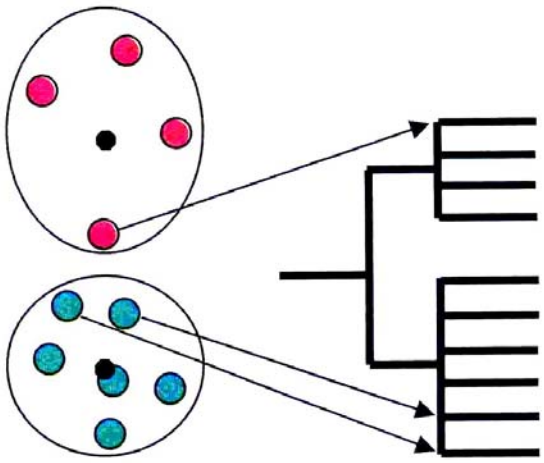
Clustering Algorithm

- Clustering Algorithm
  - k-means: *k* clusters; each cluster is represented by the center of the cluster
  - PAM : *k* clusters; each cluster is represented by one of the objects in the cluster
  - Hierarchical clustering: returns a complete tree with individual patterns as leaves and the convergence points of all branches as the root.
  - SOM

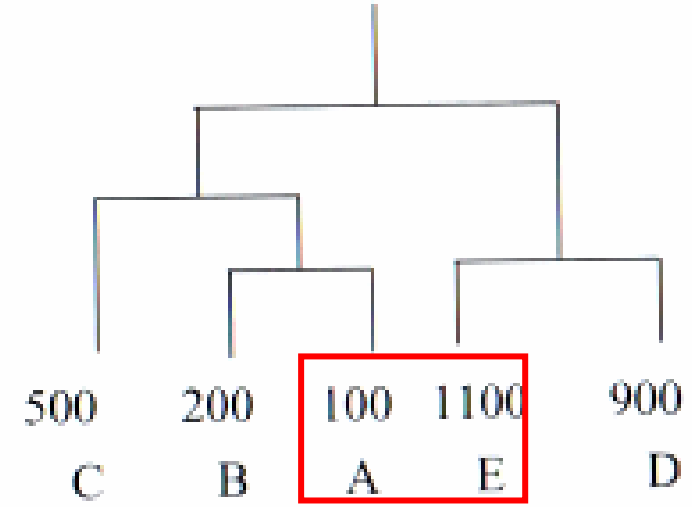
SOM: Motivation

- Misleading dendrograms:

K-means



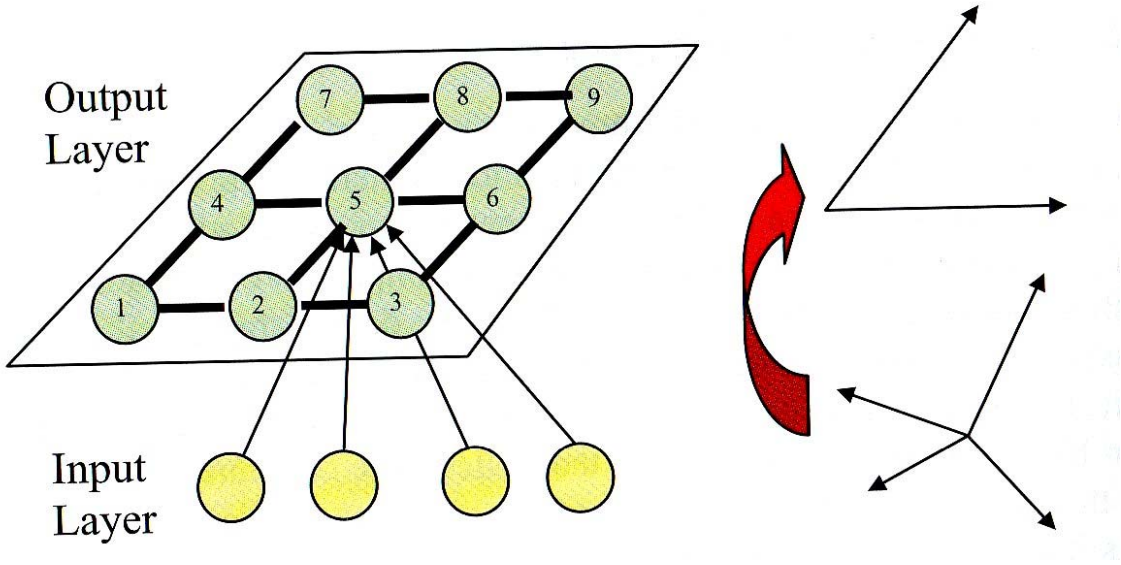
Hierarchical



- The SOM clustering is designed to create a plot in which similar patterns are plotted next to each other.

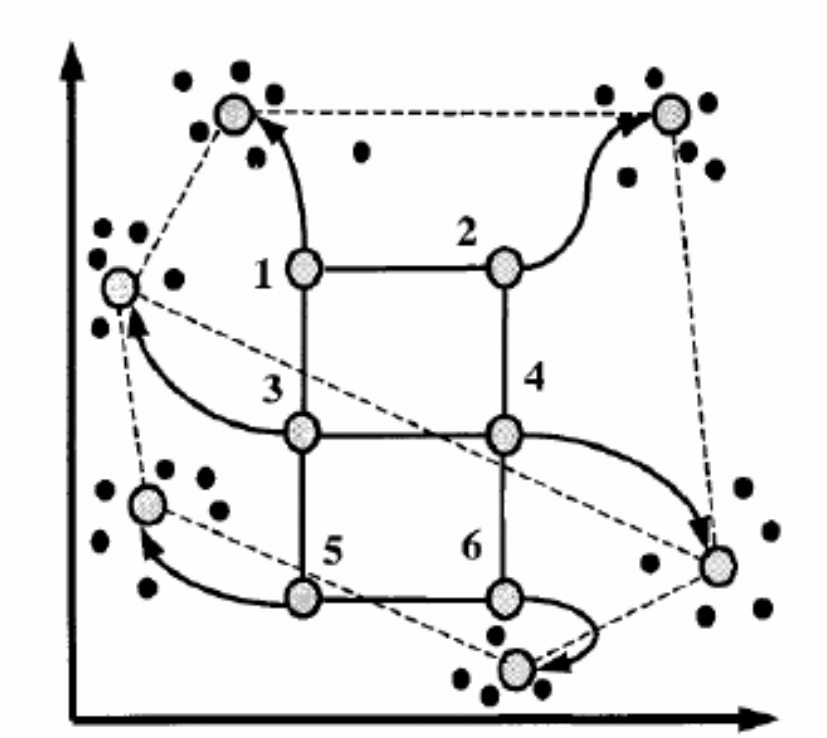
Self-Organizing Feature Maps (SOM)

- SOM: A *map* consists of many simple elements (nodes or neurons); it is constructed by training.
  - SOMs are believed to resemble processing that can occur in the brain
  - Useful for visualizing high-dimensional data in 2- or 3-D space
  - The number of groups = number of nodes



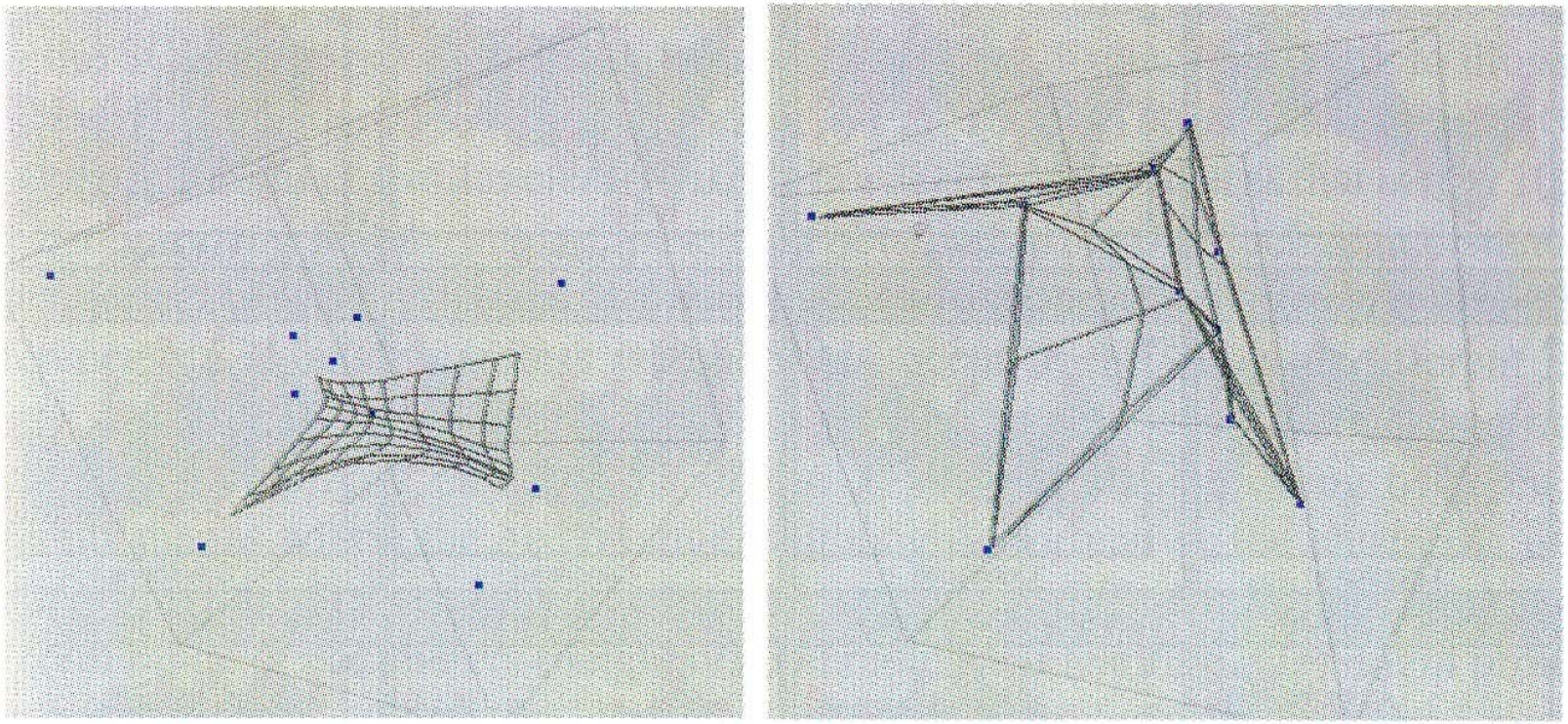
SOM: Example

1. Six nodes (N_1, N_2,…, N_6) of 3×2 grids on 2D are first selected.
2. At each iteration, a data point (gene) P is randomly selected. N_P is the node that maps nearest to P. Then the mapping of nodes is updated by moving points (all nodes) towards P. N_p is moved the most.
3. Data point P is recycled and the procedures continue for 20,000-50,000 iterations.



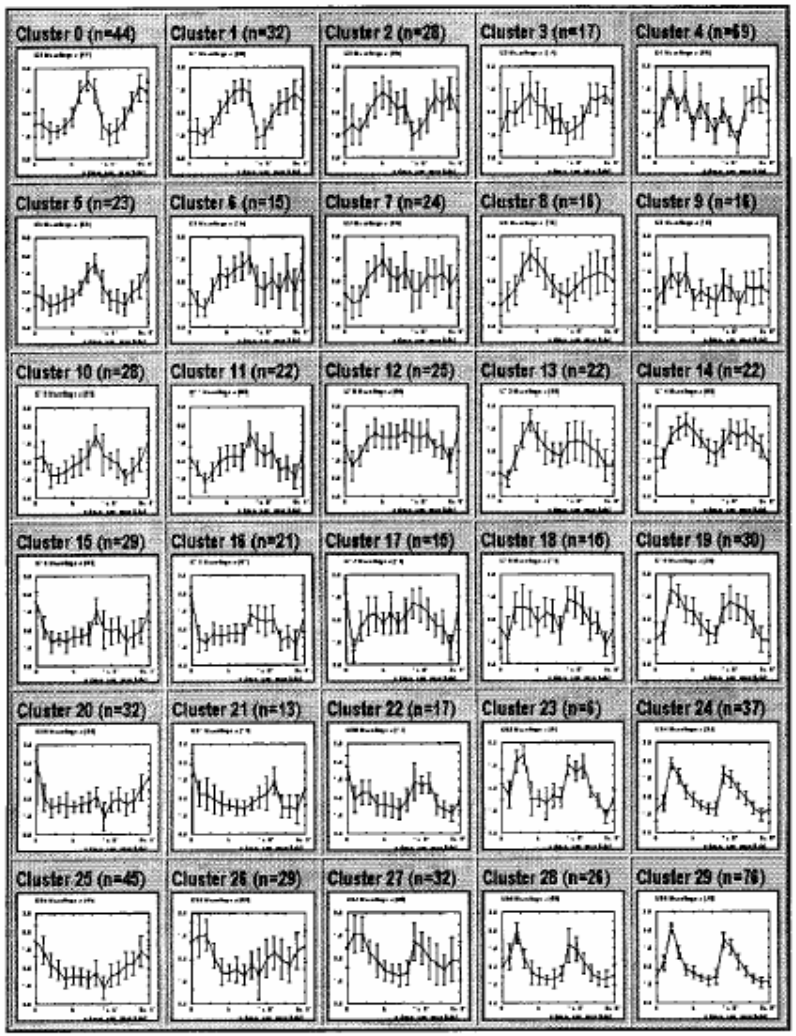
Self-Organizing Feature Maps (SOM)

- This process can be visualized by imagining all SOM units being connected to each other by rubber bands.



A 2D SOFM trained on 3-dimensional data.

Example - Tamayo et al.(1999)



6 x 5 SOM.

The 828 genes that passed the variation filter were grouped into 30 clusters.

Each cluster is represented by the centroid (average pattern) for genes in the cluster.

Expression levels are shown on *y*-axis and time points on *x*-axis. Error bars indicate the SD of average expression. *n* indicates the number of genes within each cluster.

