For written notes on this lecture, please read chapter 14 of *The Practical Bioinformatician*.

CS2220: Introduction to Computational Biology
Unit 3: Gene Expression Analysis

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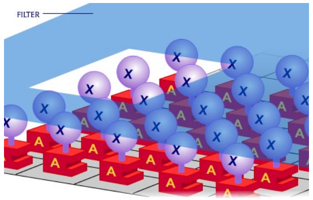
Plan

- Microarray background
- Gene expression profile classification
- Gene expression profile clustering
- Normalization
- Extreme sample selection
- Gene regulatory network inference

Background on microarrays

What is a microarray?

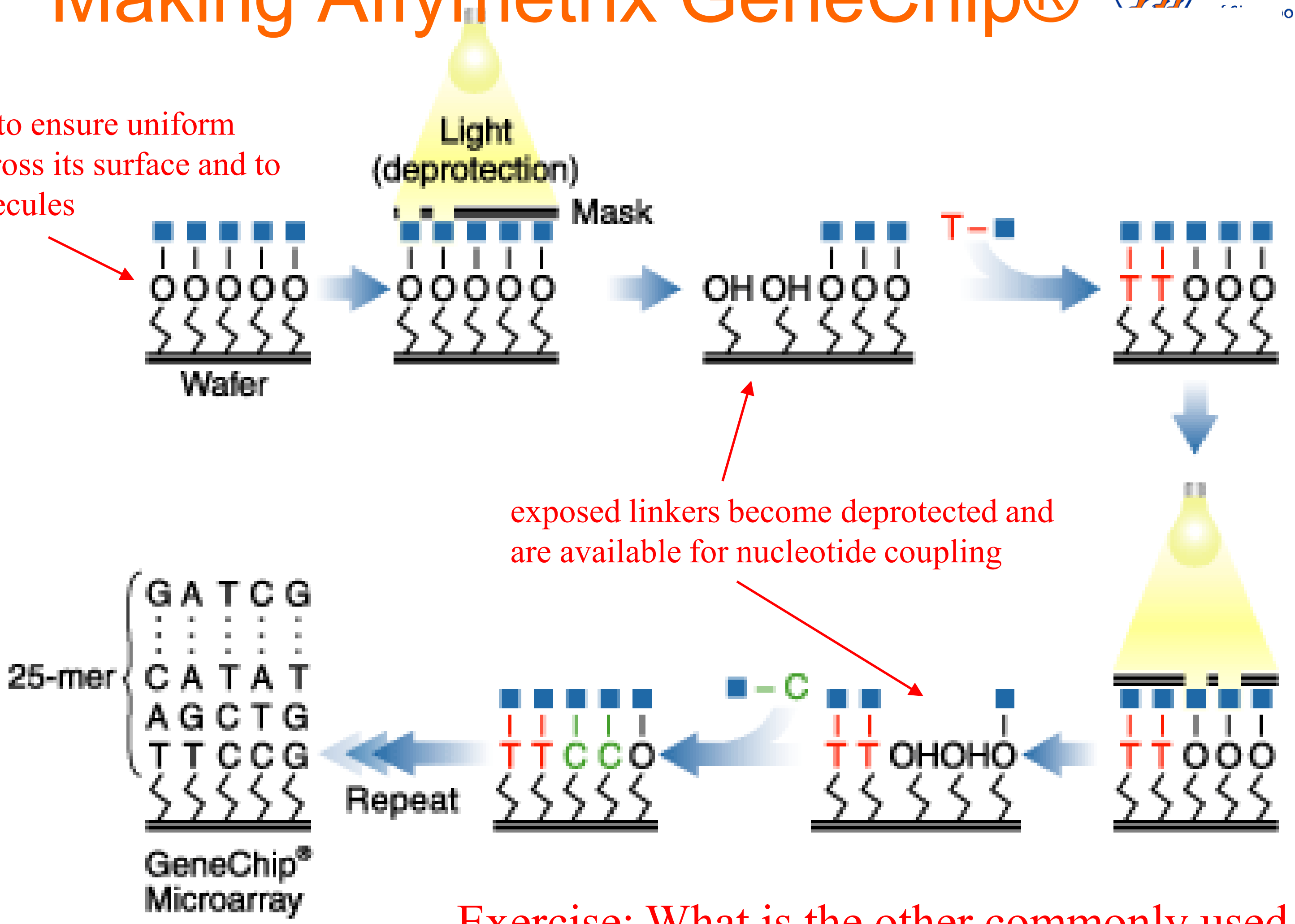
- Contain large numbers of DNA molecules spotted on glass slides, nylon membranes, or silicon wafers
- Detect what genes are being expressed or found in a cell of a tissue sample
- Measure expression of thousands of genes simultaneously



Affymetrix GeneChip®

Making Affymetrix GeneChip®

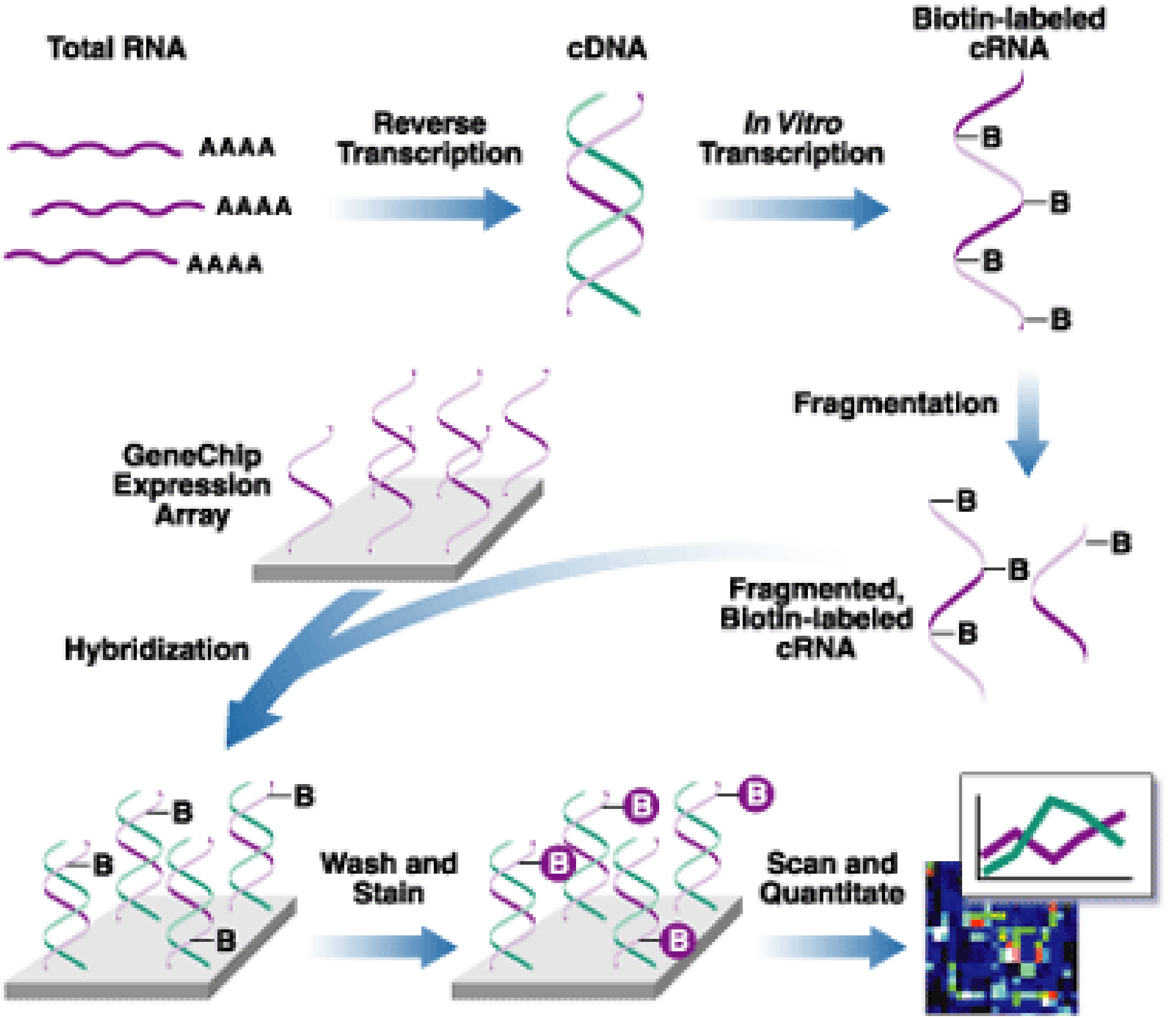
quartz is washed to ensure uniform hydroxylation across its surface and to attach linker molecules



exposed linkers become deprotected and are available for nucleotide coupling

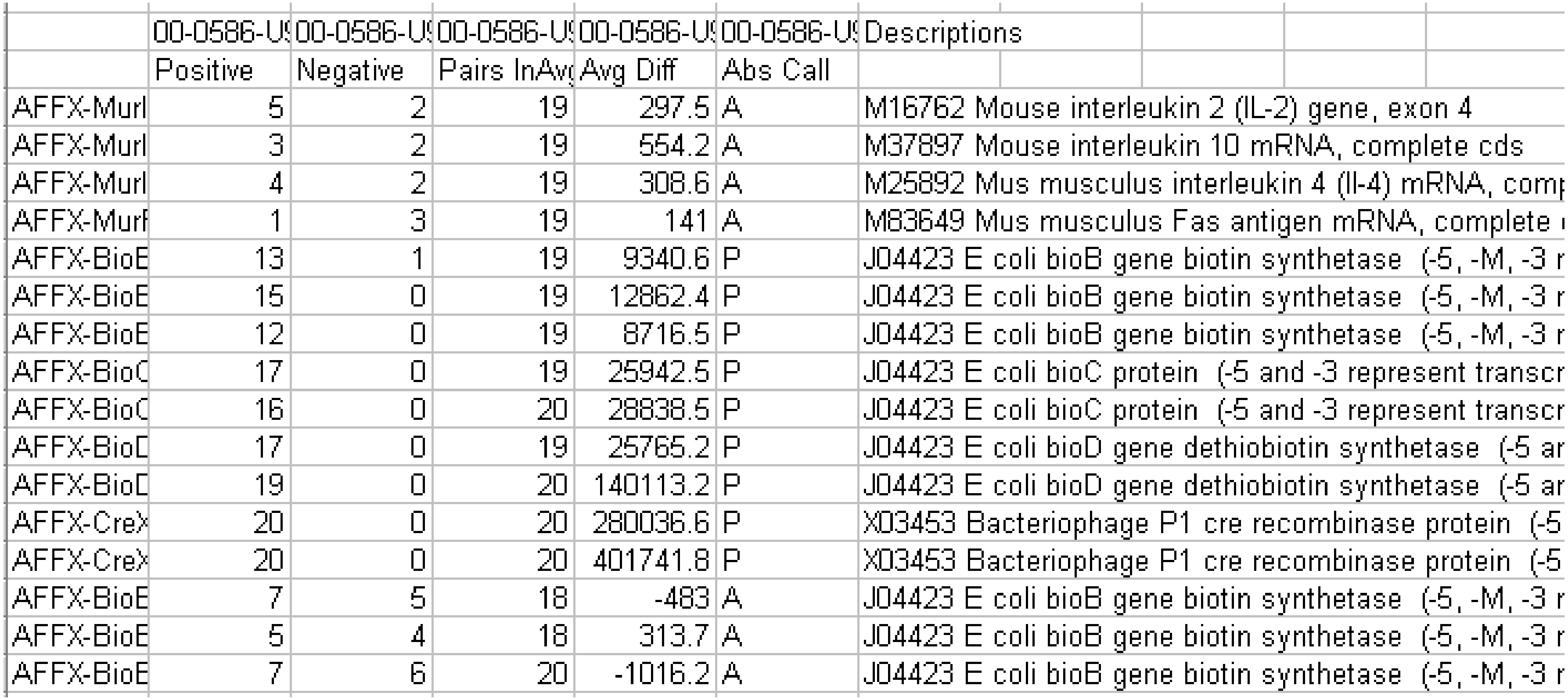
Exercise: What is the other commonly used type of microarray? How is that one different from Affymetrix's?

Gene expression measurement by Affymetrix GeneChip®



Click to watch an interesting movie explaining the working of microarray

Sample Affymetrix GeneChip® data file (U95A)



	00-0586-U	00-0586-U	00-0586-U	00-0586-U	00-0586-U	Descriptions
	Positive	Negative	Pairs InAv	Avg Diff	Abs Call	
AFFX-MurI	5	2	19	297.5	A	M16762 Mouse interleukin 2 (IL-2) gene, exon 4
AFFX-MurI	3	2	19	554.2	A	M37897 Mouse interleukin 10 mRNA, complete cds
AFFX-MurI	4	2	19	308.6	A	M25892 Mus musculus interleukin 4 (Il-4) mRNA, comp
AFFX-MurF	1	3	19	141	A	M83649 Mus musculus Fas antigen mRNA, complete
AFFX-BioB	13	1	19	9340.6	P	J04423 E coli bioB gene biotin synthetase (-5, -M, -3 r
AFFX-BioB	15	0	19	12862.4	P	J04423 E coli bioB gene biotin synthetase (-5, -M, -3 r
AFFX-BioB	12	0	19	8716.5	P	J04423 E coli bioB gene biotin synthetase (-5, -M, -3 r
AFFX-BioC	17	0	19	25942.5	P	J04423 E coli bioC protein (-5 and -3 represent transcr
AFFX-BioC	16	0	20	28838.5	P	J04423 E coli bioC protein (-5 and -3 represent transcr
AFFX-BioD	17	0	19	25765.2	P	J04423 E coli bioD gene dethiobiotin synthetase (-5 ar
AFFX-BioD	19	0	20	140113.2	P	J04423 E coli bioD gene dethiobiotin synthetase (-5 ar
AFFX-CreX	20	0	20	280036.6	P	X03453 Bacteriophage P1 cre recombinase protein (-5
AFFX-CreX	20	0	20	401741.8	P	X03453 Bacteriophage P1 cre recombinase protein (-5
AFFX-BioB	7	5	18	-483	A	J04423 E coli bioB gene biotin synthetase (-5, -M, -3 r
AFFX-BioB	5	4	18	313.7	A	J04423 E coli bioB gene biotin synthetase (-5, -M, -3 r
AFFX-BioB	7	6	20	-1016.2	A	J04423 E coli bioB gene biotin synthetase (-5, -M, -3 r

Some advice on processing Affymetrix GeneChip® data

- **Ignore AFFX genes**
  - These genes are control genes
- **Ignore genes with “Abs Call” equal to “A” or “M”**
  - Measurement quality is suspect
- **Upperbound 40000, lowerbound 100**
  - Saturation of laser scanner
- **Deal with missing values**

Exercise: Suggest 2 ways to deal with missing value

Type of gene expression datasets

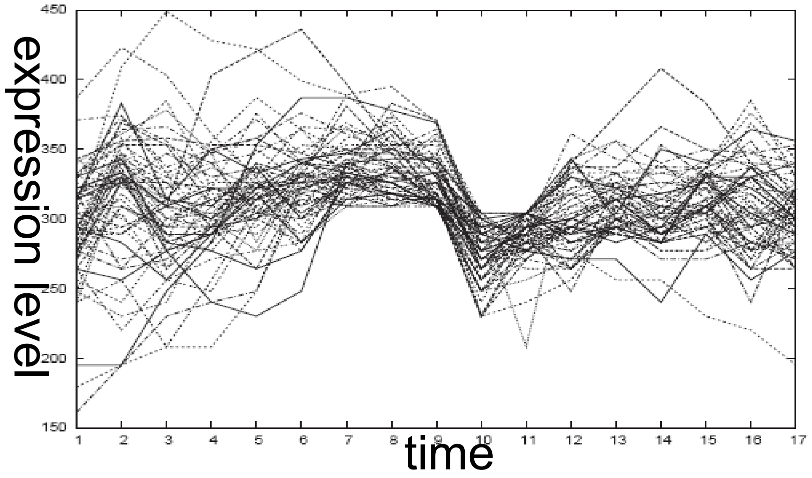
- Gene-Conditions or **Gene-Sample** (**numeric** or discretized)

1000 - 100,000 columns

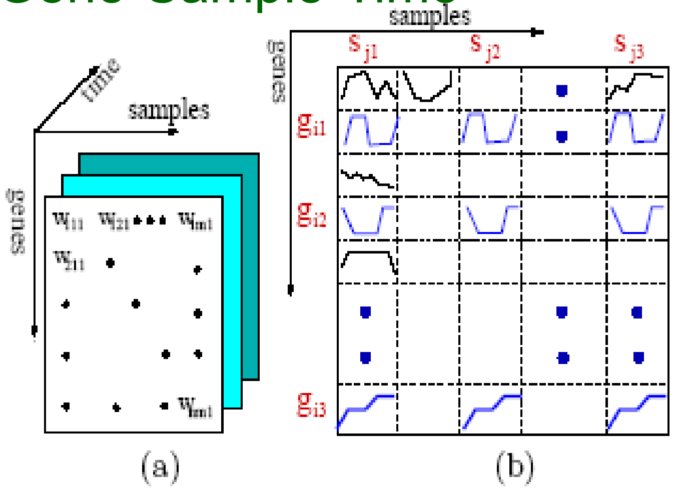
100-500 rows

	Class	Gene1	Gene2	Gene3	Gene4	Gene5	Gene6	Gene7	.....	
Sample1	Cancer	0.12	-1.3	1.7	1.0	-3.2	0.78	-0.12		
Sample2	Cancer							1.3		
.										
	~Cancer									
SampleN	~Cancer									

- Gene-Time



- Gene-Sample-Time



Type of gene expression datasets

- Gene-Conditions or **Gene-Sample** (numeric or **discretized**)

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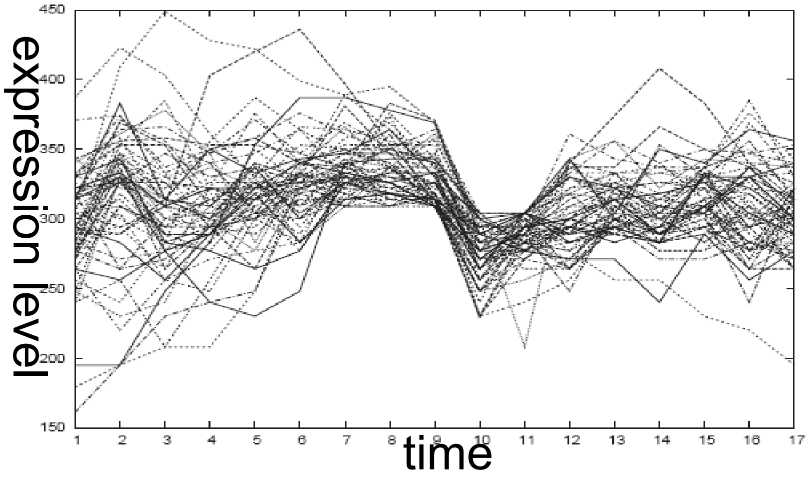
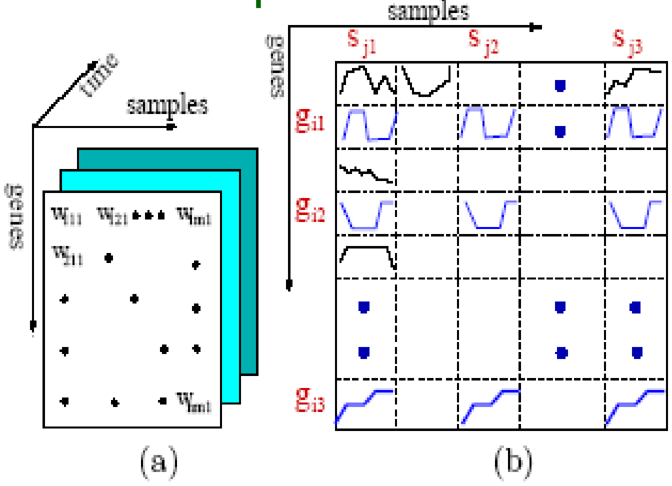
	Class	Gene1	Gene2	Gene3	Gene4	Gene5	Gene6	Gene7	.....	
Sample1	Cancer	1	0	1	1	1	0	0		
Sample2	Cancer							1		
.										
	~Cancer									
SampleN	~Cancer									

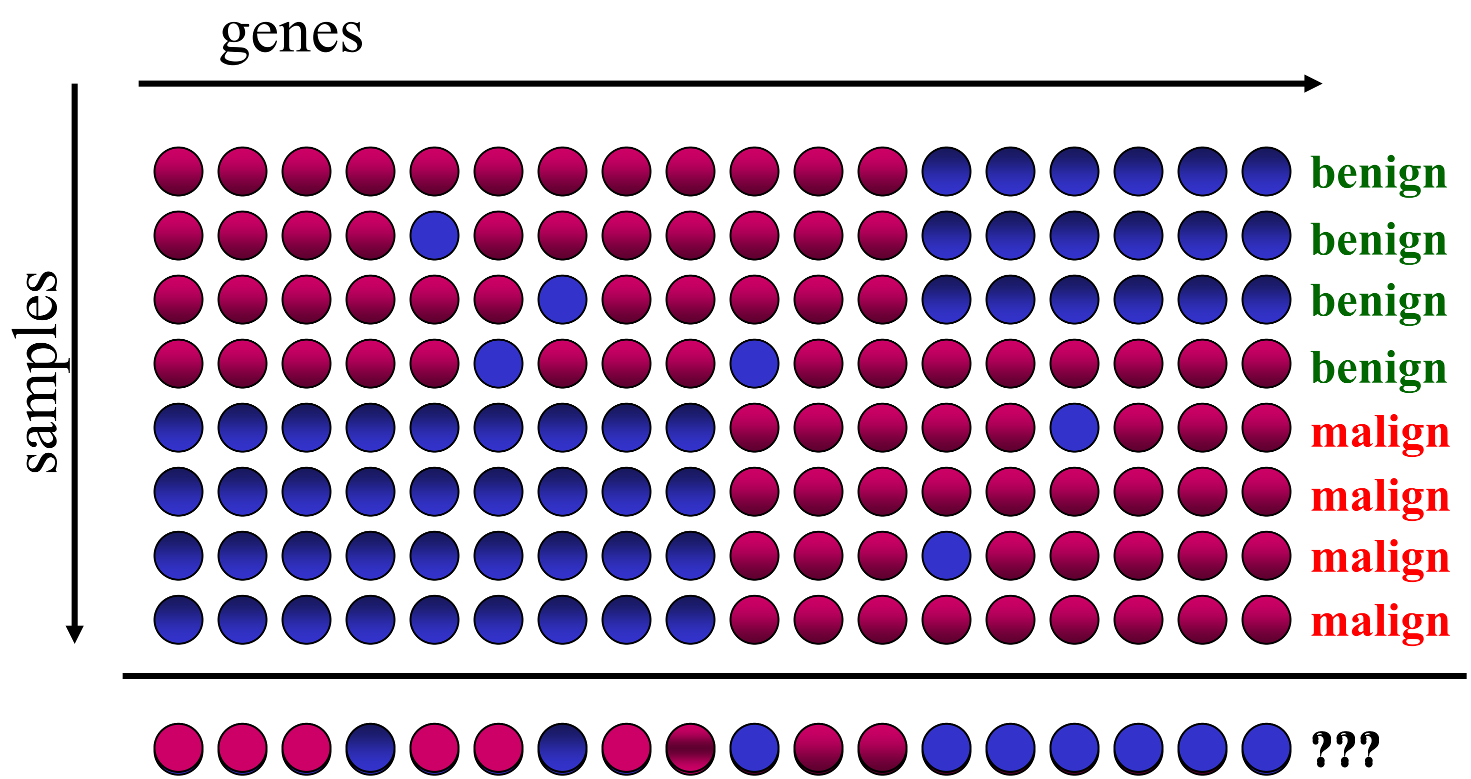
- Gene-Time



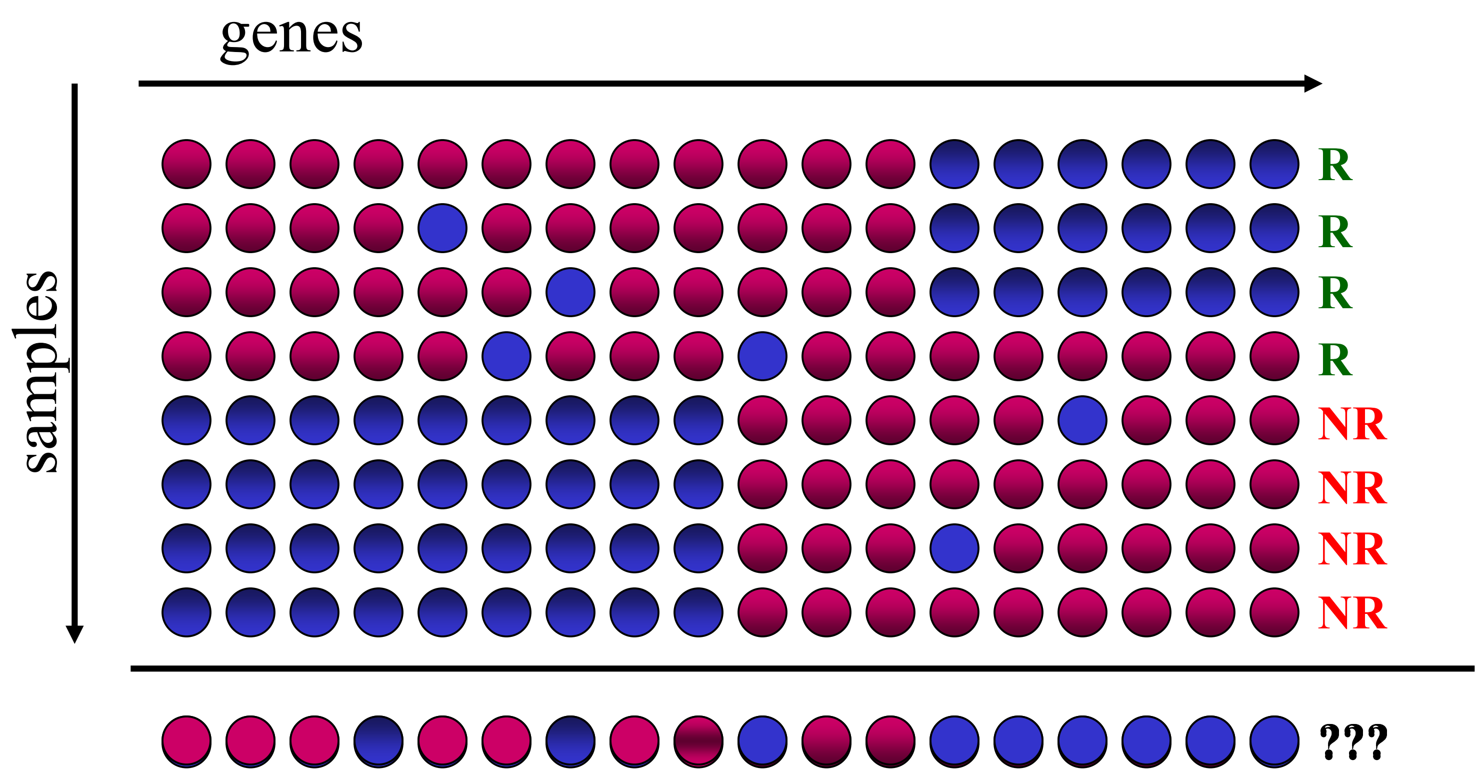
- Gene-Sample-Time



Application: Disease subtype diagnosis



Application: Treatment prognosis



Type of gene expression datasets

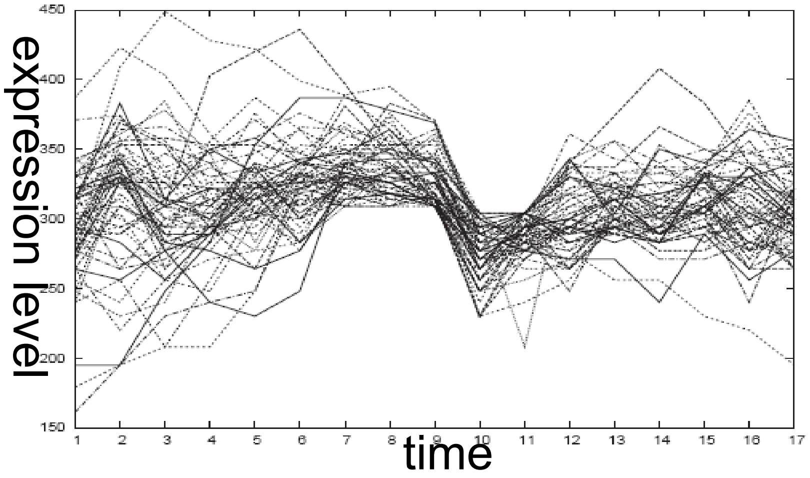
- **Gene-Conditions** or Gene-Sample (**numeric** or discretized)

