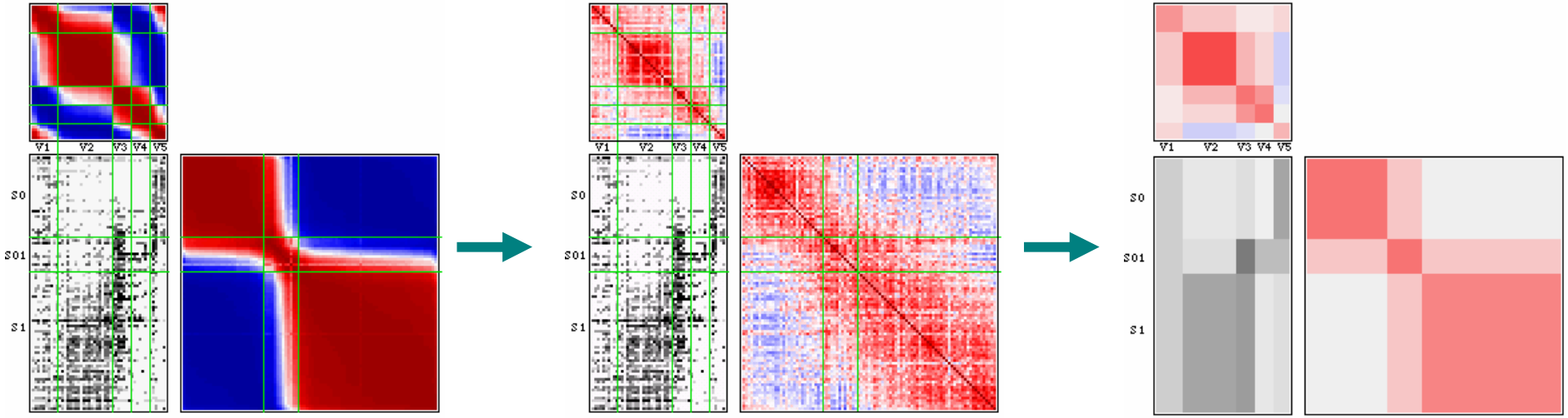
Sufficient Statistic

	小考1	期中考	小考2	期末考	報告
平均	71.77	86.54	80.38	53.46	83
低平均	65.67	81.83	73.67	53.67	72
高平均	77.83	90.67	86.67	53.67	94.17

70







Generalization and Flexibility

Sedimented MV for patients and symptoms.

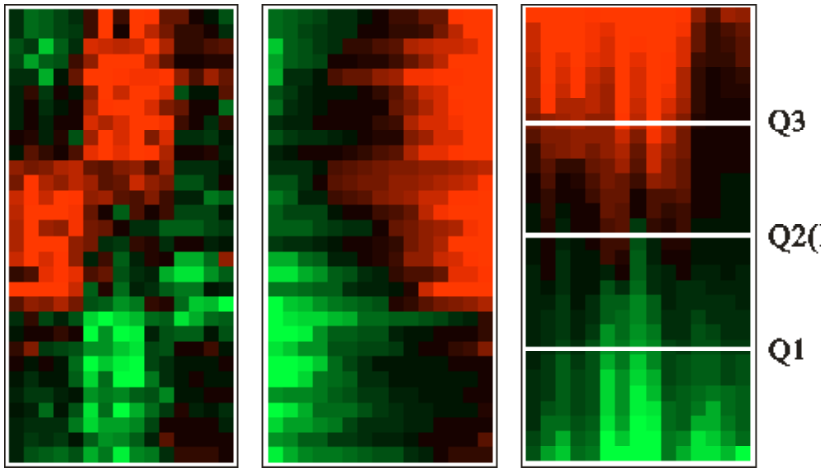
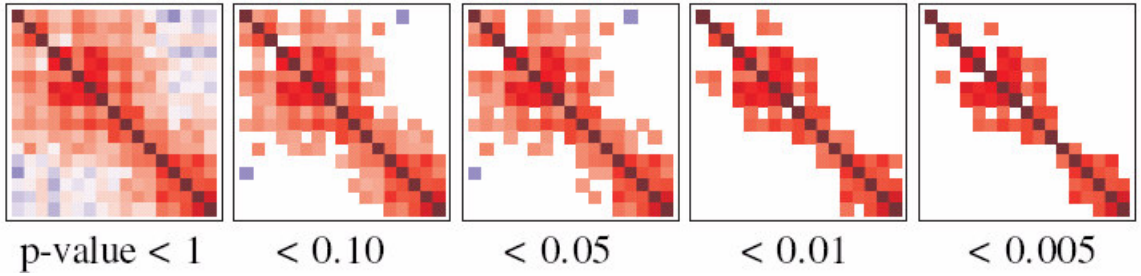


Image source: Chen etal 2004

The sediment MV for patients: express severity structure.

The sediment MV for symptoms: this is a side-by-side bar-chart and box-plot which displays the distribution structure for all symptoms simultaneously.

Sectional MV for the permuted correlation coefficient map.



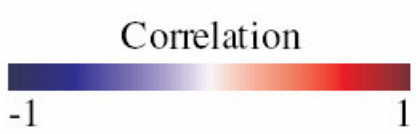
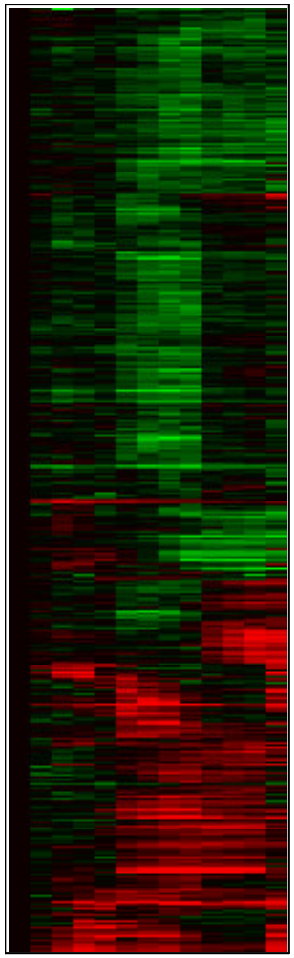