- Literature:
  - Eisen, Spellman, Browndagger, and Botstein (1998) Cluster analysis and display of genome-wide expression patterns. *PNAS*, **95**, 14863-14868
  - Algorithmic Approaches to Clustering Gene Expression Data http://citeseer.nj.nec.com/shamir01algorithmic.html
  - Tibshirani, Hastie, Narasimhan and Chu (2002) http://www.pnas.org/cgi/reprint/99/10/6567
  - Rousseeuw, P.J. (1987) Silhouettes: A graphical aid to the interpretation and validation of cluster analysis. *J. Comput. Appl. Math.*, **20**, 53–65

R: Clustering Algorithm

- Partitioning methods (PM):
  - k-means: kmeans(stats)
  - PAM: pam(cluster)
- Hierarchical clustering (HC):
  - hclust(stats), agnes(cluster), diana(cluster)
- SOM
  - som(som)
- Visualization:
  - Silhouette plot: silhouette(cluster)
  - Reordering heatmap for HC: heatmap(stats), heatmap.2(gplots)
  - (R-2.7.0) heatmaps for PM and SOM: heatmapsM(maigesPack)

Example: Apop.xls

http://homepage.ntu.edu.tw/~lyliu/IntroBioinfo/Apop.xls

save the file as comma delimitated (.csv).

```
> Apop = as.matrix(read.csv("Apop.csv",row.names=1))
> Apop = t(as.matrix(read.csv("Apop.csv",row.names=1)))
```

Partitioning Methods

kmeans:

> out.km = kmeans(Apop,3)

```
Available components:
[1] "cluster"  "centers"  "withinss" "size"
```

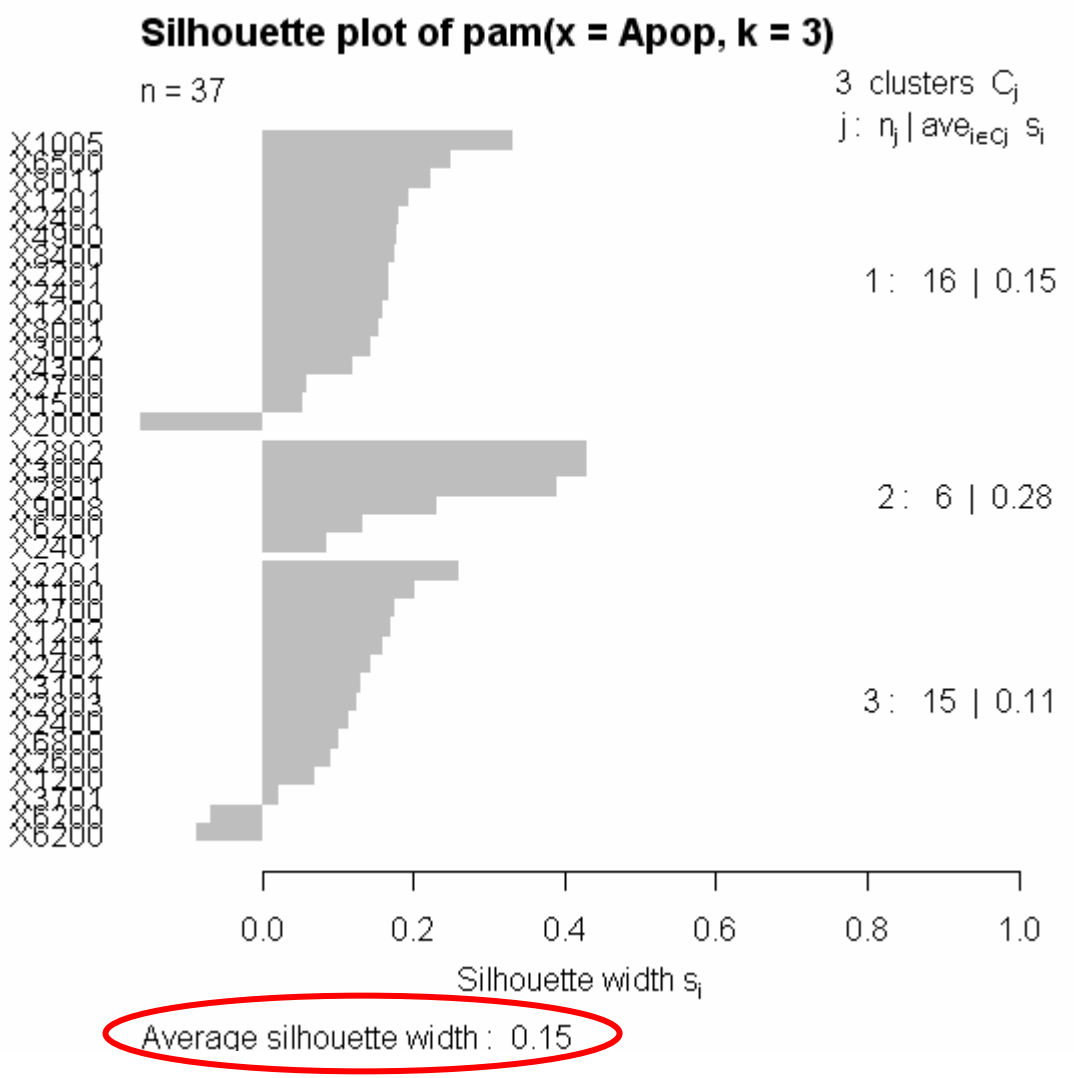
PAM:

> library(cluster)

> out.pam3 = pam(Apop,3)

```
Available components:
 [1] "medoids"    "id.med"     "clustering" "objective"  "isolation"
 [6] "clusinfo"   "silinfo"    "diss"       "call"       "data"
```

```
> si.pam3 = silhouette(out.pam3)
> plot(si.pam3)
```



Average Silhouette

- For each gene j, compute its silhouette (S_j):

$$S_j = \frac{b_j - a_j}{\max(a_j, b_j)}$$

a_j = average distance between gene j and other elements in the same group

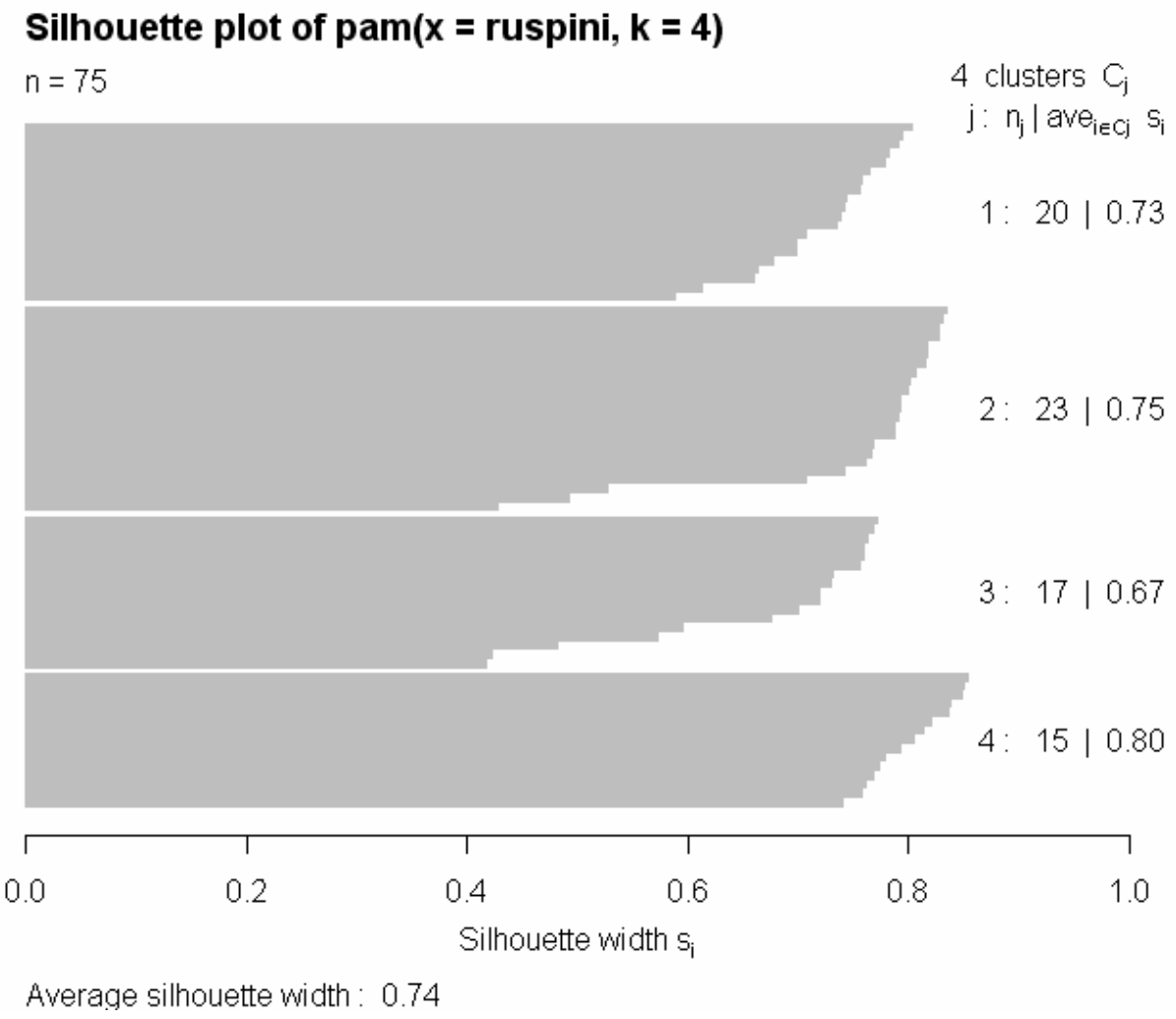
$bj = \max_k b_{jk}$

b_{jk} = average distance between gene j and the elements in the kth group ($k \neq j$)

- Average silhouette = $\frac{1}{n}\sum_{j=1}^{n} S_j$

Silhouette Plot

Each observation is represented by a horizontal bar



Average Silhouette

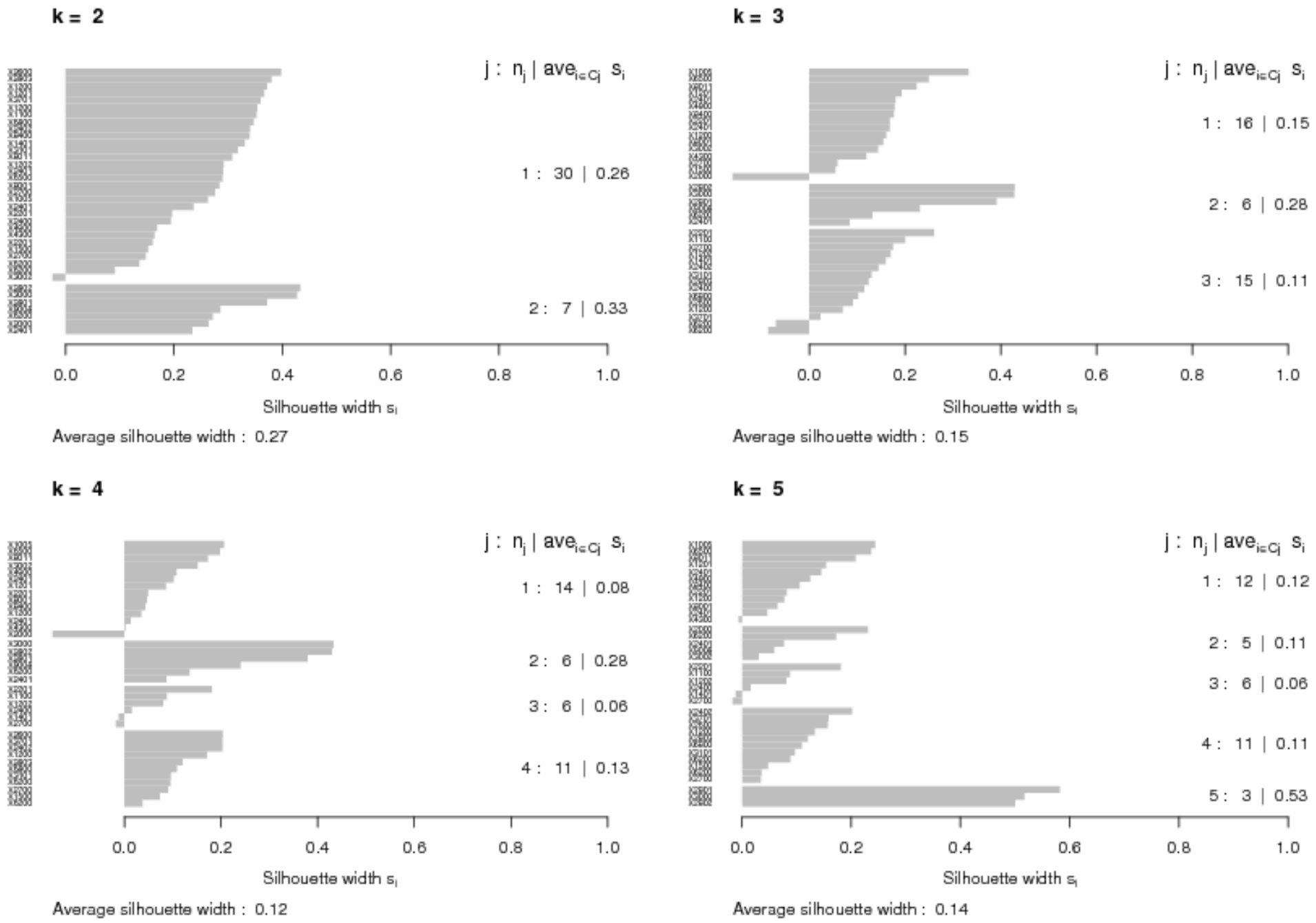
- Number of clusters, *k*:

  For different *k*, compute the average silhouette; the largest average silhouette gives the optimal number of clusters.