1000 - 100,000 columns

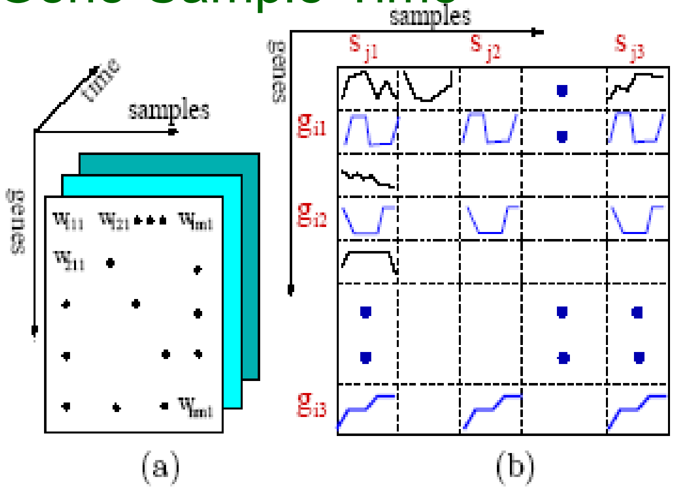
100-500 rows

	Gene1	Gene2	Gene3	Gene 4	Gene5	Gene6	Gene7		
Cond1	0.12	-1.3	1.7	1.0	-3.2	0.78	-0.12		
Cond2							1.3		
.									
CondN									

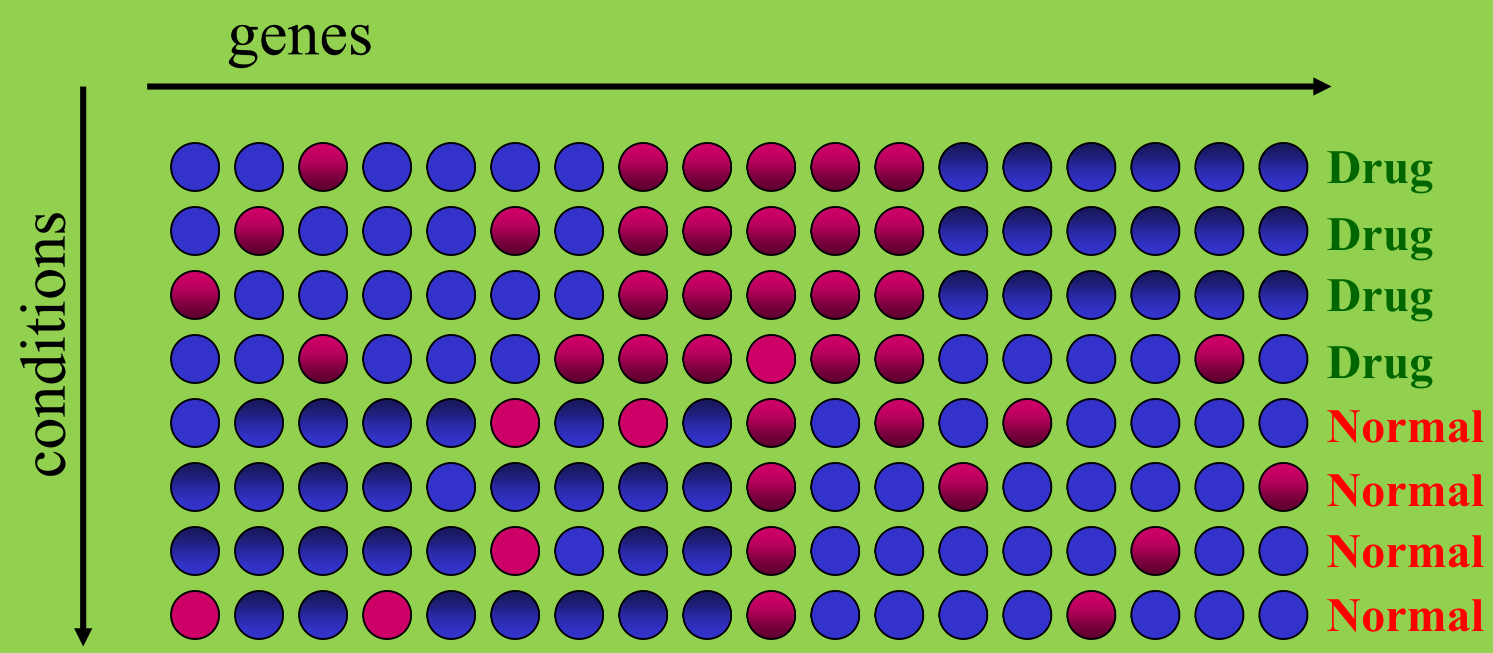
- Gene-Time



- Gene-Sample-Time



Application: Drug-action detection



- **Which group of genes does the drug affect? Why?**

Exercise #1

Gene expression profile classification

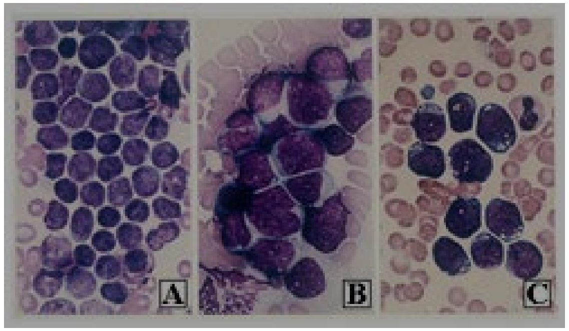
Childhood acute lymphoblastic leukemia subtype diagnosis

NUS National University of Singapore

Childhood ALL

- **Major subtypes: T-ALL, E2A-PBX, TEL-AML, BCR-ABL, MLL genome rearrangements, Hyperdiploid>50**
- **Diff subtypes respond differently to same Tx**
- **Over-intensive Tx**
  - Development of secondary cancers
  - Reduction of IQ
- **Under-intensiveTx**
  - Relapse

- **The subtypes look similar**



- **Conventional diagnosis**
  - Immunophenotyping
  - Cytogenetics
  - Molecular diagnostics
- **Unavailable in most ASEAN countries**

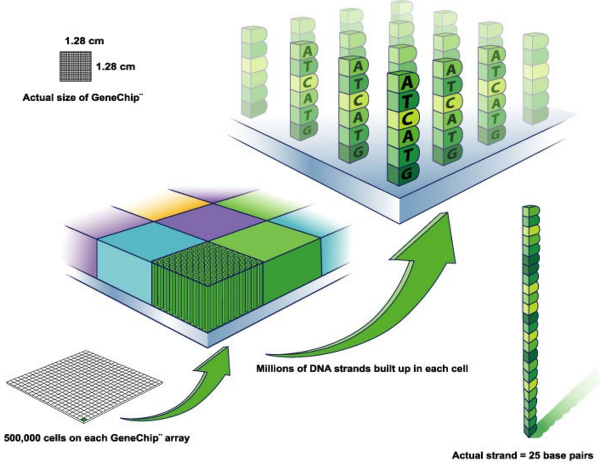
Mission

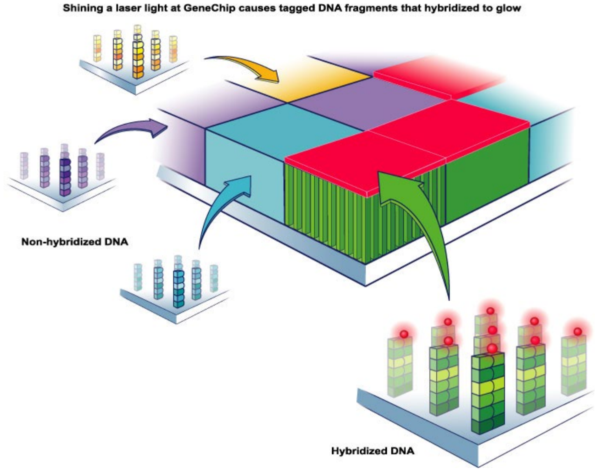
- Conventional risk assignment procedure requires difficult expensive tests and collective judgement of multiple specialists

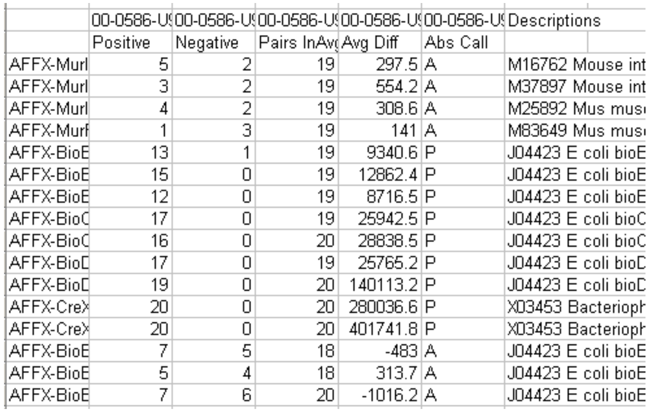
- Generally available only in major advanced hospitals

⇒ Can we have a single-test easy-to-use platform instead?

Single-test platform of microarray & machine learning


1.28 cm
1.28 cm
Actual size of GeneChip™
500,000 cells on each GeneChip™ array
Millions of DNA strands built up in each cell
Actual strand = 25 base pairs
A T C A T G


Shining a laser light at GeneChip causes tagged DNA fragments that hybridized to glow
Non-hybridized DNA
Hybridized DNA



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AFFX-BioC	17	0	19	25942.5	P	J04423 E coli bioC
AFFX-BioC	16	0	20	28838.5	P	J04423 E coli bioC
AFFX-BioD	17	0	19	25765.2	P	J04423 E coli bioD
AFFX-BioD	19	0	20	140113.2	P	J04423 E coli bioD
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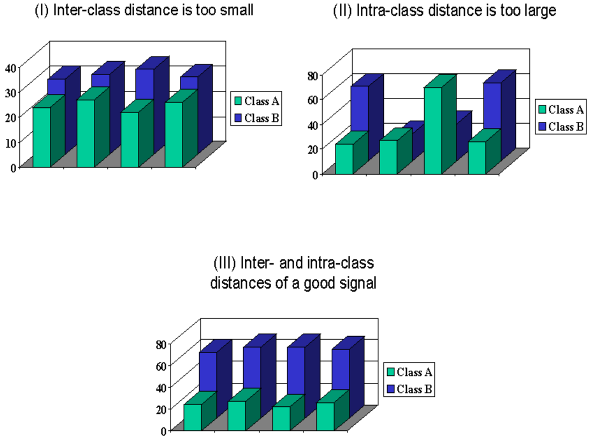
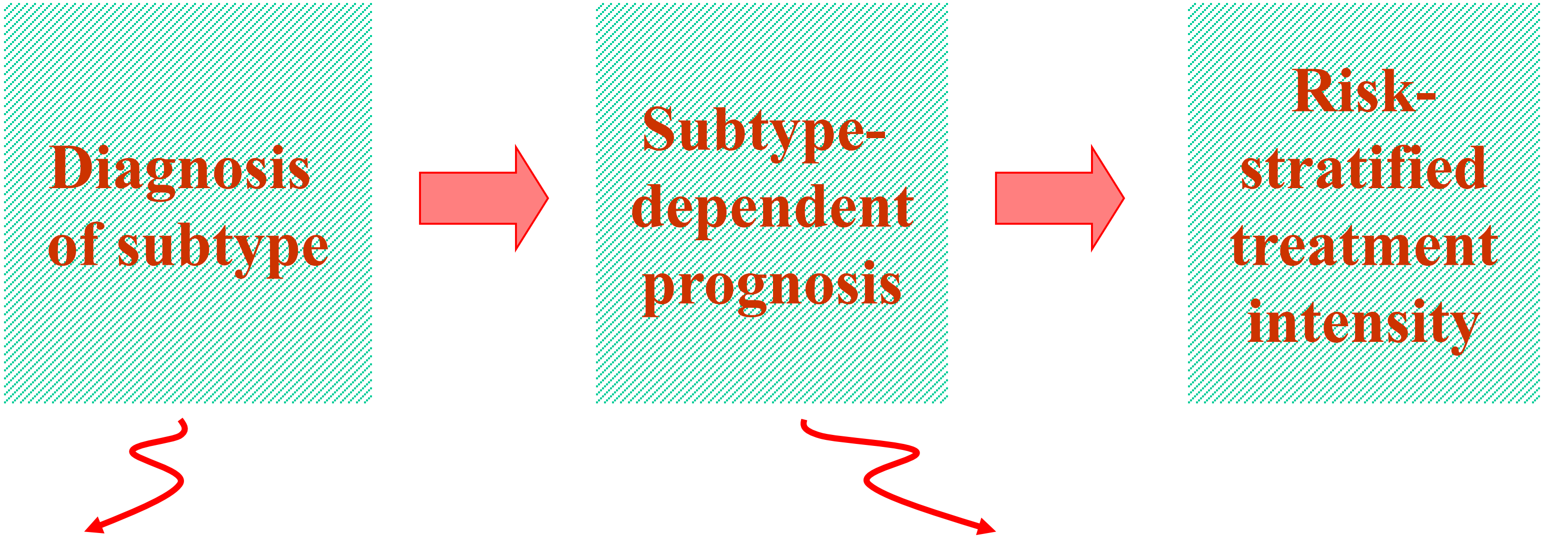

(I) Inter-class distance is too small

(II) Intra-class distance is too large

(III) Inter- and intra-class distances of a good signal

Overall strategy



Diagnosis of subtype

Subtype-dependent prognosis

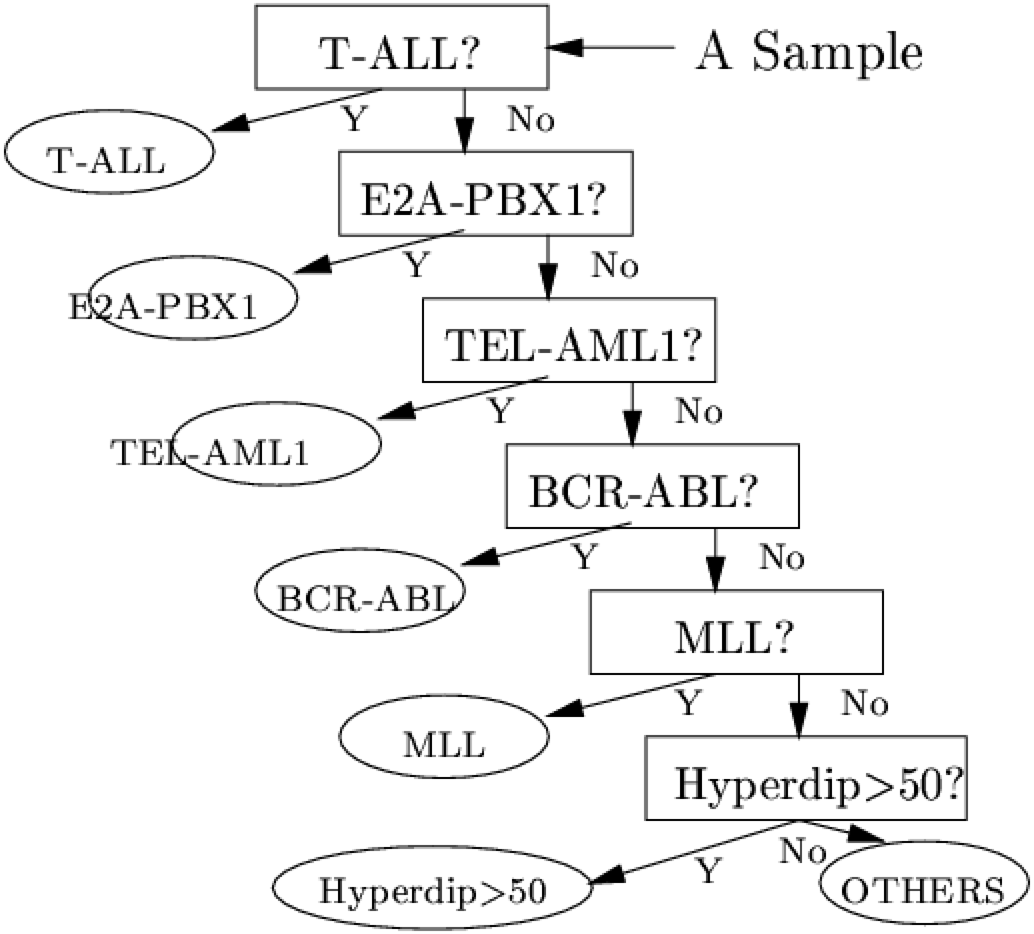
Risk-stratified treatment intensity

- For each subtype, select genes to develop classification model for diagnosing that subtype
- For each subtype, select genes to develop prediction model for prognosis of that subtype

Subtype diagnosis by PCL

- Gene expression data collection
- Gene selection by χ2
- Classifier training by emerging pattern
- ~~Classifier tuning (optional for some machine learning methods)~~
- Apply classifier for diagnosis of future cases by PCL

Childhood ALL subtype diagnosis workflow



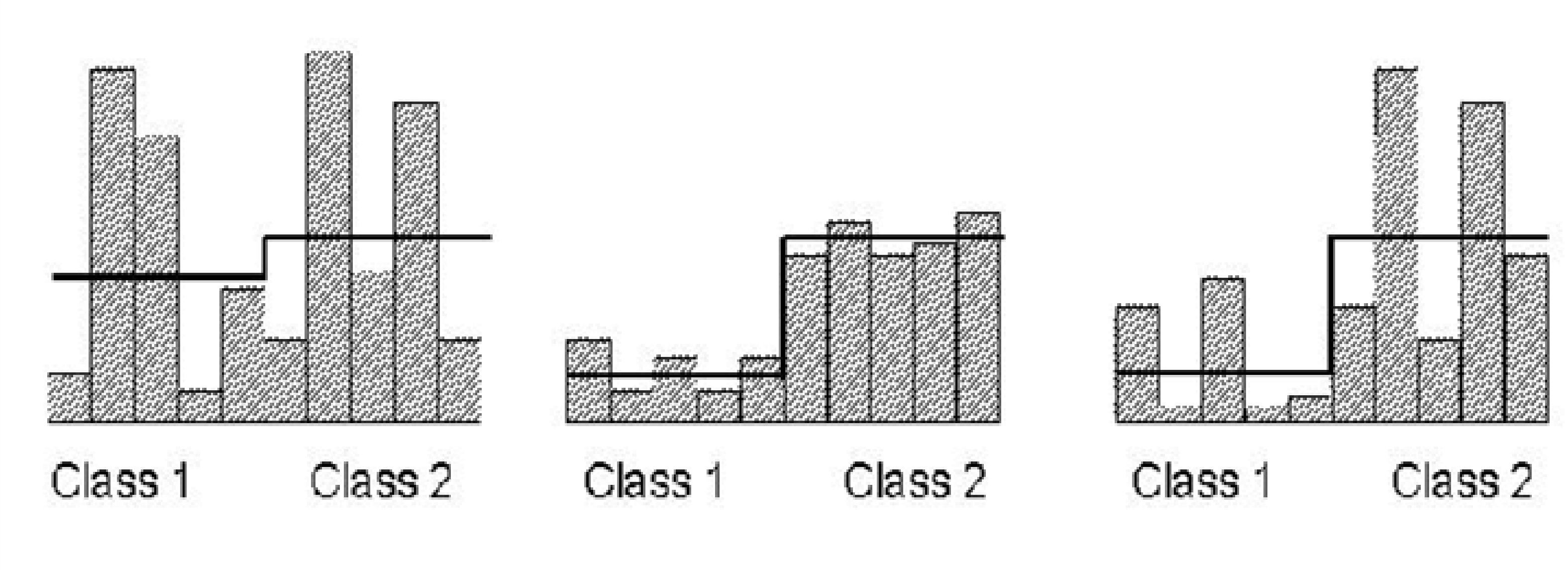
A tree-structured diagnostic workflow was recommended by our doctor collaborator

Training and testing sets

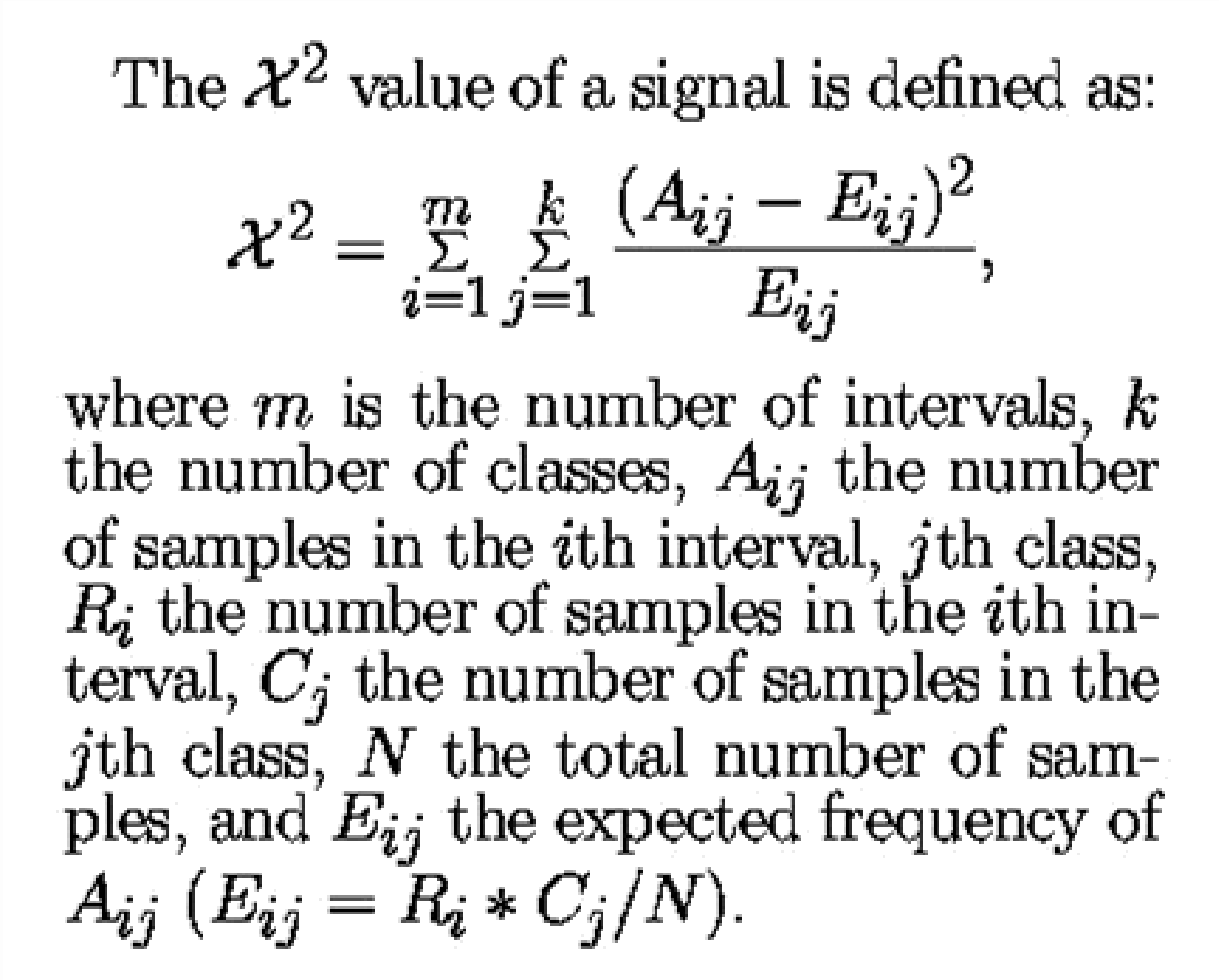
Paired datasets	Ingredients	Training	Testing
T-ALL vs OTHERS1	OTHERS1 ={E2A-PBX1, TEL-AML1, BCR-ABL, Hyperdip>50, MLL, OTHERS}	28 vs 187	15 vs 97
E2A-PBX1 vs OTHERS2	OTHERS2 = {TEL-AML1, BCR-ABL Hyperdip>50, MLL, OTHERS}	18 vs 169	9 vs 88
TEL-AML1 vs OTHERS3	OTHERS3 = {BCR-ABL Hyperdip>50, MLL, OTHERS}	52 vs 117	27 vs 61
BCR-ABL vs OTHERS4	OTHERS4 = {Hyperdip>50, MLL, OTHERS}	9 vs 108	6 vs 55
MLL vs OTHERS5	OTHERS5 = {Hyperdip>50, OTHERS}	14 vs 94	6 vs 49
Hyperdip>50 vs OTHERS	OTHERS = {Hyperdip47-50, Pseudodip, Hypodip, Normo}	42 vs 52	22 vs 27

Signal selection basic idea

- Choose a signal w/ low intra-class distance
- Choose a signal w/ high inter-class distance



Signal selection by χ2



The $\mathcal{X}^2$ value of a signal is defined as:

$$\mathcal{X}^2 = \sum_{i=1}^{m} \sum_{j=1}^{k} \frac{(A_{ij} - E_{ij})^2}{E_{ij}},$$

where m is the number of intervals, k the number of classes, A_{ij} the number of samples in the ith interval, jth class, R_i the number of samples in the ith interval, C_j the number of samples in the jth class, N the total number of samples, and E_{ij} the expected frequency of A_{ij} ($E_{ij} = R_i * C_j / N$).

