10-810: Advanced Algorithms and Models for Computational Biology

Differentially Expressed Genes

Data analysis

- Normalization
- Combining results from replicates
- Identifying differentially expressed genes
- Dealing with missing values
- Static vs. time series

Motivation

- In many cases, this is the goal of the experiment.
- Such genes can be key to understanding what goes wrong / or get fixed under certain condition (cancer, stress etc.).
- In other cases, these genes can be used as ‘features’ for a classifier.
- These genes can also serve as a starting point for a model for the system being studied (e.g. cell cycle, phermone response etc.).

Problems

- As mentioned in the previous lecture, differences in expression values can result from many different noise sources.
- Our goal is to identify the ‘real’ differences, that is, differences that can be explained by the various errors introduced during the experimental phase.
- Need to understand both the experimental protocol and take into account the underlying biology / chemistry

Hypothesis testing

- A general way of identifying differentially expressed genes is by testing two hypothesis
- Let g_A denote the mean expression of gene *g* under condition *A* (say healthy) and g_B be the mean expression under condition *B* (cancer).
- In this case we can test the following hypotheses:

H_0 (or the null hypothesis): $g_A = g_B$

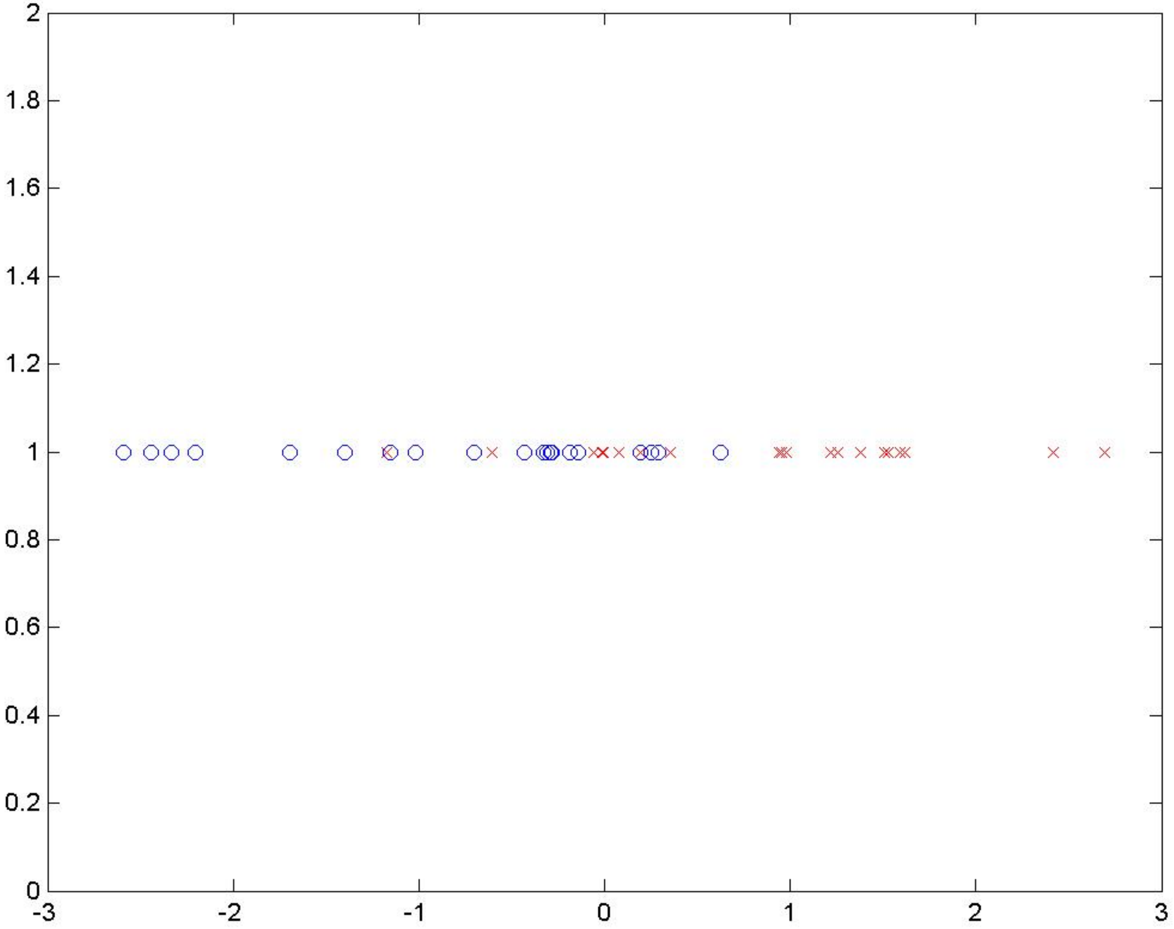
H_1 (or the alternative hypothesis): $g_A \neq g_B$

- If we *reject* **H_0** then gene g has a different mean under the two conditions, and so is *differentially expressed*

P-value

- Using hypothesis testing we need determine our confidence in the resulting decision
- This is done using a *test statistics* which indicates how strongly the data we observe supports our decision
- A p-value (or probability value) measures how likely it is to see the data we observed under the null hypothesis
- Small p-values indicate that it is very unlikely that the data was generated according to the null hypothesis