Silhouette Plots for Different *k*

將視窗分成2 x 2 = 4個小區

```
par(mfrow=c(2,2))
for(i in 2:5) {
    plot(silhouette(pam(Apop,i)),
         main = paste("k = ",i), do.n.k=FALSE,
         cex.names=0.5)
}
```


k = 2
j : n_j | ave_{i∈Cj} s_i
1 : 30 | 0.26
2 : 7 | 0.33
0.0 0.2 0.4 0.6 0.8 1.0
Silhouette width s_i
Average silhouette width : 0.27
k = 3
j : n_j | ave_{i∈Cj} s_i
1 : 16 | 0.15
2 : 6 | 0.28
3 : 15 | 0.11
0.0 0.2 0.4 0.6 0.8 1.0
Silhouette width s_i
Average silhouette width : 0.15
k = 4
j : n_j | ave_{i∈Cj} s_i
1 : 14 | 0.08
2 : 6 | 0.28
3 : 6 | 0.06
4 : 11 | 0.13
0.0 0.2 0.4 0.6 0.8 1.0
Silhouette width s_i
Average silhouette width : 0.12
k = 5
j : n_j | ave_{i∈Cj} s_i
1 : 12 | 0.12
2 : 5 | 0.11
3 : 6 | 0.06
4 : 11 | 0.11
5 : 3 | 0.53
0.0 0.2 0.4 0.6 0.8 1.0
Silhouette width s_i
Average silhouette width : 0.14

Save the plot (LaTeX user):

```
postscript("silhouette_Apop.ps")
par(mfrow=c(2,2))
for(i in 2:5) {
    plot(silhouette(pam(Apop,i)),
         main = paste("k = ",i), do.n.k=FALSE,
         cex.names=0.5)
}
dev.off()
```

存成postscript(.ps)檔
(需安裝GSview與Ghostscript)

Ghostscript: http://pages.cs.wisc.edu/~ghost/doc/AFPL/get853.htm
GSview: ftp://mirror.cs.wisc.edu/pub/mirrors/ghost/ghostgum/gsv48w32.exe

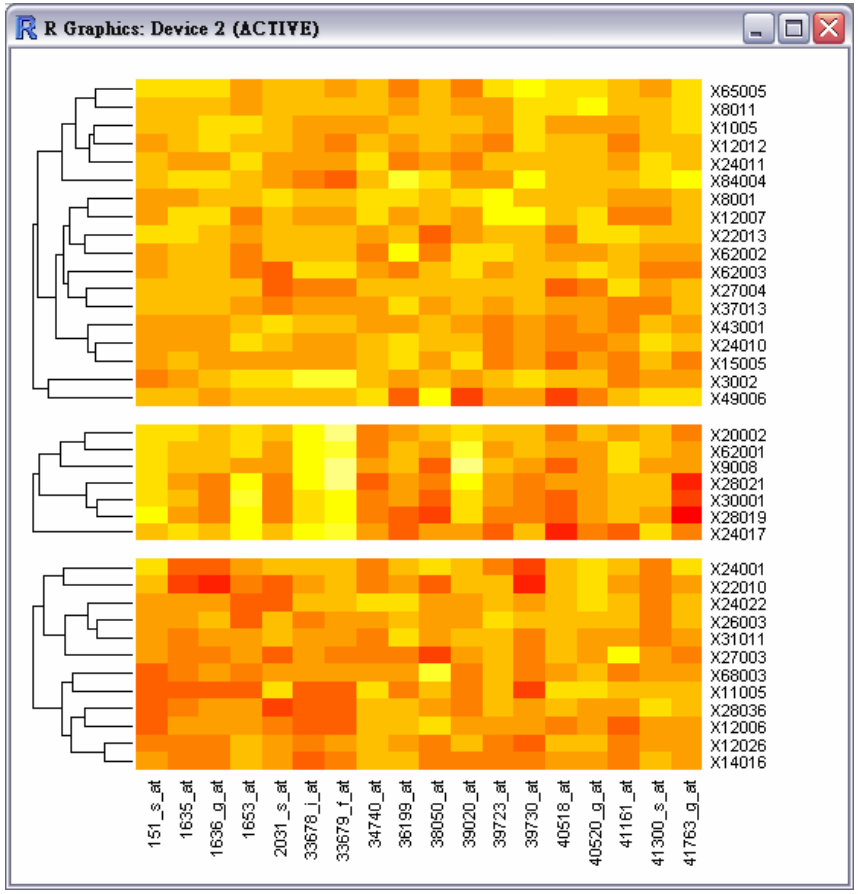
Save the plot (MS Office Word user):

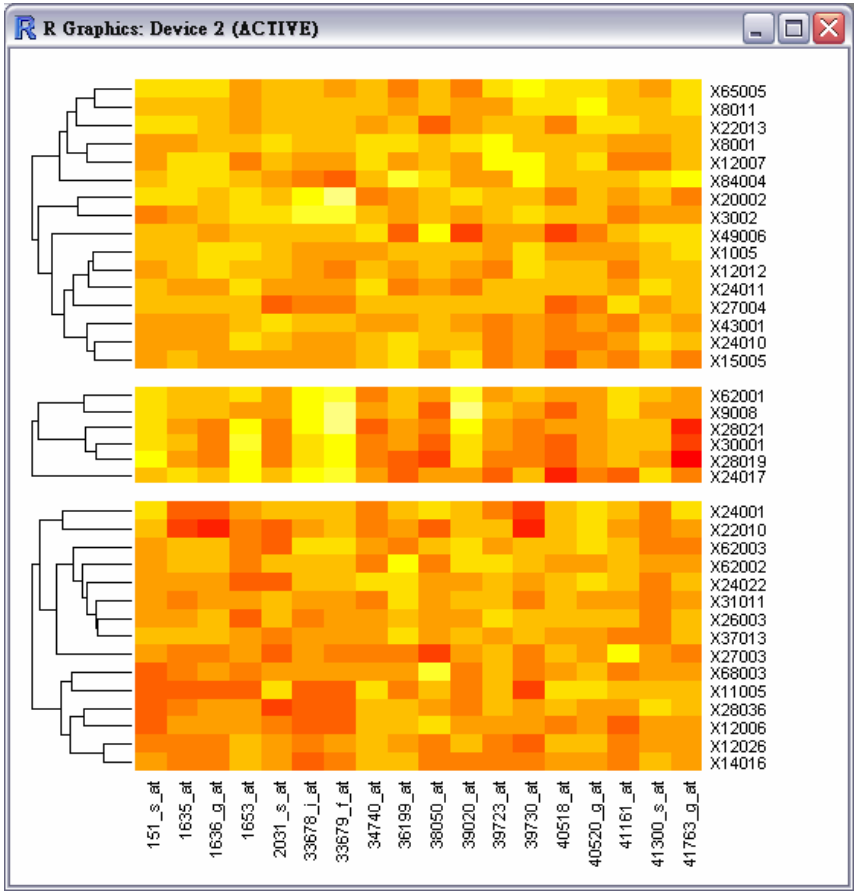
```
win.metafile("silhouette_Apop.emf")
par(mfrow=c(2,2))
for(i in 2:5) {
    plot(silhouette(pam(Apop,i)),
         main = paste("k = ",i), do.n.k=FALSE,
         cex.names=0.5)
}
dev.off()
```

存成metafile(.emf)檔

R-2.7.0 (試用版) only

```
library(maigesPack)
heatmapsM(Apop,groups=out.km$cluster)
heatmapsM(Apop,groups=out.pam3$clustering)
```





Hierarchical Clustering

- Bottom-up (agglomerative):

  > hclust(*d*, *method*)

  - d: distance matrix
  - method: "single", "complete"', "'average", "centroid"

  > agnes(*x*, *metric*, *method*)

  - x: data matrix or distance matrix
  - metric: "euclidean“, "manhattan"
  - method: "single", "complete"', "'average", "centroid“

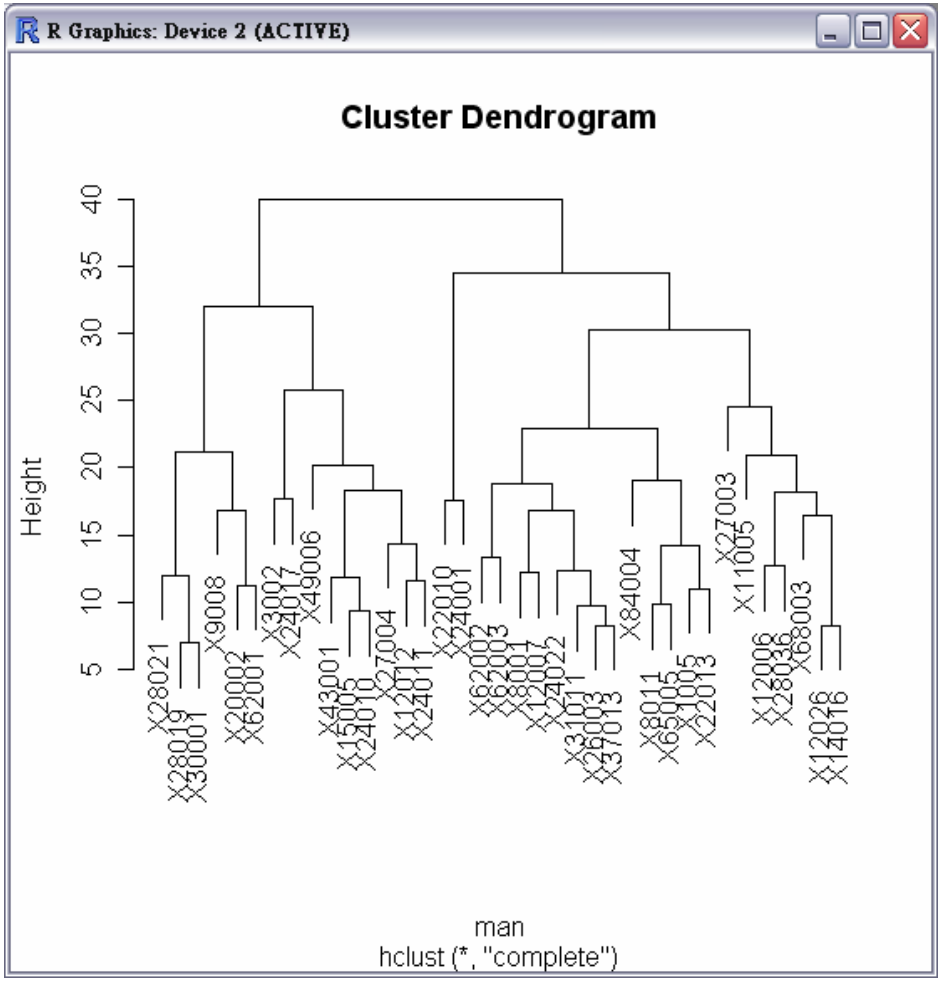
- Top-down (divisive):

  > diana(*x*, *metric*)

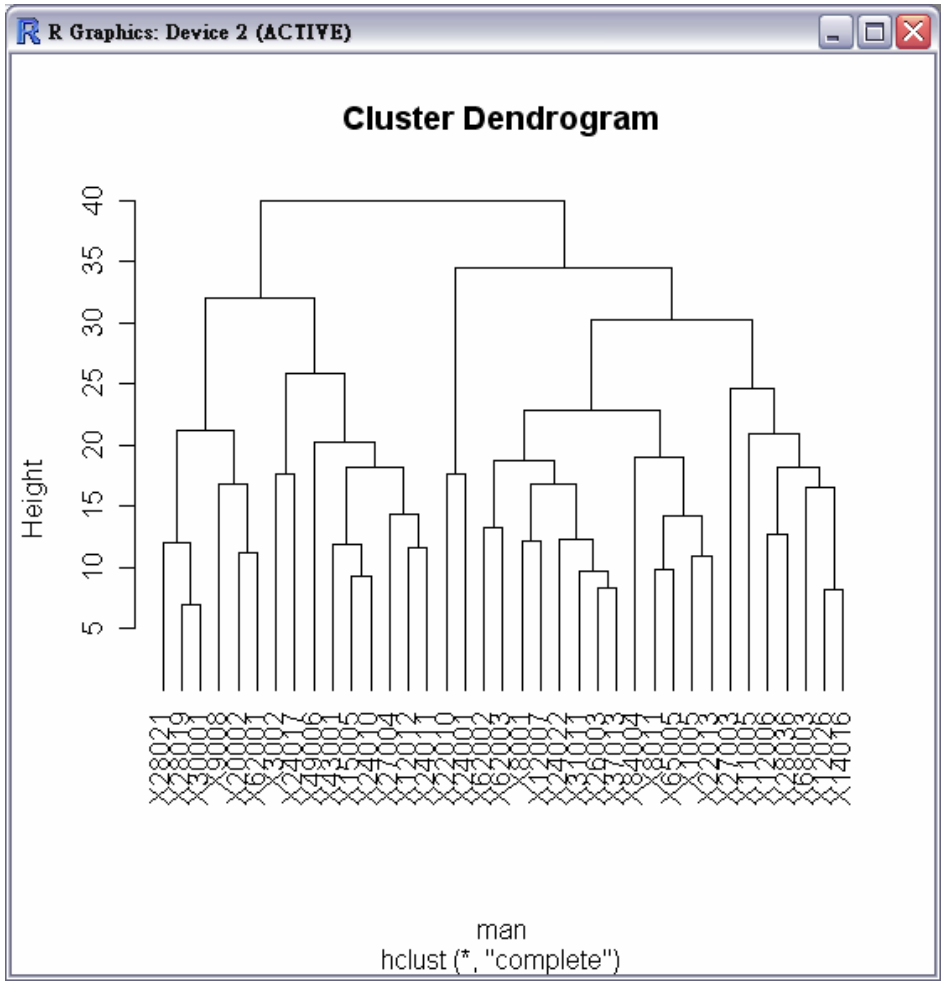
  - x: data matrix or distance matrix
  - metric: "euclidean“, "manhattan"
  - method: "single", "complete"', "'average", "centroid“

Bottom-up: hclust (stats)

