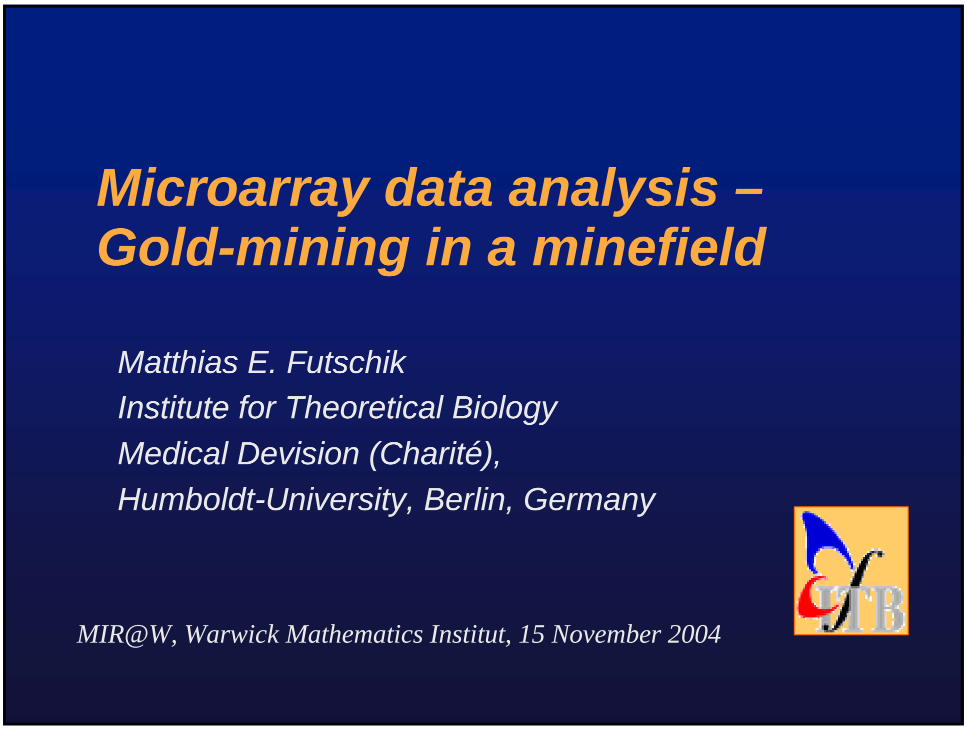
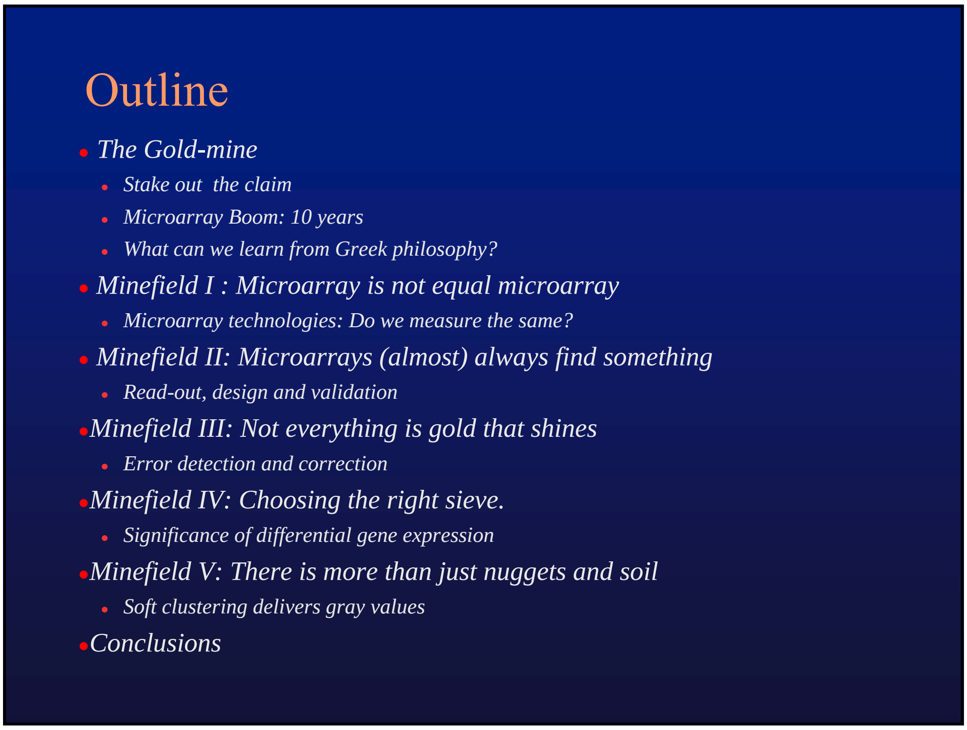


Microarray data analysis – Gold-mining in a minefield

Matthias E. Futschik
Institute for Theoretical Biology
Medical Devision (Charité),
Humboldt-University, Berlin, Germany