Example: Measurements for one gene in 40 (20+20) experiments of two conditions



Hypothesis testing: Log likelihood ratio test

- If we have a probabilistic model for gene expression we can compute the likelihood of the data given the model.
- In our case, lets assume that gene expression is normally distributed with different mean under the different conditions and the same variance.
- Thus for the alterative hypothesis we have:

$$y_A \sim N(\mu_A, \sigma^2) \quad y_B \sim N(\mu_B, \sigma^2)$$

and for the null hypothesis we have:

$$y_A \sim N(\mu, \sigma^2) \quad y_B \sim N(\mu, \sigma^2)$$

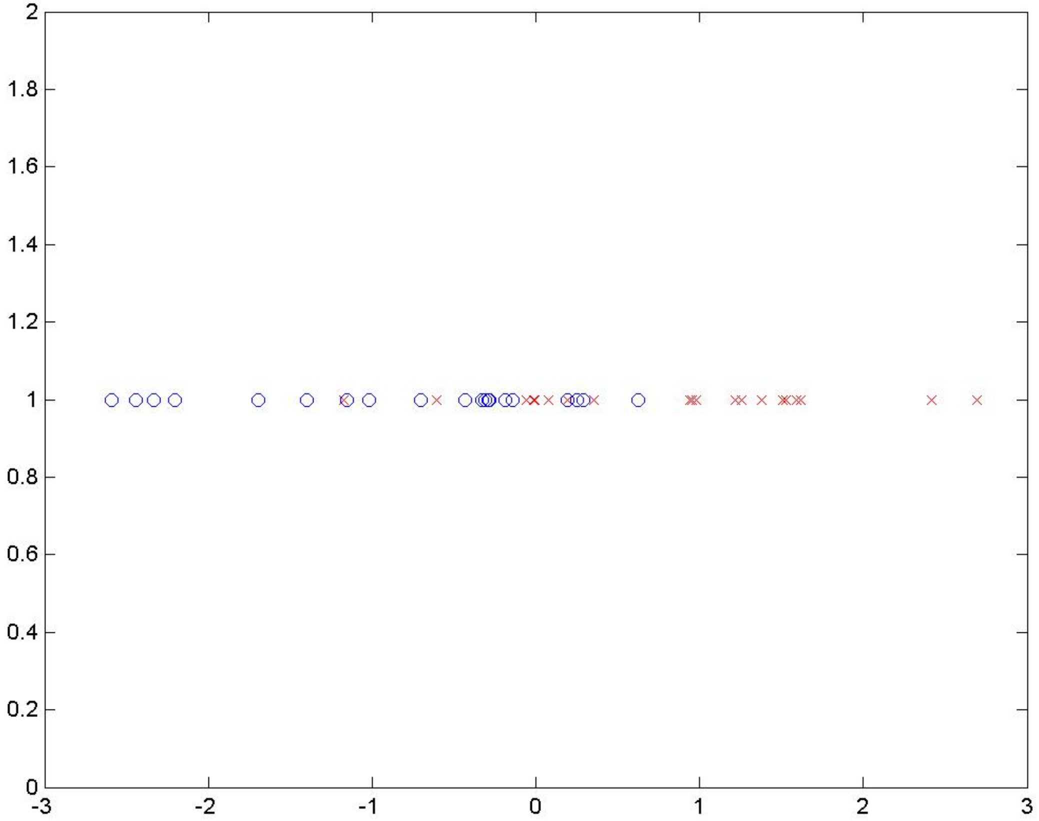
- We can compute the estimated means and variance from the data (and thus we will be using the *sample mean* and *sample variance*)

Example mean

Blue mean: -0.81

Red mean: 0.84

Combined mean: 0.02



Data likelihood

- Given our model, the likelihood of the data under the two hypothesis is:

$$L(0) = \prod_{i \in A} \frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{(y^i - \mu)^2}{2\sigma^2}} \prod_{i \in B} \frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{(y^i - \mu)^2}{2\sigma^2}}$$

$$L(1) = \prod_{i \in A} \frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{(y^i - \mu_A)^2}{2\sigma^2}} \prod_{i \in B} \frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{(y^i - \mu_B)^2}{2\sigma^2}}$$

- We can also compute the *ratio* of the likelihoods (L(1)/L(0))
- Intuitively, the higher this ratio the *more* likely it is that the data was indeed generated according to the alternative hypothesis (and thus the genes are differentially expressed).

Log likelihood ratio test

- We use the *log of the likelihood ratio*, and after simplifying arrive it:

$$T = 2\frac{\sum_{i\in A}(y^i-\mu_A)^2+\sum_{i\in B}(y^i-\mu_B)^2}{\sum_{i\in A}(y^i-\mu)^2+\sum_{i\in B}(y^i-\mu)^2}$$

- *T* is our test statistics, and in this case can be shown to be distributed as χ^2

Degrees of freedom

- We are almost done …
- We still need to determine one more value in order to use the test
- Degrees of freedom for likelihood ratio tests depends on the difference in the number of *free parameters*
- In this case, our free parameters are the mean and variance
- Thus the difference is …

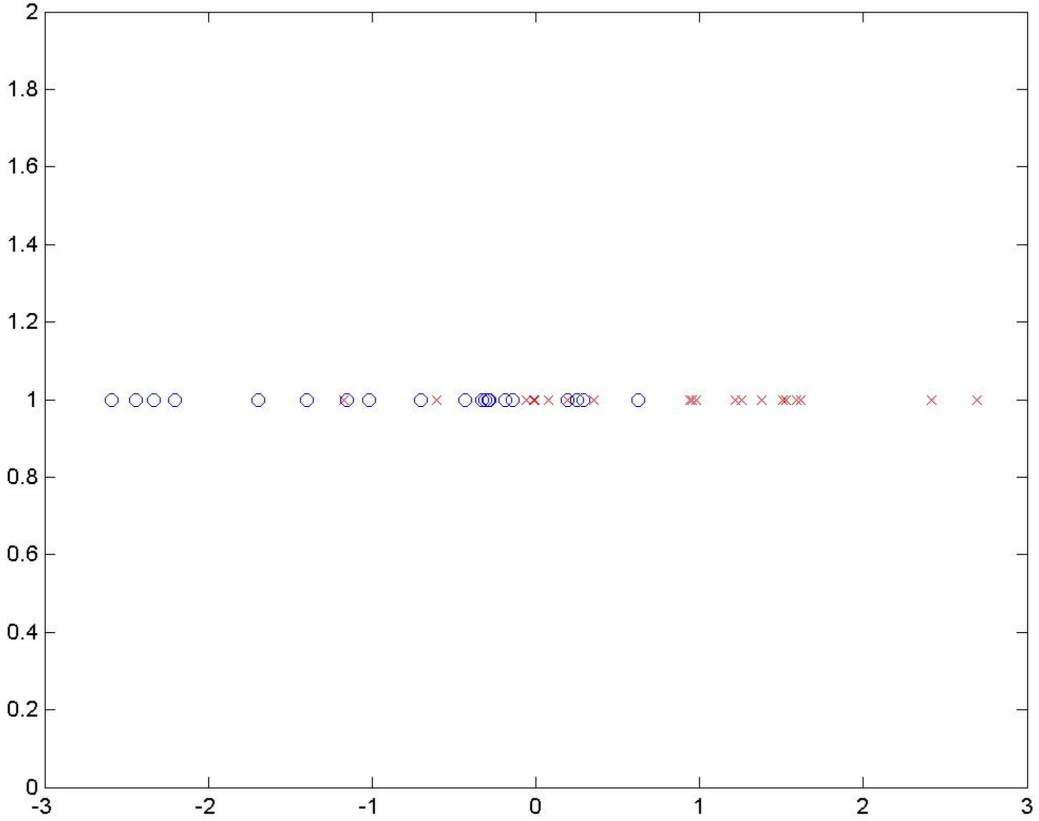
- In this case, the difference is 1 (two means vs. one)