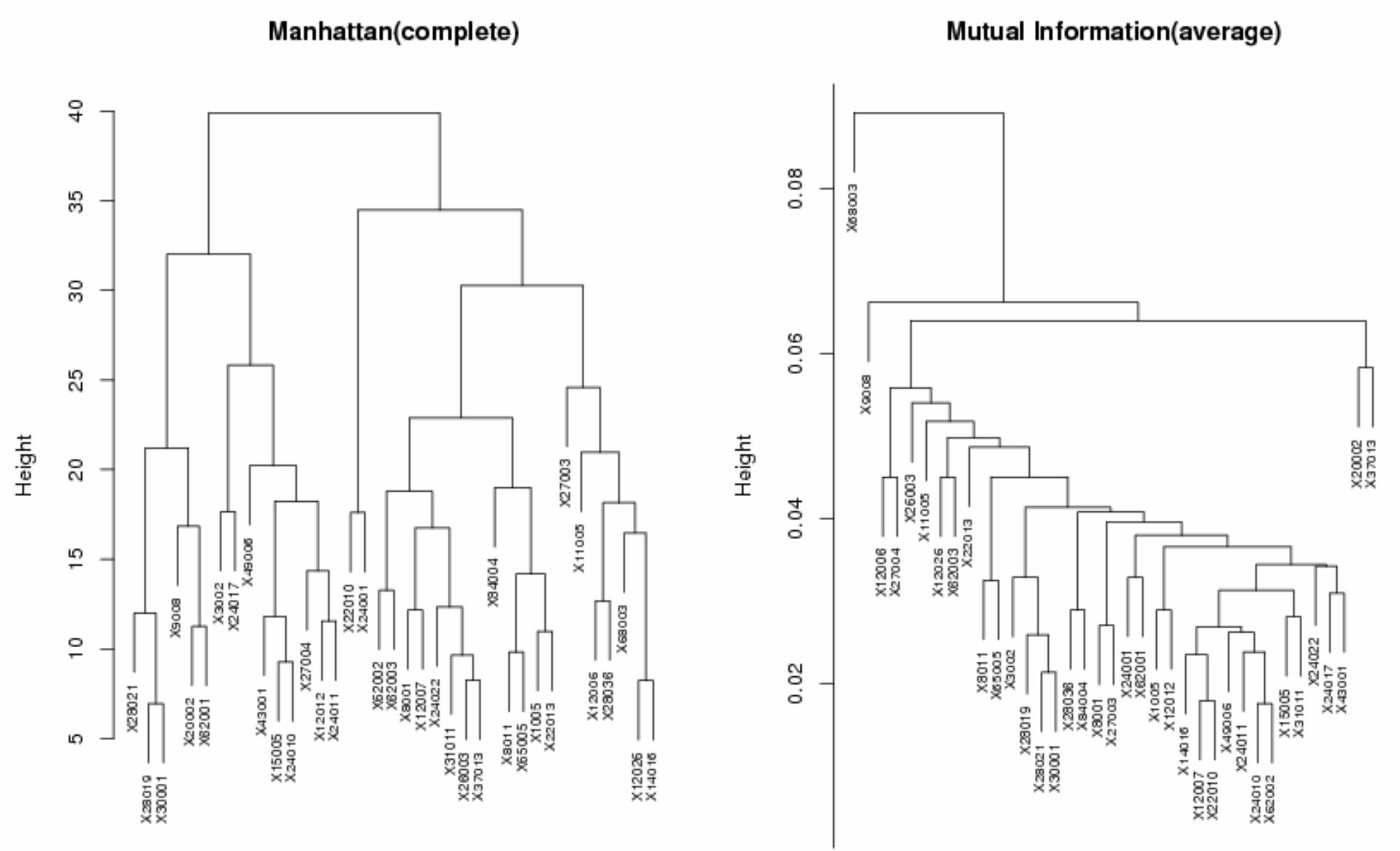
Default



hang = -1

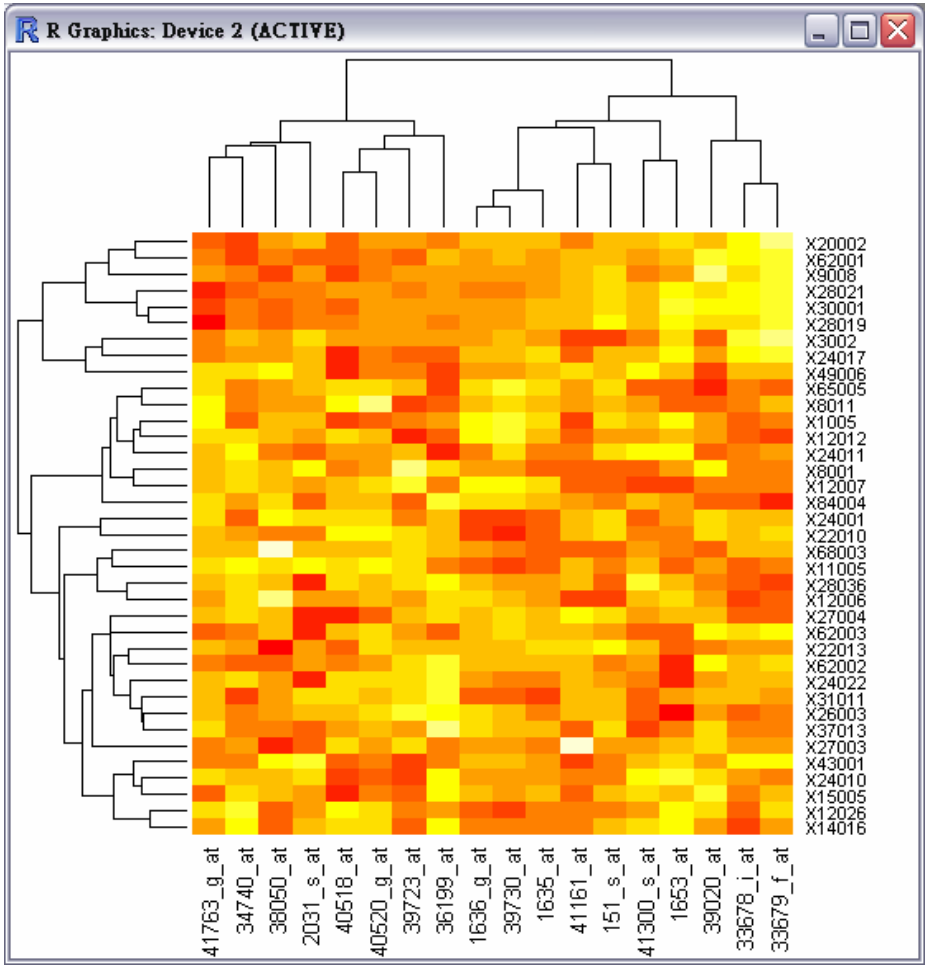


Bottom-up: hclust (stats)

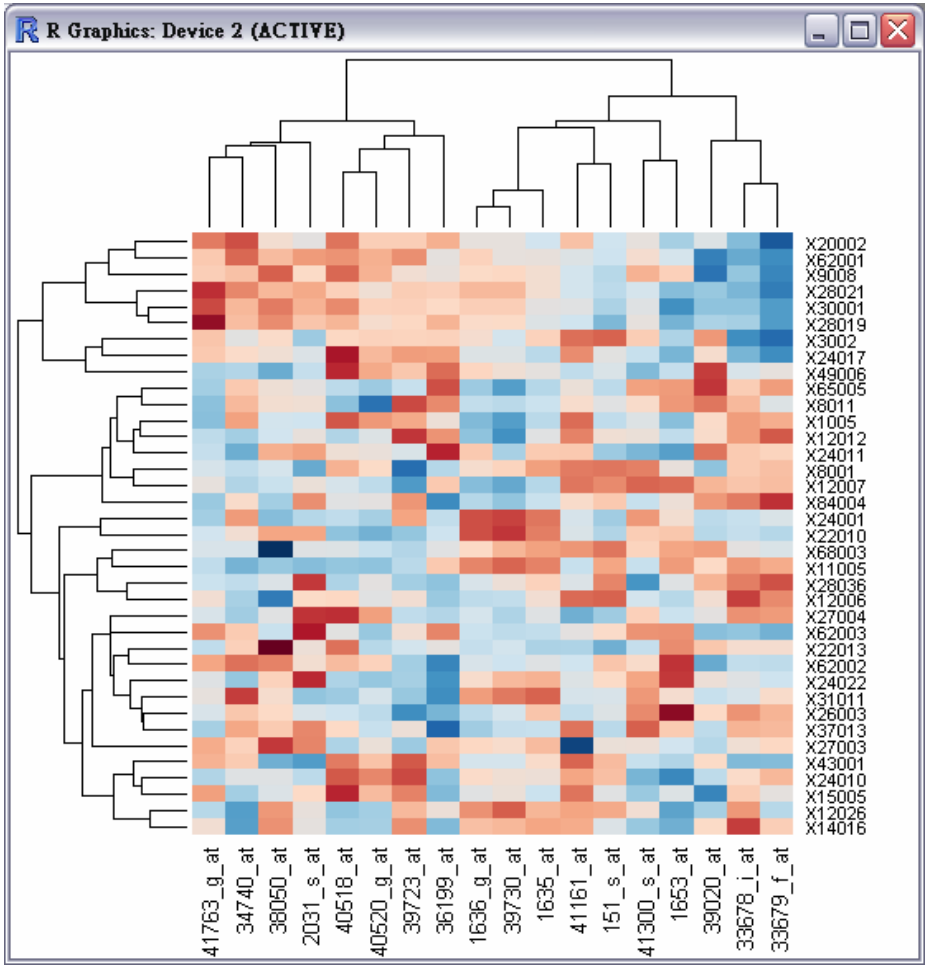


Bottom-up: hclust (stats)