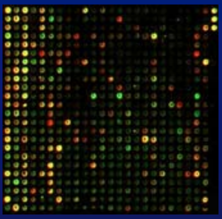
MIR@W, Warwick Mathematics Institut, 15 November 2004

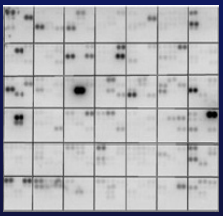


Outline

- *The Gold-mine*
  - *Stake out the claim*
  - *Microarray Boom: 10 years*
  - *What can we learn from Greek philosophy?*
- *Minefield I : Microarray is not equal microarray*
  - *Microarray technologies: Do we measure the same?*
- *Minefield II: Microarrays (almost) always find something*
  - *Read-out, design and validation*
- *Minefield III: Not everything is gold that shines*
  - *Error detection and correction*
- *Minefield IV: Choosing the right sieve.*
  - *Significance of differential gene expression*
- *Minefield V: There is more than just nuggets and soil*
  - *Soft clustering delivers gray values*
- *Conclusions*

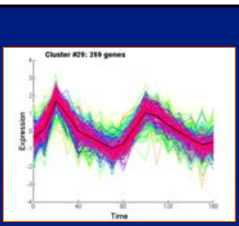
What are microarrays?

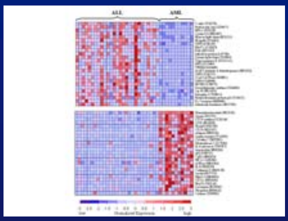




- Microarrays consist of localised spots of oligonucleotides or cDNA attached on glass surface or nylon filter
- Microarrays are based on **base-pair complementarity**
- Different production:
  - Spotted microarrays
  - Photolithographicly synthesised microarrays (Affymetrix)
- Different read-outs:
  - Two-channel (or two-colour) microarrays
  - One-channel (or one-colour) microarrays

Goldmines:



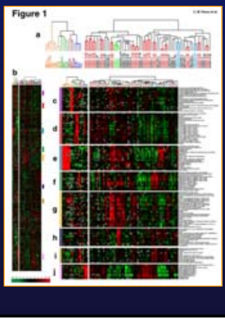


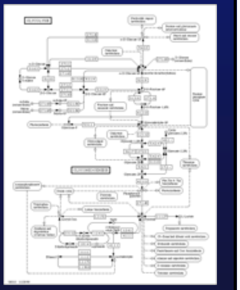
Clustering of genes:

Co-expression and co-regulation go together enabling functional annotation

Clustering of time series

Classification of tissue samples and marker gene identification

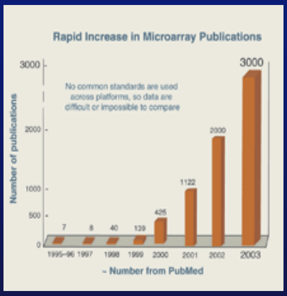




Clustering of arrays: finding new disease subclasses

Reconstruction of gene networks
(*Reverse engineering*)

10 years of microarrays

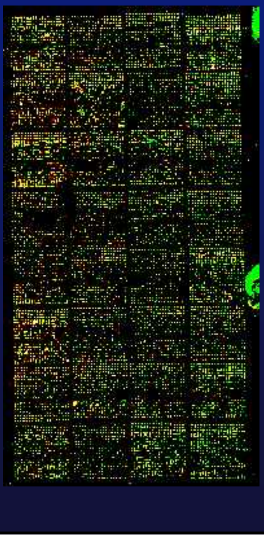


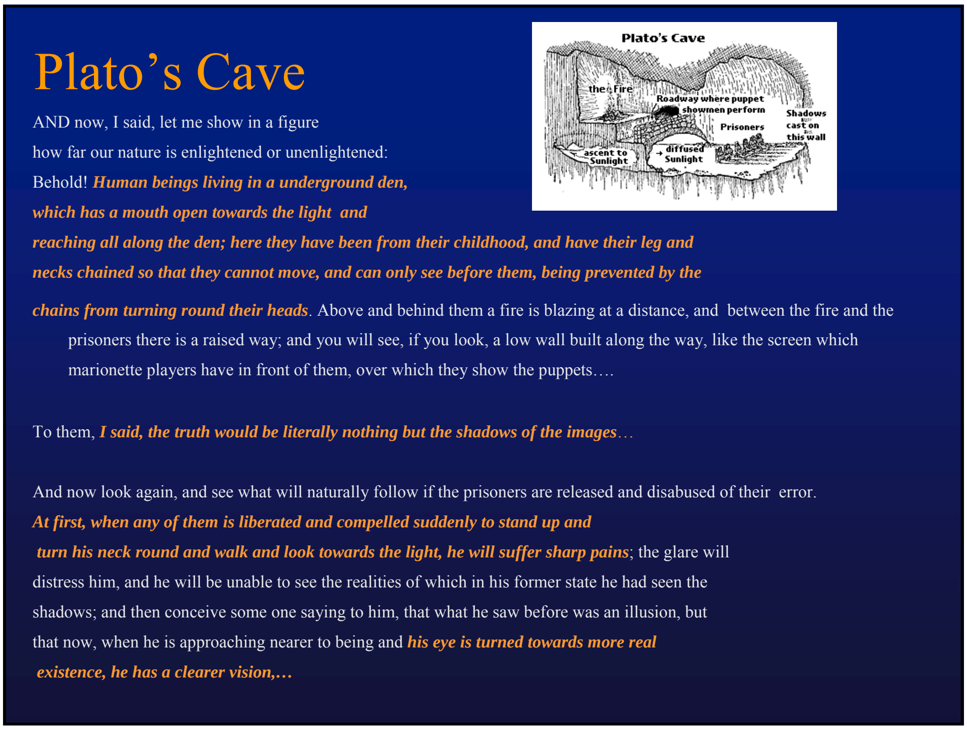
Microarrays have become a standard technology for genomics research.