Example: Log likelihood ratio

T = 2*(64.3/37.1) = 3.46

D.O.F = 1

P-value = 0.06



Limitations

- We assumed a specific probabilistic model (Gaussian noise) which may not actually capture the true noise factors
- We may need many replicates to derive significant results
- Multiple hypothesis testing

Multiple hypothesis testing

- A p-value is meaningful when one test is carried out
- However, when thousands of tests are being carried out, it is hard to determine the real significance of the results based on the p-value alone.
- Consider the following two cases:

we test **100** genes

we find **10** to be differentially expressed with a p-value **< .01**

we test **1000** genes

we find **10** to be differentially expressed with a p-value **< .01**

- We need to correct for the multiple tests we are carrying out!

Bonferroni Correction

- Bonferroni Correction is a simple and widely used method to correct for multiple hypothesis testing
- Using this approach, the significance value obtained is divided by the number of tests carried out.
- For example, if we are testing 1000 genes and are interested in a (gene specific) p-value of 0.05 we will only select genes with a p-value of 0.05/1000 = 0.00005 = $5*10^{-5}$
- Motivation: If

$$p(specific \quad T_i \quad passes \mid H_0) < \frac{\alpha}{n}$$

- Then

$$p(some \quad T_i \quad passes \mid H_0) < \alpha$$

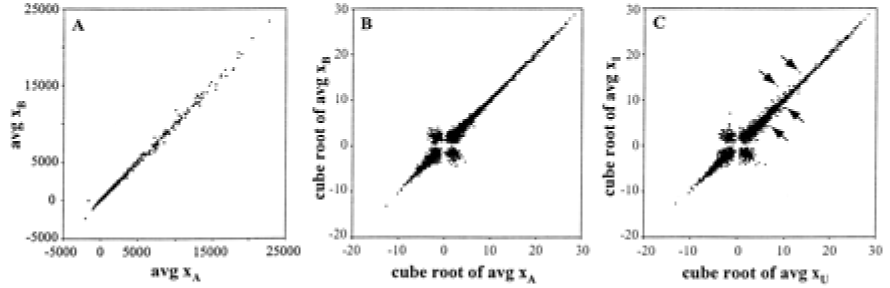
Bonferroni Correction

- The Bonferroni Correction is very conservative
- Using it may lead to missing important genes
- Other methods rely on the false discovery rate (FDR) as we discuss for SAM

SAM – Significance Analysis of Microarray

- Relies on repeats.
- Avoid using fold change alone.
- Use permutations to determine the false discovery rate.

Data



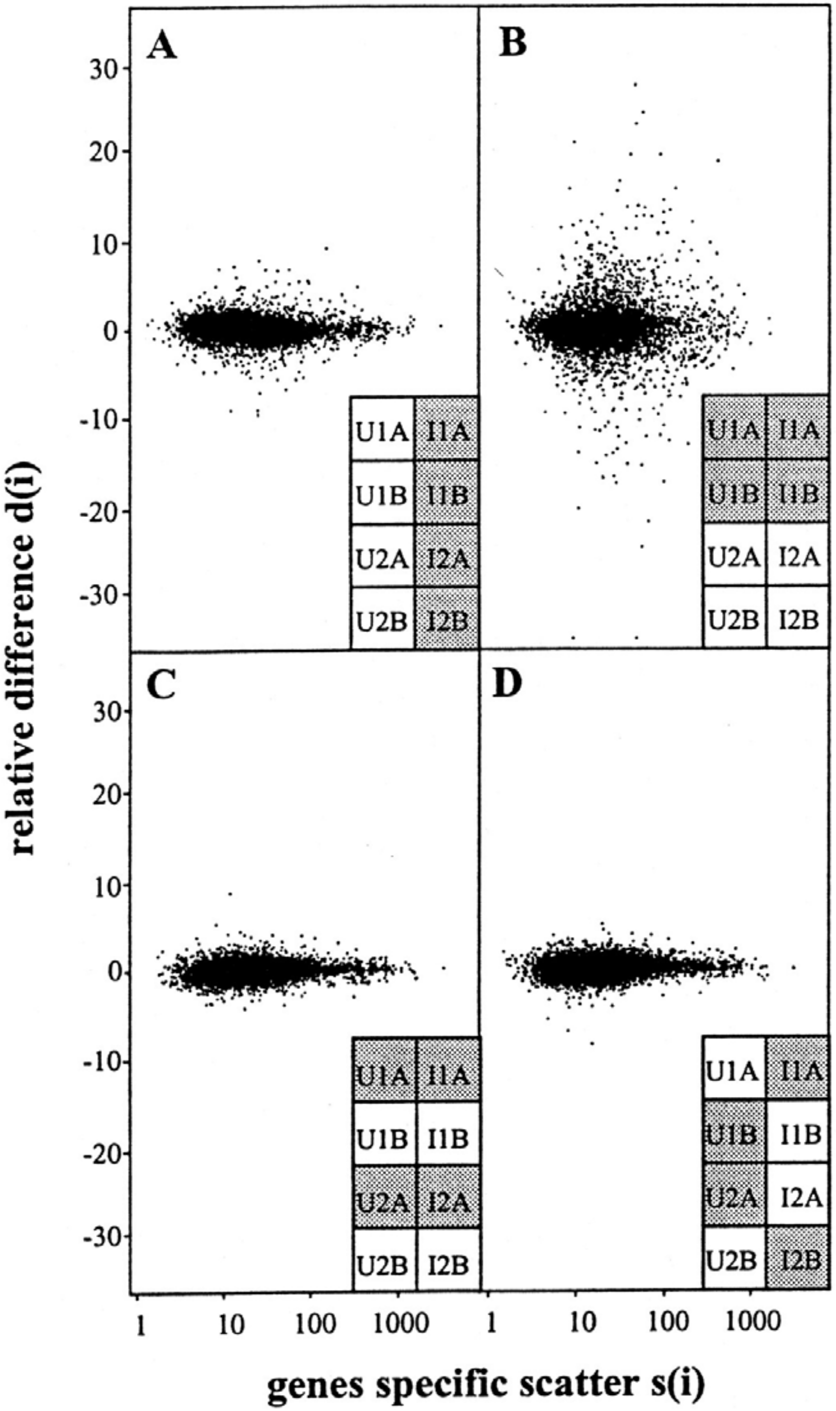
- Many gene were assigned negative values
- Many where expressed at low levels
- Noise is larger for genes expressed at low levels.