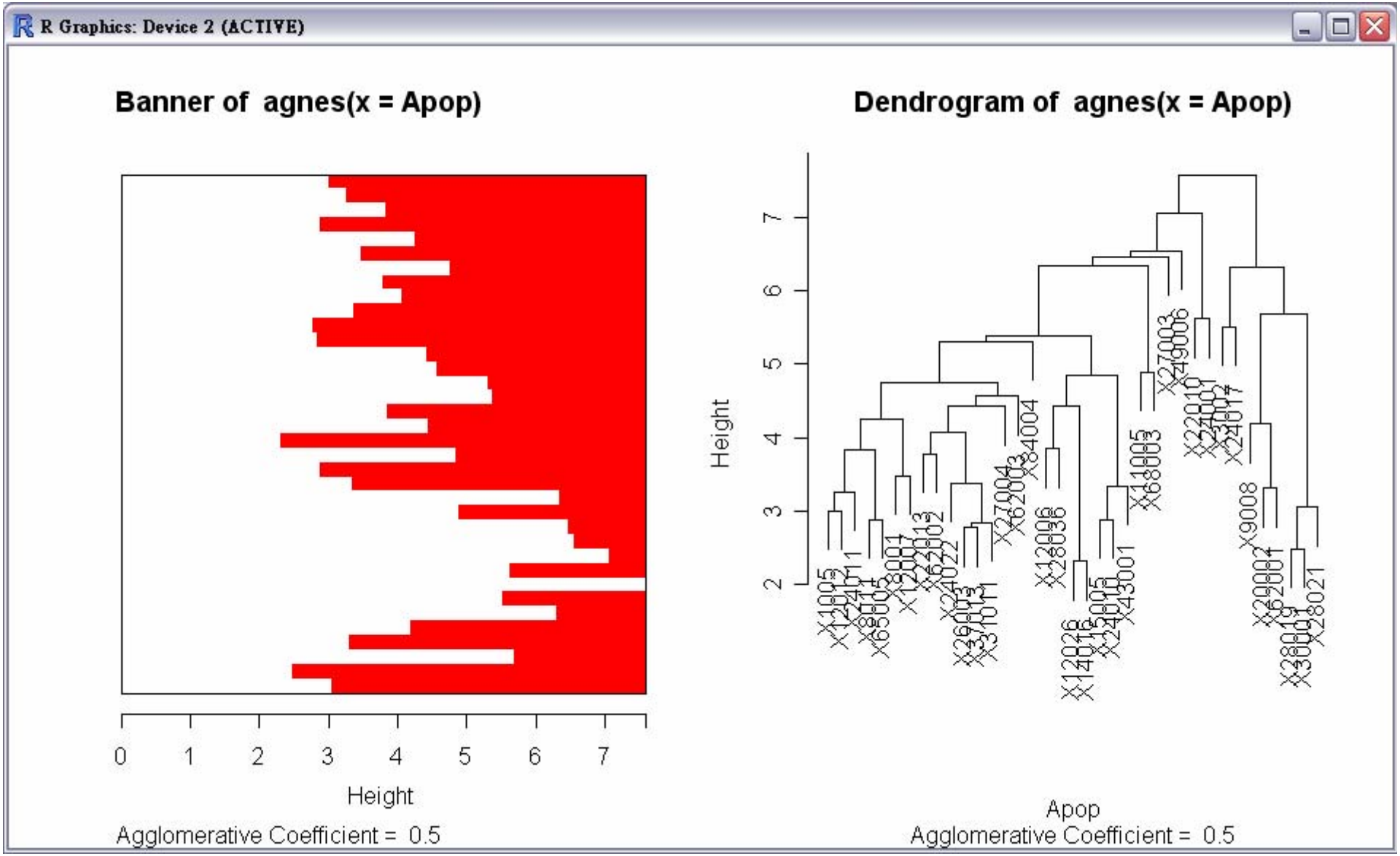
Default color



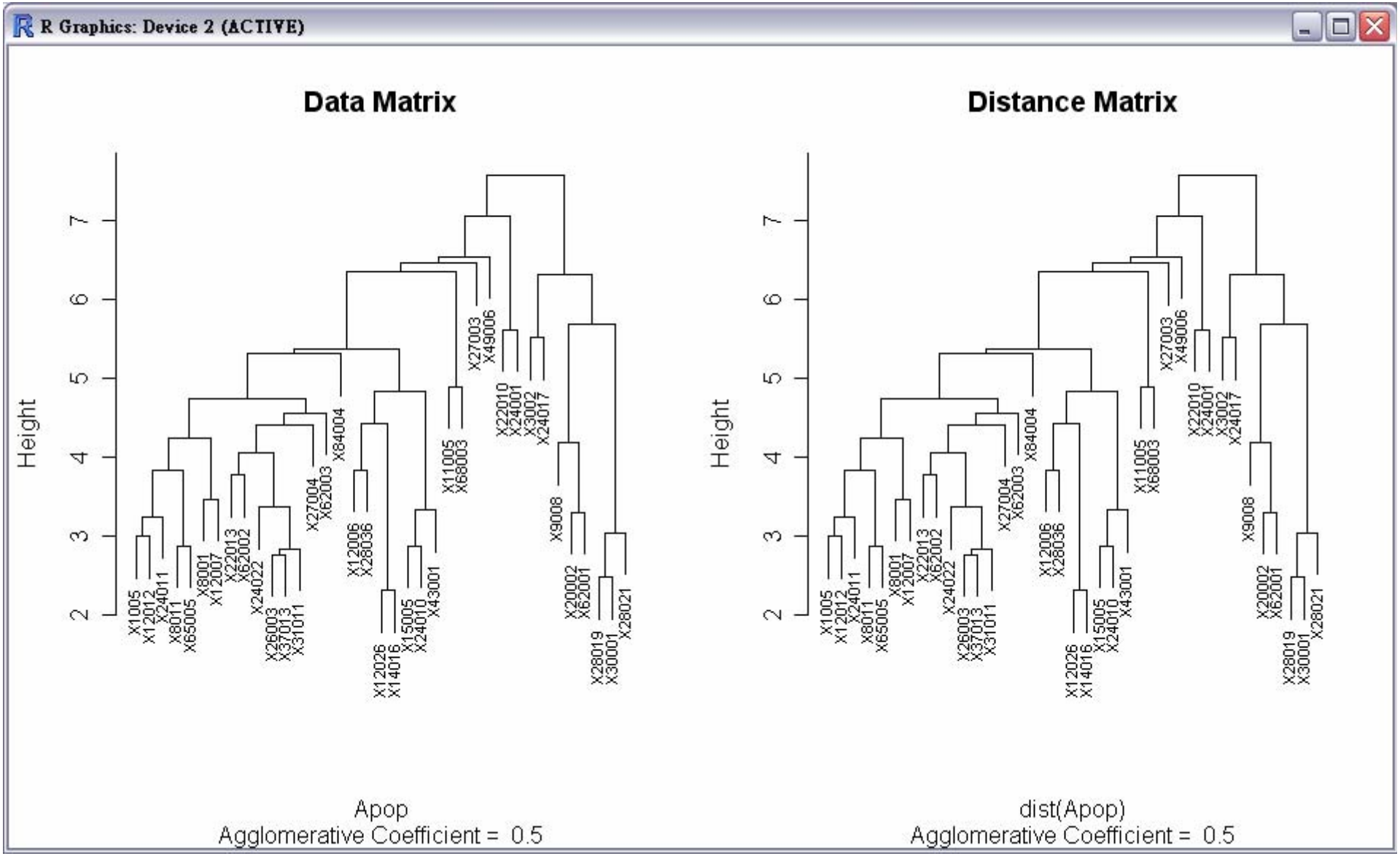
Brewer color (RdBu)



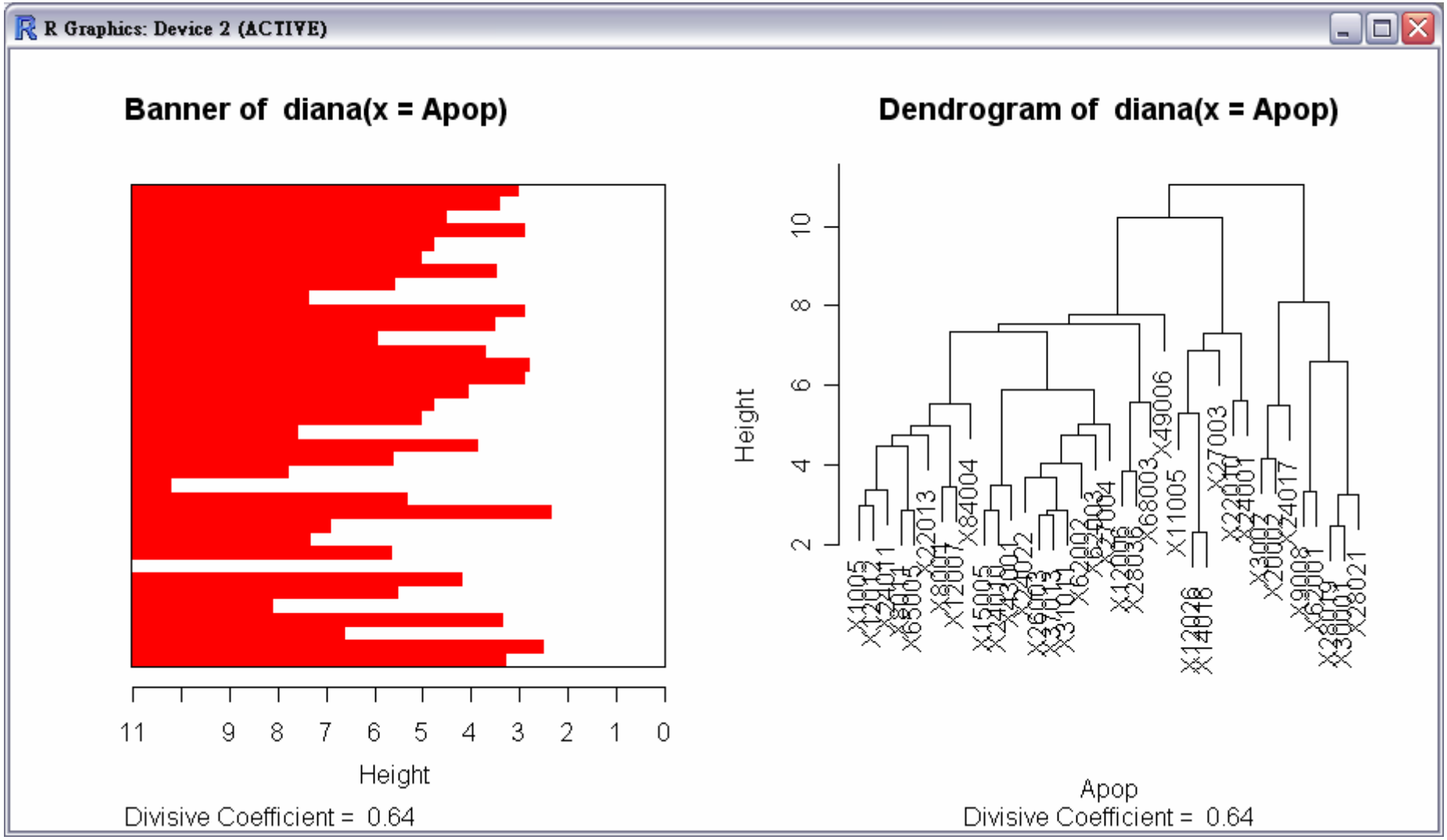
Bottom-up: agnes (cluster)



Bottom-up: agnes (cluster)



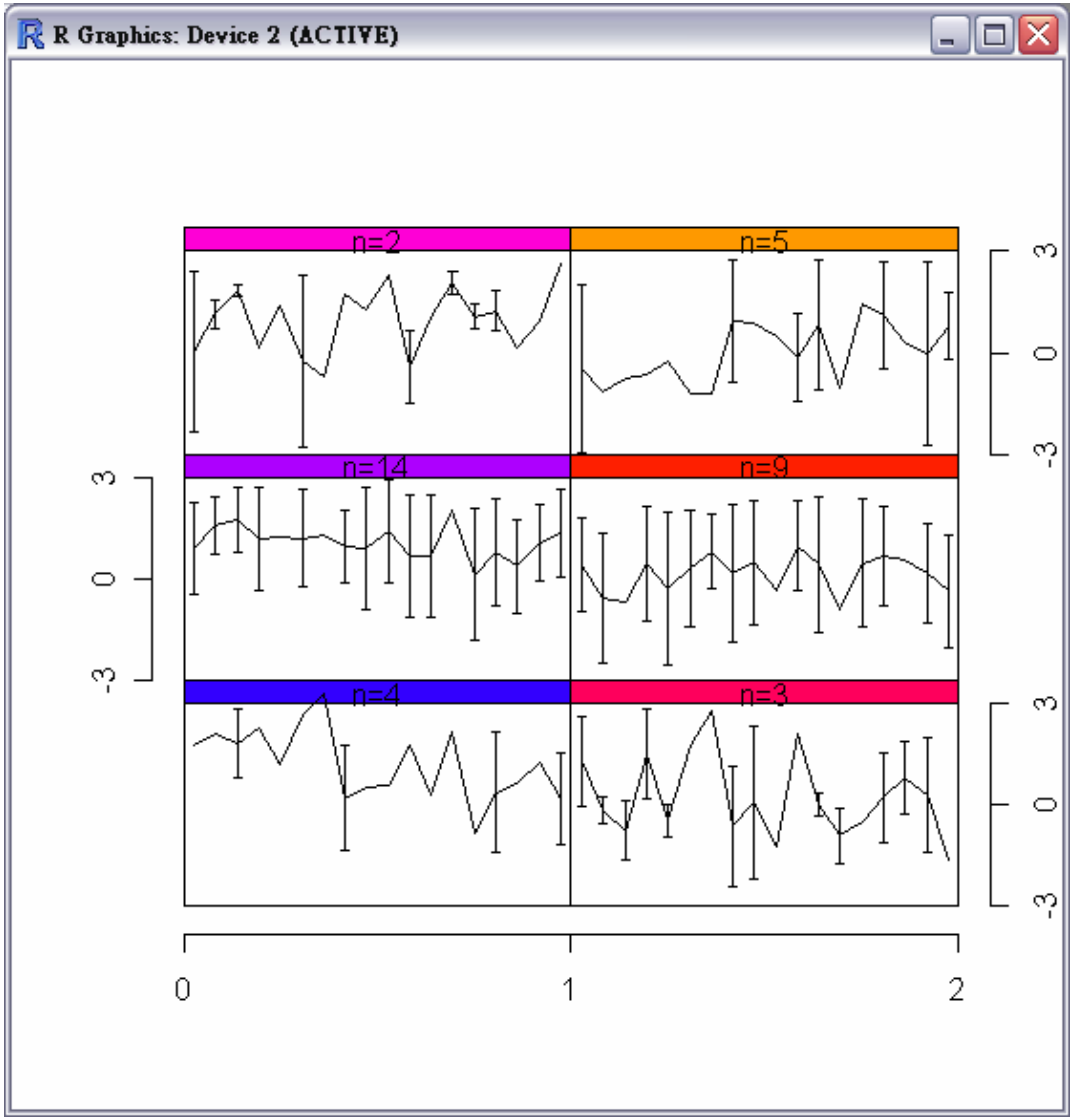
Top-down: diana (cluster)



SOM

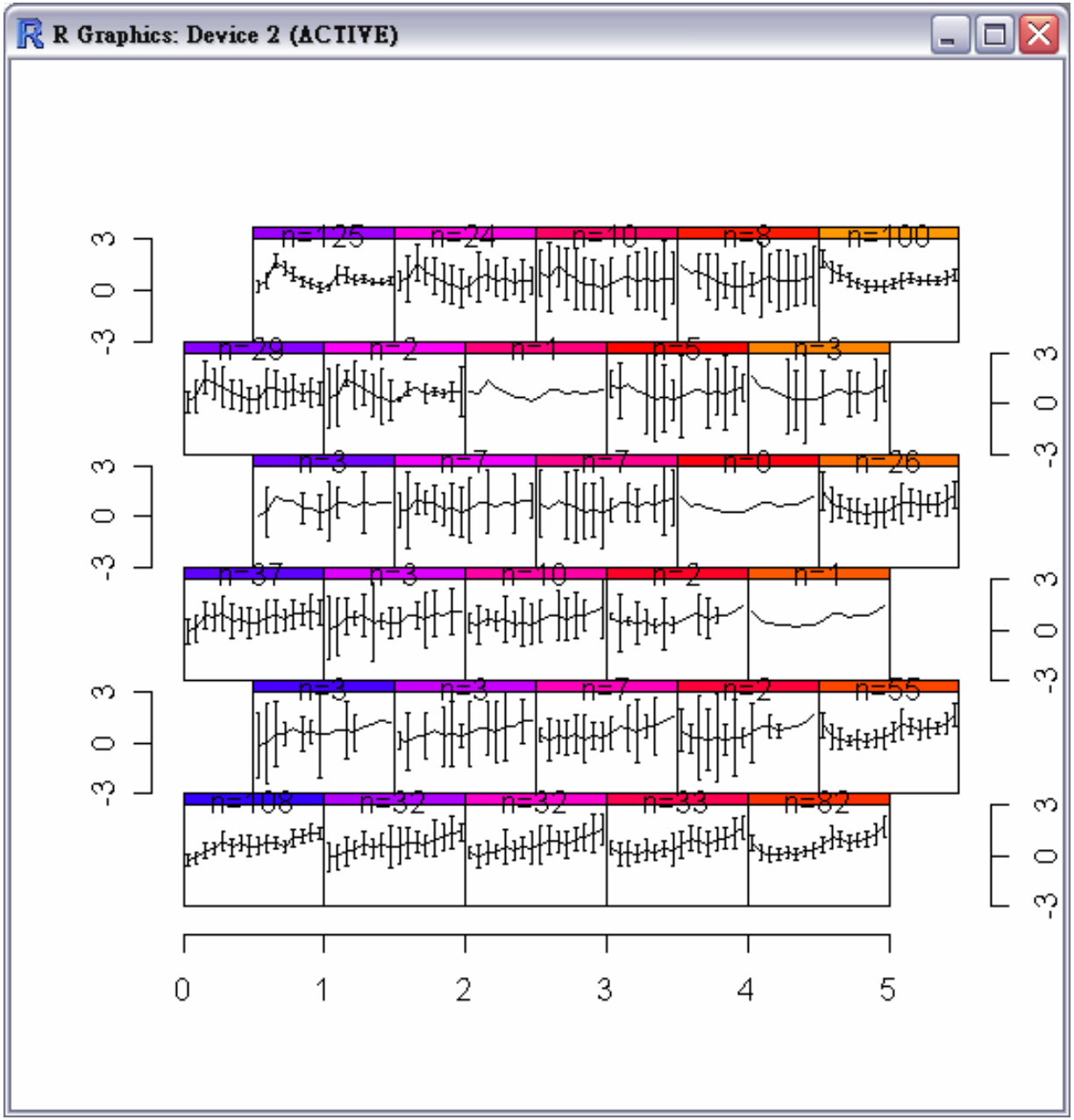
som(*data*,*xdim*,*ydim*)

> library(som)
> som(Apop,2,3)



> example(som)

Note: The data contains 6601 genes, measured at 18 time points.