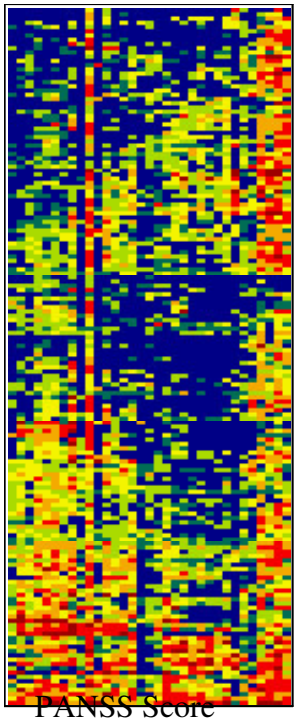
Image source: Chen etal 2004

Visualization of Data Matrices

Simple ← Information Visualization of Data Matrices → **Difficult**

Continuous (Gene/Time)	Ordinal (Patient/Symptom)	Binary (Mouse/Tumor)	Categorical (Subject/SNP)

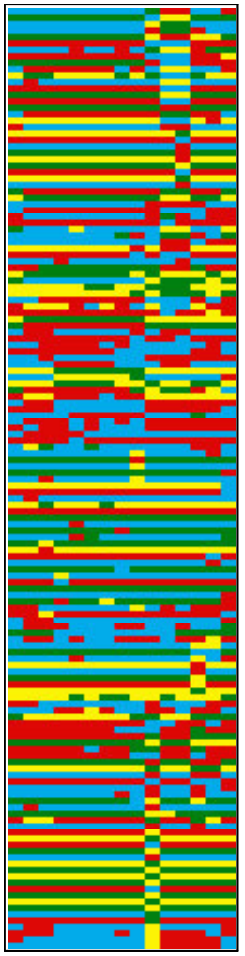




PANSS Score

1 2 3 4 5 6 7

0 1



>8 >6 >4 >2 1:1 >2 >4 >6 >8 Log2ratio

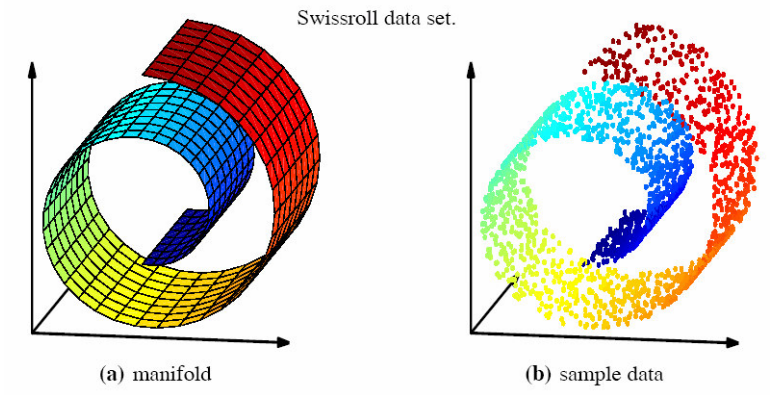
Image source: Chen Chun-houh's slide

A C G T

Concept of Manifolds and Nonlinearity

- A manifold is a topological space which is locally Euclidean. (i.e., around every point, there is a neighborhood that is topologically the same as the open unit ball in).
- In general, any object which is nearly "flat" on small scales is a manifold.
- Euclidean space is a simplest example of a manifold.
- More formally, any object that can be "charted" is a manifold.
- Intuitively, a manifold can be considered as a ``nice'' topological space that behaves at every point like our intuitive notion of a surface
- Manifolds arise naturally whenever there is a smooth variation of parameters [like pose of the face]

- The dimension of a manifold is the minimum integer number of co-ordinates necessary to identify each point in that manifold.



Isometric Mapping (isomap)

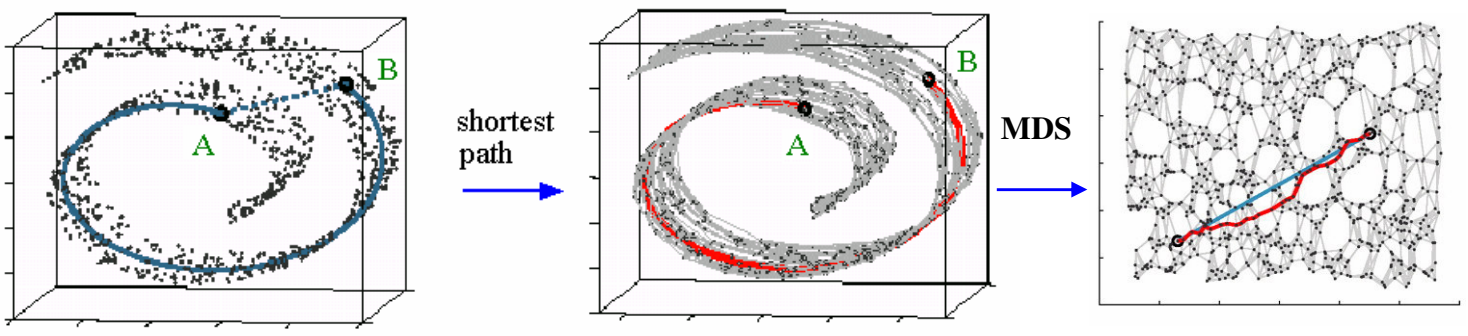
Isomap finds the projection that preserves the global, nonlinear geometry of the data by preserving the geodesic manifold interpoint distances.

- For neighboring points Euclidean distance is a good approximation to the geodesic distance.
- For farway points estimate the distance by a series of short hops between neighboring points.
- Find shortest paths in a graph with edges connecting neighboring data points.
- Once we have all pairwise geodesic distances use classical metric MDS

Algorithm of Isomap (Tenenbaum *et al.*, 2000)

1. Calculate the distance $d_X(i,j)$ between all pairs i,j from n data points in the p-dimensional input space.
2. Construct the graph by determining the neighbors for each data point with ϵ-Isomap or k-Isomap.
3. Pursue the shortest paths in the graph G. Initialize $d_G(i,j) = d_X(i,j)$ if i,j are neighbors; otherwise, set $d_G(i,j) = \infty$. For each value of $l = 1, 2, \cdots, n$ and for all i,j, $d_G(i,j)$ are replaced by $\min\{d_G(i,j), d_G(i,l) + d_G(l,j)\}$.
4. Apply classical MDS to D_G.

What is important is the geodesic distance!