Relative difference

$$d(i) = \frac{\hat{x}_1(i) - \hat{x}_2(i)}{s(i) + s_0}$$

- Where x_1 and x_2 are the observed means and *s(i)* is the observed standard deviation.
- S_0 is chosen so that *d(i)* is consistent across the different expression levels.

Different comparisons of repeated experiments.



Identifying differentially expressed genes

- Using the normalized *d(i)* we can detect differentially expressed genes by selecting a cutoff above (or below for negative values) which we will declare this gene to be differentially expressed.
- However selecting the cutoff is still a hard problem.
- Solution: use the False Discovery Rate (FDR) to choose the best cutoff.

False Discovery Rate

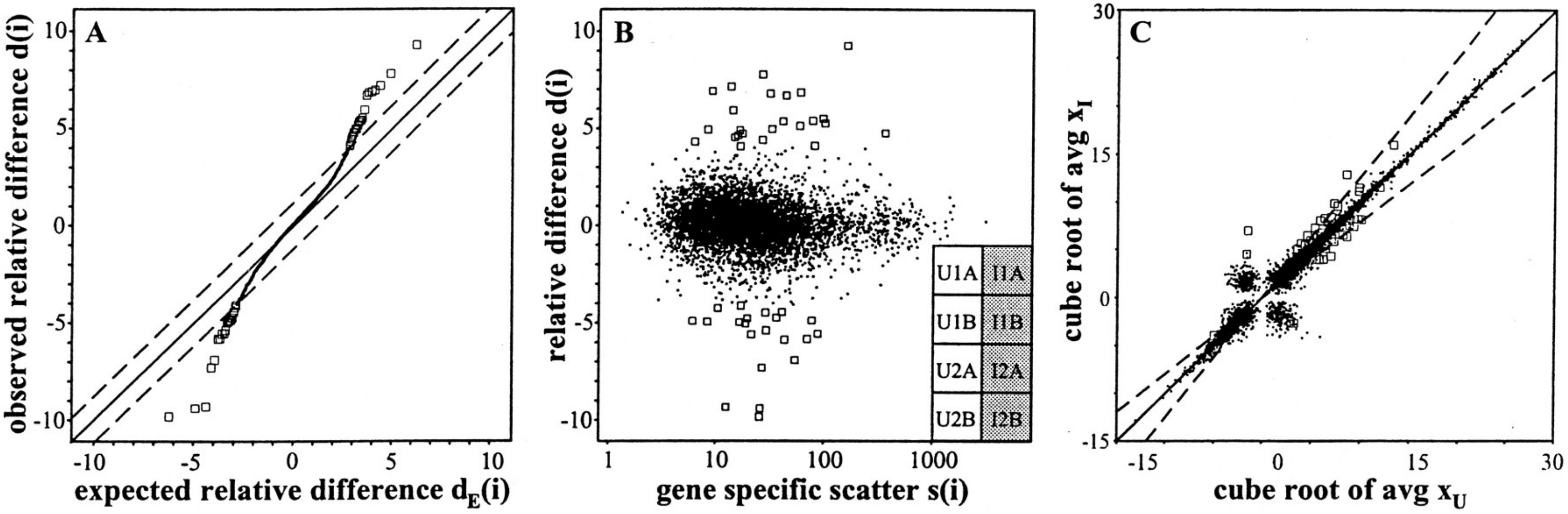
- Percentage of genes wrongly identifies / total gene identified.
- What is the difference between this and a p-value ?

P-value: probability under the null hypothesis for observing this value

Determining the FDR

- A permutation based method.
- Use all 36 permutations (why 36 ?).
- For each one compute the $d_p(i)$ for all genes.
- Scatter plot observed *d(i)* vs. expected *d(i).*

Selecting differentially expressed genes



Extensions

- Can be extended to multiple labels.
- Compute average for each label.
- Compute difference between specific class average and global average and corresponding variance.
- As before, adjust variance to correct for low / high level of expression.

