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

Wong Limsoon
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
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)

<table>
<thead>
<tr>
<th>Description</th>
<th>Positive</th>
<th>Negative</th>
<th>Pairs</th>
<th>Avg</th>
<th>Abs Call</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFFX-Mur1</td>
<td>5</td>
<td>2</td>
<td>19</td>
<td>297.5</td>
<td>M16762 Mouse interleukin 2 (IL-2) gene, exon 4</td>
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<tr>
<td>AFFX-Mur1</td>
<td>3</td>
<td>2</td>
<td>19</td>
<td>554.2</td>
<td>M37897 Mouse interleukin 10 mRNA, complete cds</td>
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<td>2</td>
<td>19</td>
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<td>M25892 Mus musculus interleukin 4 (II-4) mRNA, complete</td>
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<tr>
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<td>3</td>
<td>19</td>
<td>141</td>
<td>M83649 Mus musculus Fas antigen mRNA, complete</td>
</tr>
<tr>
<td>AFFX-BioE</td>
<td>13</td>
<td>1</td>
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</tr>
<tr>
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<td>0</td>
<td>20</td>
<td>28838.5</td>
<td>J04423 E.coli bioC protein (-5 and -3 represent transcr</td>
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<tr>
<td>AFFX-BioD</td>
<td>17</td>
<td>0</td>
<td>19</td>
<td>25765.2</td>
<td>J04423 E.coli bioD gene dethiobiotin synthetase (-5 ar</td>
</tr>
<tr>
<td>AFFX-BioD</td>
<td>19</td>
<td>0</td>
<td>20</td>
<td>140113.2</td>
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<tr>
<td>AFFX-CreX</td>
<td>20</td>
<td>0</td>
<td>20</td>
<td>280036.6</td>
<td>XD3453 Bacteriophage P1 cre recombinase protein (-5</td>
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<tr>
<td>AFFX-CreX</td>
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<td>20</td>
<td>401741.8</td>
<td>XD3453 Bacteriophage P1 cre recombinase protein (-5</td>
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<td>5</td>
<td>18</td>
<td>-483</td>
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<td>4</td>
<td>18</td>
<td>313.7</td>
<td>J04423 E.coli bioB gene biotin synthetase (-5, -M, -3 r</td>
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<tr>
<td>AFFX-BioE</td>
<td>7</td>
<td>6</td>
<td>20</td>
<td>-1016.2</td>
<td>J04423 E.coli bioB gene biotin synthetase (-5, -M, -3 r</td>
</tr>
</tbody>
</table>
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)*

<table>
<thead>
<tr>
<th></th>
<th>Class</th>
<th>Gene1</th>
<th>Gene2</th>
<th>Gene3</th>
<th>Gene4</th>
<th>Gene5</th>
<th>Gene6</th>
<th>Gene7</th>
<th>.....</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample1</td>
<td>Cancer</td>
<td>0.12</td>
<td>-1.3</td>
<td>1.7</td>
<td>1.0</td>
<td>-3.2</td>
<td>0.78</td>
<td>-0.12</td>
<td></td>
</tr>
<tr>
<td>Sample2</td>
<td>Cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>SampleN</td>
<td>~Cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

100-500 rows

- **Gene-Time**

- **Gene-Sample-Time**

![Graph showing expression level over time](image)

![Graph showing sample expression over time](image)
### Type of gene expression datasets

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

<table>
<thead>
<tr>
<th>Class</th>
<th>Gene1</th>
<th>Gene2</th>
<th>Gene3</th>
<th>Gene4</th>
<th>Gene5</th>
<th>Gene6</th>
<th>Gene7</th>
<th>.....</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample1</td>
<td>Cancer</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sample2</td>
<td>Cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>SampleN</td>
<td>~Cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Gene-Time**

- **Gene-Sample-Time**

![Gene-Time Diagram](image1)

![Gene-Sample-Time Diagram](image2)
Application: Disease subtype diagnosis

samples

genes

benign
benign
benign
malign
malign
malign
malign
???