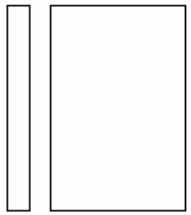
Tenenbaum , J. B., Silva, V. de, and Langford, J. C. (2000). A Global Geometric Framework for Nonlinear Dimensionality Reduction, Science 290, 2319-2323.

Example

lymphoma dataset

Alizadeh *et al.* (2000)

96 samples



854 genes

9 diagnostic classes

defined by Alizadeh *et al.* (2000).

- DLBCL
- Germinal Centre B
- NI Lymph Node/Tonsil
- Activated blood B
- Resting/activated T
- Transformed cell lines
- Follicular lymphoma
- Resting blood B
- CLL

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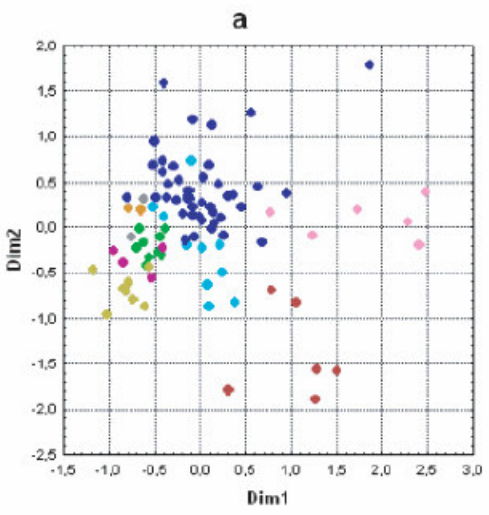
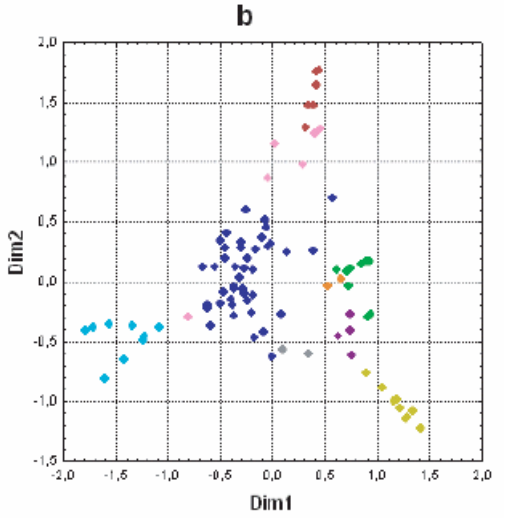
Vol. 20 no. 6 2004, pages 874–880
DOI: 10.1093/bioinformatics/btg496

Approximate geodesic distances reveal biologically relevant structures in microarray data

Jens Nilsson[1,], Thoas Fioretos[2], Mattias Höglund[2] and Magnus Fontes[1]*

[1]Centre for Mathematical Sciences, Lund University, Box 118, SE-221 00 Lund, Sweden and [2]Department of Clinical Genetics, Lund University Hospital, SE-221 85 Lund, Sweden



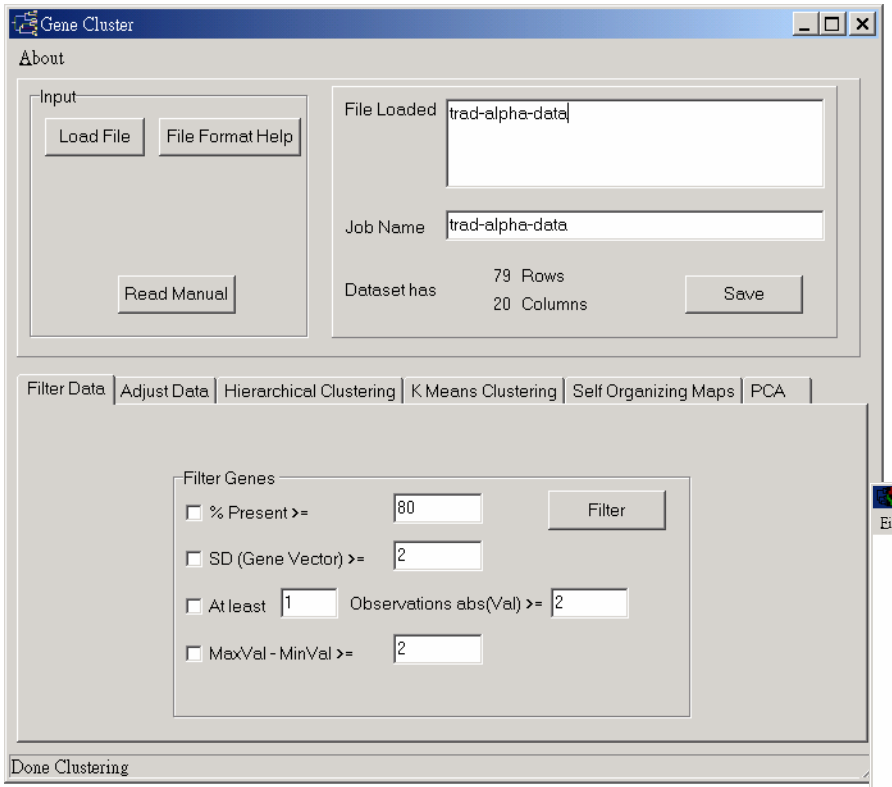


Software

- Cluster and TreeView
- Bioconductor: Limma, LimmaGUI, LimmaAffy, gclus
- PermutMatrix
- GAP (Generalized Association Plots)