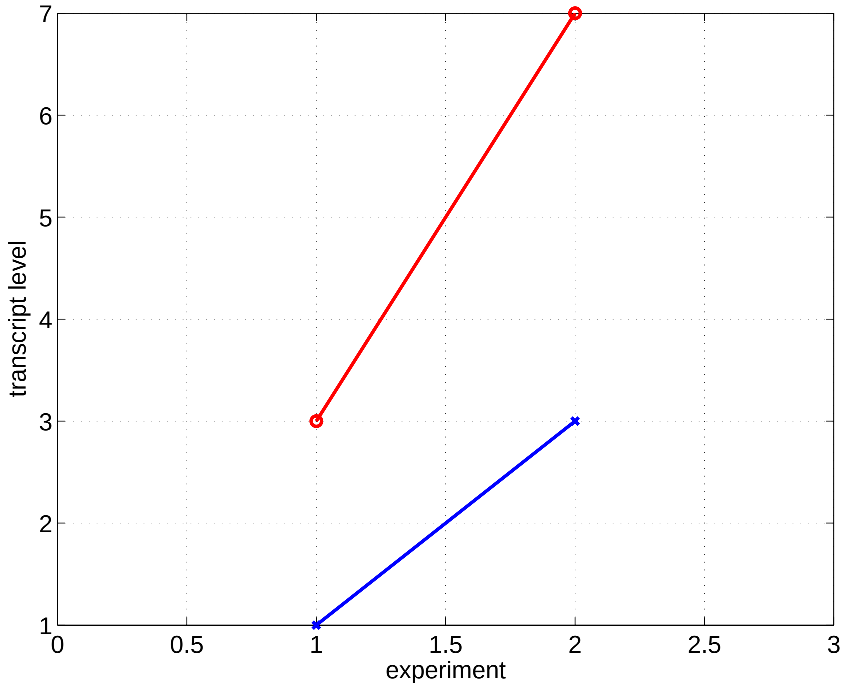
Mixture populations

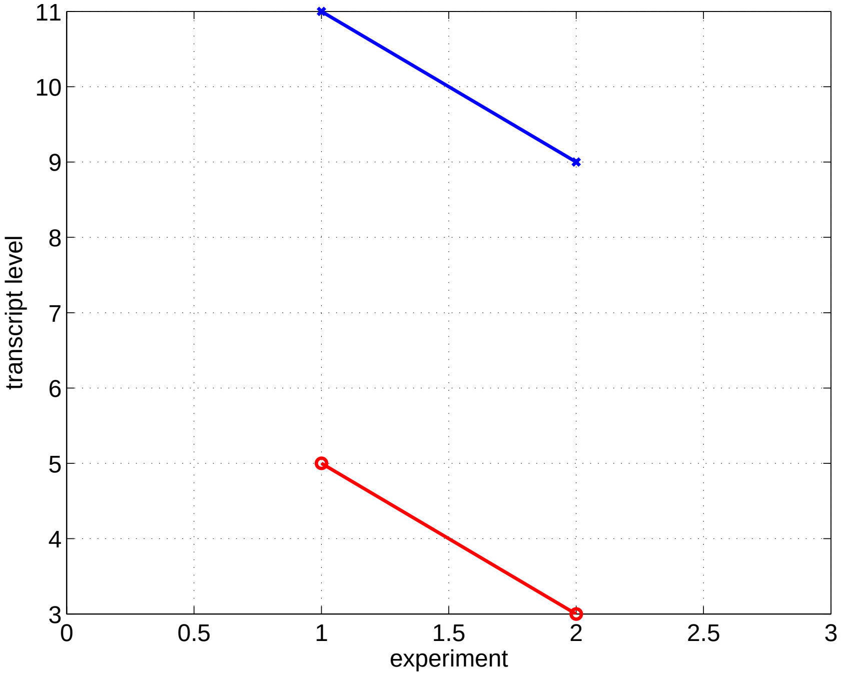
- We may be measuring the transcript levels in a heterogeneous (mixture) cell population
- There are a few surprises:

  - genes co-expressed (correlated) in each cell type may appear uncorrelated in the mixture

  - genes uncorrelated in each cell type may appear perfectly correlated in the mixture

Example


7
6
5
4
3
2
1
transcript level
0
0.5
1
1.5
2
2.5
3
experiment


11
10
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transcript level
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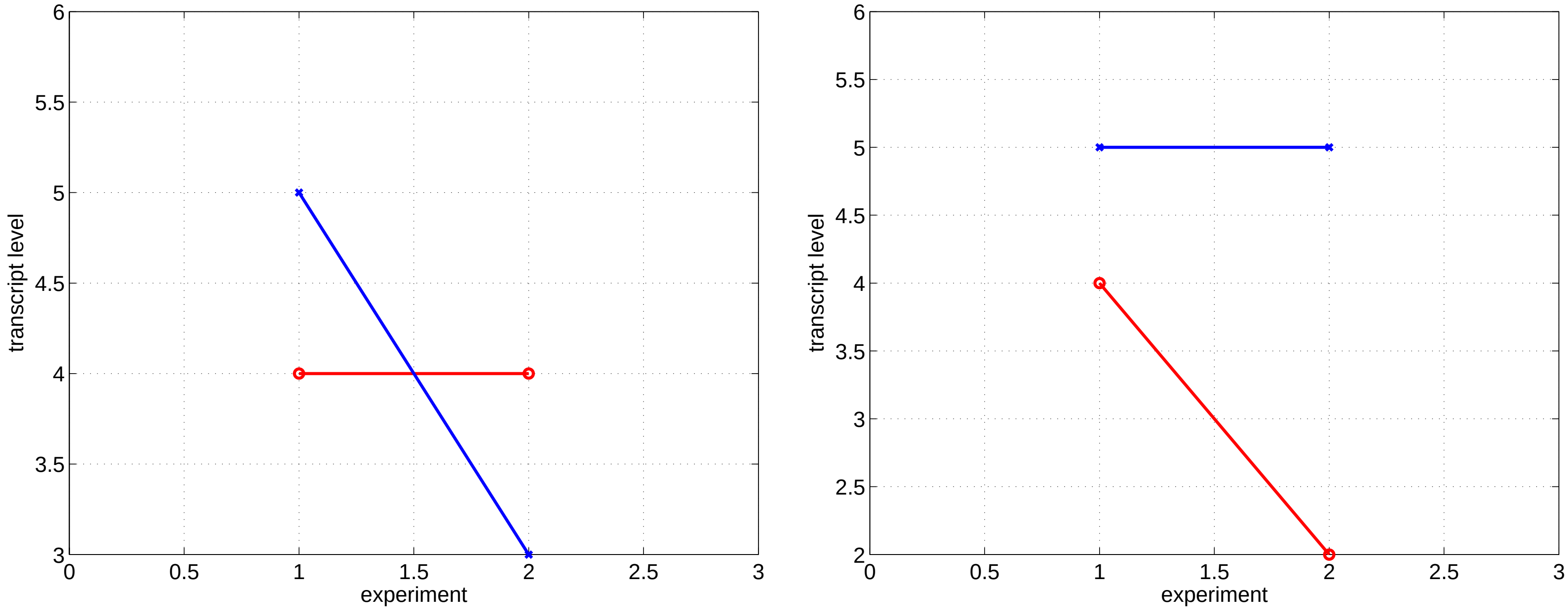
experiment