Emerging patterns

- **An emerging pattern is a set of conditions**
  - usually involving several features
  - that most members of a class satisfy
  - but none or few of the other class satisfy
- **A jumping emerging pattern is an emerging pattern that**
  - some members of a class satisfy
  - but no members of the other class satisfy
- **We use only jumping emerging patterns**

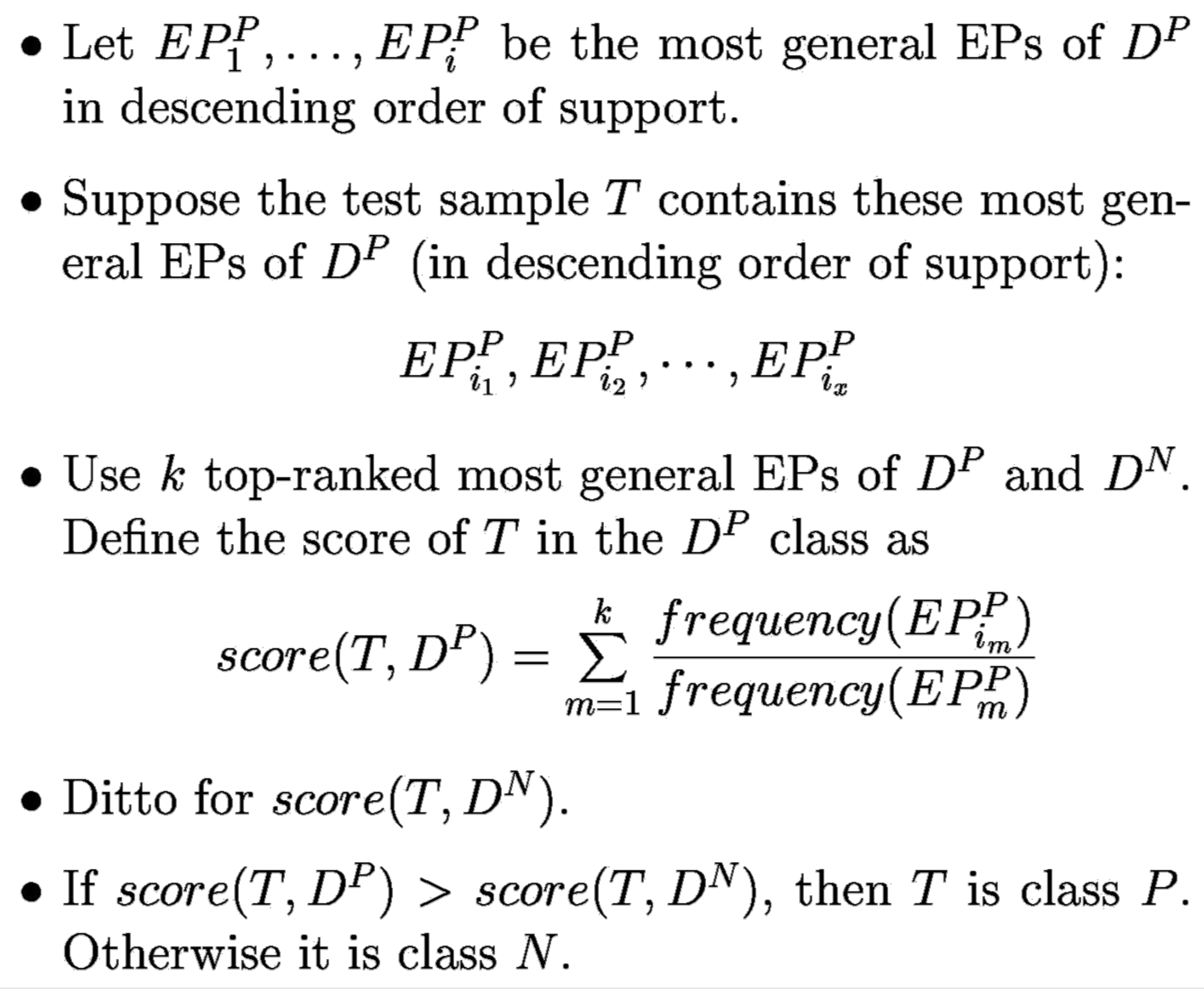
Examples

Patterns	Frequency (P)	Frequency(N)
{9, 36}	38 instances	0
{9, 23}	38	0
{4, 9}	38	0
{9, 14}	38	0
{6, 9}	38	0
{7, 21}	0	36
{7, 11}	0	35
{7, 43}	0	35
{7, 39}	0	34
{24, 29}	0	34

Easy interpretation

Reference number 9: the expression of gene 37720_at > 215
Reference number 36: the expression of gene 38028_at ≤ 12

PCL: Prediction by Collective Likelihood



- Let $EP_1^P, \ldots, EP_i^P$ be the most general EPs of D^P in descending order of support.
- Suppose the test sample T contains these most general EPs of D^P (in descending order of support):

$$EP_{i_1}^P, EP_{i_2}^P, \cdots, EP_{i_x}^P$$

- Use k top-ranked most general EPs of D^P and D^N. Define the score of T in the D^P class as

$$score(T, D^P) = \sum_{m=1}^{k} \frac{frequency(EP_{i_m}^P)}{frequency(EP_m^P)}$$

- Ditto for $score(T, D^N)$.
- If $score(T, D^P) > score(T, D^N)$, then T is class P. Otherwise it is class N.

PCL learning

Top-Ranked EPs in Positive class

EP_1^P (90%)
EP_2^P (86%)
.
.
EP_n^P (68%)

Top-Ranked EPs in Negative class

EP_1^N (100%)
EP_2^N (95%)
.
.
EP_n^N (80%)

The idea of summarizing multiple top-ranked EPs is intended to avoid some rare tie cases

PCL testing

Most freq EP of pos class in the test sample

$$\text{Score}^{P} = EP_1^{P'} / EP_1^{P} + \ldots + EP_k^{P'} / EP_k^{P}$$

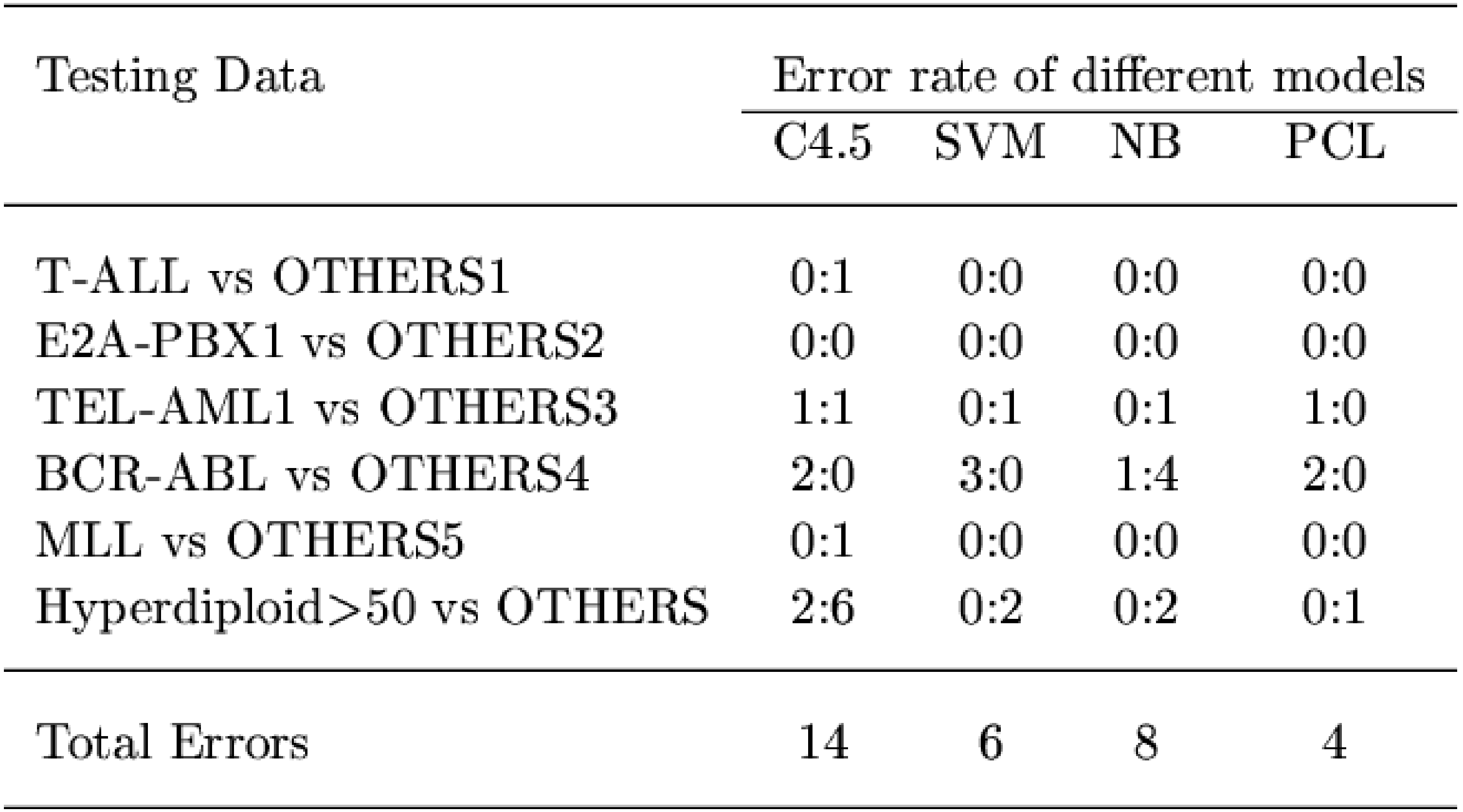
Most freq EP of pos class

Similarly,

$$\text{Score}^{N} = EP_1^{N'} / EP_1^{N} + \ldots + EP_k^{N'} / EP_k^{N}$$

If $\text{Score}^{P} > \text{Score}^{N}$, then positive class, Otherwise negative class

Accuracy of PCL (vs. other classifiers)



Testing Data	Error rate of different models			
	C4.5	SVM	NB	PCL
T-ALL vs OTHERS1	0:1	0:0	0:0	0:0
E2A-PBX1 vs OTHERS2	0:0	0:0	0:0	0:0
TEL-AML1 vs OTHERS3	1:1	0:1	0:1	1:0
BCR-ABL vs OTHERS4	2:0	3:0	1:4	2:0
MLL vs OTHERS5	0:1	0:0	0:0	0:0
Hyperdiploid>50 vs OTHERS	2:6	0:2	0:2	0:1
Total Errors	14	6	8	4

The classifiers are all applied to the 20 genes selected by $\chi 2$ at each level of the tree

Understandability of PCL

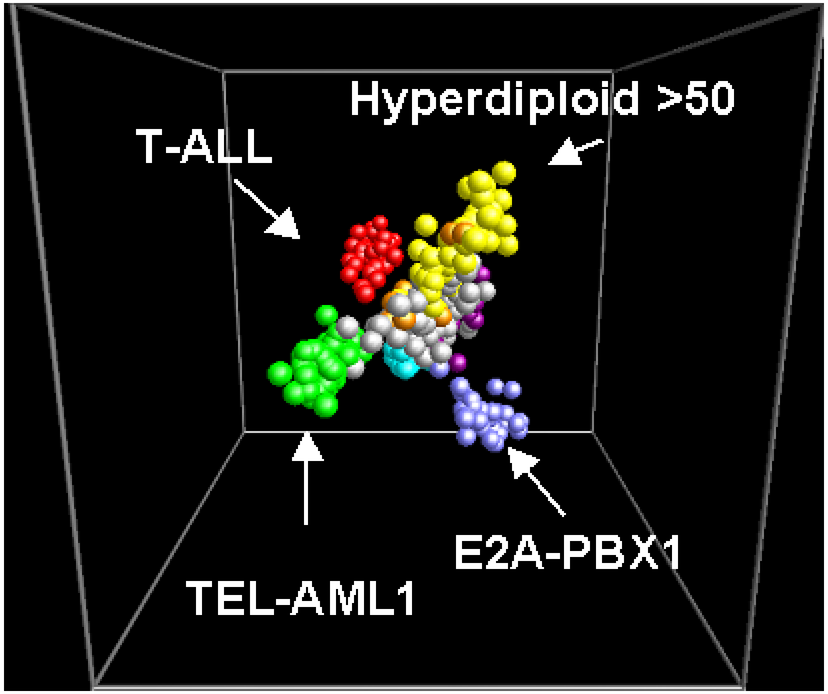
- E.g., for T-ALL vs. OTHERS, one ideally discriminatory gene 38319_at was found, inducing these 2 EPs

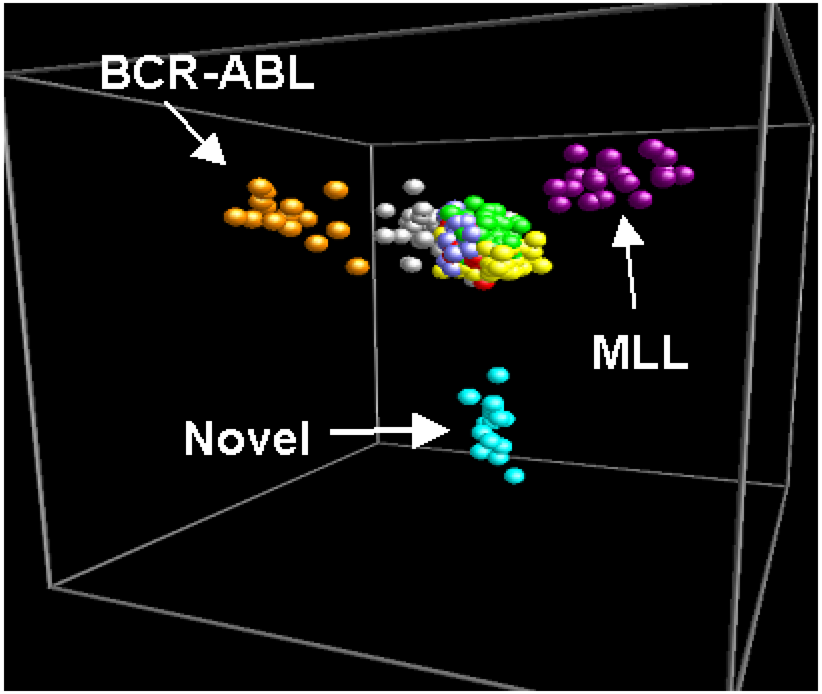
$$\{gene_{-(38\,319\_at)}@(-\infty, 15\,975.6)\} \text{ and}$$
$$\{gene_{-(38\,319\_at)}@[15\,975.6, +\infty)\}.$$

- These give us the diagnostic rule

If the expression of 38 319_*at* is less than 15 975.6, then
this ALL sample must be a T-ALL.
Otherwise it must be a subtype in OTHERS1.

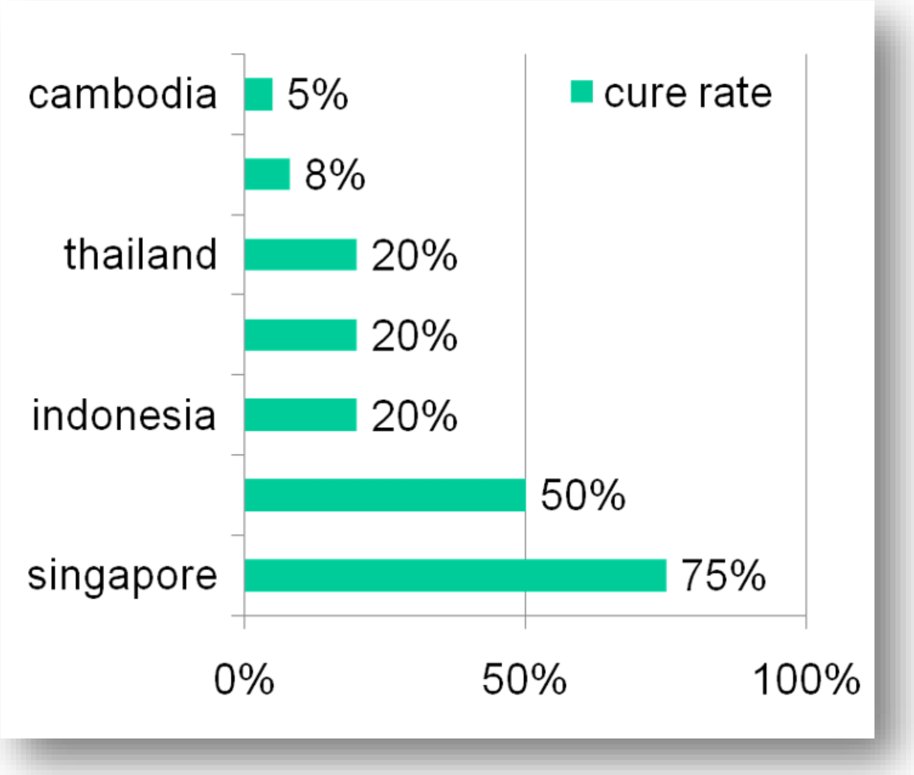
Multidimensional scaling plot for subtype diagnosis





Obtained by performing PCA on the 20 genes chosen for each level

Childhood ALL cure rates



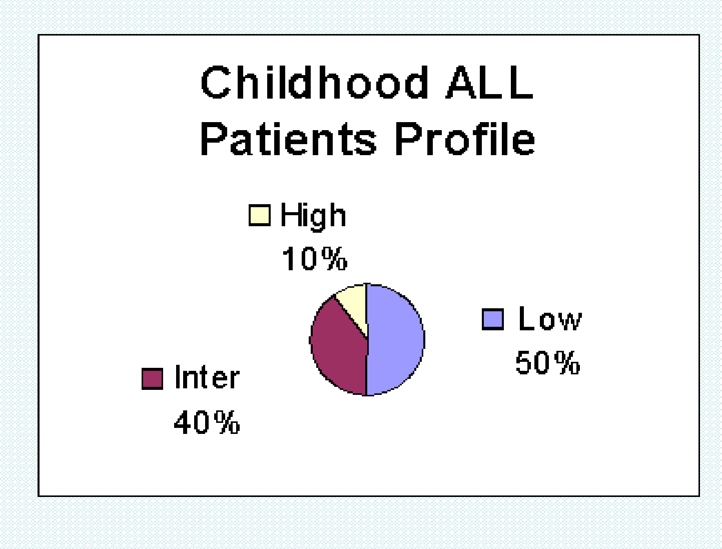
- **Conventional risk assignment procedure requires difficult expensive tests and collective judgement of multiple specialists**

⇒ **Not available in less advanced ASEAN countries**

Childhood ALL treatment cost

- **Treatment for childhood ALL over 2 yrs**
  - Intermediate intensity: US$60k
  - Low intensity: US$36k
  - High intensity: US$72k
- **Treatment for relapse: US$150k**
- **Cost for side-effects: Unquantified**

Current situation (2000 new cases / yr in ASEAN)



- Intermediate intensity conventionally applied in less advanced ASEAN countries

- Over intensive for 50% of patients, thus more side effects
- Under intensive for 10% of patients, thus more relapse

- US$120m (US$60k * 2000) for intermediate intensity tx
- US$30m (US$150k * 2000 * 10%) for relapse tx
- Total US$150m/yr plus un-quantified costs for dealing with side effects

Using our platform

- Low intensity applied to 50% of patients
- Intermediate intensity to 40% of patients
- High intensity to 10% of patients

⇒ Reduced side effects

⇒ Reduced relapse

⇒ 75-80% cure rates

- US$36m (US$36k * 2000 * 50%) for low intensity
- US$48m (US$60k * 2000 * 40%) for intermediate intensity
- US$14.4m (US$72k * 2000 * 10%) for high intensity

- Total US$98.4m/yr

⇒ Save US$51.6m/yr

A nice ending…

- **Asian Innovation Gold Award 2003**

The Winners 2003

SOLID GOLD: Allen Yeoh (*front*) with teammates (*from left*) Huiqing Liu, Limsoon Wong and Jinyan Li

GOLD AWARD

Kids' Cancer Heroes

A Singapore team has invented a simple test for childhood leukaemia that promises safer treatments and higher cure rates for kids in the developing world

By Trish Saywell/SINGAPORE

Photos by Munshi Ahmed/REVIEW

Gene expression profile clustering

Novel disease subtype discovery

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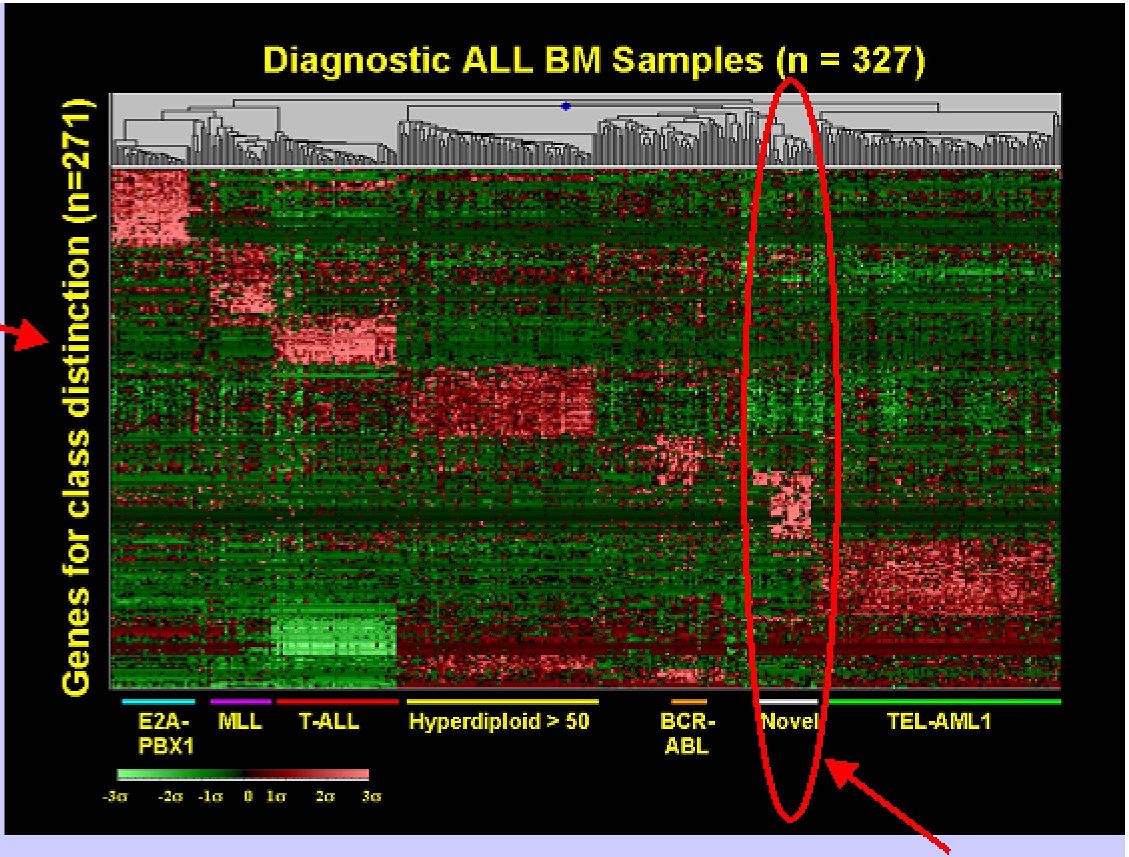
Is there a new subtype?