???
Application: Treatment prognosis

genes

samples

R
R
R
R
NR
NR
NR
NR

???
Type of gene expression datasets

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

  1000 - 100,000 columns

<table>
<thead>
<tr>
<th>Gene1</th>
<th>Gene2</th>
<th>Gene3</th>
<th>Gene4</th>
<th>Gene5</th>
<th>Gene6</th>
<th>Gene7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cond1</td>
<td>0.12</td>
<td>-1.3</td>
<td>1.7</td>
<td>1.0</td>
<td>-3.2</td>
<td>0.78</td>
</tr>
<tr>
<td>Cond2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.3</td>
</tr>
<tr>
<td>CondN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

  100-500 rows

- **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
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-intensive Tx
  - 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
Overall strategy

1. Diagnosis of subtype
2. Subtype-dependent prognosis
3. 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 $\chi^2$
- 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

<table>
<thead>
<tr>
<th>Paired datasets</th>
<th>Ingredients</th>
<th>Training vs Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-ALL vs OTHERS1</td>
<td>OTHERS1 = {E2A-PBX1, TEL-AML1, BCR-ABL, Hyperdip&gt;50, MLL, OTHERS}</td>
<td>28 vs 187, 15 vs 97</td>
</tr>
<tr>
<td>E2A-PBX1 vs OTHERS2</td>
<td>OTHERS2 = {TEL-AML1, BCR-ABL, Hyperdip&gt;50, MLL, OTHERS}</td>
<td>18 vs 169, 9 vs 88</td>
</tr>
<tr>
<td>TEL-AML1 vs OTHERS3</td>
<td>OTHERS3 = {BCR-ABL, Hyperdip&gt;50, MLL, OTHERS}</td>
<td>52 vs 117, 27 vs 61</td>
</tr>
<tr>
<td>BCR-ABL vs OTHERS4</td>
<td>OTHERS4 = {Hyperdip&gt;50, MLL, OTHERS}</td>
<td>9 vs 108, 6 vs 55</td>
</tr>
<tr>
<td>MLL vs OTHERS5</td>
<td>OTHERS5 = {Hyperdip&gt;50, OTHERS}</td>
<td>14 vs 94, 6 vs 49</td>
</tr>
<tr>
<td>Hyperdip&gt;50 vs OTHERS</td>
<td>OTHERS = {Hyperdip47-50, Pseudodip, Hypodip, Normo}</td>
<td>42 vs 52, 22 vs 27</td>
</tr>
</tbody>
</table>
Signal selection basic idea

- Choose a signal with low intra-class distance
- Choose a signal with high inter-class distance
Signal selection by $\chi^2$

The $\chi^2$ value of a signal is defined as:

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

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

<table>
<thead>
<tr>
<th>Patterns</th>
<th>Frequency (P)</th>
<th>Frequency (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>{9, 36}</td>
<td>38 instances</td>
<td>0</td>
</tr>
<tr>
<td>{9, 23}</td>
<td>38</td>
<td>0</td>
</tr>
<tr>
<td>{4, 9}</td>
<td>38</td>
<td>0</td>
</tr>
<tr>
<td>{9, 14}</td>
<td>38</td>
<td>0</td>
</tr>
<tr>
<td>{6, 9}</td>
<td>38</td>
<td>0</td>
</tr>
<tr>
<td>{7, 21}</td>
<td>0</td>
<td>36</td>
</tr>
<tr>
<td>{7, 11}</td>
<td>0</td>
<td>35</td>
</tr>
<tr>
<td>{7, 43}</td>
<td>0</td>
<td>35</td>
</tr>
<tr>
<td>{7, 39}</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td>{24, 29}</td>
<td>0</td>
<td>34</td>
</tr>
</tbody>
</table>

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):
  
  \[ EP_{i_1}^P, EP_{i_2}^P, \ldots, EP_{i_x}^P \]

- Use $k$ top-ranked most general EPs of $D^P$ and $D^N$. Define the score of $T$ in the $D^P$ class as
  
  \[ score(T, D^P) = \sum_{m=1}^{k} \frac{frequency(EP_{i_m}^P)}{frequency(EP_{P_m}^N)} \]

- 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

<table>
<thead>
<tr>
<th>EP _P</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>EP _1 _P</td>
<td>90%</td>
</tr>
<tr>
<td>EP _2 _P</td>
<td>86%</td>
</tr>
<tr>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>EP _n _P</td>
<td>68%</td>
</tr>
</tbody>
</table>

#### Top-Ranked EPs in Negative class

<table>
<thead>
<tr>
<th>EP _N</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>EP _1 _N</td>
<td>100%</td>
</tr>
<tr>
<td>EP _2 _N</td>
<td>95%</td>
</tr>
<tr>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>EP _n _N</td>
<td>80%</td>
</tr>
</tbody>
</table>