Example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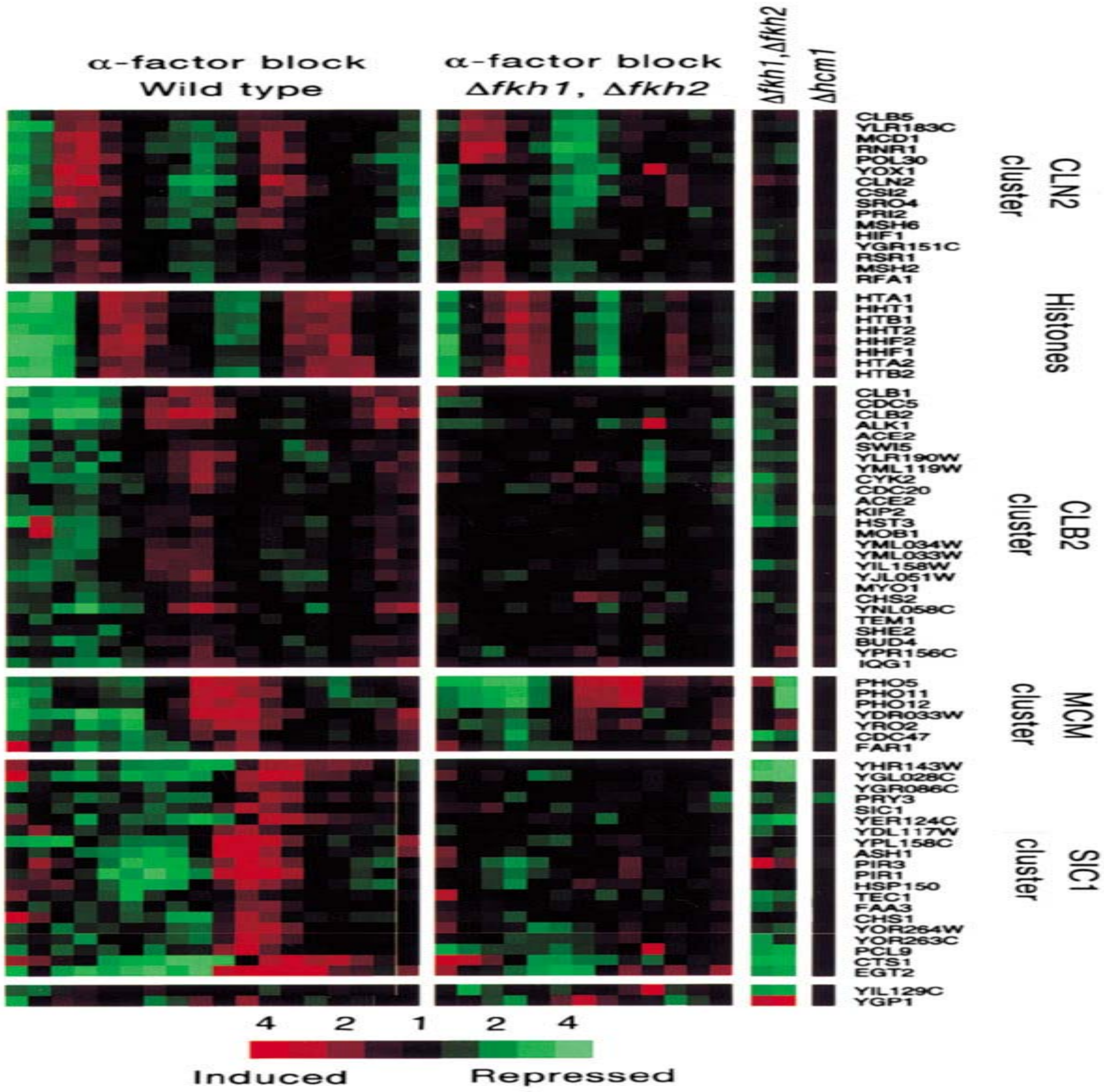
0.5
1
1.5
2
2.5
3
experiment
transcript level

What about time series ?

- Comparing time points is not always possible (different sampling rates).
- Even if sampling rates are the same, there are differences in the *timing* of the system under different conditions.
- Another problem is lack of repeats.