Genes selected by χ2



New subtype discovered

- **Hierarchical clustering of gene expression profiles reveals a novel subtype of childhood ALL**

Exercise: Name and describe one bi-clustering method

More about this in a moment

Hierarchical clustering

- **Assign each item to its own cluster**
  - If there are N items initially, we get N clusters, each containing just one item

- **Find the "most similar" pair of clusters, merge them into a single cluster, so we now have one less cluster**

- **Repeat previous step until all items are clustered into a single cluster of size N**

Gene expression profile clustering

Diagnosis via guilt-by-association

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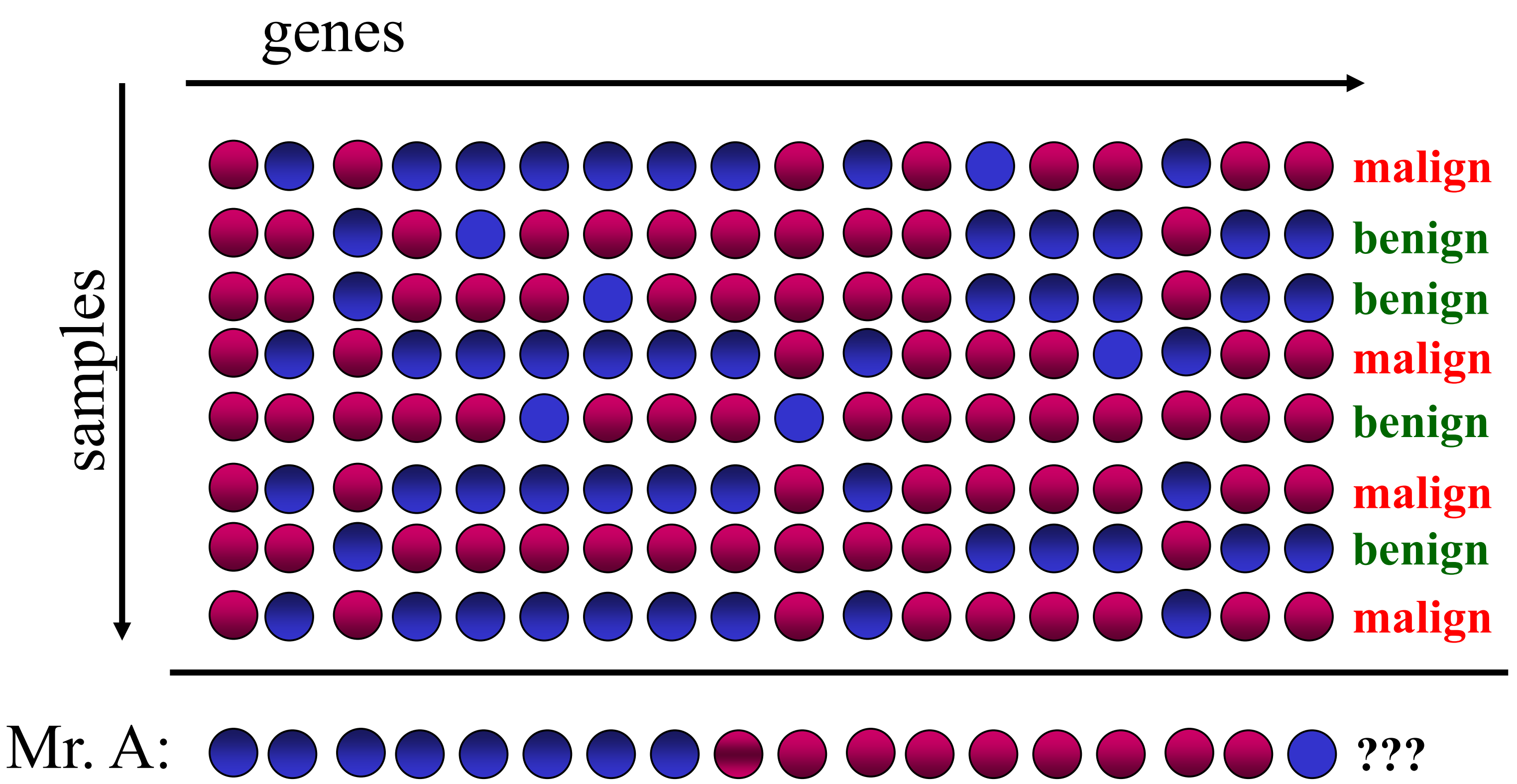
Some patient samples



genes

samples

malign

benign

benign

malign

benign

malign

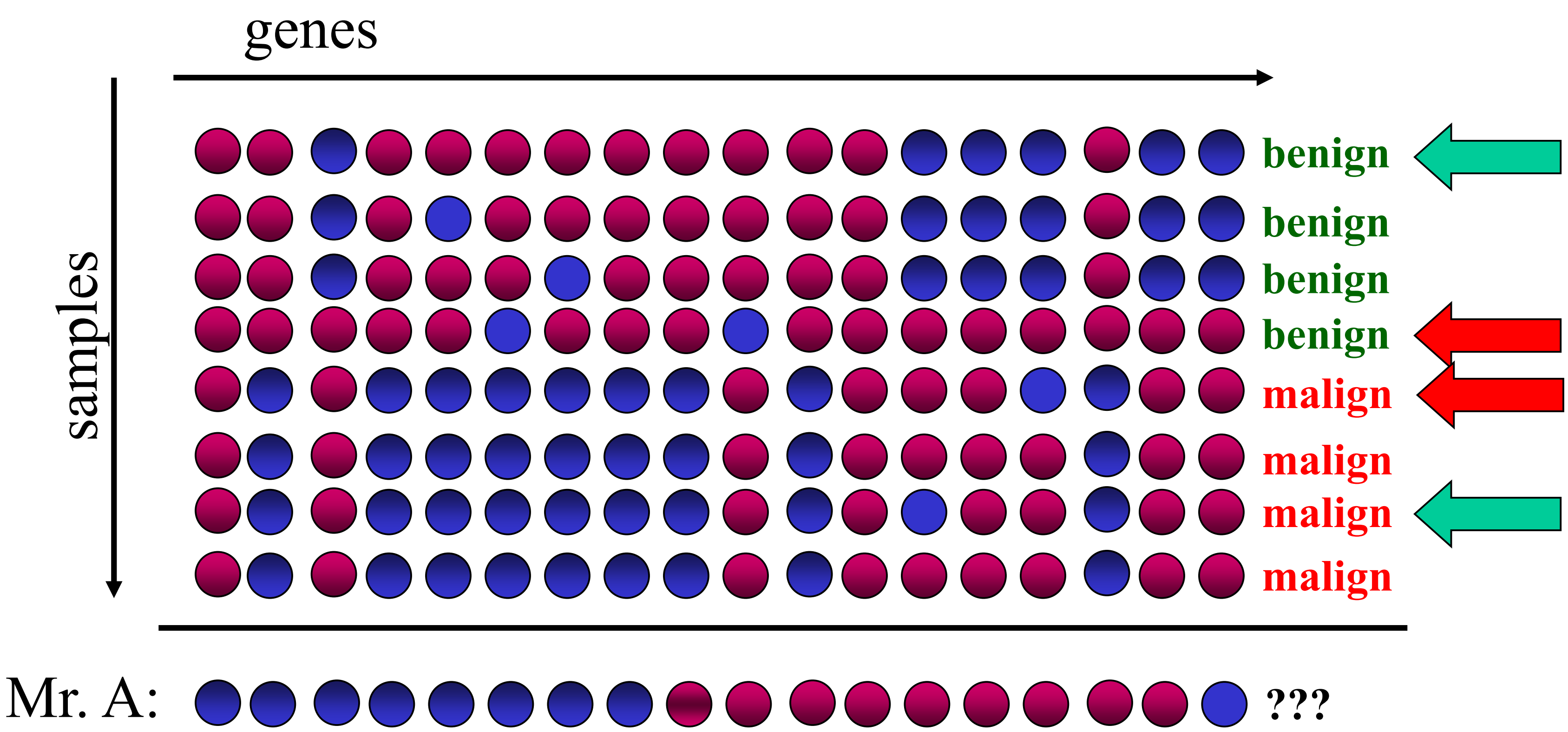
benign

malign

Mr. A: ???

- **Does Mr. A have cancer?**

Let’s rearrange the rows…



genes

samples

benign

benign

benign

benign

malign

malign

malign

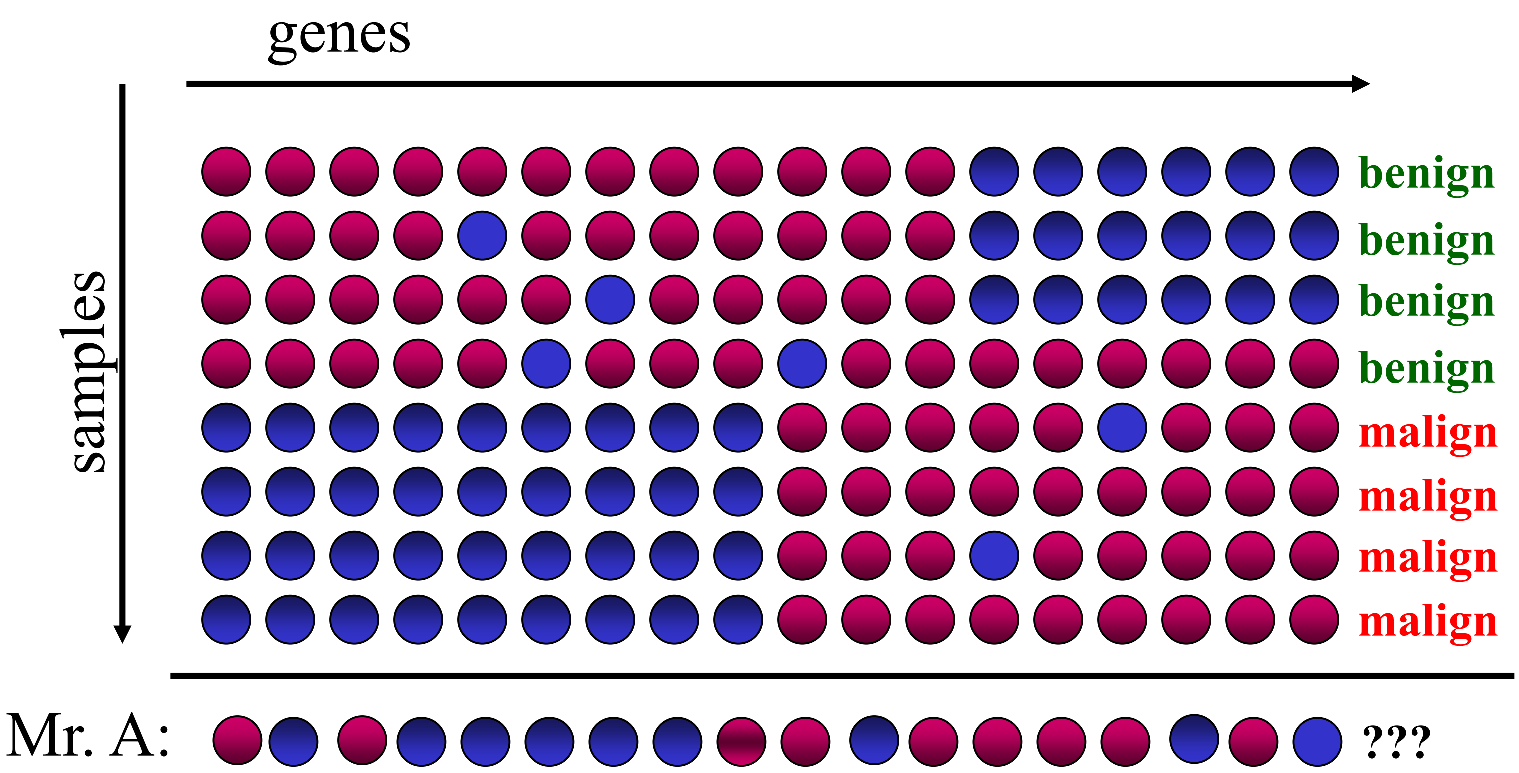
malign

Mr. A:

???

- **Does Mr. A have cancer?**

and the columns too…



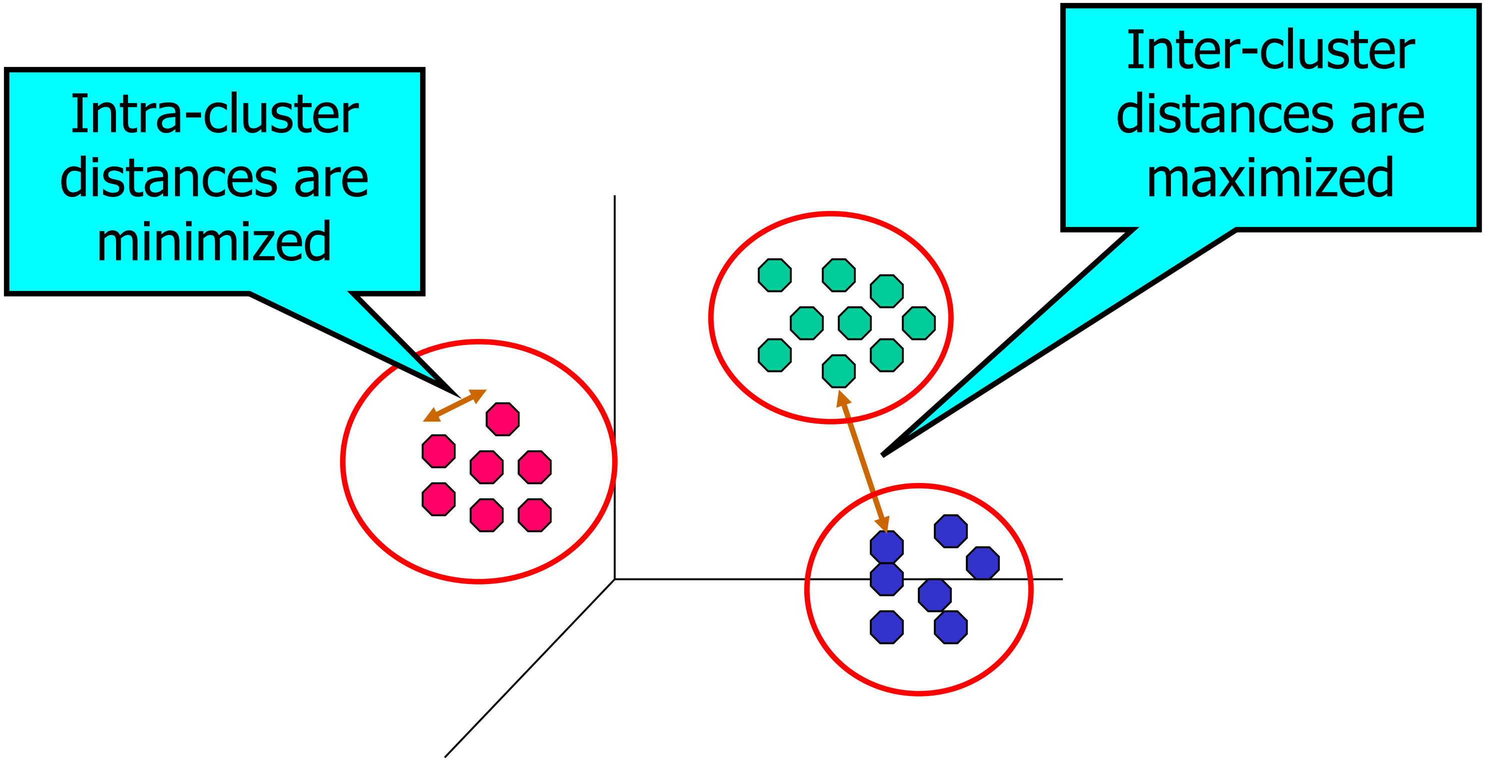
- **Does Mr. A have cancer?**

Introduction to simple clustering methods

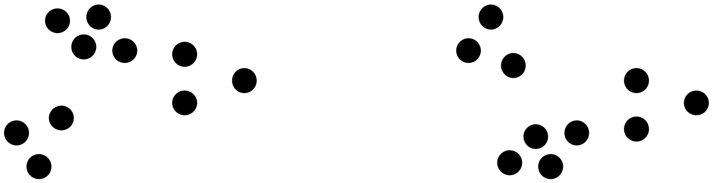
National University of Singapore

What is cluster analysis?

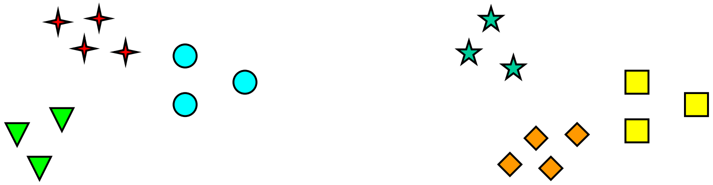
- **Finding groups of objects such that objects in a group are similar to one another and different from objects in other groups**


Intra-cluster distances are minimized
Inter-cluster distances are maximized

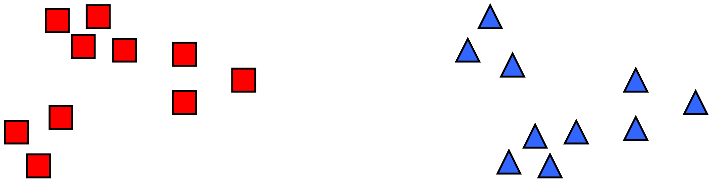
Notion of a cluster can be ambiguous



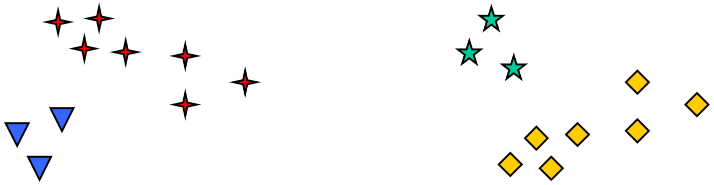
How many clusters?



Six Clusters

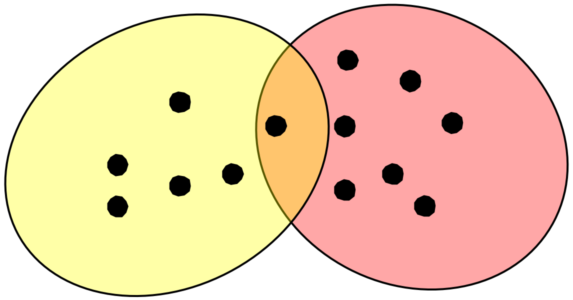


Two Clusters



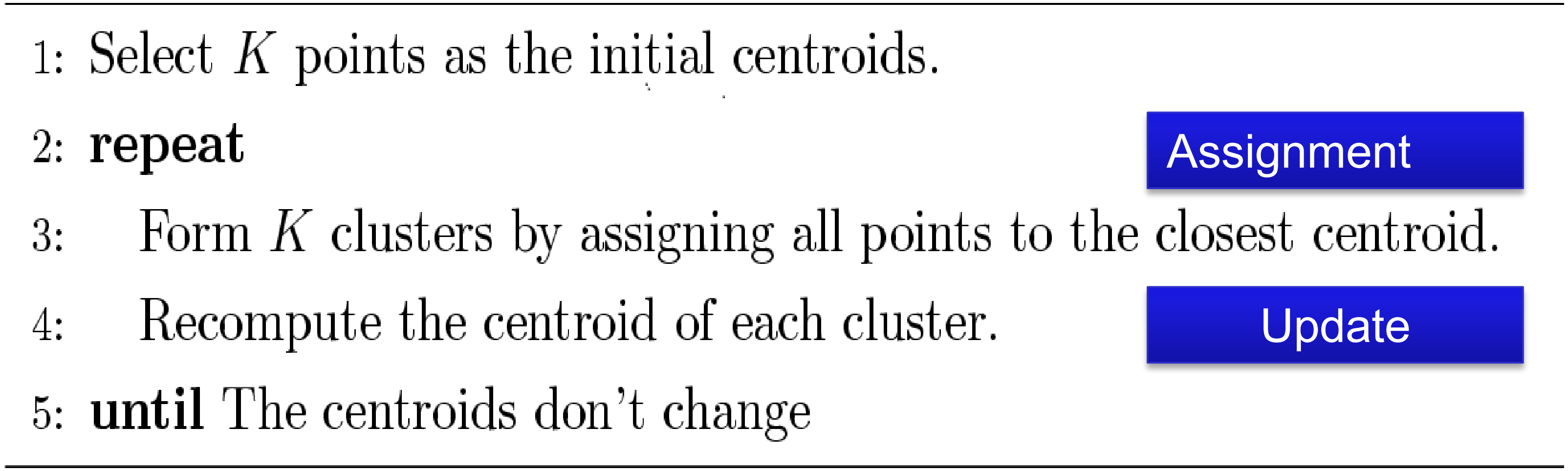
Four Clusters

We can also have



K-means clustering

- **Partitional clustering approach**
- **Each cluster is associated with a centroid**
- **Each point is assigned to the cluster with the closest centroid**
- **# of clusters, K, must be specified**

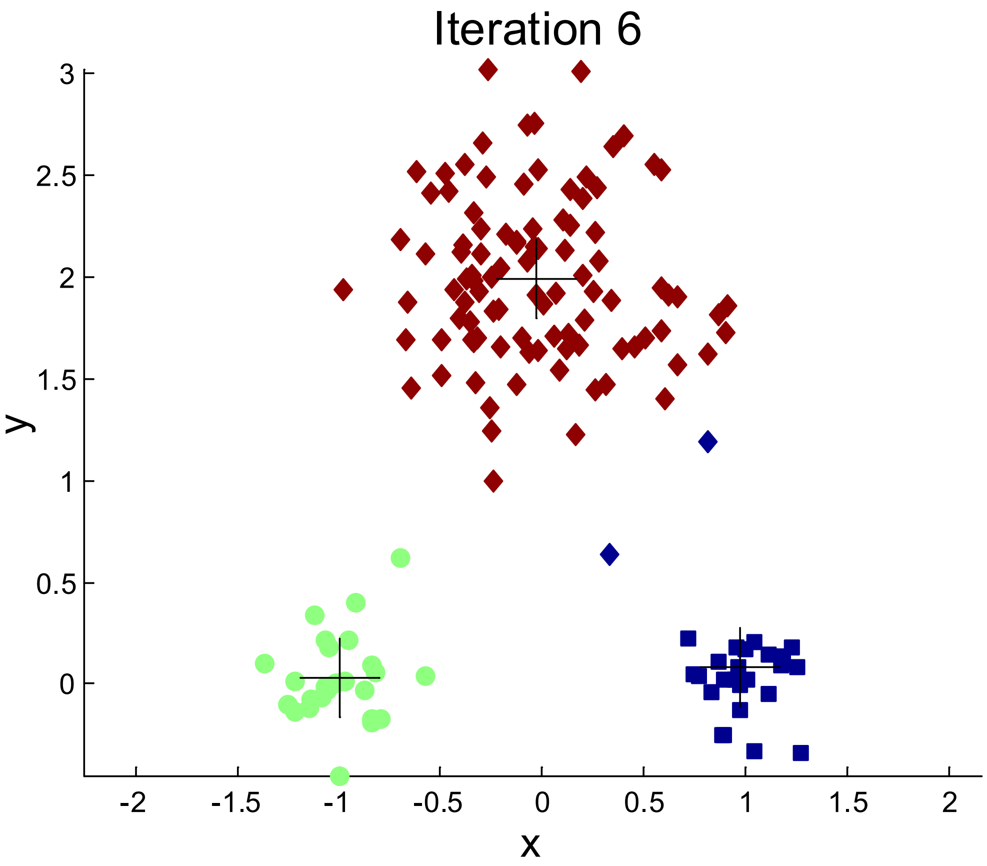


```
Select $K$ points as the initial centroids.
repeat
    Form $K$ clusters by assigning all points to the closest centroid.
    Recompute the centroid of each cluster.
until The centroids don't change
```