- GeneSpring GX v7.3

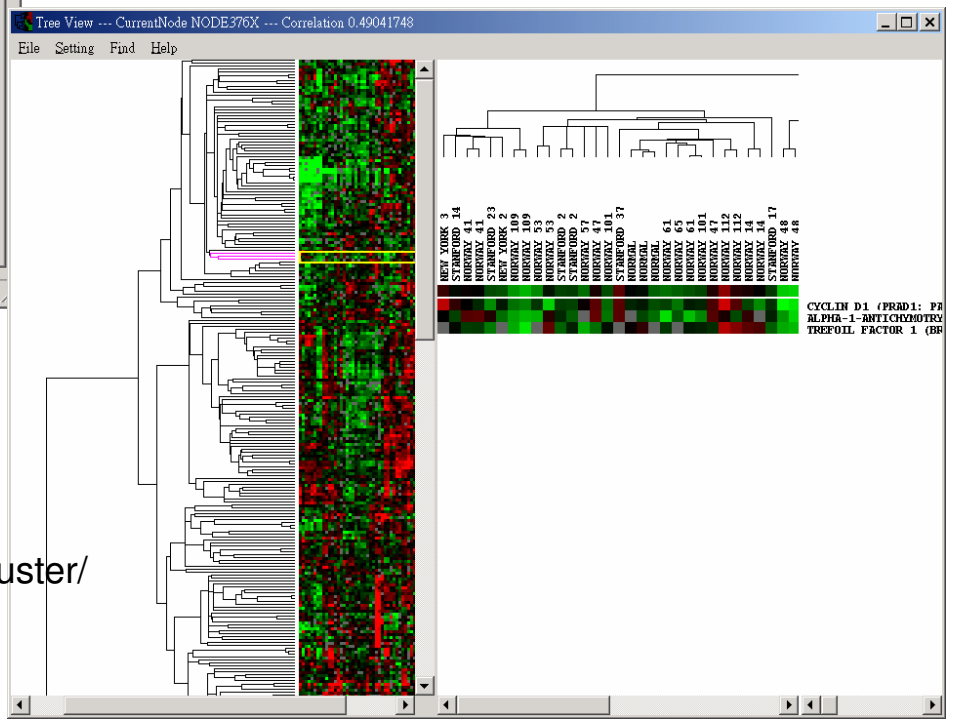
Cluster and TreeView



Eisen Lab

http://rana.lbl.gov/EisenSoftware.htm

Eisen MB, Spellman PT, Brown PO, Botstein D. (1998) **Cluster analysis and display of genome-wide expression patterns**. *Proc Natl Acad Sci.* 95(25):14863-8.



De Hoon, M.J.L.; Imoto, S.; Nolan, J.; Miyano, S.; "**Open source clustering software**". Bioinformatics, 20 (9): 1453--1454 (2004)

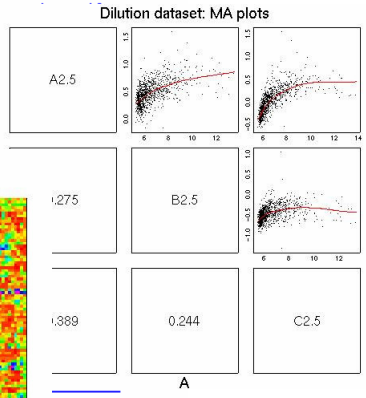
http://bonsai.ims.u-tokyo.ac.jp/~mdehoon/software/cluster/

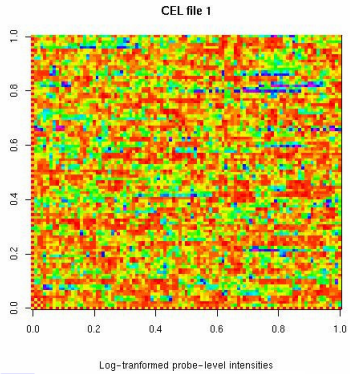
Bioconductor

Package

AnnBuilder
Biobase
DynDoc
MAGEML
MeasurementError.cor
RBGL
ROC
RdbiPgSQL
Rdbi
Rgraphviz
Ruuid

genefilter
geneplotter
globaltest
gpls
graph
hexbin
limma





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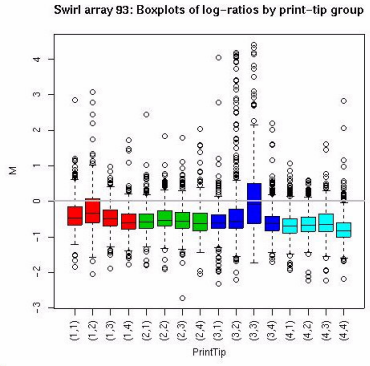
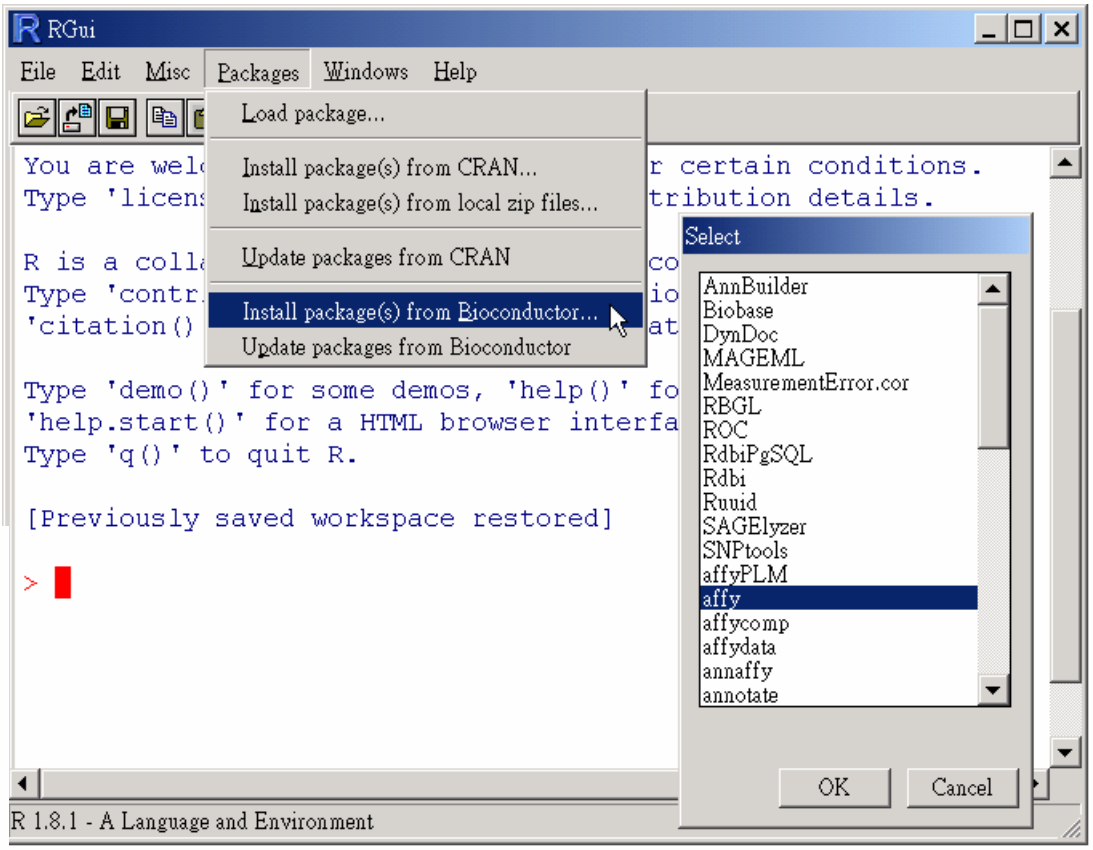