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

\[ \text{Score}^P = \frac{E_P^{1P'}}{E_P^P} + \ldots + \frac{E_P^{kP'}}{E_P^P} \]

Most freq EP of pos class

Similarly,

\[ \text{Score}^N = \frac{E_P^{1N'}}{E_P^N} + \ldots + \frac{E_P^{kN'}}{E_P^N} \]

If \( \text{Score}^P > \text{Score}^N \), then positive class,
Otherwise negative class
Accuracy of PCL (vs. other classifiers)

<table>
<thead>
<tr>
<th>Testing Data</th>
<th>Error rate of different models</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C4.5</td>
</tr>
<tr>
<td>T-ALL vs OTHERS1</td>
<td>0:1</td>
</tr>
<tr>
<td>E2A-PBX1 vs OTHERS2</td>
<td>0:0</td>
</tr>
<tr>
<td>TEL-AML1 vs OTHERS3</td>
<td>1:1</td>
</tr>
<tr>
<td>BCR-ABL vs OTHERS4</td>
<td>2:0</td>
</tr>
<tr>
<td>MLL vs OTHERS5</td>
<td>0:1</td>
</tr>
<tr>
<td>Hyperdiploid&gt;50 vs OTHERS</td>
<td>2:6</td>
</tr>
<tr>
<td>Total Errors</td>
<td>14</td>
</tr>
</tbody>
</table>

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

\[
\{gene_{-(38319\_at)} @ (-\infty, 15975.6)\} \text{ and } \{gene_{-(38319\_at)} @ [15975.6, +\infty)\}.
\]

- These give us the diagnostic rule

If the expression of 38319_at is less than 15975.6, 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 unquantified 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

⇒ 75-80% cure rates
⇒ Save US$51.6m/yr

⇒ Total US$98.4m/yr
A nice ending…

- Asian Innovation Gold Award 2003
Gene expression profile clustering

Novel disease subtype discovery
Is there a new subtype?

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

Exercise: Name and describe one bi-clustering method
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
Some patient samples

- Does Mr. A have cancer?
Let’s rearrange the rows…

• Does Mr. A have cancer?
and the columns too…

<table>
<thead>
<tr>
<th>samples</th>
<th>genes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>benign</td>
<td></td>
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<tr>
<td>benign</td>
<td>benign</td>
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<td>benign</td>
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<td>malign</td>
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<td>malign</td>
<td>malign</td>
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<tr>
<td>malign</td>
<td>malign</td>
</tr>
</tbody>
</table>

Mr. A: ???

- **Does Mr. A have cancer?**
Introduction to simple clustering methods
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?
  - Two Clusters
  - Four Clusters
  - Six 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

1: Select $K$ points as the initial centroids.
2: repeat
3: Form $K$ clusters by assigning all points to the closest centroid.
4: Recompute the centroid of each cluster.
5: until The centroids don’t change
K-means clustering illustration
K-means clustering illustration

Iteration 1

Iteration 2

Iteration 3

Iteration 4

Iteration 5

Iteration 6
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

• Key is computation of proximity of two clusters
  
  – Different approaches to defining the distance / similarity between clusters
Visualization of agglomerative hierarchical clustering
Single, complete, & average Linkage

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

\[ d(r, s) = \min \{ \text{dist}(x_{rj}, x_{sj}) \} \]

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

\[ d(r, s) = \max \{ \text{dist}(x_{rj}, x_{sj}) \} \]

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

<table>
<thead>
<tr>
<th></th>
<th>p1</th>
<th>p2</th>
<th>p3</th>
<th>p4</th>
<th>p5</th>
<th>...</th>
</tr>
</thead>
<tbody>
<tr>
<td>p1</td>
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Proximity Matrix
Intermediate situation

- After some merging steps, we have some clusters

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Proximity Matrix
Intermediate situation

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

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

<table>
<thead>
<tr>
<th></th>
<th>C2</th>
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Proximity Matrix

C1 - C3 - C4 - C2 U C5

p1 - p2 - p3 - p4 - p9 - p10 - p11 - p12
How to define inter-cluster similarity

- Min
- Max
- Group average
- Distance between centroids

### Proximity Matrix

<table>
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<tr>
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Similarity?
How to define inter-cluster similarity