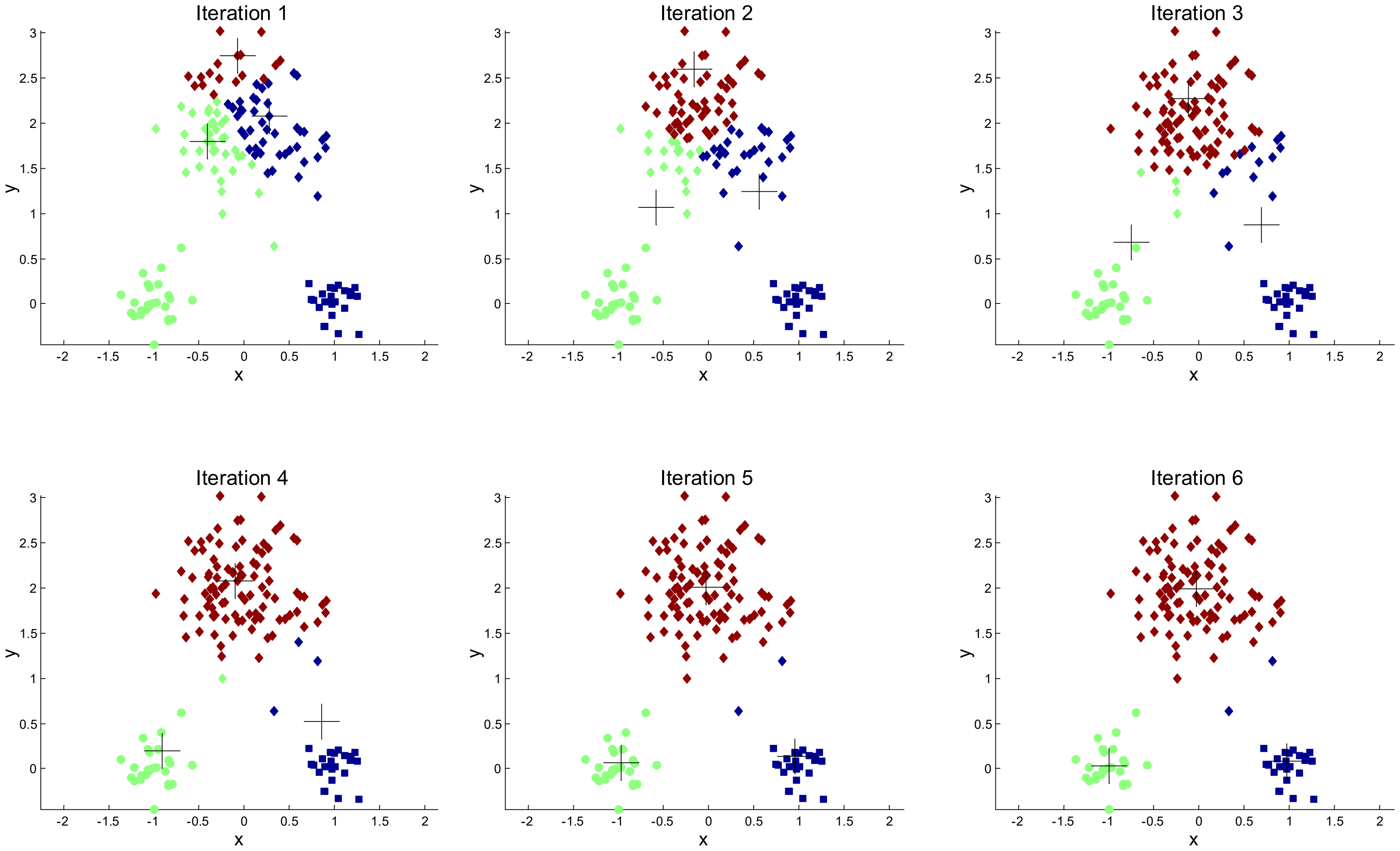
Assignment

Update

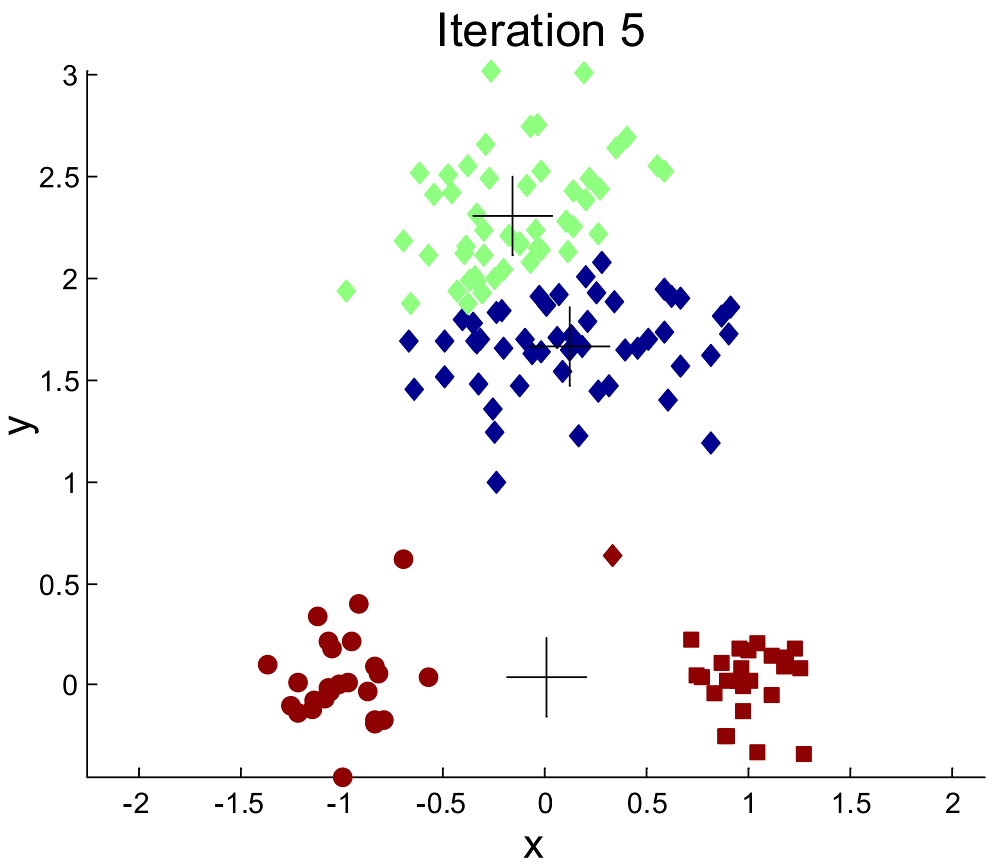
K-means clustering illustration



K-means clustering illustration



Importance of choosing initial centroids



Hierarchical clustering

- **Two main types of hierarchical clustering**
  - Agglomerative:
    - **Start with the points as individual clusters**
    - **At each step, merge the closest pair of clusters until only one cluster (or k clusters) left**
  - Divisive:
    - **Start with one, all-inclusive cluster**
    - **At each step, split a cluster until each cluster contains a point (or there are k clusters)**
- **Traditional hierarchical algorithms use a similarity or distance matrix**
  - Merge or split one cluster at a time

Agglomerative hierarchical clustering

- **More popular hierarchical clustering technique**

- **Basic algorithm**

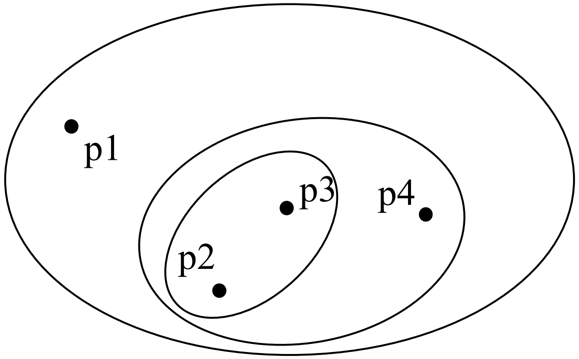
```
Compute the proximity matrix
Let each data point be a cluster
Repeat
    Merge the two closest clusters
    Update the proximity matrix
Until only a single cluster remains
```

Merge

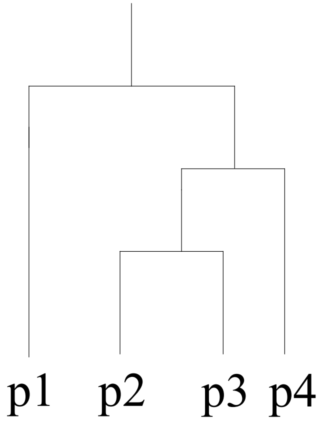
Update

- **Key is computation of proximity of two clusters**
  - Different approaches to defining the distance / similarity between clusters

Visualization of agglomerative hierarchical clustering

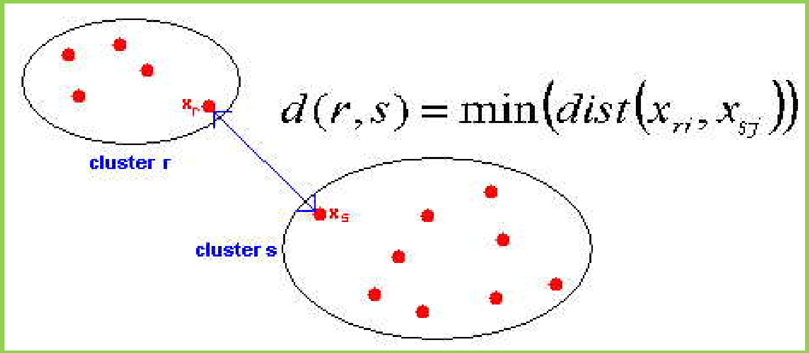


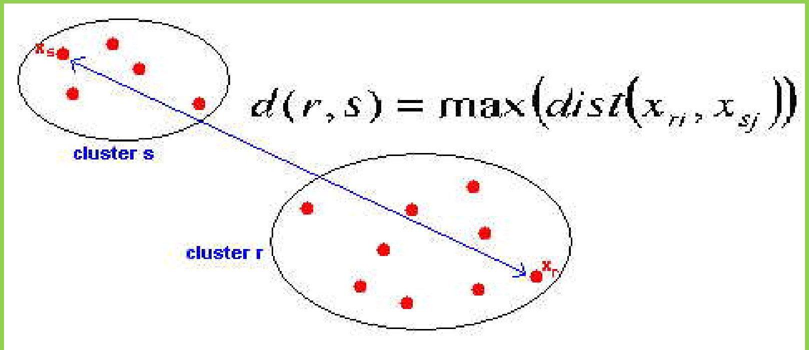
Traditional Hierarchical Clustering



Traditional Dendrogram

Single, complete, & average Linkage





Single linkage defines distance betw two clusters as min distance betw them

Complete linkage defines distance betw two clusters as max distance betw them

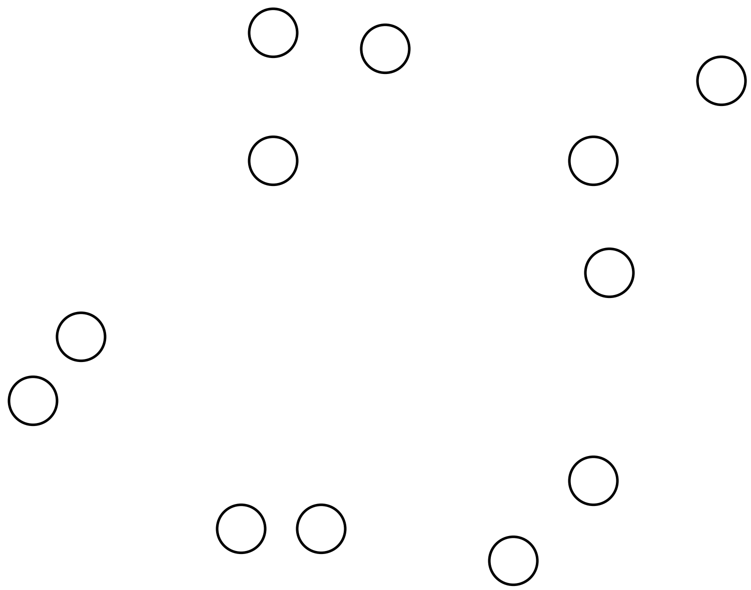
Exercise: Give definition of "average linkage"

Exercise #2

Image source: UCL Microcore Website

Simulation: Starting situation

- **Start with clusters of individual points and a proximity matrix**

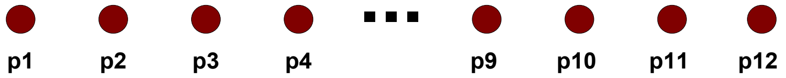


	p1	p2	p3	p4	p5	. . .
p1						
p2						
p3						
p4						
p5						
.						

.

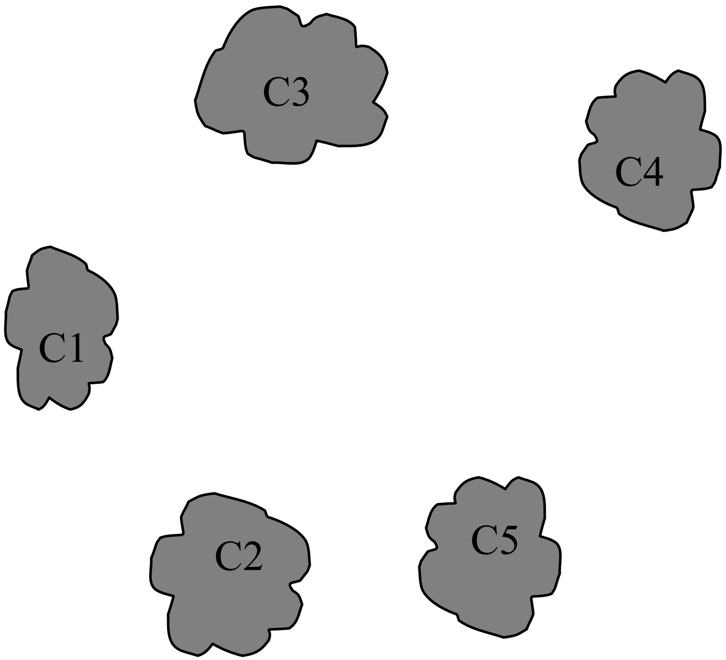
.

Proximity Matrix



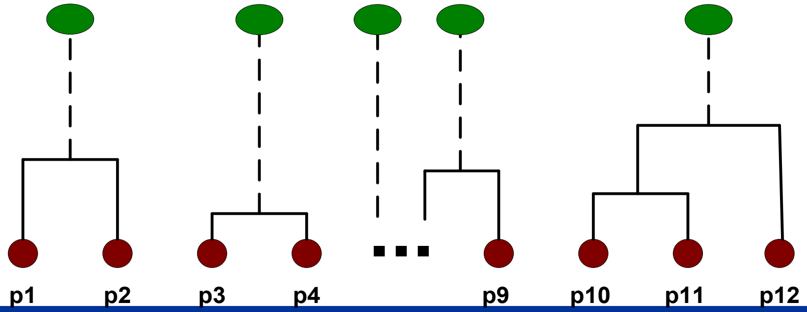
Intermediate situation

- **After some merging steps, we have some clusters**



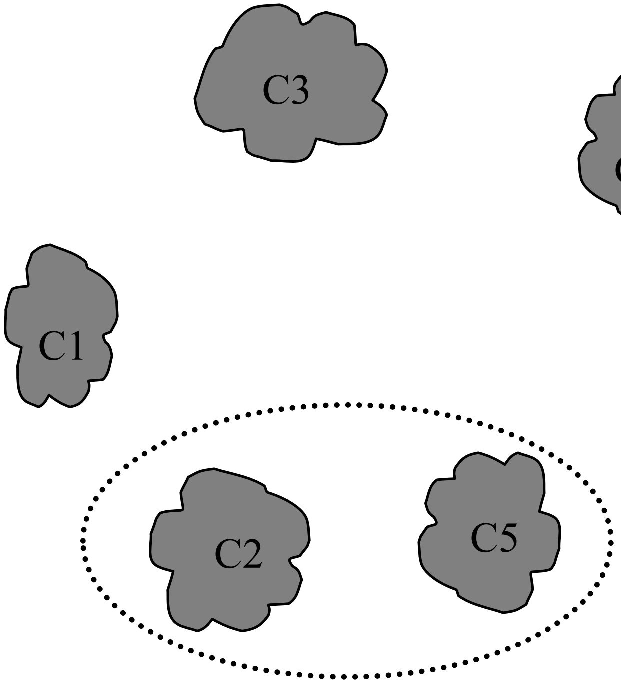
	C1	C2	C3	C4	C5
C1					
C2					
C3					
C4					
C5					

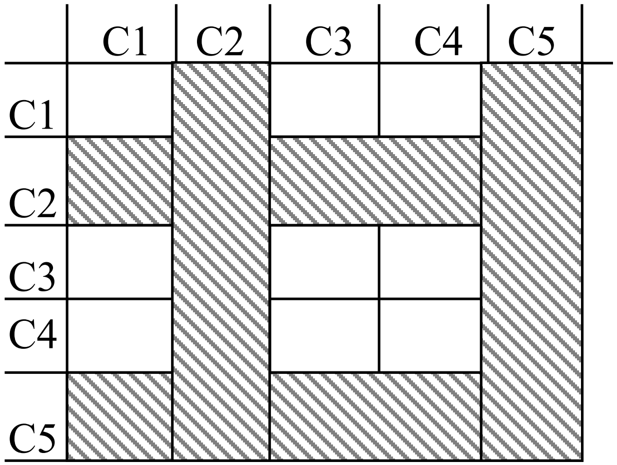
Proximity Matrix



Intermediate situation

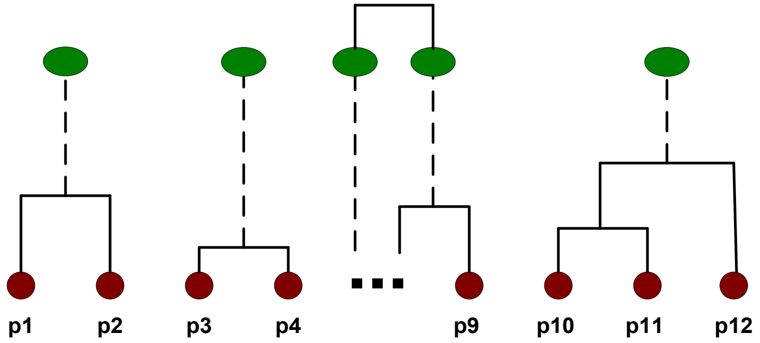
- We want to **merge** the two **closest** clusters (C2 and C5) and update the proximity matrix.





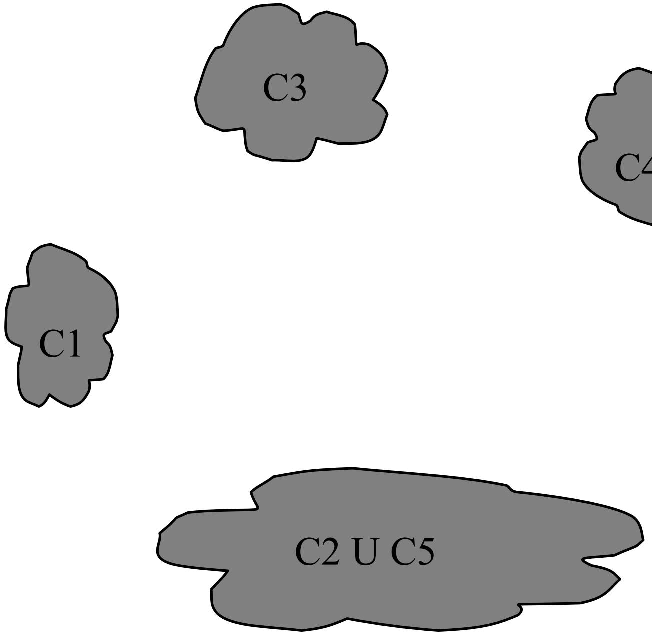
	C1	C2	C3	C4	C5
C1		▨			▨
C2	▨	▨	▨	▨	▨
C3		▨			▨
C4		▨			▨
C5	▨	▨	▨	▨	▨

Proximity Matrix



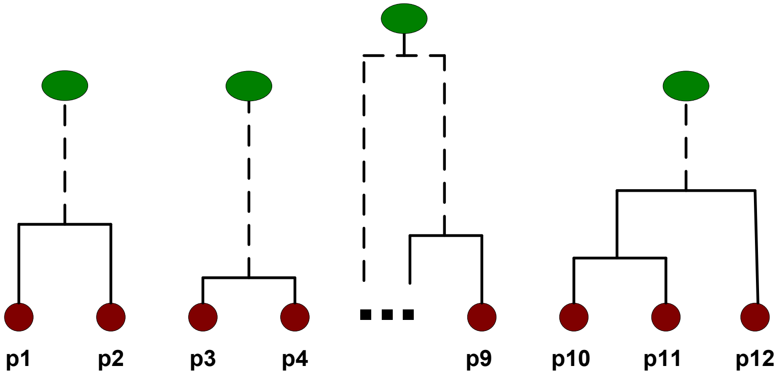
After merging

- The question is “How do we **update** the proximity matrix?”

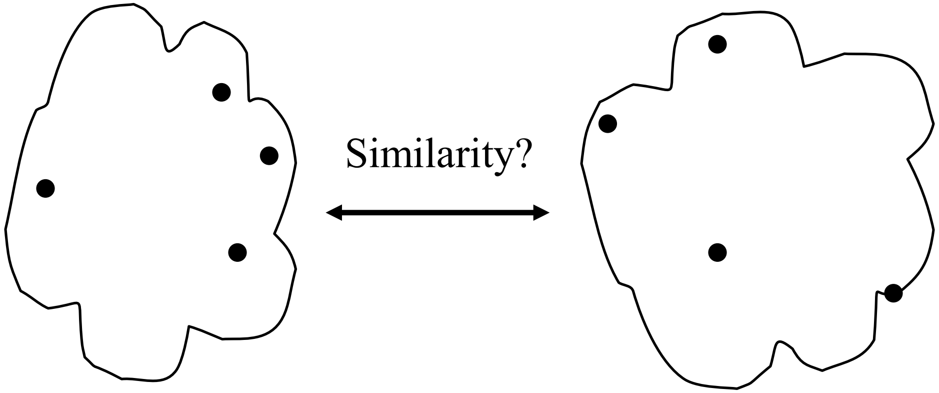


	C1	C2 U C5	C3	C4
C1		?		
C2 U C5	?	?	?	?
C3		?		
C4		?		

Proximity Matrix



How to define inter-cluster similarity

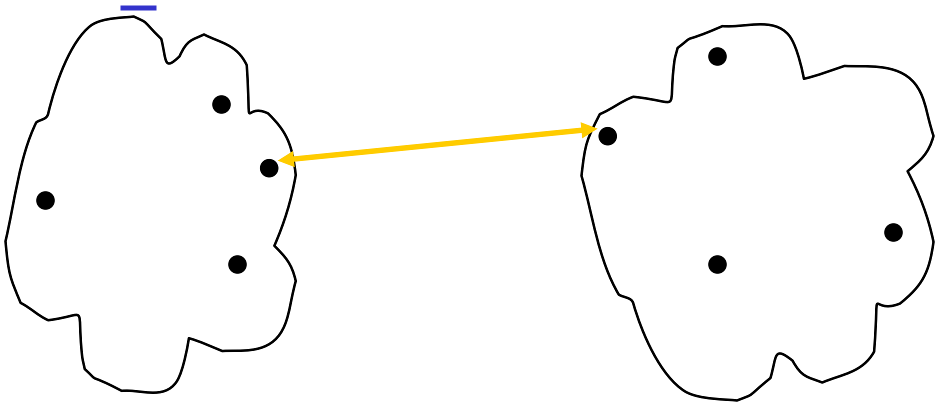


	p1	p2	p3	p4	p5	. . .
p1						
p2						
p3						
p4						
p5						
.						
.						
.						

Proximity Matrix

- Min
- Max
- Group average
- Distance between centroids

How to define inter-cluster similarity



	p1	p2	p3	p4	p5	. . .
p1						
p2						
p3						
p4						
p5						

.

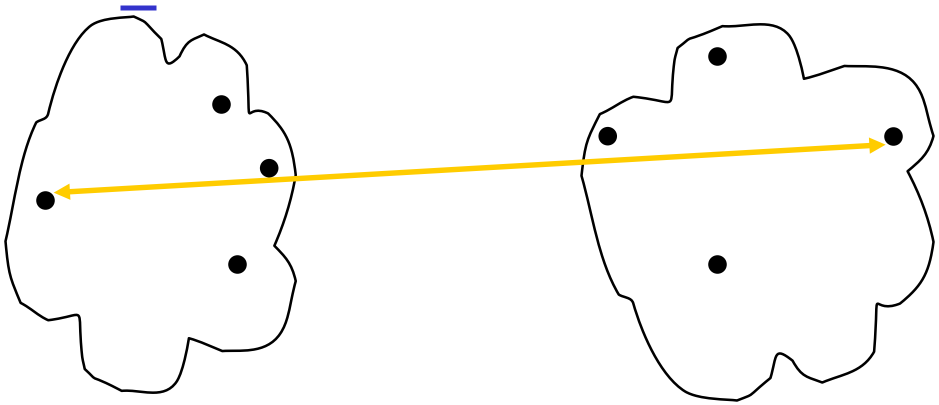
.

.

Proximity Matrix

- Min
- Max
- Group average
- Distance between centroids

How to define inter-cluster similarity

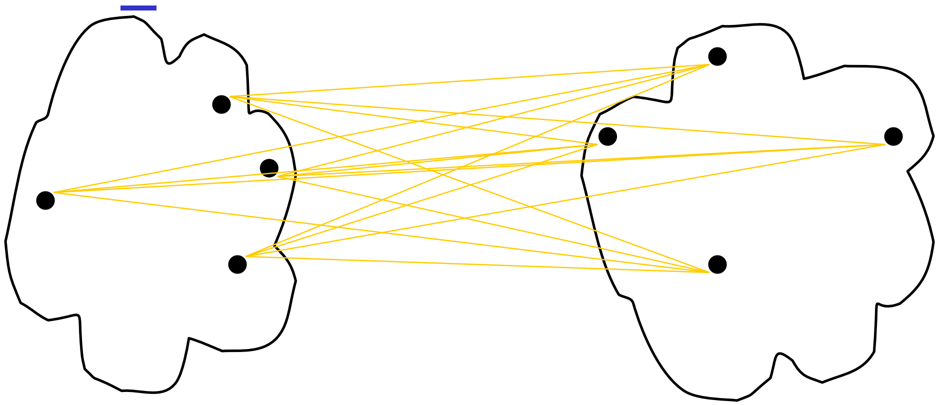


	p1	p2	p3	p4	p5	. . .
p1						
p2						
p3						
p4						
p5						
.						
.						
.						