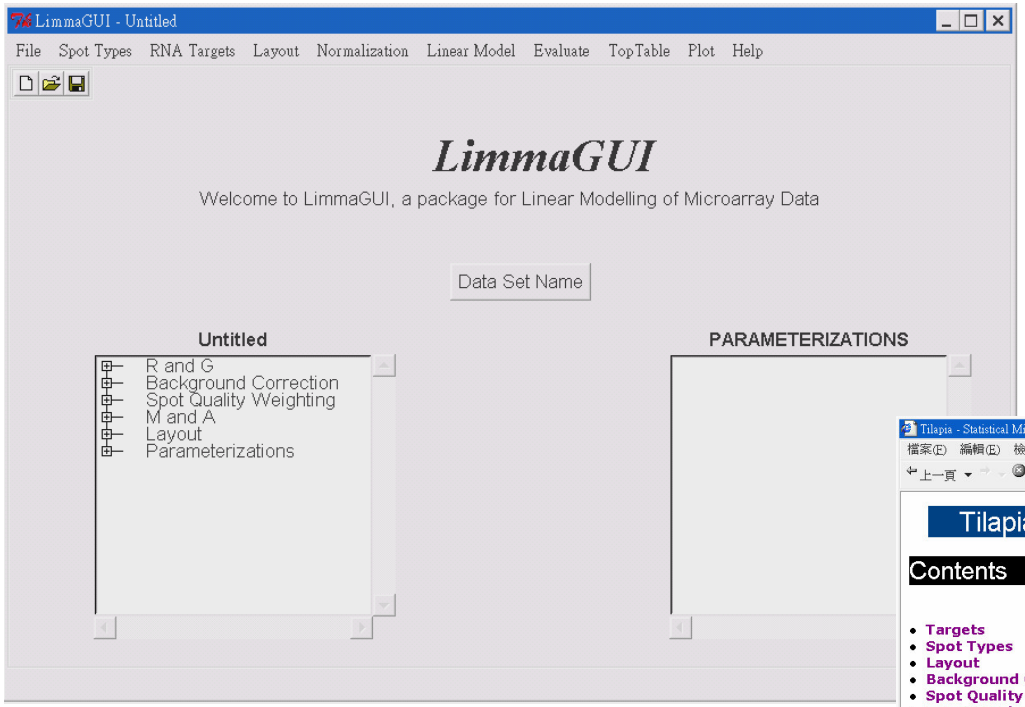
The Bioconductor
version 1.6
http://www.bioconductor.org

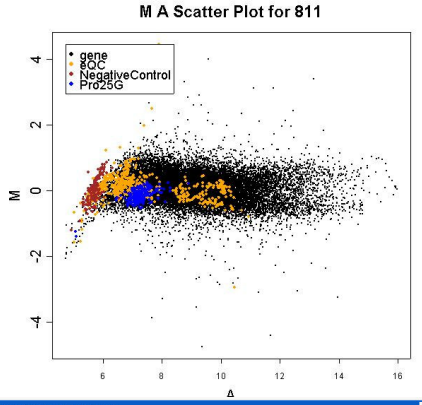
The R Project for Statistical Computing

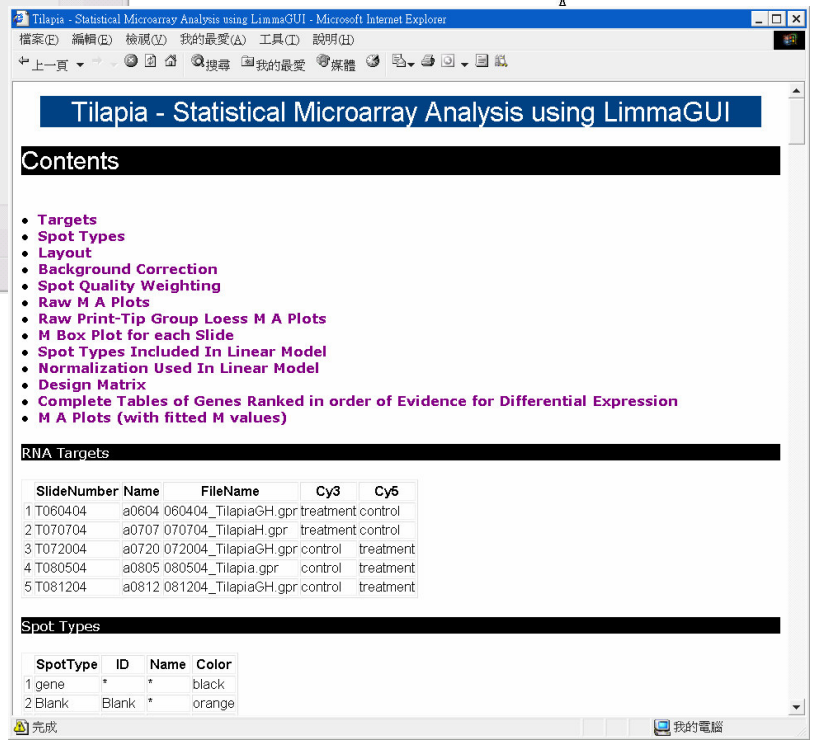
R version 2.1.1 (**2005-06-20**)
http://www.r-project.org