- Min
- Max
- Group average
- Distance between centroids

Proximity Matrix

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Proximity Matrix
How to define inter-cluster similarity

- Min
- Max
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- Distance between centroids

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Proximity Matrix
How to define inter-cluster similarity

- Min
- Max
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Proximity Matrix
How to define inter-cluster similarity

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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

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<thead>
<tr>
<th></th>
<th>p1</th>
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<th>p3</th>
<th>p4</th>
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</table>

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?
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

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<td>0.39</td>
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Table 8.4. Euclidean distance matrix for 6 points.
Hierarchical clustering: Max

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?
Cluster similarity: Group average

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

\[
\text{proximity}(\text{Cluster}_i, \text{Cluster}_j) = \frac{\sum_{\substack{p_i \in \text{Cluster}_i, \ p_j \in \text{Cluster}_j \ \text{proximity}(p_i, p_j)}}}{|\text{Cluster}_i| \times |\text{Cluster}_j|}
\]

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<td>0.00</td>
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*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?
Normalization
Sometimes, a gene expression study may involve batches of data collected over a long period of time…
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
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., \( Z = \frac{(x - \mu)}{\sigma} \)

• Quantile normalization
Quantile normalization

- Given \( n \) arrays of length \( p \), form \( X \) of size \( p \times n \) where each array is a column.

- Sort each column of \( X \) to give \( X_{\text{sort}} \).

- Take means across rows of \( X_{\text{sort}} \) and assign this mean to each element in the row to get \( X'_{\text{sort}} \).

- Get \( X_{\text{normalized}} \) by arranging each column of \( X'_{\text{sort}} \) to have same ordering as \( X \).

- Implemented in some microarray s/w, e.g., EXPANDER.
Sometimes, a gene expression study may involve batches of data collected over a long period of time...

After quantile normalization

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

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?
Selection of patient samples and genes for disease prognosis
Gene expression profile + clinical data \[\Rightarrow\] 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)
- ......
Our approach

Extreme sample selection

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

Short-term survivors
who died within a short period
\[ F(T) < c_1 \text{ and } E(T) = 1 \]

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
ERCOF
Entropy-Based Rank Sum Test & Correlation Filtering

- 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 $w(x) \in [c_{lower}, c_{upper}]$
- Group features by Pearson Correlation For each group, retain the top 50% wrt class entropy
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 \((\text{posterior probability})\)

\[ S(T) = \frac{1}{1 + e^{-G(T)}} \quad (S(T) \in (0,1)) \]

\(S(T)\): \textit{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

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

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<th>Data set</th>
<th>Status</th>
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<td>Alive</td>
</tr>
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<td>Original</td>
<td>88</td>
<td>72</td>
</tr>
<tr>
<td>DLBCL</td>
<td>Informative</td>
<td>47+1(*)</td>
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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

<table>
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<tr>
<th>Gene selection</th>
<th>DLBCL</th>
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<tbody>
<tr>
<td>Original</td>
<td>4937(*)</td>
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<tr>
<td>Phase I</td>
<td>132(2.7%)</td>
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<tr>
<td>Phase II</td>
<td>84(1.7%)</td>
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</table>

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, 
\[ p\text{-value} = 0.0063 \]

(B) IPI intermediate, 
\[ p\text{-value} = 0.0003 \]
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
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
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** $j$,
  - $s_{ij} = \text{up}$ if $x_{ij} > a_i$
  - $s_{ij} = \text{down}$ if $x_{ij} \leq a_i$
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• 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
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 (i.e., Philadelphia chromosome, Ph)
  – NCI summary of clinical trial of imatinib for ALL at
    [http://www.cancer.gov/cancertrials/results/ALLimatinib1109/print](http://www.cancer.gov/cancertrials/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](http://acgt.cs.tau.ac.il/expander)

- **BRB-Array Tools**

- **NetProt**
  - [http://rpubs.com/gohwils/204259](http://rpubs.com/gohwils/204259)
  - [https://github.com/gohwils/NetProt/releases/](https://github.com/gohwils/NetProt/releases/)
Any question?
References

• E.-J. Yeoh et al., “Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling”, Cancer Cell, 1:133--143, 2002


• J. Li, L. Wong, “Techniques for Analysis of Gene Expression”, The Practical Bioinformatician, Chapter 14, pages 319—346, WSPC, 2004