Proximity Matrix

- Min
- Max
- Group average
- Distance between centroids

How to define inter-cluster similarity

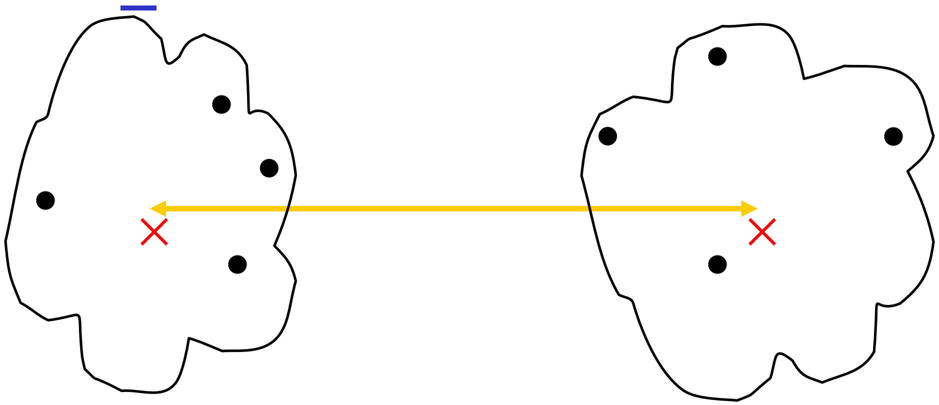


	p1	p2	p3	p4	p5	. . .
p1						
p2						
p3						
p4						
p5						
. . .						

Proximity Matrix

- Min
- Max
- Group average
- Distance between centroids

How to define inter-cluster similarity



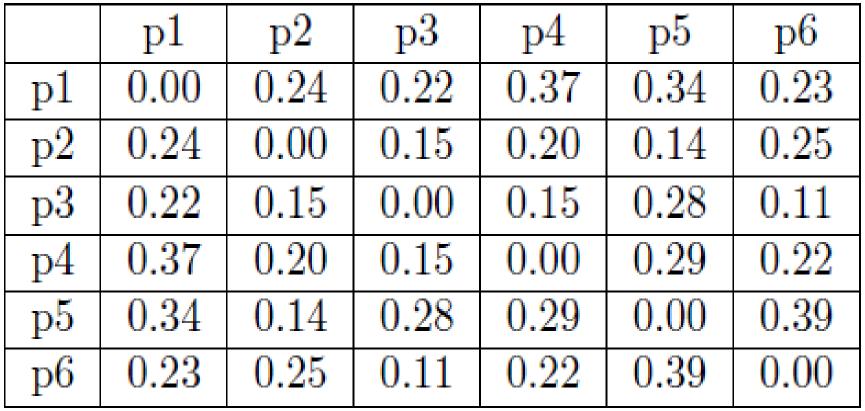
	p1	p2	p3	p4	p5	. . .
p1						
p2						
p3						
p4						
p5						
. . .						

Proximity Matrix

- Min
- Max
- Group average
- Distance between centroids

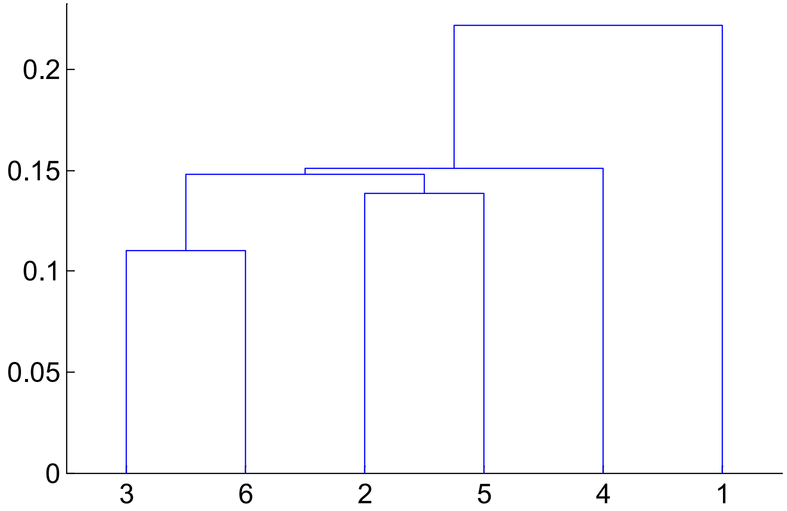
Cluster similarity: Min / single linkage

- **Similarity of two clusters is based on the two most similar (closest) points in the different clusters**
  - Determined by one pair of points, i.e., by one link in the proximity graph

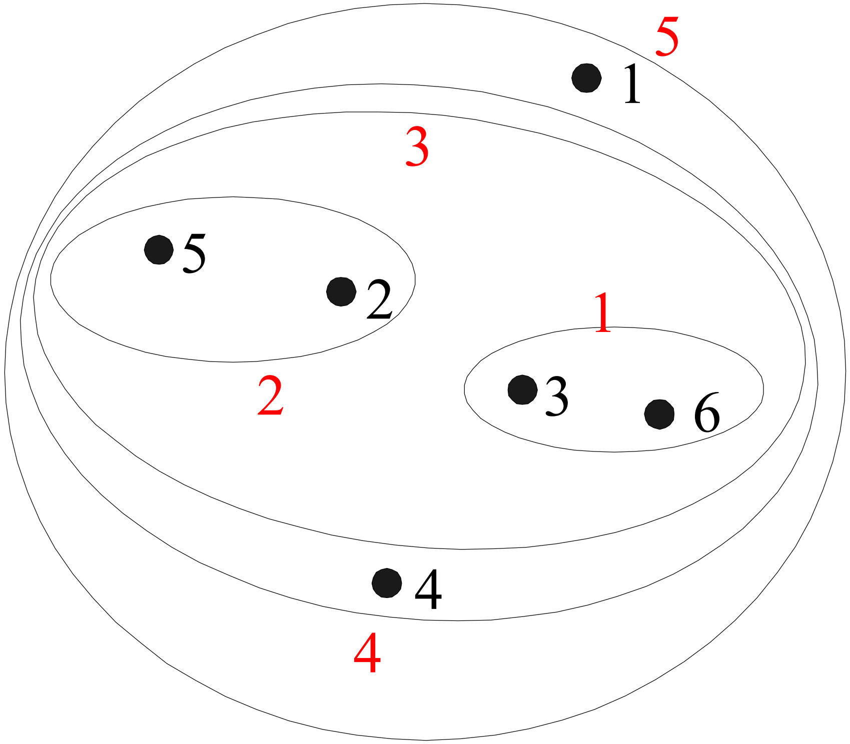


	p1	p2	p3	p4	p5	p6
p1	0.00	0.24	0.22	0.37	0.34	0.23
p2	0.24	0.00	0.15	0.20	0.14	0.25
p3	0.22	0.15	0.00	0.15	0.28	0.11
p4	0.37	0.20	0.15	0.00	0.29	0.22
p5	0.34	0.14	0.28	0.29	0.00	0.39
p6	0.23	0.25	0.11	0.22	0.39	0.00

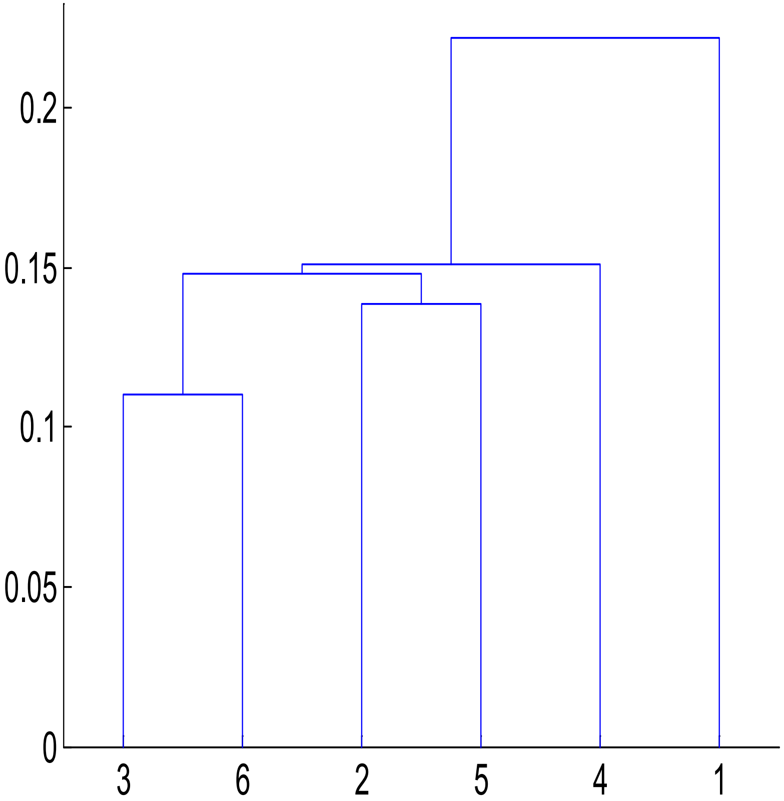
Table 8.4. Euclidean distance matrix for 6 points.



Hierarchical clustering: Min



Single-linkage clustering



Single-linkage dendrogram

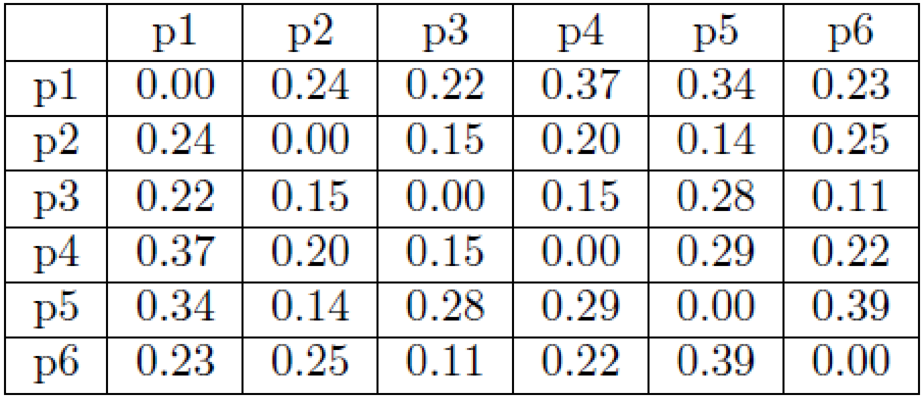
Food for thought

- What are the key strengths of single-linkage clustering?
- What are the key weaknesses of single-linkage clustering?

Exercise #3

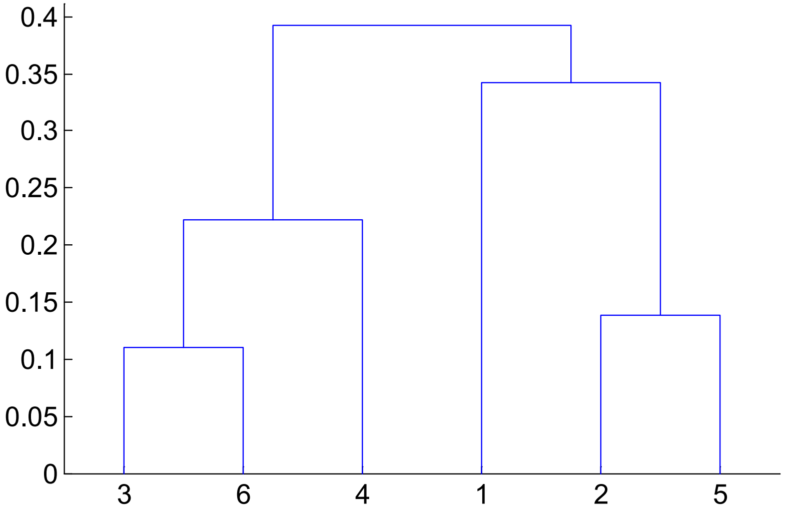
Cluster similarity: Max / complete linkage

- **Similarity of two clusters is based on the two least similar (most distant) points in the different clusters**
  - Determined by all pairs of points in the two clusters

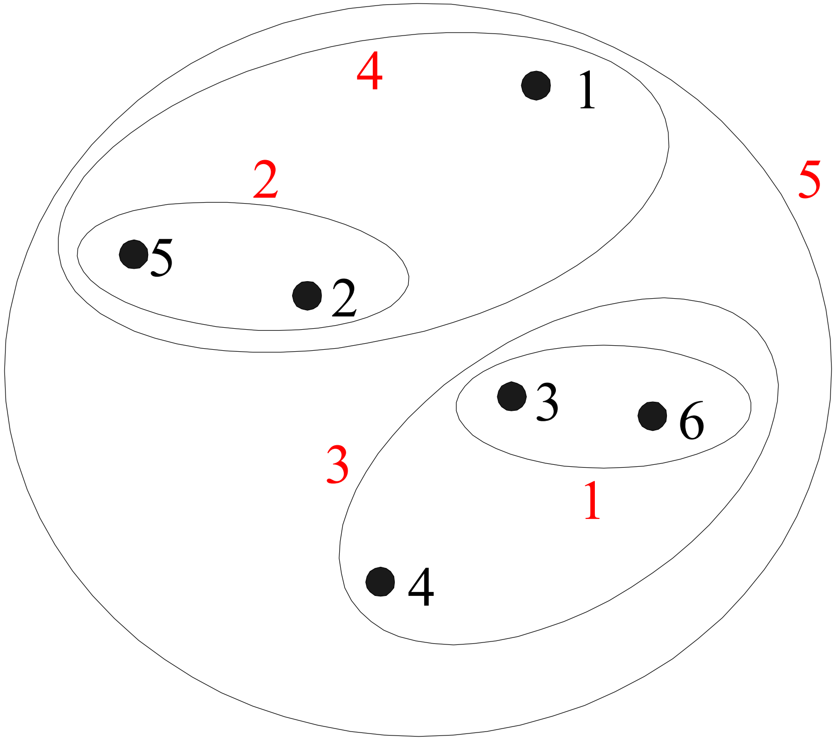


	p1	p2	p3	p4	p5	p6
p1	0.00	0.24	0.22	0.37	0.34	0.23
p2	0.24	0.00	0.15	0.20	0.14	0.25
p3	0.22	0.15	0.00	0.15	0.28	0.11
p4	0.37	0.20	0.15	0.00	0.29	0.22
p5	0.34	0.14	0.28	0.29	0.00	0.39
p6	0.23	0.25	0.11	0.22	0.39	0.00

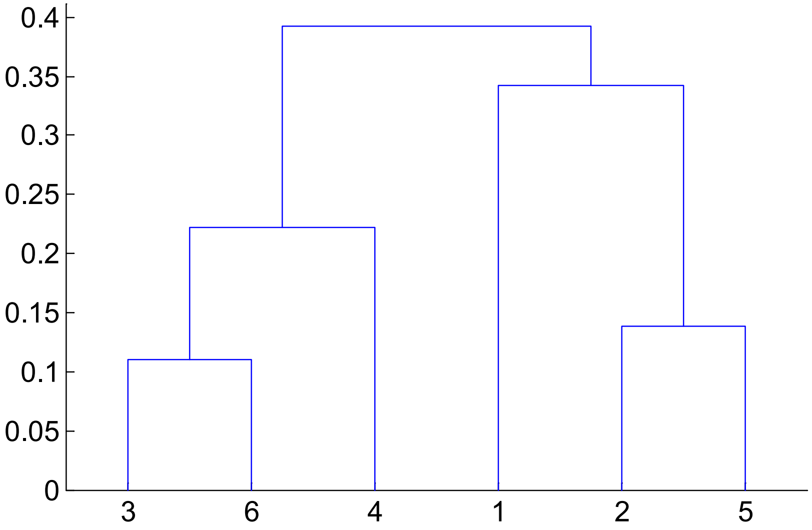
Table 8.4. Euclidean distance matrix for 6 points.



Hierarchical clustering: Max



Nested Clusters



Dendrogram

We still want to merge two most similar clusters each time.
But we define the distance between clusters based on MAX

Food for thought

- **What are the key strengths of complete-linkage clustering?**

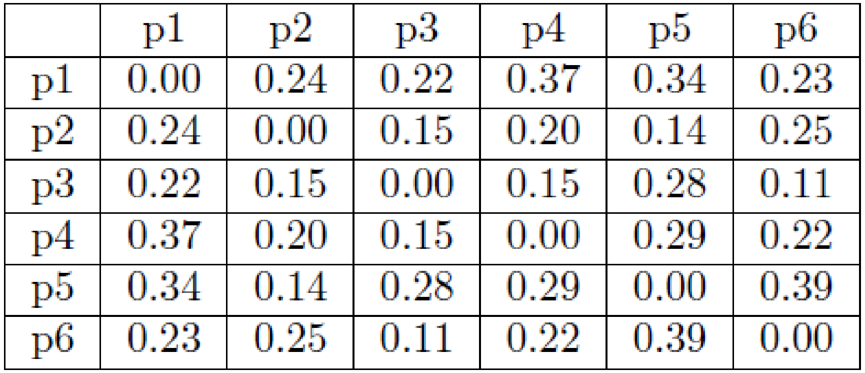
- **What are the key weaknesses of complete-linkage clustering?**

Exercise #4

Cluster similarity: Group average

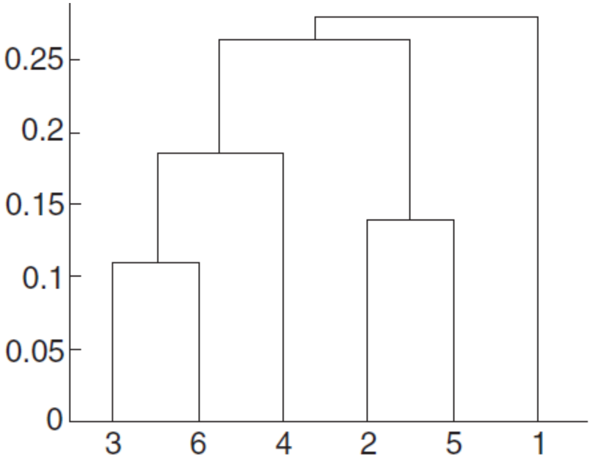
- **Proximity of two clusters is the average of pairwise proximity between points in the two clusters**

$$\textbf{proximity}(\textbf{Cluster}_i, \textbf{Cluster}_j) = \frac{\sum\limits_{\substack{p_i \in \textbf{Cluster}_i \\ p_j \in \textbf{Cluster}_j}} \textbf{proximity}(p_i, p_j)}{|\textbf{Cluster}_i| * |\textbf{Cluster}_j|}$$

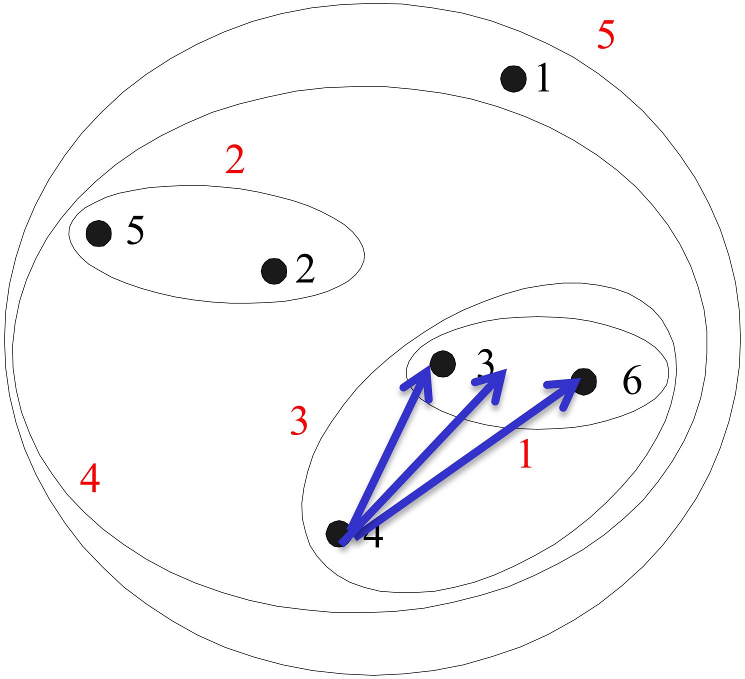


	p1	p2	p3	p4	p5	p6
p1	0.00	0.24	0.22	0.37	0.34	0.23
p2	0.24	0.00	0.15	0.20	0.14	0.25
p3	0.22	0.15	0.00	0.15	0.28	0.11
p4	0.37	0.20	0.15	0.00	0.29	0.22
p5	0.34	0.14	0.28	0.29	0.00	0.39
p6	0.23	0.25	0.11	0.22	0.39	0.00

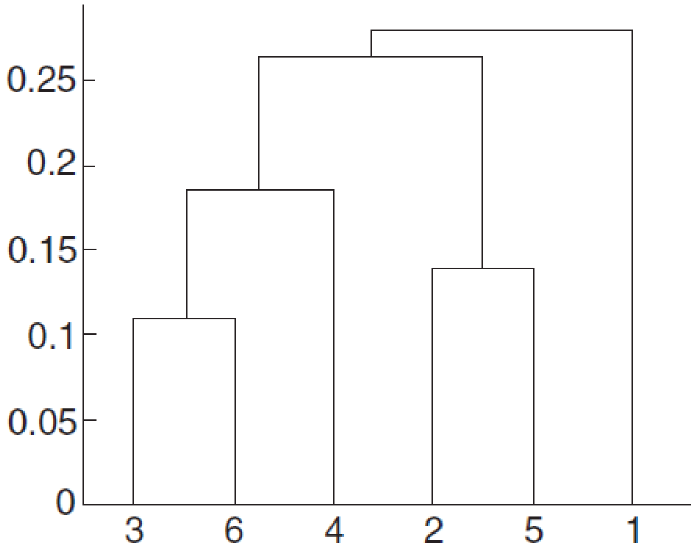
Table 8.4. Euclidean distance matrix for 6 points.



Hierarchical clustering: Group average



Group Average Clustering

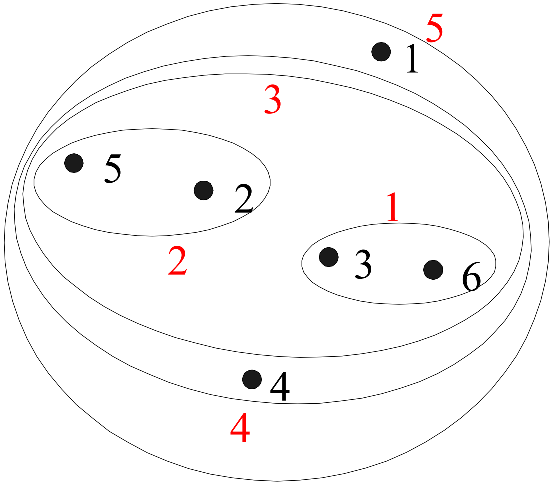


Group Average Dendrogram

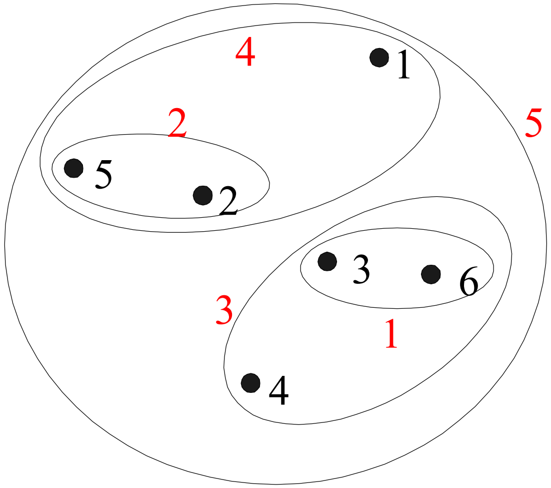
Hierarchical clustering: Group average

- **Compromise between single and complete linkage**
- **Strengths**
  - Less susceptible to noise and outliers
- **Limitations**
  - Biased towards globular clusters

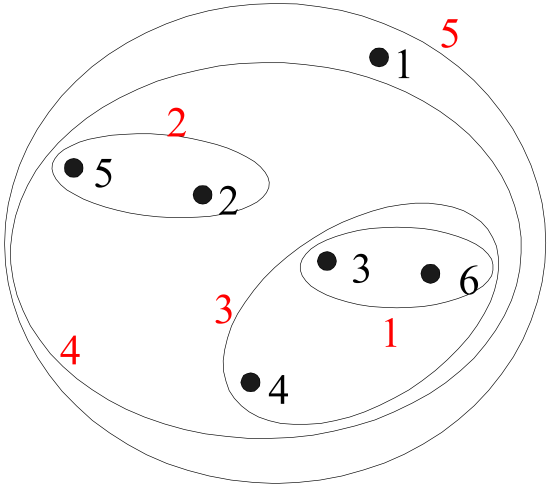
Hierarchical clustering: Comparison



Min



Max



Group average

Food for thought

- **What are the space and time complexity of hierarchical clustering?**

Exercise #5

Normalization

Sometimes, a gene expression study may involve batches of data collected over a long period of time…

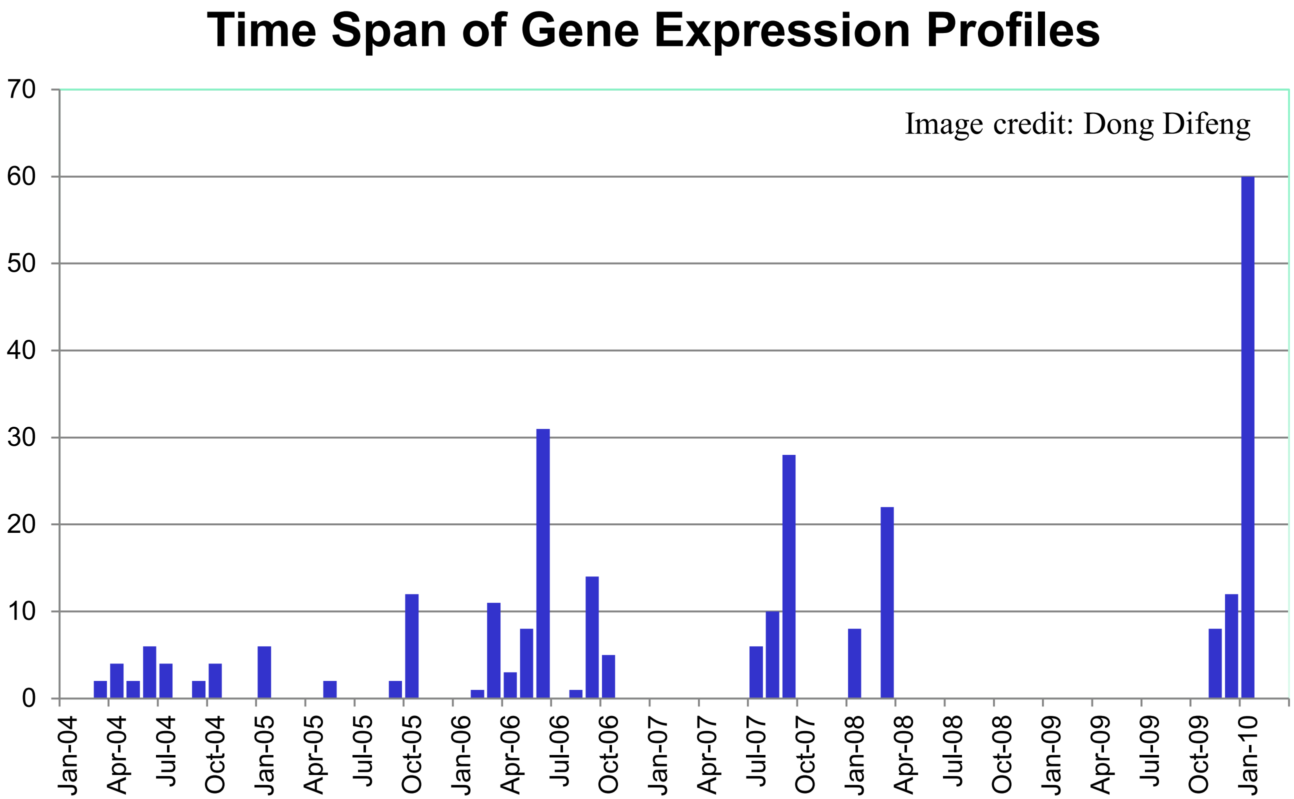


Image credit: Dong Difeng

In such a case, batch effect may be severe… to the extent that you can predict the batch that each sample comes!