Limma, LimmaGUI, LimmaAffy







Limma: Linear Models for Microarray Data

http://bioinf.wehi.edu.au/limma/

LimmaGUI: a menu driven interface of Limma

http://bioinf.wehi.edu.au/limmaGUI

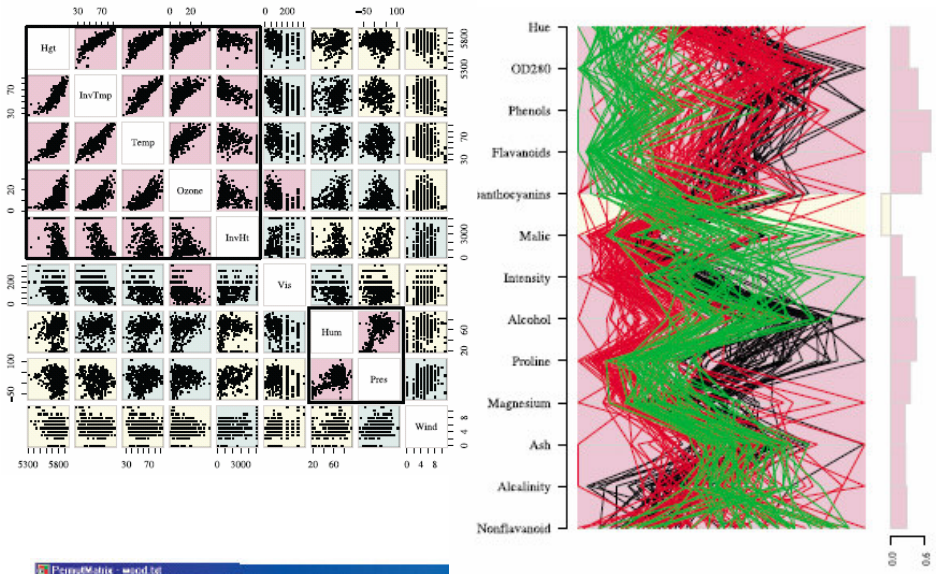
- Smyth, G. K. (2005). Limma: linear models for microarray data. In: Bioinformatics and Computational Biology Solutions using R and Bioconductor, R. Gentleman, V. Carey, S. Dudoit, R. Irizarry, W. Huber (eds.), Springer, New York, Chapter 23. (To be published in 2005)
- Smyth, G. K. (2004). Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. Statistical Applications in Genetics and Molecular Biology 3, No. 1, Article 3.

Gclus, PermutMatrix

gclus: Clustering Graphics

(R package)
http://cran.r-project.org/src/contrib/Descriptions/gclus.html

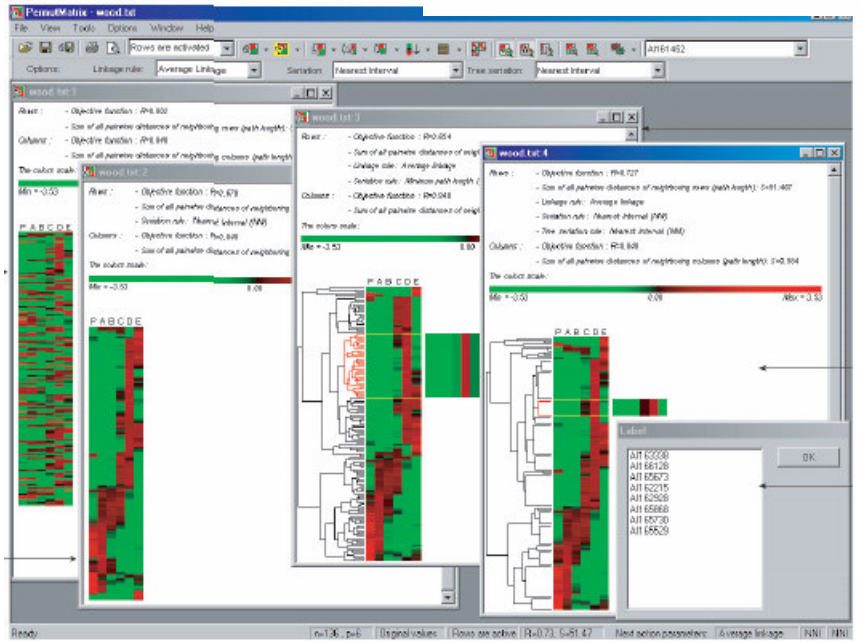
Catherine B. Hurley, (2004), Clustering Visualizations of Multidimensional Data, Journal of Computational & Graphical Statistics, Vol. 13, No. 4, pp.788-806



PermutMatrix

http://www.lirmm.fr/~caraux/PermutMatrix

Caraux, G., and Pinloche, S. (2005), "Permutmatrix: A Graphical Environment to Arrange Gene Expression Profiles in Optimal Linear Order," Bioinformatics, 21, 1280-1281.



GAP (Generalized Association Plots)

Generalized Association Plots

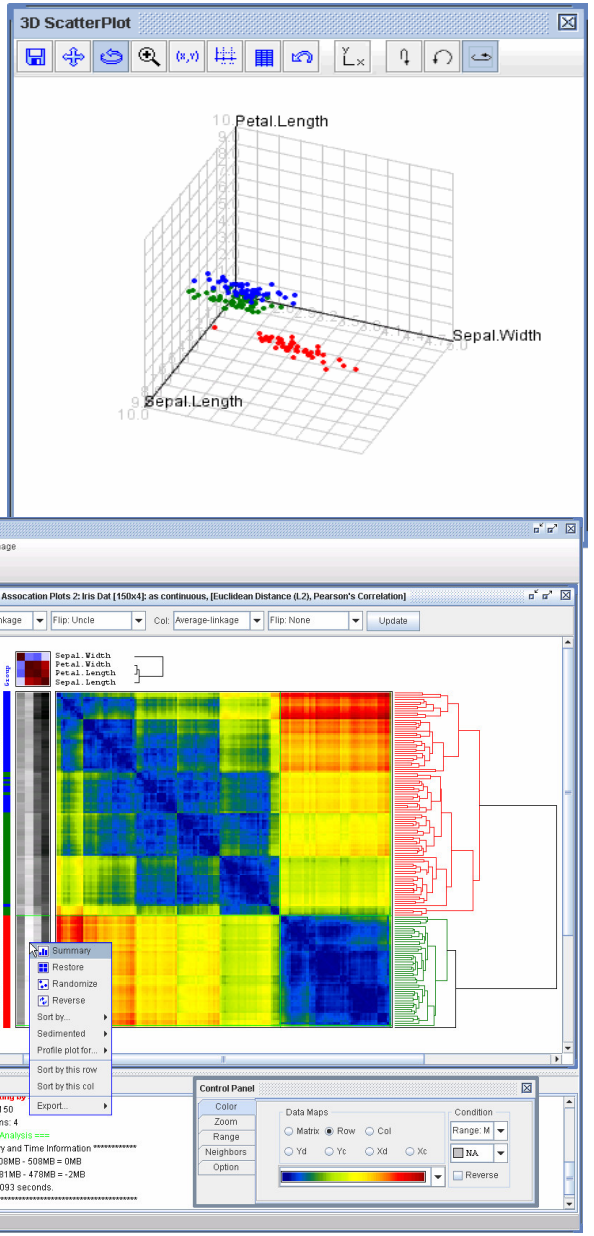
- Input Data Type: continuous or binary.
- Various seriation algorithms and **clustering analysis**.
- Various display conditions.
- GAP with Covaraite Adjusted, Nonlinear Association Analysis, Missing Value Imputation.