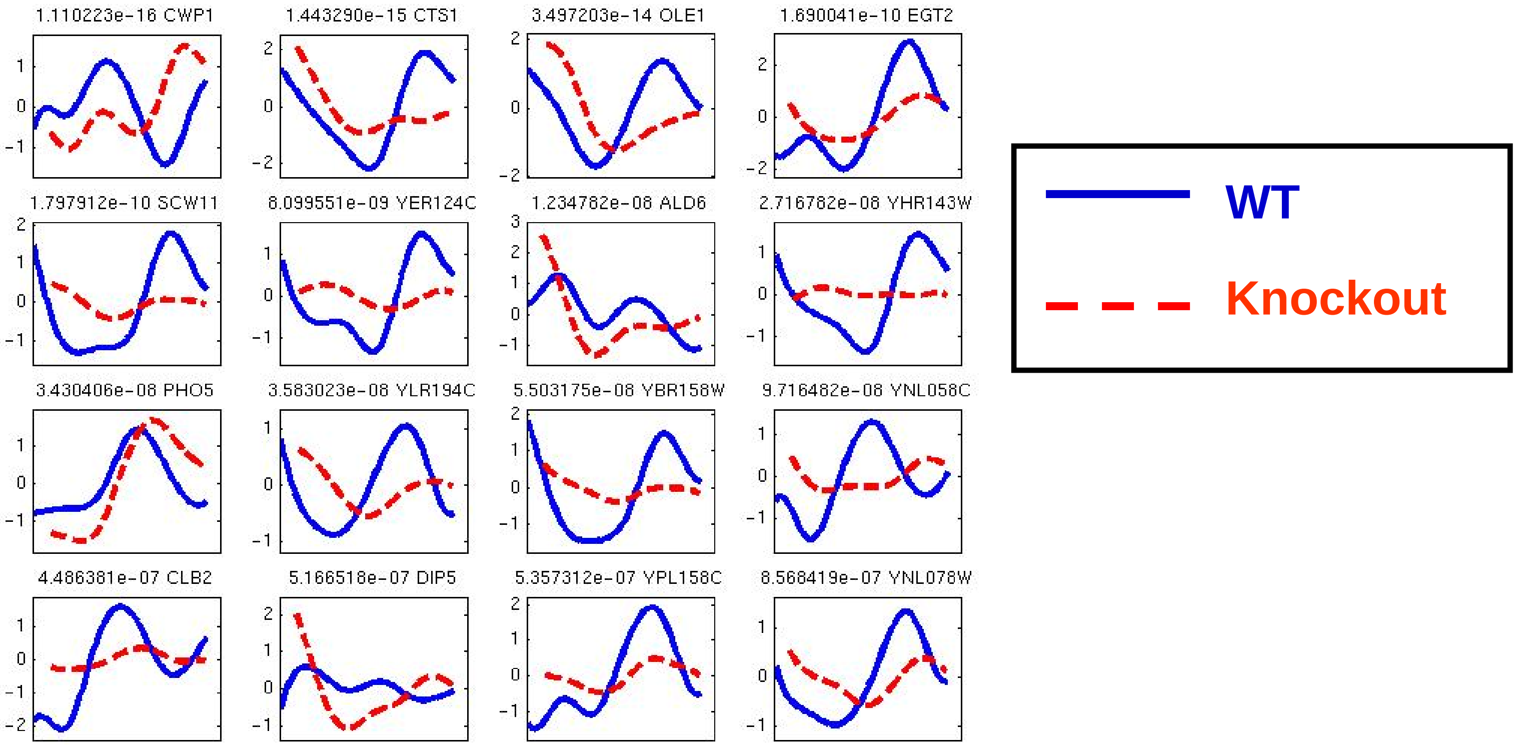
Time series comparison



knockout = deletion of gene(s) from the sequence

Zhu *et al*, Nature 2000

Results for the Fkh1/2 Knockout



Clustering expression data

Goal

- Data organization (for further study)
- Functional assignment
- Determine different patterns

Genes

- Classification
- Relations between experimental conditions

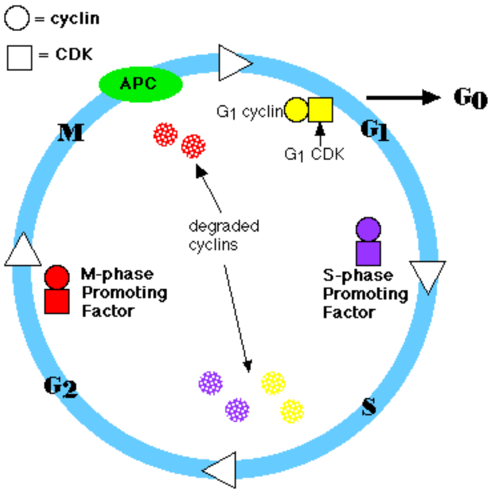
Experiments

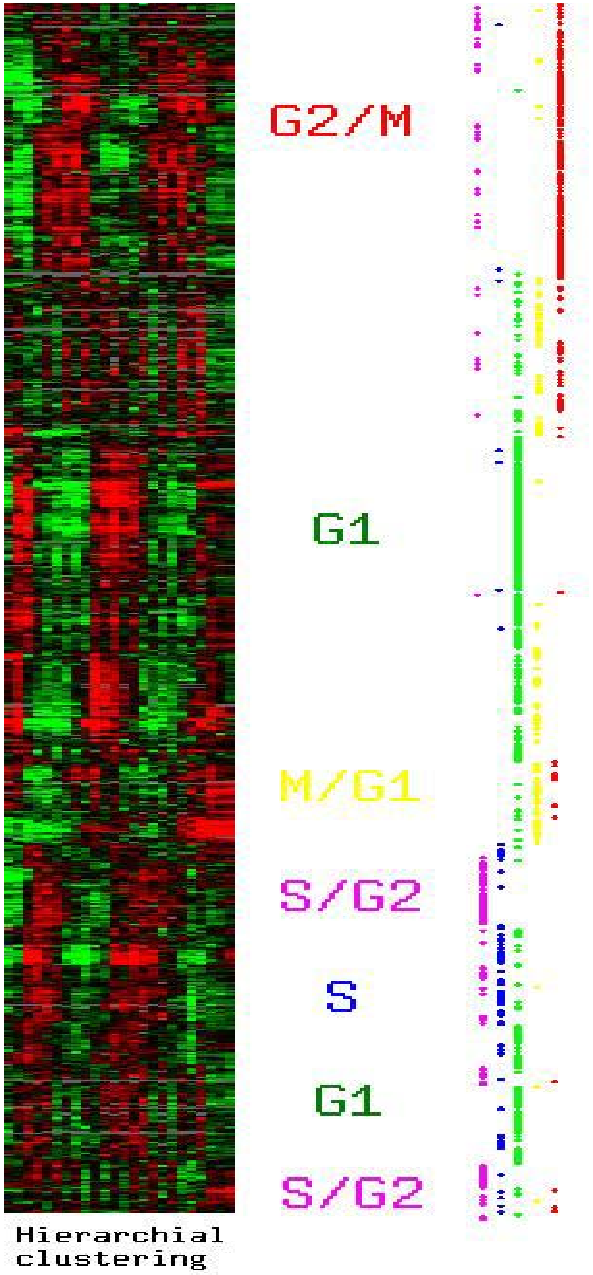
- Subsets of genes related to subset of experiments