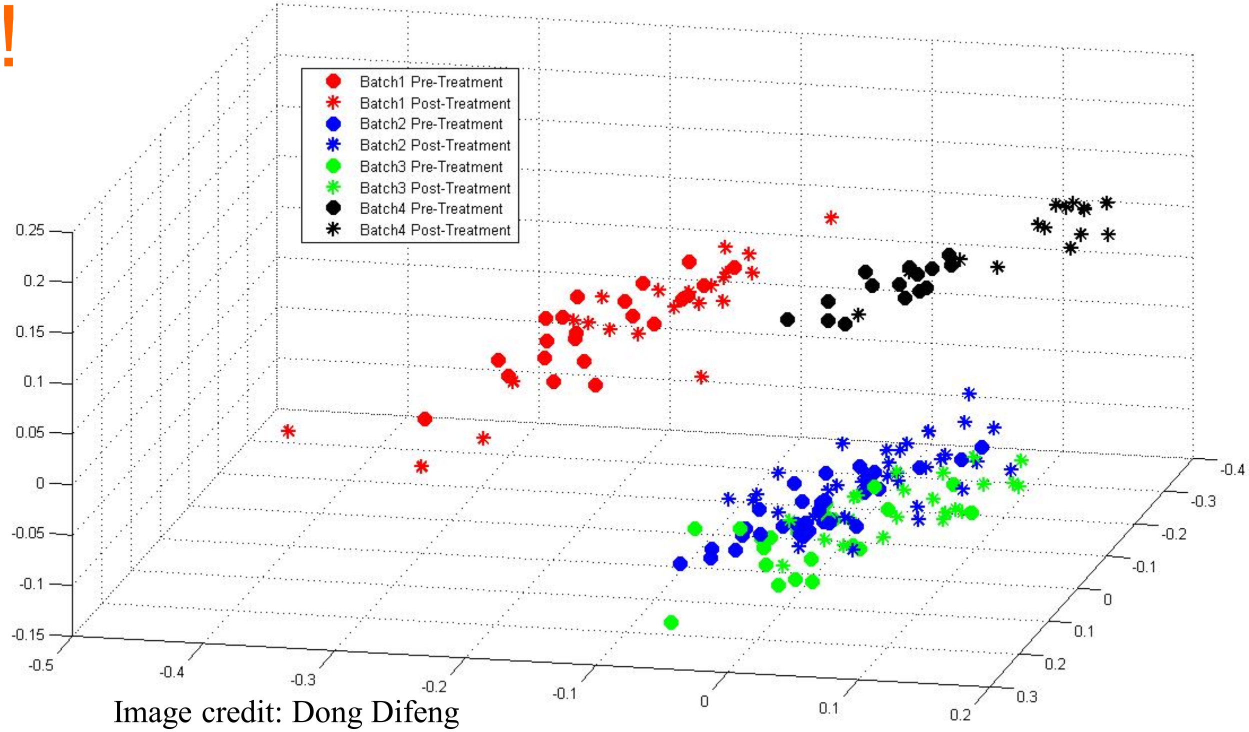


Image credit: Dong Difeng

⇒ **Need normalization to correct for batch effect**

Normalization approaches

- **Aim of normalization:**
  Reduce variance w/o increasing bias

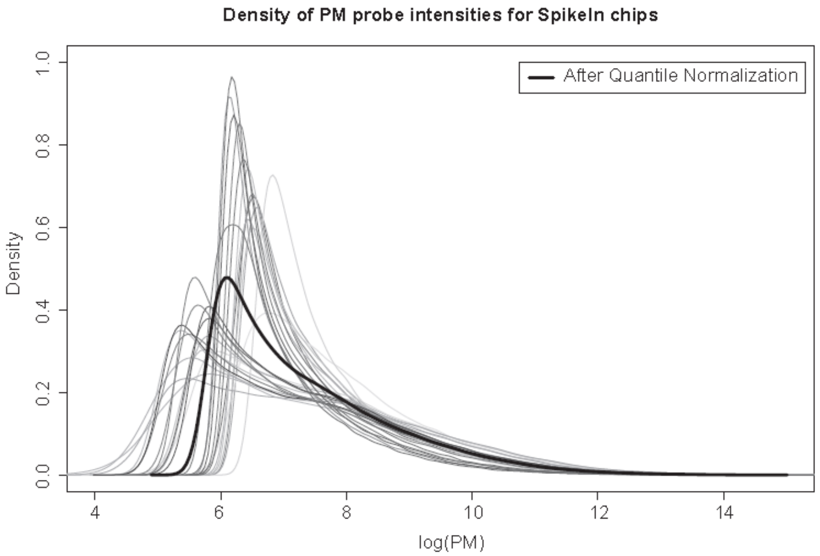
- **Scaling method**
  - Intensities are scaled so that each array has same ave value
  - E.g., Affymetrix's

- **Xform data so that distribution of probe intensities is same on all arrays**
  - E.g., $Z = (x - \mu) / \sigma$

- **Quantile normalization**

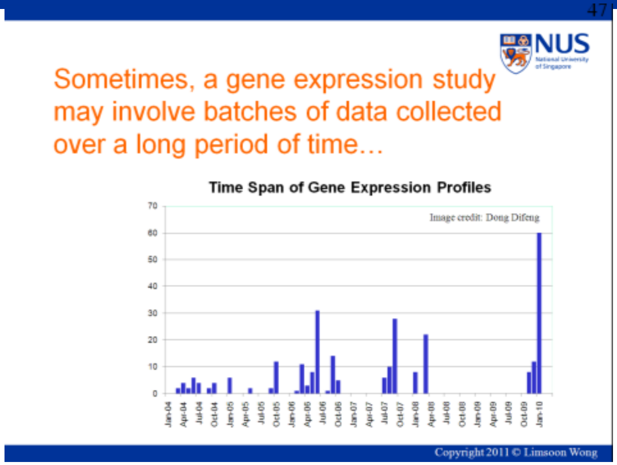
Quantile normalization

- Given *n arrays of length p, form X of size p × n where each array is a column*
- Sort each column of *X to give* X_{sort}
- Take means across rows of X_{sort} *and assign this* mean to each elem in the row to get X'_{sort}
- Get $X_{normalized}$ *by arranging each column of* X'_{sort} to have same ordering as *X*



- Implemented in some microarray s/w, e.g., EXPANDER

After quantile normalization



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Sometimes, a gene expression study may involve batches of data collected over a long period of time…

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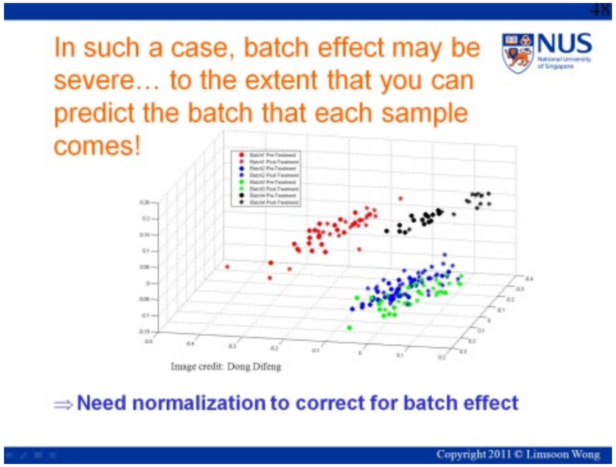

48

In such a case, batch effect may be severe… to the extent that you can predict the batch that each sample comes!

⇒ Need normalization to correct for batch effect

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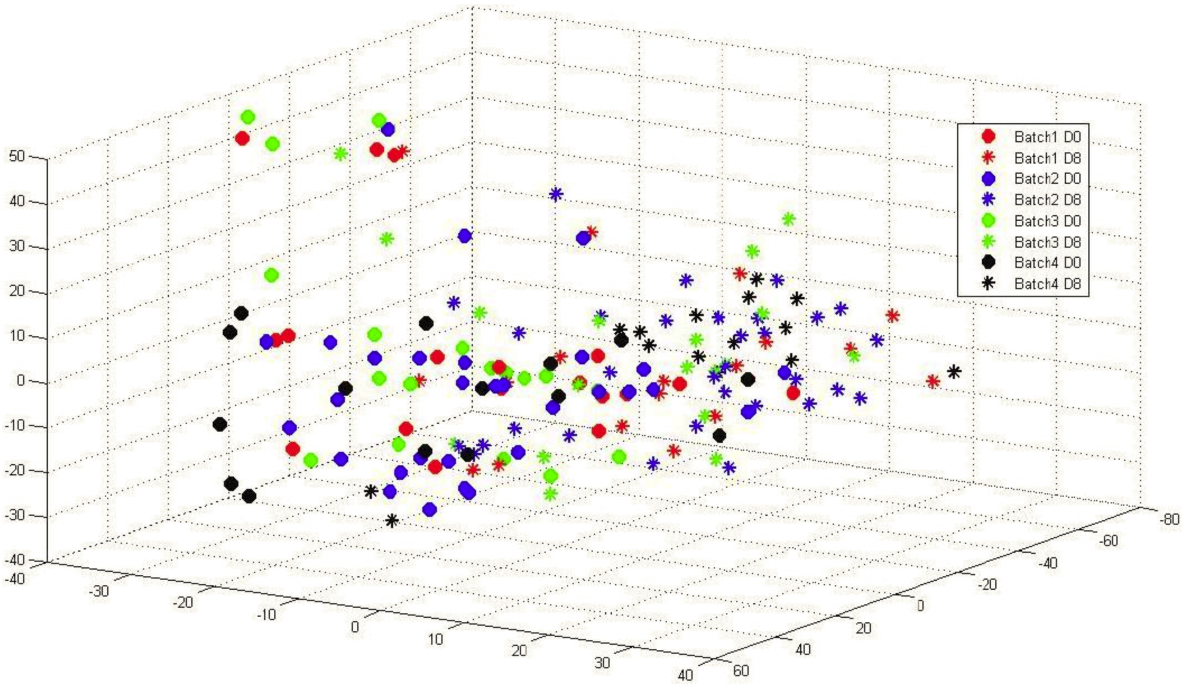
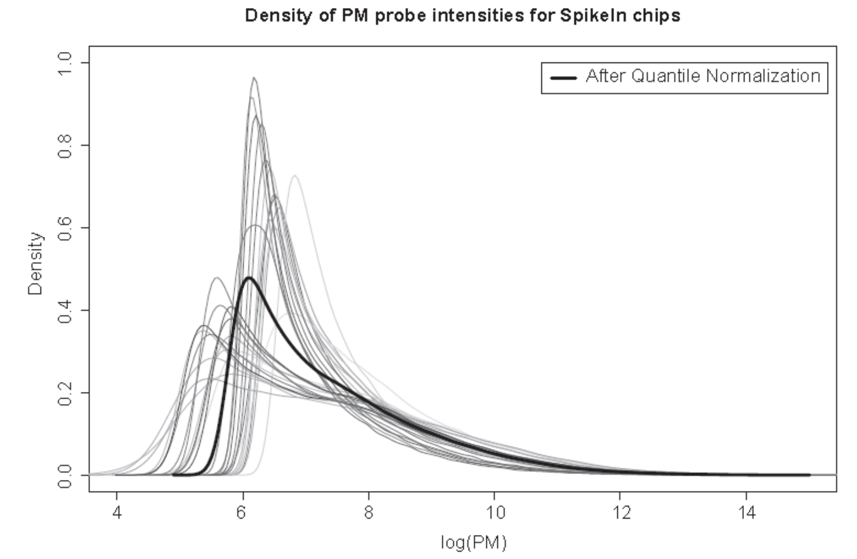


Figure 3.6: GEPs after the batch effects removing.

Food for thought

- Given a cancer vs normal dataset
- Should you apply quantile normalization to the dataset as a whole or should you apply quantile normalization to the cancer and the normal part separately? Why?



Exercise #6

Food for thought

- Given a cancer vs normal dataset
- Should you apply Z-normalization to each phenotype separately or to the whole dataset in one go?
- Should you apply Z-normalization in a patient-wise or gene-wise manner? Why?

Exercise #7

Selection of patient samples and genes for disease prognosis

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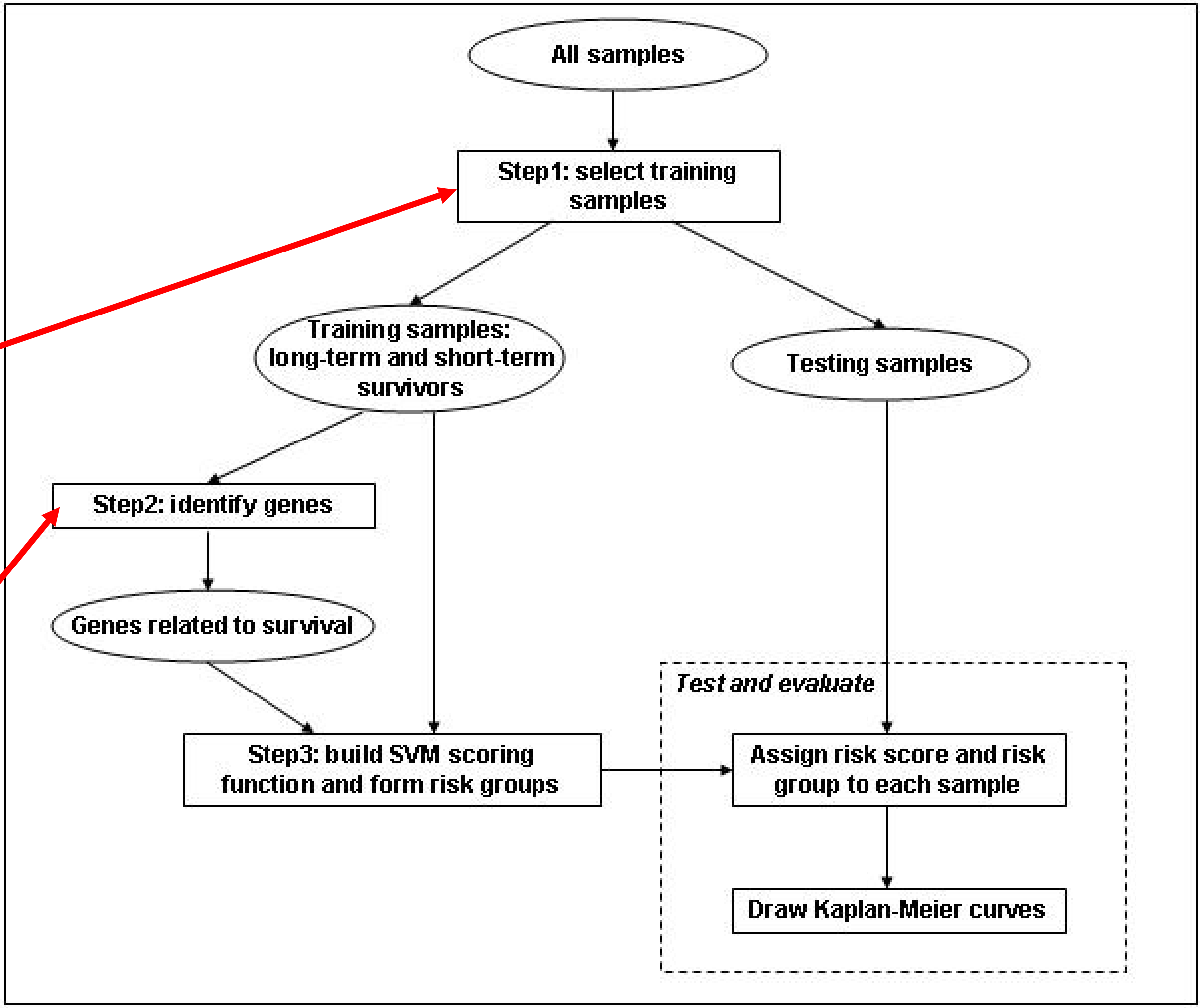
Gene expression profile + clinical data ⇒ outcome prediction

- **Univariate & multivariate Cox survival analysis** (Beer et al 2002, Rosenwald et al 2002)
- **Fuzzy neural network** (Ando et al 2002)
- **Partial least squares regression** (Park et al 2002)
- **Weighted voting algorithm** (Shipp et al 2002)
- **Gene index and "reference gene"** (LeBlanc et al 2003)
- ……

Liu et al. "Use of extreme patient samples for outcome prediction from gene expression data. *Bioinformatics*, 21(16):3377--3384, 2005

Our approach


All samples
Step1: select training samples
Training samples: long-term and short-term survivors
Testing samples
Step2: identify genes
Genes related to survival
Test and evaluate
Step3: build SVM scoring function and form risk groups
Assign risk score and risk group to each sample
Draw Kaplan-Meier curves

"extreme" sample selection

ERCOF

Extreme sample selection

Short-term Survivors *v.s.* Long-term Survivors

Short-term survivors who died within a *short* period

⇓

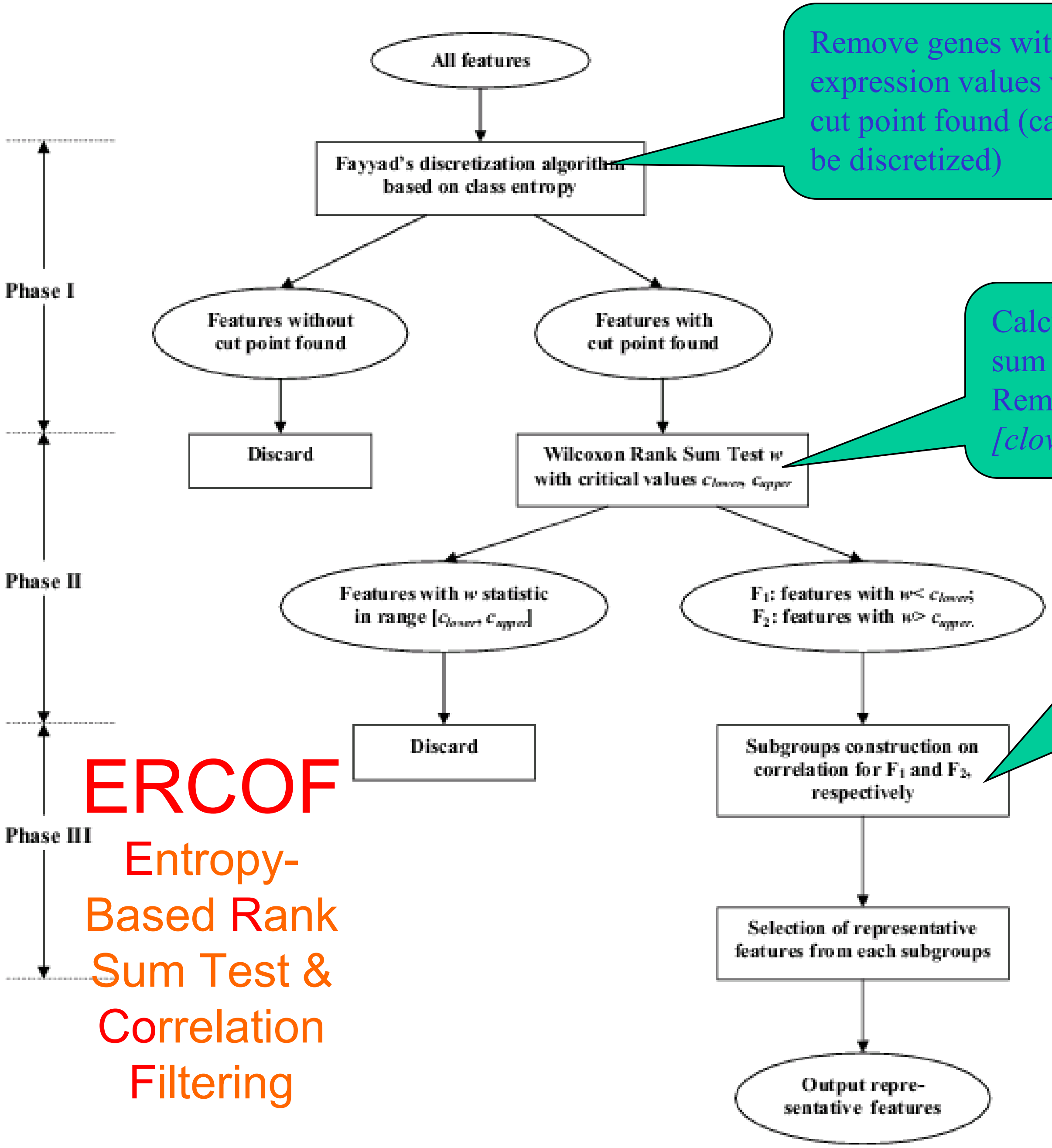
$F(T) < c_1$ and $E(T) = 1$

Long-term survivors who were alive after a *long* follow-up time

⇓

$F(T) > c_2$

T: sample
$F(T)$: follow-up time
$E(T)$: status (1:unfavorable; 0: favorable)
c_1 and c_2: thresholds of survival time



All features

Fayyad's discretization algorithm based on class entropy

Features without cut point found

Features with cut point found

Discard

Wilcoxon Rank Sum Test w with critical values c_{lower}, c_{upper}

Features with w statistic in range $[c_{lower}, c_{upper}]$

F_1: features with $w < c_{lower}$;
F_2: features with $w > c_{upper}$.

Discard

Subgroups construction on correlation for F_1 and F_2, respectively

Selection of representative features from each subgroups

Output representative features

Phase I

Phase II

Phase III

Remove genes with expression values w/o cut point found (can't be discretized)

Calculate Wilcoxon rank sum *w(x)* for gene *x*. Remove gene *x* if *w(x)* ∈ *[clower, cupper]*

Group features by Pearson Correlation
For each group, retain the top 50% wrt class entropy

ERCOF

Entropy-Based Rank Sum Test & Correlation Filtering

Risk score construction

Linear Kernel SVM regression function

$$G(T) = \sum_i a_i y_i K(T, x(i)) + b$$

T: test sample, *x*(*i*): support vector,
y_i: class label (1: short-term survivors; -1: long-term survivors)

Transformation function (*posterior probability*)

$$S(T) = \frac{1}{1 + e^{-G(T)}} \qquad (S(T) \in (0,1))$$

S(*T*): ***risk score*** of sample *T*

Diffuse large B-cell lymphoma

- **DLBC lymphoma is the most common type of lymphoma in adults**
- **Can be cured by anthracycline-based chemotherapy in 35 to 40 percent of patients**

⇒ DLBC lymphoma comprises several diseases that differ in responsiveness to chemotherapy

- **Intl Prognostic Index (IPI)**
  - age, “Eastern Cooperative Oncology Group” Performance status, tumor stage, lactate dehydrogenase level, sites of extranodal disease, ...
- **Not very good for stratifying DLBC lymphoma patients for therapeutic trials**

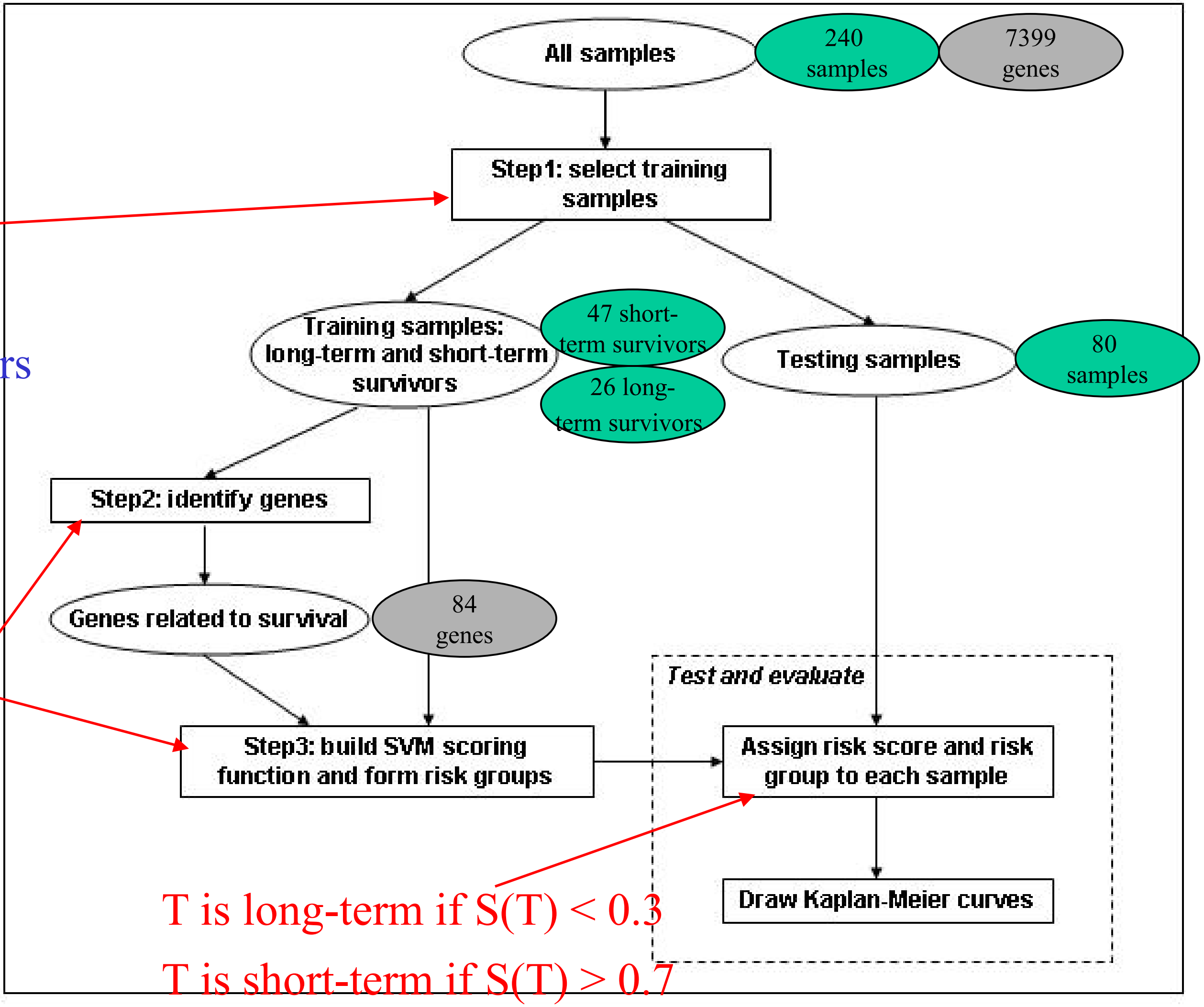
⇒ Use gene-expression profiles to predict outcome of chemotherapy?