Statistical Plots

- 2D Scatterplot, 3D Scatterplot (Rotatable)

Web Site

http://gap.stat.sinica.edu.tw/Software/GAP



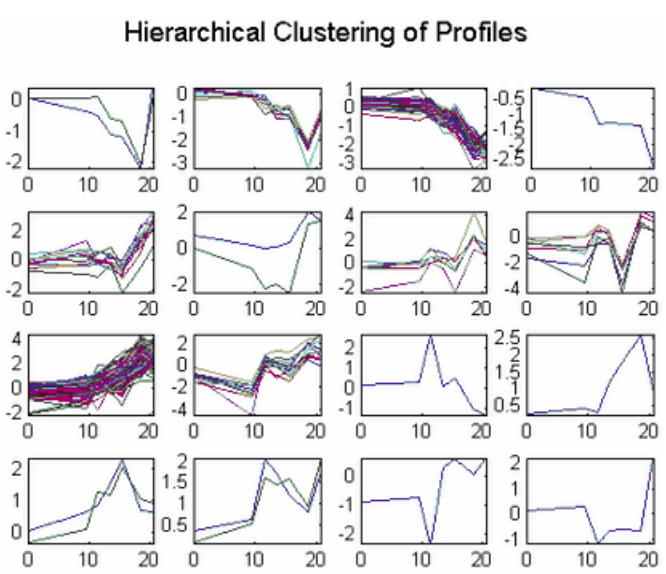
Chen, C. H. (2002). Generalized Association Plots: Information Visualization via Iteratively Generated Correlation Matrices. Statistica Sinica 12, 7-29.
Wu, H. M., Tien, Y. J. and Chen, C. H. (2006). GAP: a Graphical Environment for Matrix Visualization and Information Mining.

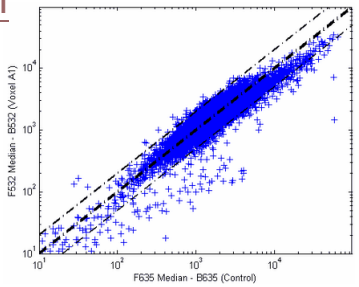
Matlab: Bioinformatics ToolBox

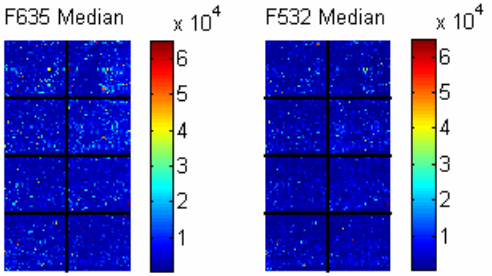
The MathWorks
Bioinformatics Toolbox

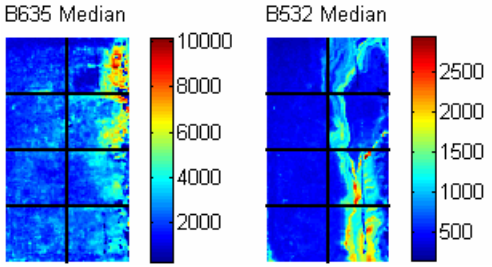
http://www.mathworks.com/access/helpdesk/help/toolbox/bioinfo/index.html

- Data Formats and Databases — Access online databases, read and write to files with standard genome and proteome formats such as FASTA and PDB.
- Sequence Alignments — Compare nucleotide or amino acid sequences using pairwise and multiple sequence alignment functions.
- Sequence Utilities and Statistics — Manipulate sequences and determine physical, chemical, and biological characteristics.
- Microarray Analysis — Read, filter, normalize, and visualize microarray data.
- Protein Structure Analysis — Determine protein characteristics and simulate enzyme cleavage reactions.