Detecting differentially expressed genes in microarray data

Introduction

- In many cases, the purpose of microarray experiment is to compare the gene expression levels in two or several predetermined classes.
  - The comparison is often performed under gene-by-gene basis.
  - However, the genes are rarely independent.
  - For the convenient interpretability, differentially expression analysis usually ignore the dependencies between genes.

Fold Change

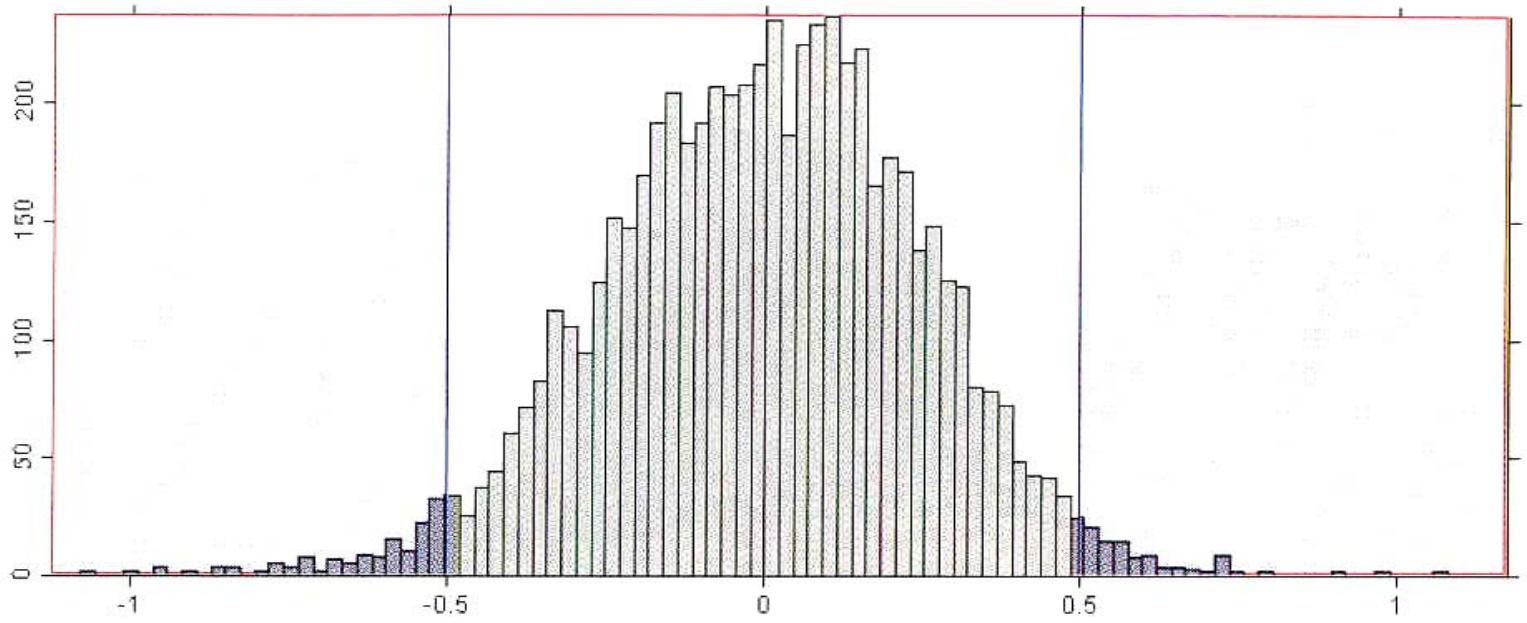
- Fold change is the important and intuitive approach to find differentially regulated genes:

$$\text{Fold change (FC)} = \frac{\text{Expression of Experimental Sample}}{\text{Expression of Reference Sample}}$$

$$\log_2(\text{FC}) = \log_2(\text{Expression of experimental sample}) - \log_2(\text{Expression of reference sample})$$

Fold Change

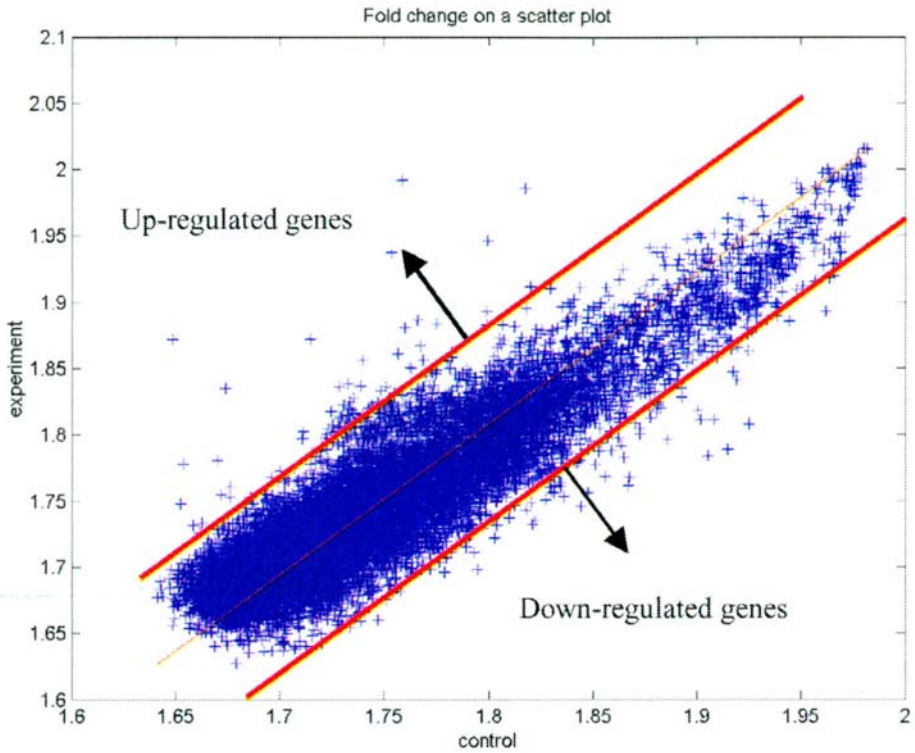
- Histogram of $\log_2$(fold-change):

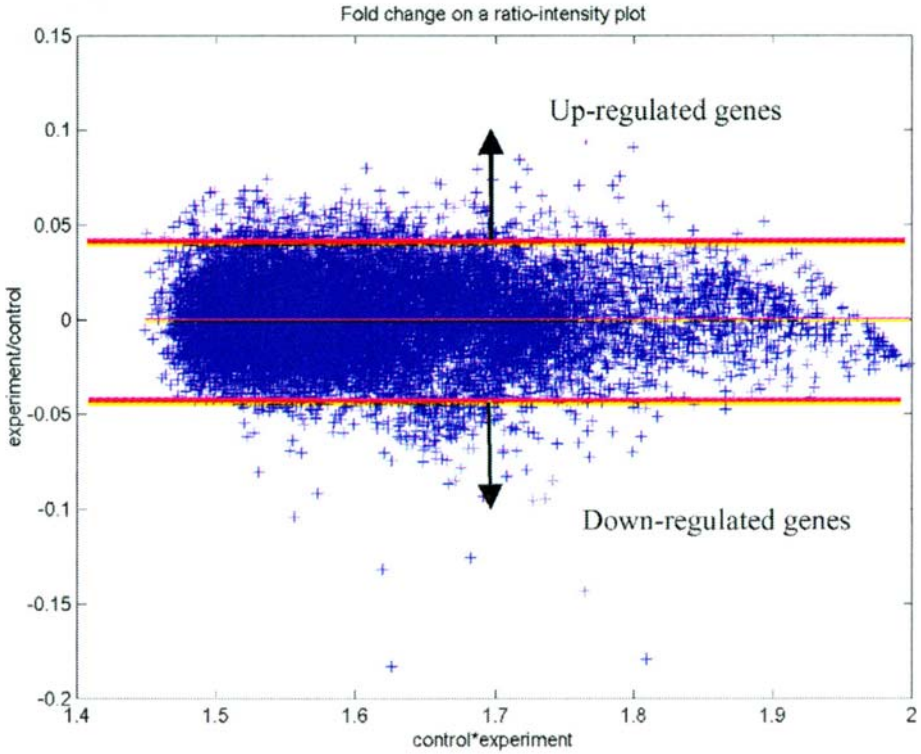


Selects genes in the tails of the histogram by setting thresholds at the desired minimum fold change. For example, FC > $2^{0.5}$ → $\log_2$(FC) > 0.5

Fold Change

- Fold change method can also be visualized on scatter plots and MA-plots.





Fold Change

- It may be the only possibility in cases where no, or very few replicates, are available.
- The fold change is chosen arbitrarily and cannot access the level of significance.

$\Rightarrow$ statistical tests!

Standard Statistical Tests

Decide which genes are significantly regulated in a microarray experiment.

Microarray Data	Paired data *Dependent samples*	Unpaired data *Independent samples*	Complex data *More than two Groups*
Parametric Hypothesis Testing	• ***z-test*** • ***t-test***	• ***two-sample t-test***	• One-Way Analysis of Variance (ANOVA)
	Assumptions and Test for Normality • QQplot • Shapiro-Wilk Normality Test		
Non-Parametric Hypothesis Testing	• Wilcoxon signed-rank test	• Wilcoxon rank-sum test, (Mann-Whitney U test).	• Kruskal-Wallis test

Terminology in Hypothesis Testing

- The null hypothesis:
  - H_0: $\mu = 1.15$. (the average price of a gallon of gas is $1.15)
- The alternative hypothesis:
  - H_1: $\mu > 1.15$. (gas prices were actually higher)
  - H_1: $\mu < 1.15$.
  - H_1: $\mu \neq 1.15$.

Terminology in Hypothesis Testing

- The significance level (α) is related to the degree of certainty you require in order to reject the null hypothesis in favor of the alternative.
  - Decide in advance
  - Reject the null hypothesis if the probability of observing a more extreme result than your sampled one (p-value) is less than the significance level.
  - The probability of incorrectly rejecting the null hypothesis when it is actually true (Type I error) is 100(1- α)%.
  - If you need more protection from this error, then choose a lower value of α.

Terminology in Hypothesis Testing

- P-value:
  - Definition: P(observing at least this level of differential gene expression by random chance)
  - The smaller the p-value, the less likely it is that the observed data have occurred by chance, and the more significant the result.

Terminology in Hypothesis Testing

- Confidence intervals: a range of values that have a chosen probability of containing the true hypothesized quantity.
  - Suppose, in our example, 1.15 is inside a 95% confidence interval for the mean, μ. That is equivalent to being unable to reject the null hypothesis at a significance level of 0.05.
  - Conversely if the 100(1-α)% confidence interval does not contain 1.15, then you reject the null hypothesis at the alpha level of significance.

Steps of Hypothesis Testing

1. Determine the null and alternative hypothesis, using mathematical expressions if applicable.
2. Select a significance level (α).
3. Take a random sample from the population of interest.
4. Calculate a test statistic from the sample that provides information about the null hypothesis.
5. Decision
   - If the value of the statistic is consistent with the null hypothesis then do not reject H0.
   - If the value of the statistic is not consistent with the null hypothesis, then reject H0 and accept the alternative hypothesis.

Hypothesis Testing in Microarray Study

- In all of the Microarray datasets, we are interested in identifying differentially expressed genes.
- The method would then be applied to every gene (one gene at a time) on the microarray in order to identify those genes that are differentially expressed.
- If the null hypothesis were true, then the variability in the data does not represent the biological effect under study, but instead results from difference between individuals or measurement error.
- We then select differentially expressed genes not on the basis of their fold ratio, but on the basis of their p-value.

Hypothesis Testing in Microarray Study

- Hypothesis test for two groups:
  - Two sample means: t-test (paired or independent)
- Hypothesis test for more than two groups:
  - One-Way Analysis of Variance (ANOVA)

Paired Data

- Paired data: there are two measurements from each object. We are interested in the difference between the two measurements

Example: Samples are taken from 20 breast cancer patients, before and after a 16 week course of doxorubicin chemotherapy, and analyzed using microarray. There are 9216 genes.

$\Rightarrow$ Has a gene been up-regulated or down-regulated in breast cancer following doxoruicin chemotherapy?

Paired Data

- For each object, calculate the difference between the two measurements :

$$D_i = X_{i1} - X_{i2}$$

- The D_i's can be viewed as a new set of independent sample and can be tested whether the population mean of D_i's is equal to 0!

$$H_0: \mu_D = 0 \qquad H_a: \mu_D \neq 0$$

Paired Data

Note that $\frac{\overline{D} - \mu_D}{\sqrt{S_D^2 / n}} \sim t(n-1)$

Under H_0: $\mu_D = 0$, $t_0 = \frac{\overline{D} - 0}{\sqrt{S_D^2 / n}} = \frac{\overline{D}}{\sqrt{S_D^2 / n}} \sim t(n-1)$

Reject H_0 if $|t_0| < t_{\alpha/2,n-1}$ or if p-value < α

Paired Data

Example (cont.): Gene ACAT2

$$\overline{D} = 0.346955, \quad S_D^2 = 0.2315987$$

$$t_0 = \frac{\overline{D}}{\sqrt{S_D^2 / n}} = 3.2242, \qquad p - value = 0.004465$$

Reject H_0!