Rosenwald et al., *NEJM* 2002

- **240 data samples**
  - – 160 in preliminary group
  - – 80 in validation group
  - – each sample described by 7399 microarray features
- **Rosenwald et al.'s approach**
  - – identify gene: Cox proportional-hazards model
  - – cluster identified genes into four gene signatures
  - – calculate for each sample an outcome-predictor score
  - – divide patients into quartiles according to score

Knowledge discovery from gene expression of "extreme" samples

"extreme" sample selection:
< 1 yr vs > 8 yrs


All samples
240 samples
7399 genes
Step1: select training samples
Training samples: long-term and short-term survivors
47 short-term survivors
26 long-term survivors
Testing samples
80 samples
Step2: identify genes
Genes related to survival
84 genes
Step3: build SVM scoring function and form risk groups
Test and evaluate
Assign risk score and risk group to each sample
Draw Kaplan-Meier curves

knowledge discovery from gene expression

T is long-term if S(T) < 0.3

T is short-term if S(T) > 0.7

Discussions: Sample selection

Application	Data set	Status		Total
		Dead	Alive	
DLBCL	Original	88	72	160
	Informative	47+1(*)	25	73

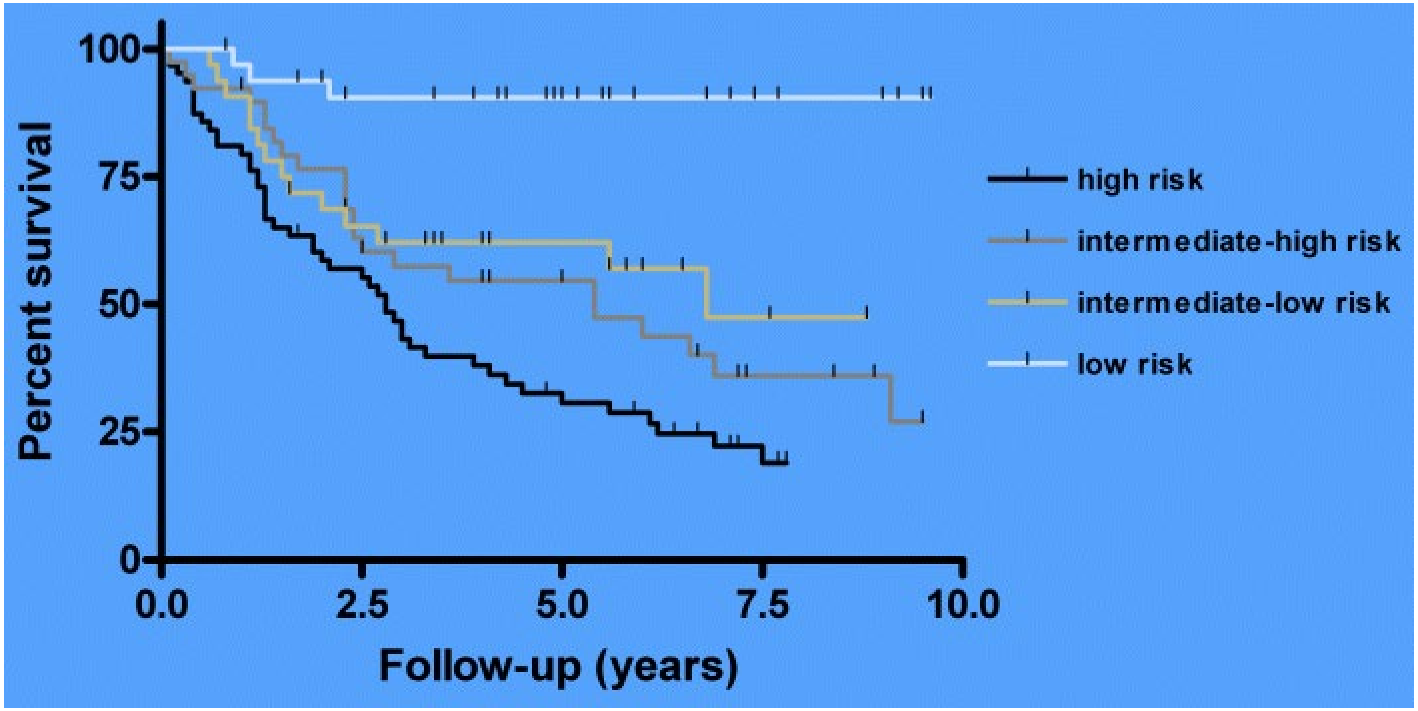
Number of samples in original data and selected informative training set. (*): Number of samples whose corresponding patient was dead at the end of follow-up time, but selected as a long-term survivor.

Discussions: Gene identification

Gene selection	DLBCL
Original	4937(*)
Phase I	132(2.7%)
Phase II	84(1.7%)

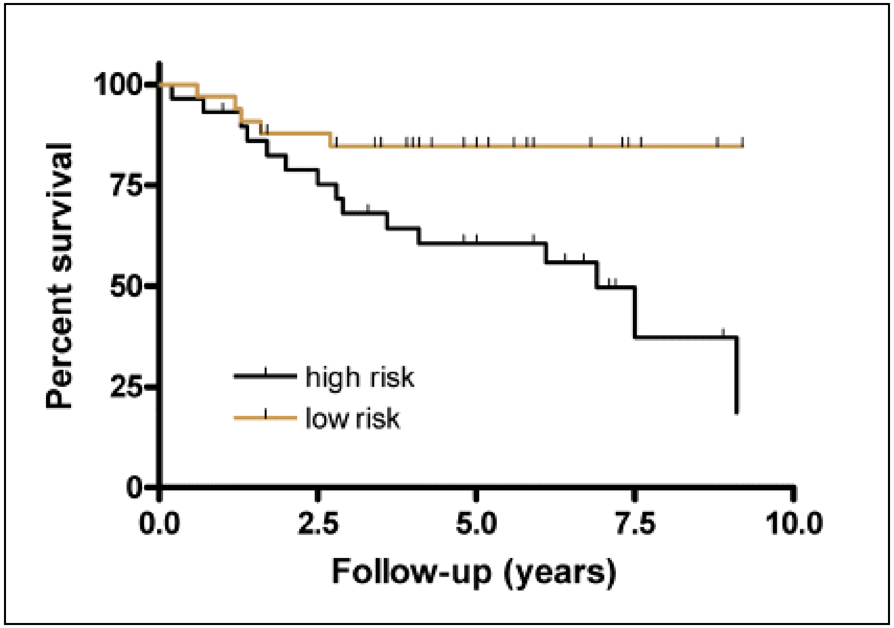
Number of genes left after feature filtering for each phase.
(*): number of genes after removing those genes who were absent in more than 10% of the experiments.

Kaplan-Meier plot for 80 test cases

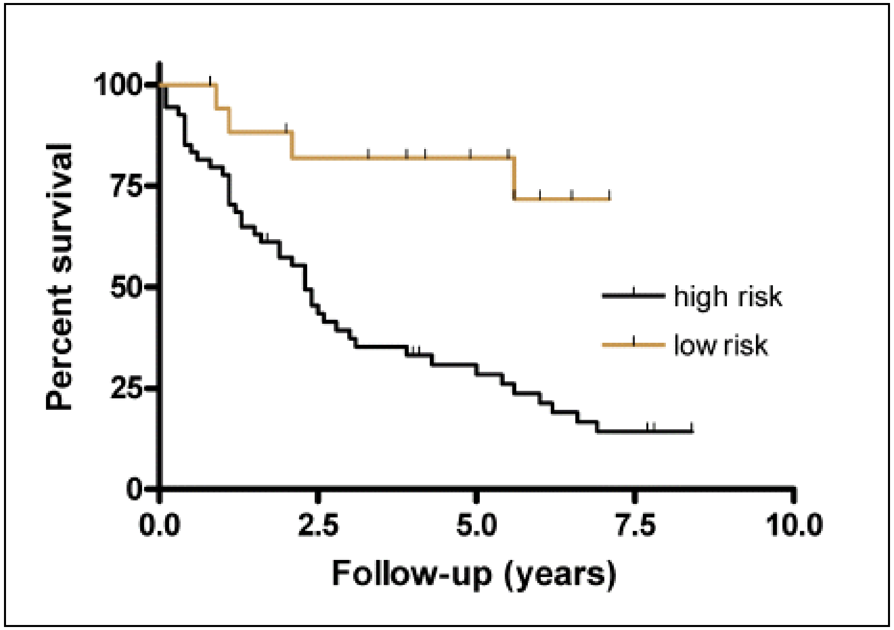


p-value of log-rank test: < 0.0001
Risk score thresholds: 0.7, 0.3

Improvement over IPI

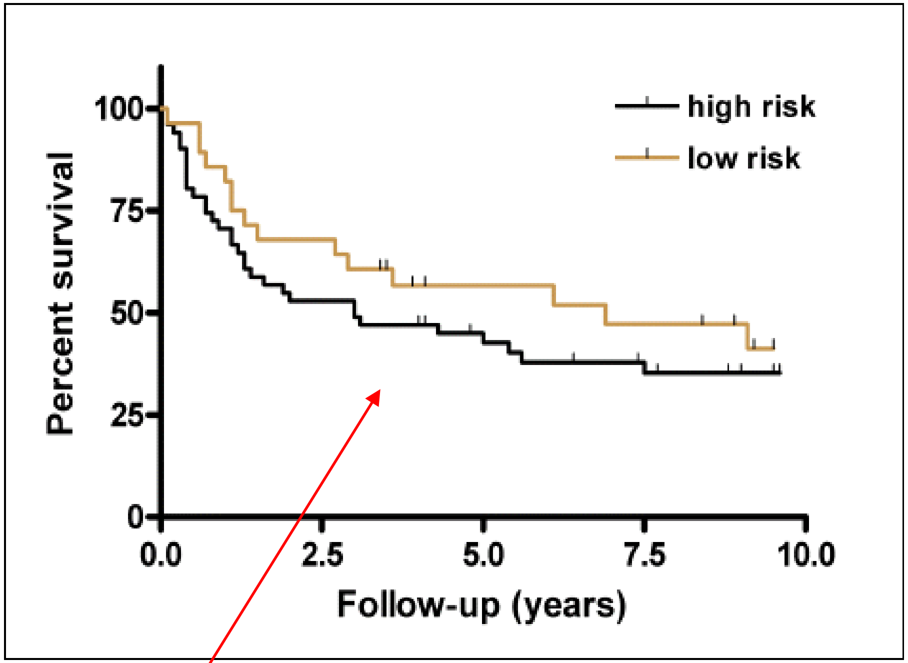


(A) IPI low,
p-value = 0.0063

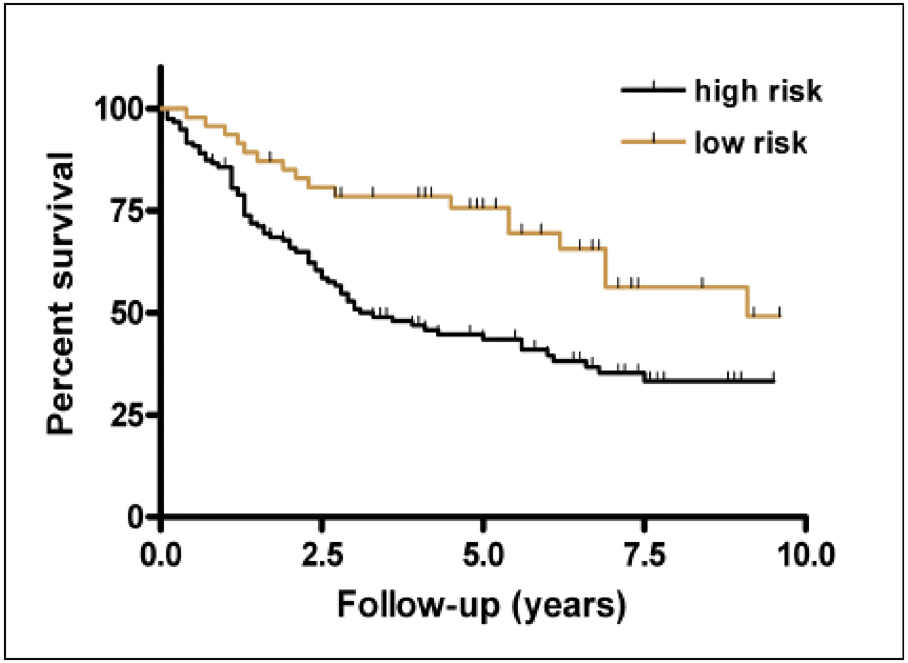


(B) IPI intermediate,
p-value = 0.0003

Merit of "extreme" samples



(A) W/o sample selection (*p* =0.38)



(B) With sample selection (p=0.009)

No clear difference on the overall survival of the 80 samples in the validation group of DLBCL study, if **no training sample selection conducted**

About the inventor: Huiqing Liu

- **Huiqing Liu**
  - PhD, NUS, 2004
  - Currently PI at Incyte
  - Asian Innovation Gold Award 2003
  - New Jersey Cancer Research Award for Scientific Excellence 2008
  - Gallo Prize 2008

Beyond disease diagnosis & prognosis

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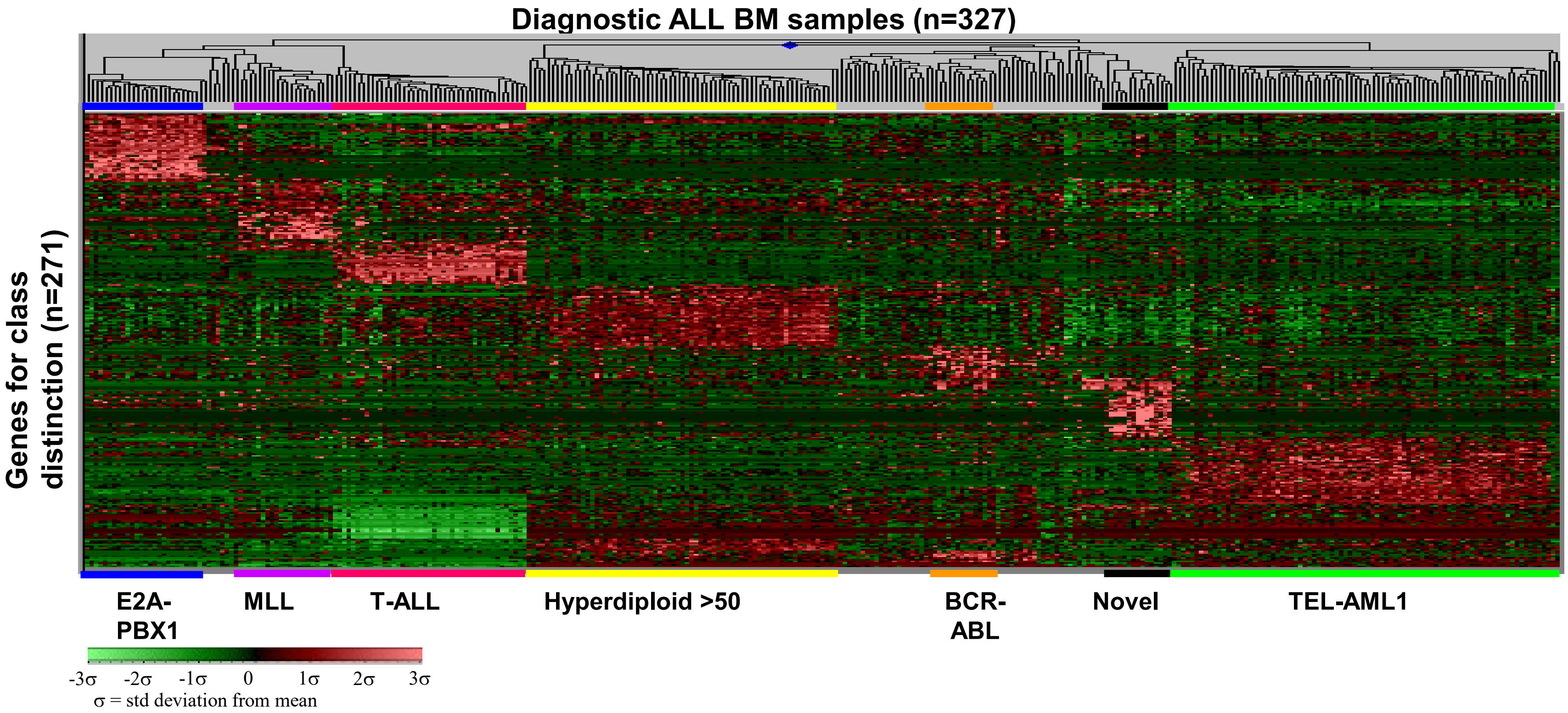
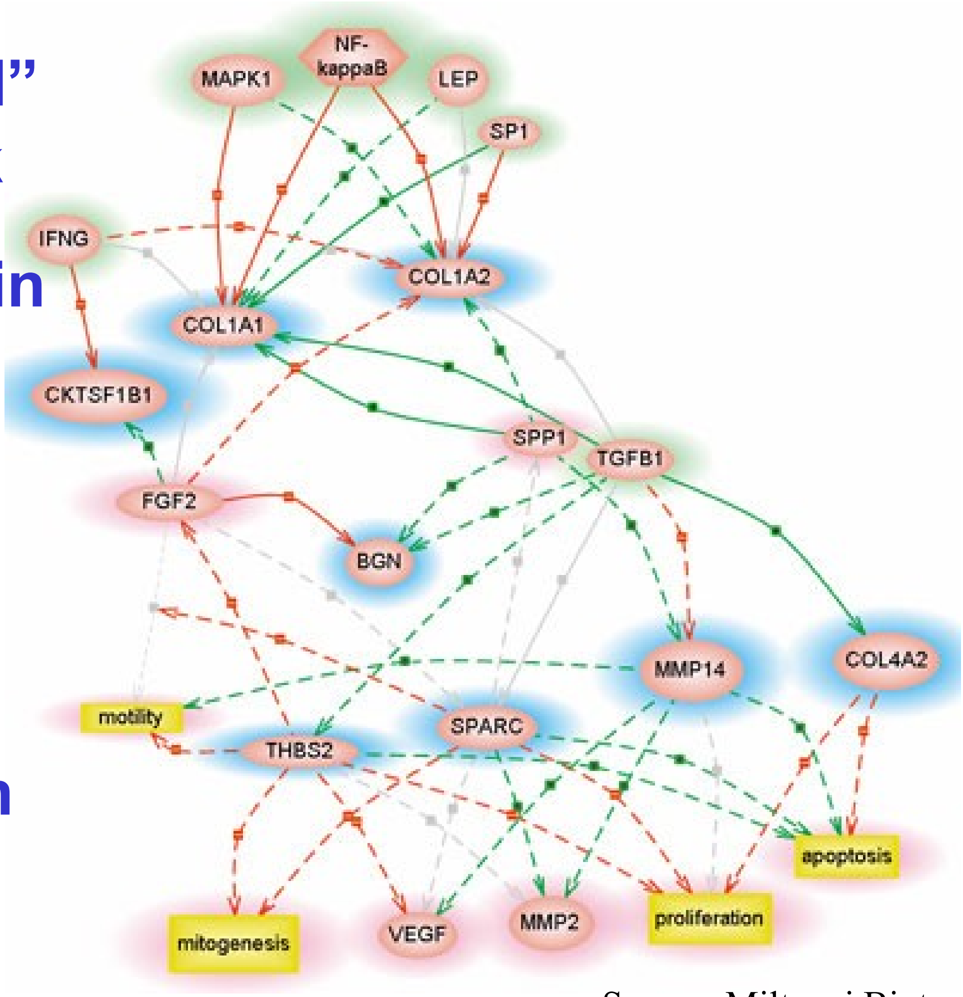
Beyond classification of gene expression profiles

- **After identifying the candidate genes by feature selection, do we know which ones are causal genes, which ones are surrogates, and which are noise?**



Gene regulatory circuits

- **Genes are "connected" in "circuit" or network**
- **Expression of a gene in a network depends on expression of some other genes in the network**
- **Can we "reconstruct" the gene network from gene expression and other data?**



Source: Miltenyi Biotec

Key questions

For each gene in the network:

- **Which genes affect it?**
- **How they affect it?**
  - Positively?
  - Negatively?
  - More complicated ways?

Some techniques

- **Bayesian Networks**
  - Friedman et al., *JCB* 7:601--620, 2000
- **Boolean Networks**
  - Akutsu et al., *PSB* 2000, pages 293--304
- **Differential equations**
  - Chen et al., *PSB* 1999, pages 29--40
- **Classification-based method**
  - Soinov et al., "Towards reconstruction of gene network from expression data by supervised learning", *Genome Biology* 4:R6.1--9, 2003

A classification-based technique

Soinov et al., *Genome Biology* 4:R6.1-9, 2003

- **Given a gene expression matrix X**
  - each row is a gene
  - each column is a sample
  - each element x_{ij} is expression of gene i in sample j

- **Find the average value a_i of each gene i**

- **Denote s_{ij} as state of gene i in sample j,**
  - s_{ij} = up if $x_{ij} > a_i$
  - s_{ij} = down if $x_{ij} \leq a_i$

A classification-based technique

Soinov et al., *Genome Biology* 4:R6.1-9, Jan 2003

- **To see whether the state of gene g is determined by the state of other genes**
  - See whether $\langle s_{ij} \mid i \neq g \rangle$ can predict s_{gj}
  - If can predict with high accuracy, then "yes"
  - Any classifier can be used, such as C4.5, PCL, SVM, etc.

- **To see how the state of gene g is determined by the state of other genes**
  - Apply C4.5 (or PCL or other "rule-based" classifiers) to predict s_{gj} from $\langle s_{ij} \mid i \neq g \rangle$
  - Extract the decision tree or rules used

Advantages of this method

- Can identify genes affecting a target gene
- Don’t need discretization thresholds?
- Each data sample is treated as an example
- Explicit rules can be extracted from the classifier (assuming C4.5 or PCL)
- Generalizable to time series

- Discuss the point “Don’t need discretization thresholds”. Is it true?

Exercise #8

Concluding remarks

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Bcr-Abl

- **Targeted drug dev**
  - Know what molecular effect you want to achieve
    - **E.g., inhibit a mutated form of a protein**
  - Engineer a compound that directly binds and causes the desired effect

- **Gleevec (imatinib)**
  - 1st success for real drug
  - Targets Bcr-Abl fusion protein (ie, Philadelphia chromosome, Ph)
  - NCI summary of clinical trial of imatinib for ALL at http://www.cancer.gov/clinicaltrials/results/ALLimatinib1109/print

What have we learned?

- **Technologies**
  - Microarray
  - PCL, ERCOF
- **Microarray applications**
  - Disease diagnosis by supervised learning
  - Subtype discovery by unsupervised learning
  - Disease diagnosis via guilt-by-association
  - Gene network reconstruction
- **Important tactic**
  - Extreme sample selection

Useful packages

- **EXPANDER (EXPression Analyser & DisplayER)**
  - http://acgt.cs.tau.ac.il/expander
- **BRB-Array Tools**
  - http://linus.nci.nih.gov/BRB-ArrayTools.html
- **NetProt**
  - http://rpubs.com/gohwils/204259
  - https://github.com/gohwils/NetProt/releases/

Any question?

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