Both

Example: co-expression

For example: grouping together genes active in the same phase of the cell cycle

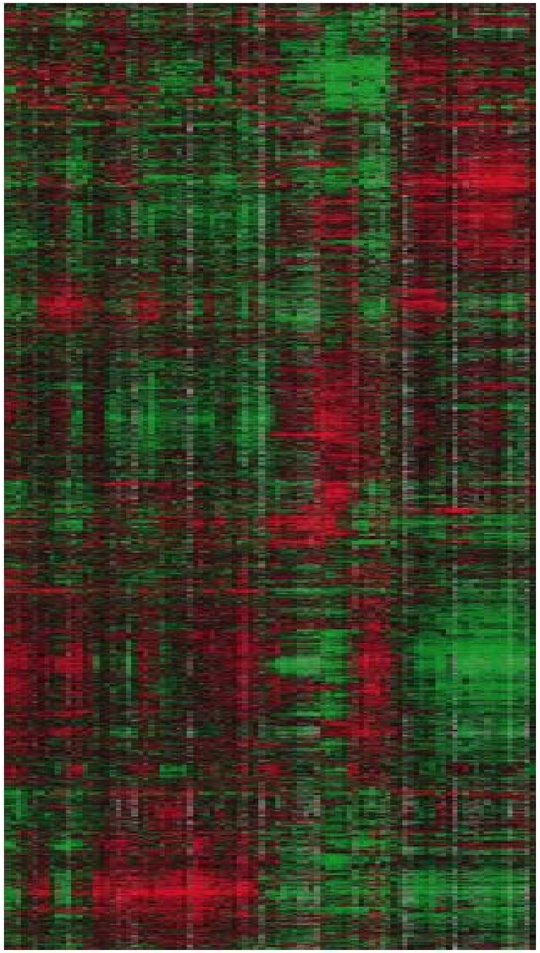




Clustering experiments

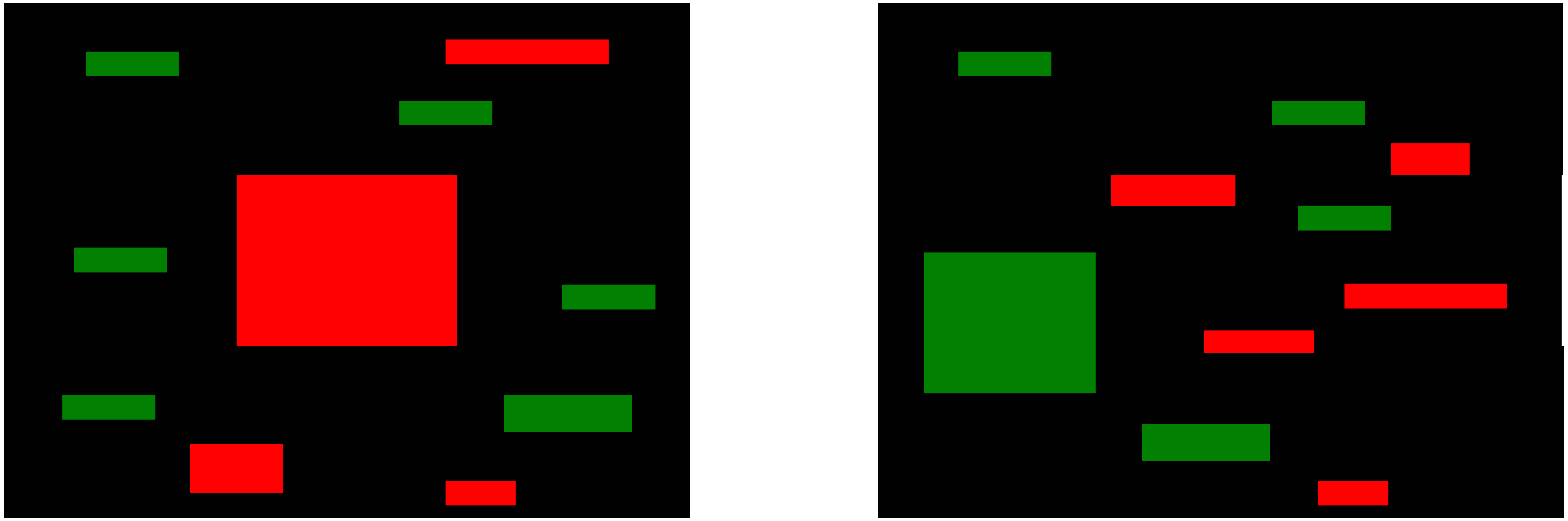
- For example: clustering genes on the basis of how similar their effects are if they are knocked out.
- The ``profiles'' associated with the genes in this case are

  the knock-out responses.



Bi-clustering

- Find subsets of genes and experiments such that the genes in the subset behave similarly across the subset of the experiments



What you should know

- Statistical hypothesis testing
- Log likelihood ratio test
- Why SAM is successful:

  - No need to model expression distribution

  - Handles Excel data well