Note: we can rank the genes based on their p-values.

R: Paired Data

- Test by R:

t.test(x, y, paired = TRUE,

alternative = c("two.sided", "less", "greater"))

```
> dd = read.delim("perou.tab")
> ACAT2 = as.numeric(dd[which(dd$Gene == "ACAT2"),-1])
> t.test(ACAT2[1:20],ACAT2[21:40],paired=T)

        Paired t-test

data:  ACAT2[1:20] and ACAT2[21:40]
t = -3.2242, df = 19, p-value = 0.004465
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -0.5721855 -0.1217245
sample estimates:
mean of the differences
              -0.346955
```

Unpair Data

- Unpaired data: two measurements are taken from two objects independently.

Example: Samples are taken from 37 patients suffering from B-cell acute lymphoblastic leukemia (BCR/ABL) and 42 normal samples (NEG) and analyzed using Affymetrix arrays. There are 12625 genes.

$\Rightarrow$ We wish to identify the genes that are up- or down-regulated in BCR/ABL relative to NEG. (i.e., to see if a gene is differentially expressed between the two groups.)

Unpair Data

(1) if $\sigma_1^2 = \sigma_2^2 = \sigma^2$,

Statistic: $$\frac{\overline{X}_1 - \overline{X}_2}{\sqrt{S_p^2(1/n_1 + 1/n_2)}} \sim t(n_1 + n_2 - 2)$$

where $$S_p^2 = \frac{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2}{n_1 + n_2 - 2}$$

(2) if $\sigma_1^2 \neq \sigma_2^2 \Rightarrow$ Welch's Approximation!

Statistics: $$\frac{\overline{X}_1 - \overline{X}_2}{\sqrt{S_1^2/n_1 + S_2^2/n_2}} \sim t(\upsilon), \quad \upsilon = \frac{\left(\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}\right)^2}{\frac{S_1^2/n_1}{n_1 - 1} + \frac{S_2^2/n_2}{n_2 - 1}}$$

Unpaired Data

- To test whether $\sigma_1^2 = \sigma_2^2$:
  Compute $F_0 = s_1^2/s_2^2$;
  we claim that $\sigma_1^2 \neq \sigma_2^2$ if

$$F_0 > F_{\alpha/2,n_1-1,n_2-1} \quad \text{or} \quad F_0 < F_{1-\alpha/2,n_1-1,n_2-1}$$

R: Unpaired Data

- Test for equal variance:

```
var.test(x, y)
```

- if $\sigma_{12} = \sigma_{22}$:

```
t.test(x, y, var.equal = TRUE,
    alternative = c("two.sided", "less", "greater"))
```

- if $\sigma_{12} \neq \sigma_{22}$:

```
t.test(x, y, var.equal = FALSE,
    alternative = c("two.sided", "less", "greater"))
```

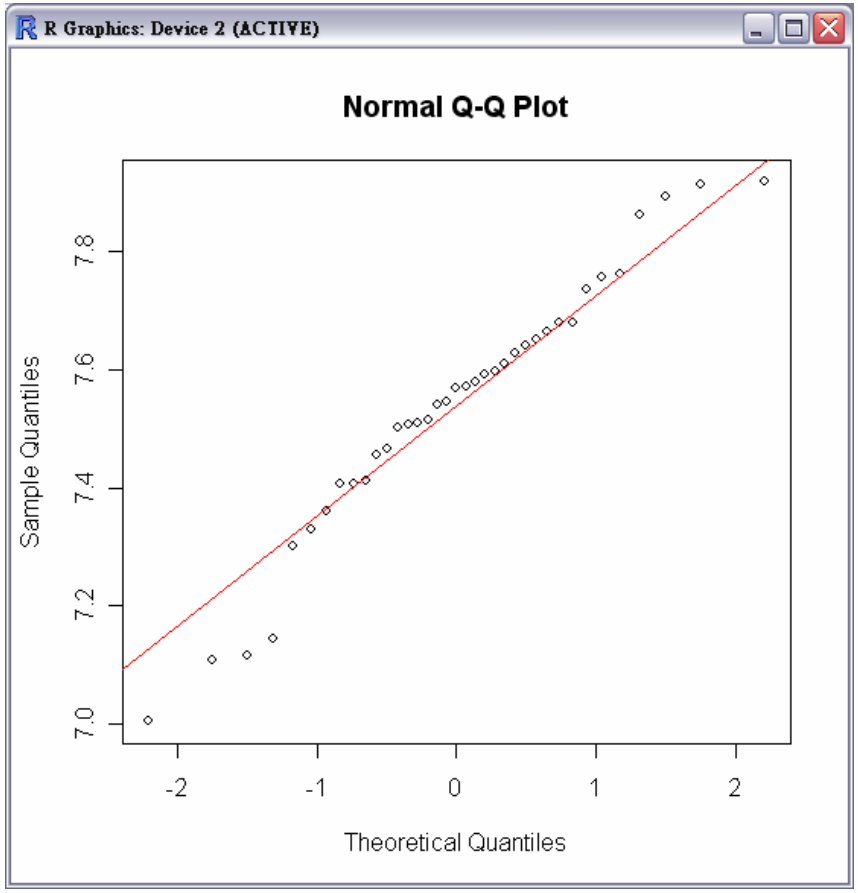
```
> var.test(exprs(eset)[1,]~cl)

        F test to compare two variances

data:  exprs(eset)[1, ] by cl
F = 0.5856, num df = 36, denom df = 41, p-value = 0.1052
alternative hypothesis: true ratio of variances is not equal to 1
95 percent confidence interval:
 0.3100400 1.1209797
sample estimates:
ratio of variances
         0.5855576

> t.test(exprs(eset)[1,]~cl,var.eq=T)

        Two Sample t-test

data:  exprs(eset)[1, ] by cl
t = 0.7365, df = 77, p-value = 0.4637
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -0.07320496  0.15914468
sample estimates:
mean in group BCR/ABL     mean in group NEG
             7.538354              7.495384
```

Note: we can rank the genes based on their p-values.

Assumption of t-test

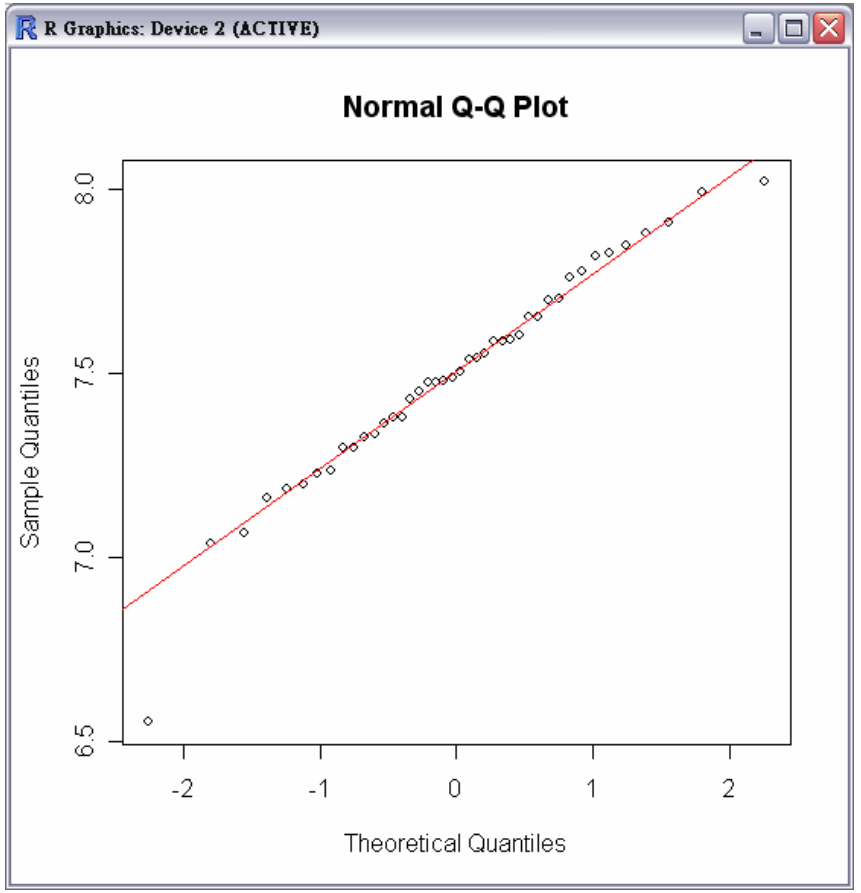
- Normality assumption:
  - For paried t-test, it is the distribution of the subtracted data that must be normal.
  - For unpaired t-test, the distribution of both data sets must be normal.
- To test normality:
  - Visualization: normal probability plot
  - Hypothesis test: Shapiro-Wilk Normality Test
- If the assumption is not held $\Rightarrow$ nonparametric methods!

BCR/ABL



p-value = 0.2441

NEG



p-value = 0.2548

Non-parametric Statistics

- Two good reasons to use non-parametric statistic.
  - *Microarray data is noisy:*
    - There are many sources of variability in a microarray experiment and outliers are frequent.
    - The distribution of intensities of many genes may not be normal.
    - Non-parametric methods are robust to outliers and noisy data.
  - *Microarray data analysis is high throughput:*
    - When analyzing the many thousands of genes on a microarray, we would need to check the normality of every gene in order to ensure that t-test is appropriate.
    - Those genes with outliers or which were not normally distributed would then need a different analysis.
    - It makes more sense to apply a test that is distribution free and thus can be applied to all genes in a single pass.

Wilcoxon Signed-Rank Test (paired data)

- Hypothesis: median(D) = 0 .
- Statistic:

$$z = \frac{T - [n(n+1)/4]}{\sqrt{[n(n+1)(2n+1)]/24}} \sim N(0,1) \text{ under } H_0$$

T = min(T^+, T^-)

T^+= sum of the ranks for the “positive” values

T^-= sum of the ranks for the “negative” values

R: Wilcoxon Signed-Rank Test

- Test by R:

wilcox.test(x, y, paired = TRUE,
alternative = c("two.sided", "less", "greater"))

```
> wilcox.test(ACAT2[1:20],ACAT2[21:40],paired=T)

        Wilcoxon signed rank test

data:  ACAT2[1:20] and ACAT2[21:40]
V = 33, p-value = 0.005581
alternative hypothesis: true location shift is not equal to 0
```

Wilcoxon Rank-Sum Test (unpaired data)

- Compute the rank sums:
  - Rank the observations in the combined sample from the smallest (1) to the largest (n1+n2)
  - T_1 = the rank sum for samples 1
  - T_2 = the rank sum for samples 2
- Statistic: $$U_1 = n_1 n_2 + \frac{n_1(n_1+1)}{2} - T_1$$

$$U_2 = n_1 n_2 + \frac{n_2(n_2+1)}{2} - T_2$$

  - one-tailed test statistic: $U = U_1$
  - two-tailed test statistic: $U = \min(U_1, U_2)$

$$Z = \frac{U - (n_1 n_2 / 2)}{\sqrt{n_1 n_2 (n_1 + n_2 + 1)/12}} \sim N(0,1) \text{ under } H_0$$

R: Wilcoxon Rank-Sum Test

- Test by R:

wilcox.test(x, y,
alternative = c("two.sided", "less", "greater"))

```
> # rank-sum test
> wilcox.test(exprs(eset)[1,]~cl)

        Wilcoxon rank sum test

data:  exprs(eset)[1, ] by cl
W = 856, p-value = 0.4427
alternative hypothesis: true location shift is not equal to 0
```

One-Way Analysis of Variance (ANOVA)

- The cases you need ANOVA:
  - when you need to compare more than two groups (e.g., drug 1, drug 2, and placebo)
  - when you need to compare groups created by more than one independent variable while controlling for the separate influence of each of them (e.g., Gender, type of Drug, and size of Dose).
- In fact, for two group comparisons, ANOVA will give results identical to a t-test.

One-Way Analysis of Variance (ANOVA)

- Example: ALL dataset

Type	ALL1/AF4	BCR/ABL	E2A/PBX1	NEG
Size	10	37	5	42

  - We want to identify genes that are differentially expressed in one or more of these four groups.