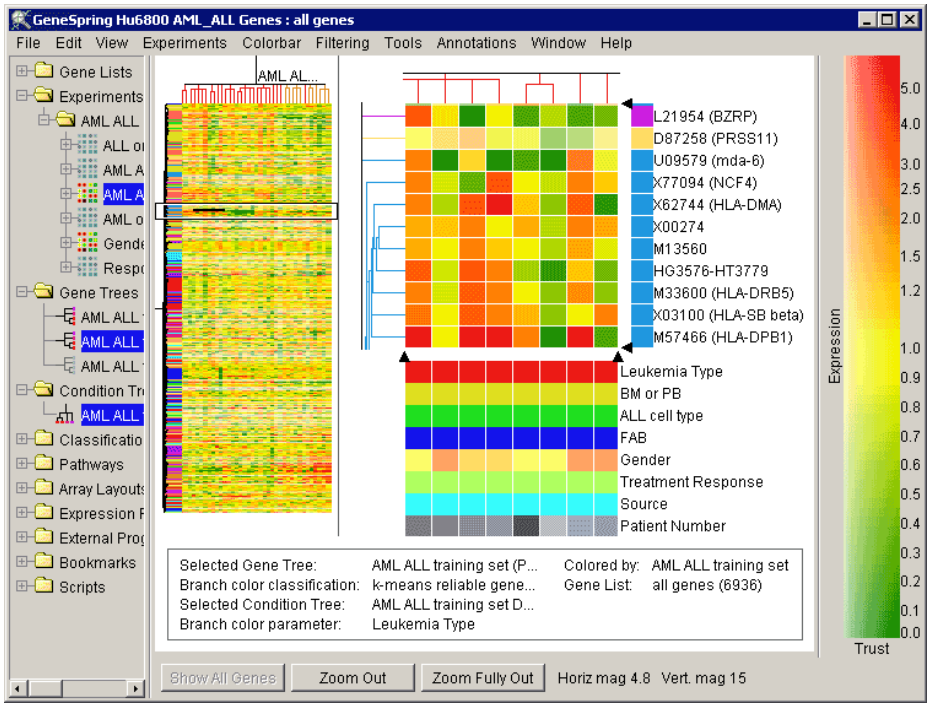


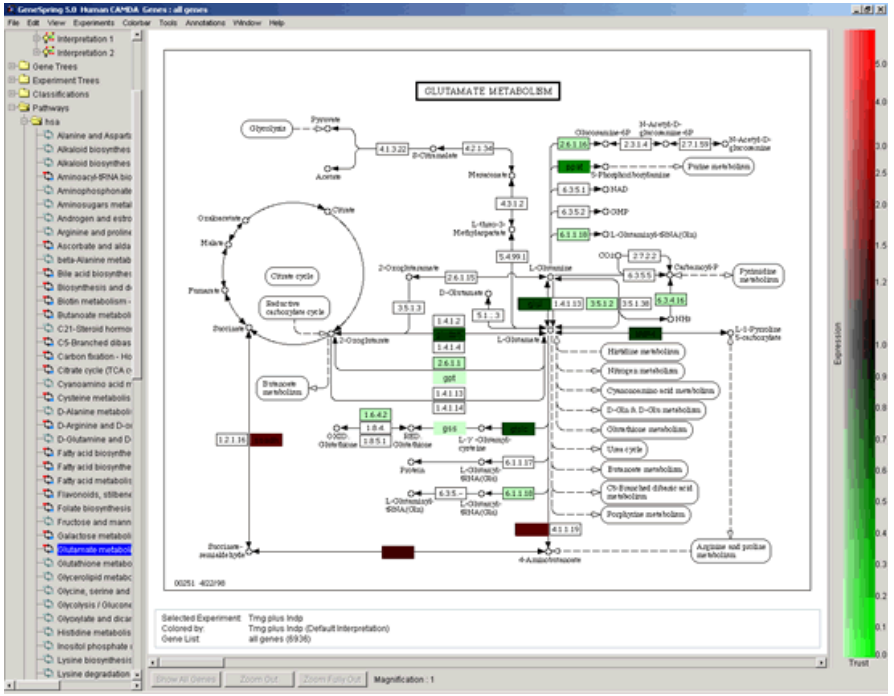


- Prototype and Development Environment — Create new algorithms, try new ideas, and compare alternatives.
- Share Algorithms and Deploy Applications — Create GUIs and stand-alone applications.

GeneSpring GX v7.3

- RMA or GC-RMA probe level analysis
- Advanced Statistical Tools
- Data Clustering
- Visual Filtering
- 3D Data Visualization
- Data Normalization (Sixteen)
- Pathway Views
- Search for Similar Samples
- Support for MIAME Compliance
- Scripting
- MAGE-ML Export





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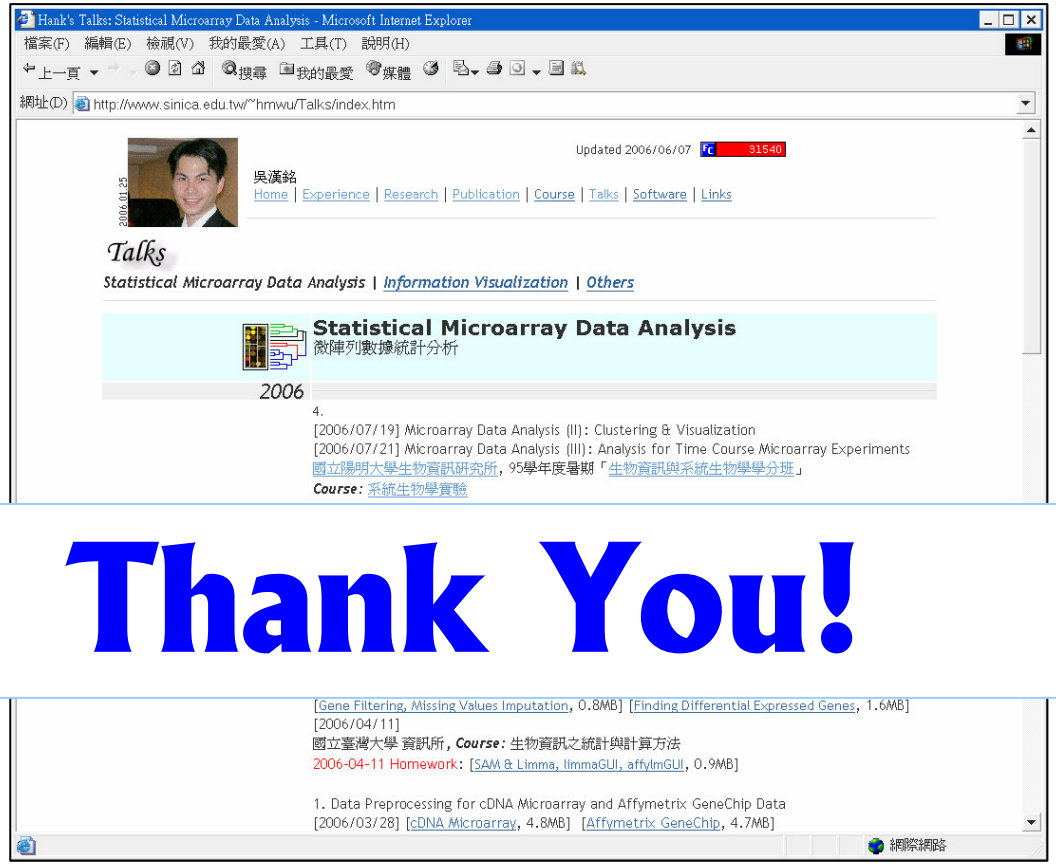
More than 700 papers

Questions?



Thank You!

Reference: http://www.sinica.edu.tw/~hmwu/MADA/index.htm

吳漢銘
hmwu@stat.sinica.edu.tw
http://www.sinica.edu.tw/~hmwu
中央研究院 統計科學研究所
Institute of Statistical Science, Academia Sinica