ANOVA

Treatment

$i=1$	$i=2$	...	$i=p$	
y_{11}	y_{21}		y_{p1}	
y_{12}	y_{22}	...	y_{p2}	
$\vdots$	$\vdots$		$\vdots$	
y_{1n_1}	y_{2n_2}		y_{pn_p}	Overall Mean
Means $\bar{y}_{1\cdot}$	$\bar{y}_{2\cdot}$	...	$\bar{y}_{p\cdot}$	$\bar{y}$

One-Way ANOVA

$$y_{ij} = \mu + \alpha_{i\cdot} + \epsilon_{ij},$$

$$i = 1, \cdots, p.$$

$$j = 1, \cdots, n_i.$$

$$\epsilon_{ij} \sim N(0, \sigma^2)$$

$$\mu_j = \mu + \alpha_j$$

$$H_0 : \mu_1 = \mu_2 = \cdots = \mu_p$$

Reject H_0 if $F_0 > F_{(\alpha, p-1, n-p)}$

The ANOVA Table for Comparing Means

Source	SS *(Sum of Squares)*	DF	MS *(Mean Square)*	F	*Prob* > F
Treatment	$SST = \sum_{i=1}^{p} \sum_{j=1}^{n_i} (\bar{y}_{i\cdot} - \bar{y})^2$	*p-1*	$MST = \frac{SST}{p-1}$	$F_0 = \frac{MST}{MSE}$	*p*-value
Error	$SSE = \sum_{i=1}^{p} \sum_{j=1}^{n_i} (y_{ij} - \bar{y}_{i\cdot})^2$	*n-p*	$MSE = \frac{SSE}{n-p}$		
Total	$TSS = \sum_{i=1}^{p} \sum_{j=1}^{n_i} (y_{ij} - \bar{y})^2$	*n-1*			

R: ANOVA

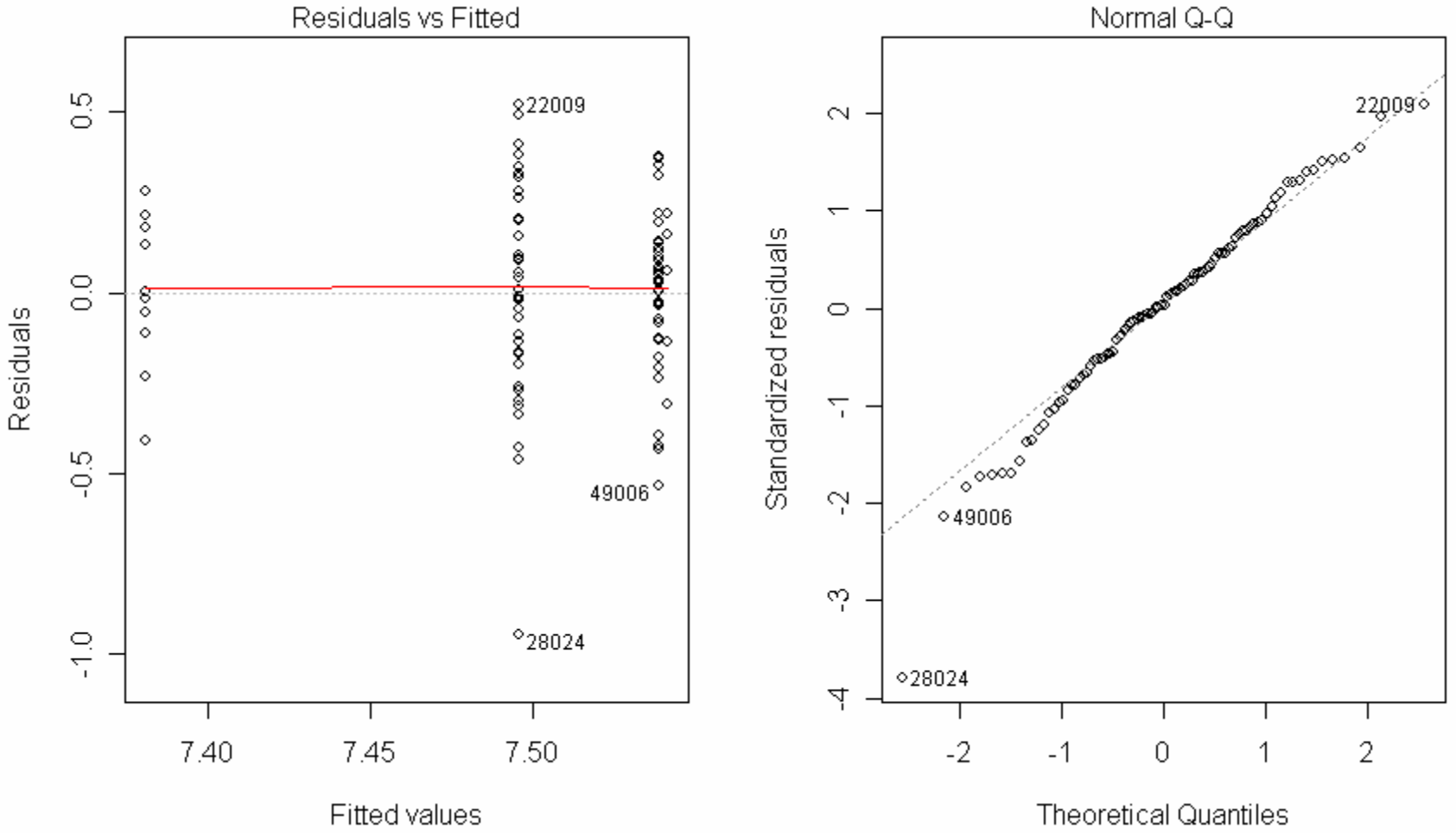
```
> y = drop(exprs(eset[1,]))
> out = lm(y~factor(cl))
> anova(out)
Analysis of Variance Table

Response: y
            Df Sum Sq Mean Sq F value Pr(>F)
factor(cl)   3 0.2048  0.0683  1.0664 0.3675
Residuals   90 5.7604  0.0640
```

Assumption of ANOVA

- Two assumptions for the residuals (observed value – fitted value):
  - Normality assumption:
    - Visualization: normal probability plot
    - Hypothesis test: Shapiro-Wilk Normality Test
  - Equal variance:
    - Visualization: plot of residuals versus fitted values (means)
    - Hypothesis test: Bartlett's Test
- If the assumption is not held $\Rightarrow$ nonparametric methods!

Check Assumptions

plot(out,which=c(1:2))



Check Assumptions

```
> shapiro.test(out$residuals)

        Shapiro-Wilk normality test

data:  out$residuals
W = 0.9759, p-value = 0.07968

> bartlett.test(out$residuals~cl)

        Bartlett test of homogeneity of variances

data:  out$residuals by cl
Bartlett's K-squared = 3.2183, df = 3, p-value = 0.3592
```

Nonparametric ANOVA

Kruskal-Wallis Test:

> kruskal.test(y ~ factor(trt))

```
> kruskal.test(y ~ factor(cl))

        Kruskal-Wallis rank sum test

data:  y by factor(cl)
Kruskal-Wallis chi-squared = 3.7234, df = 3, p-value = 0.2929
```

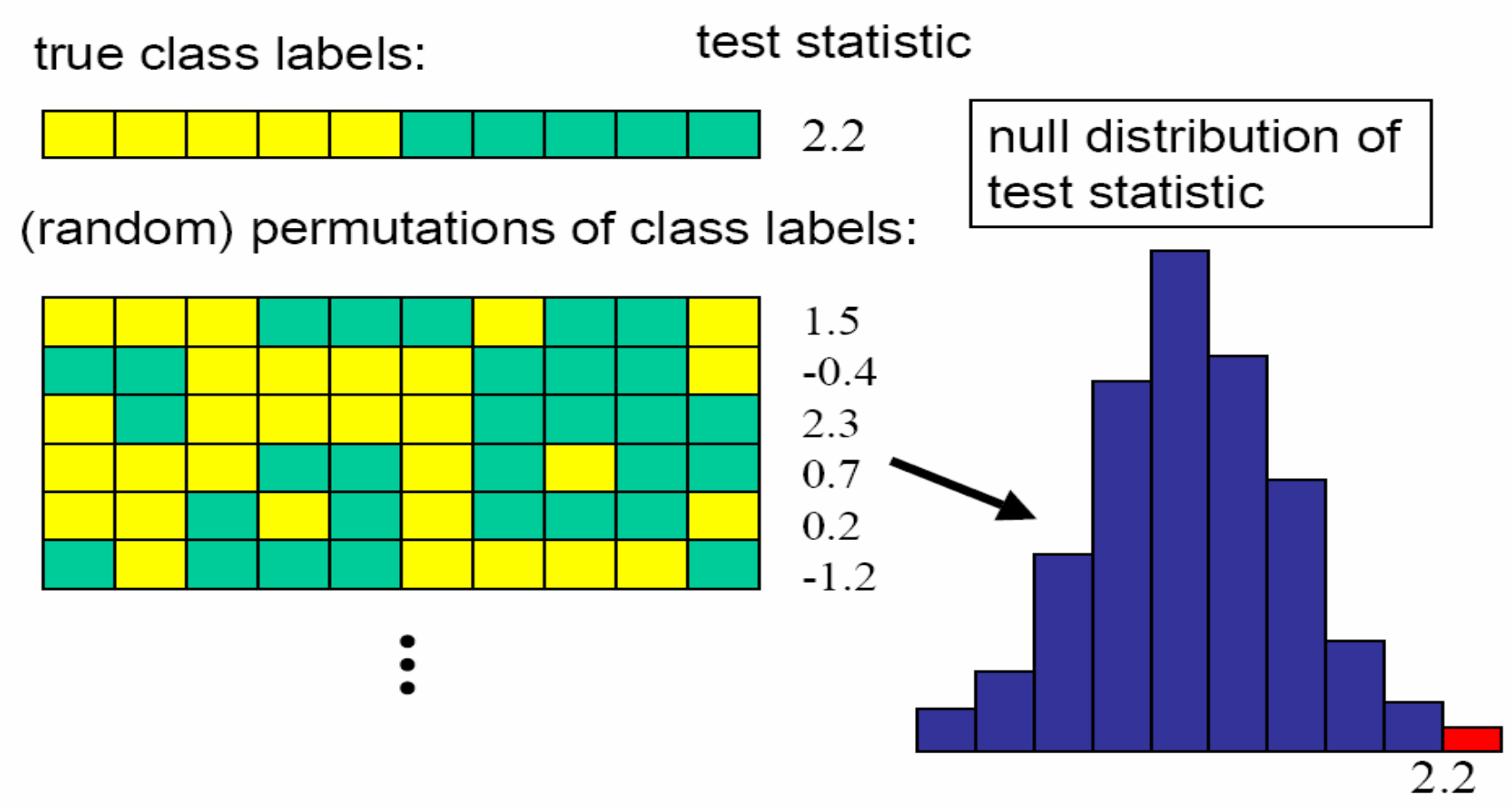
Comments

- The main hazard in using standard statistical tests occurs when there are too few replicates to obtain an accurate estimate of experimental variances. In such cases, modeling methods that use pooled variance estimates may be helpful.
- Standard interpretations t and F tests assume that the data are sampled from normal populations with equal variances. Expression data may fail to satisfy either or both of these constraints.

Permutation Tests

- Permutation tests carried out by repeatedly scrambling the samples' class labels and computing statistic for all genes in the scrambled data.
- Find the likelihood of the observed statistic based on the distribution of statistics from the permuted samples.

Permutation Tests



Permutation Tests

- Step 1: Permute the sample columns. Recalculate the statistic for the permuted sample.
- Step 2: Repeat Step 1 for all possible permutations.
  - # of permutations: B = n!/(n1! n2!)
- Step 3: Use the all permuted statistics to get the distribution
- Step 4: Step 4: Get the p-value:
  - P-value = (# of permuted statistics the same as or *more extreme* than observed one) / B.

Permutation Tests

- Example:

Class I				Class II				*t*-Statistics
a	b	c	d	e	f	g	h	
12	7	15	10	7	2	10	5	2.1004
a	f	c	d	e	b	g	h	
12	2	15	10	7	7	10	5	0.8431
h	d	f	b	a	c	g	e	
5	10	2	7	12	15	10	7	-2.1004

⋮

# of possible combinations = 70

R: Permutation Tests

- "multtest" package:
  - mt.sample.teststat: to compute permuted statistics

```
> args(mt.sample.teststat)
function (V, classlabel, test = "t", fixed.seed.sampling = "y",
    B = 10000, na = .mt.naNUM, nonpara = "n")
```

  - mt.sample.rawp: to compute the p-values

```
> args(mt.sample.rawp)
function (V, classlabel, test = "t", side = "abs", fixed.seed.sampling = "y",
    B = 10000, na = .mt.naNUM, nonpara = "n")
```

Note: "test" includes

t, t.equalvar, pairt, wilcoxon, f

Comment

- Generally best capture the unknown structure of the data.
- It is ideal when the number of arrays is sufficient to offer the desired degree of confidence.
- May be computationally expensive.

Bootstrap

- The bootstrap method attempts to determine the probability distribution from the data itself.

  Step 1: One computes a statistic from the current list.

  Step 2: Create an artificial list by randomly drawing elements from the current list. Some elements will be picked more than once.

  Step 3: Compute a new statistic.

  Step 4: Repeat 100-1000 times and look at the distribution of these objects.

Bootstrap

- Example (Hjorth, 1994):

  Eleven life lengths of an engine part were measured as

5700	36300	12400	28000	19300	21500
12900	4100	91400	7600	1600	

Step 1: Estimate the population median by the sample median $\hat{\theta} = x_{(6)} = 12900$

Bootstrap

Steps 2 & 3: Bootstrap simulations:

Table 5.1 *Data drawn in the first five bootstrap samples.*

Original data ordered	Bootstrap sample number 1	2	3	4	5
1600			+		+ +
4100	+ + +	+ +	+	+	
5700	+	+	+	+ + +	+
7600				+ +	+ +
12400	+	+	+		+
12900	+		+ +		
19300	+	+	+	+ +	
21500		+ + +	+	+	+
28000	+	+ +	+	+ +	+
36300	+	+			+
91400	+ +		+ +		+ +
$\hat{\theta}^*$	12900	21500	12900	7600	12400

Bootstrap

- After 200 simulations:

  average: 14843

  standard deviation: 5737

  bias = 14843 – 12900 = 1943

  A bias adjusted estimate of the population median: 12900 – 1942 = 10957

- This method can be applied to compute *p*-values:
  - P-value = (# of permuted statistics the same as or *more extreme* than observed one) / (Total # of simulations).

R: Bootstrap

```
> library(boot)
> englife = c(5700, 36300, 12400, 28000, 19300,
+ 21500, 12900, 4100, 91400, 7600, 1600)
> boot.out = boot(englife,function(x,id){median(x[id])},1000)
```

```
ORDINARY NONPARAMETRIC BOOTSTRAP


Call:
boot(data = englife, statistic = function(x, id) {
    median(x[id])
}, R = 1000)


Bootstrap Statistics :
    original  bias    std. error
t1*    12900  2162.5    5861.693
```

How many bootstraps?

- No clear answer to this.
- Rule of thumb : try it 100 times, then 1000 times, and see if your answers have changed by much.
- Totally have N^N possible subsamples.

Summary

- Non statistical method: fold change
- Standard statistical methods:
  - parametric
  - nonparametric
- Computation-intensive methods: permutation; bootstrap.

References

t-like tests:

- Tusher, V.G., Tibshirani, R. & Chu, G. Significance analysis of microarrays applied to the ionizing radiation response. *Proc. Natl Acad. Sci. USA* 98, 5116-5121 (2001).
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Nonparametric rank-based statistics

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Permutation tests:

- Tusher, V.G., Tibshirani, R. & Chu, G. Significance analysis of microarrays applied to the ionizing radiation response. *Proc. Natl Acad. Sci. USA* 98, 5116-5121 (2001).
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