However, standards are only slowly developing...

... and many minefields remain.

Yeast cDNA microarray





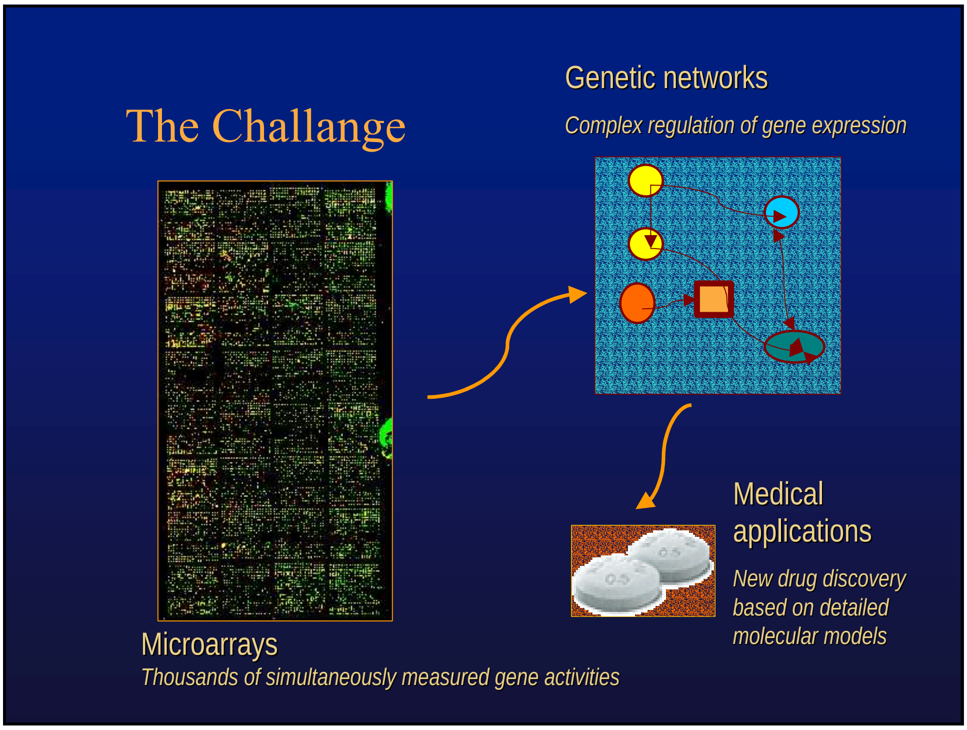
Plato’s Cave

Plato's Cave
the Fire
Roadway where puppet showmen perform
Prisoners
Shadows cast on this wall
ascent to Sunlight
diffused Sunlight

AND now, I said, let me show in a figure how far our nature is enlightened or unenlightened: Behold! ***Human beings living in a underground den, which has a mouth open towards the light and reaching all along the den; here they have been from their childhood, and have their leg and necks chained so that they cannot move, and can only see before them, being prevented by the chains from turning round their heads***. Above and behind them a fire is blazing at a distance, and between the fire and the prisoners there is a raised way; and you will see, if you look, a low wall built along the way, like the screen which marionette players have in front of them, over which they show the puppets….

To them, ***I said, the truth would be literally nothing but the shadows of the images***…

And now look again, and see what will naturally follow if the prisoners are released and disabused of their error. ***At first, when any of them is liberated and compelled suddenly to stand up and turn his neck round and walk and look towards the light, he will suffer sharp pains***; the glare will distress him, and he will be unable to see the realities of which in his former state he had seen the shadows; and then conceive some one saying to him, that what he saw before was an illusion, but that now, when he is approaching nearer to being and ***his eye is turned towards more real existence, he has a clearer vision,…***



The Challange

Genetic networks

Complex regulation of gene expression

Medical applications

New drug discovery based on detailed molecular models

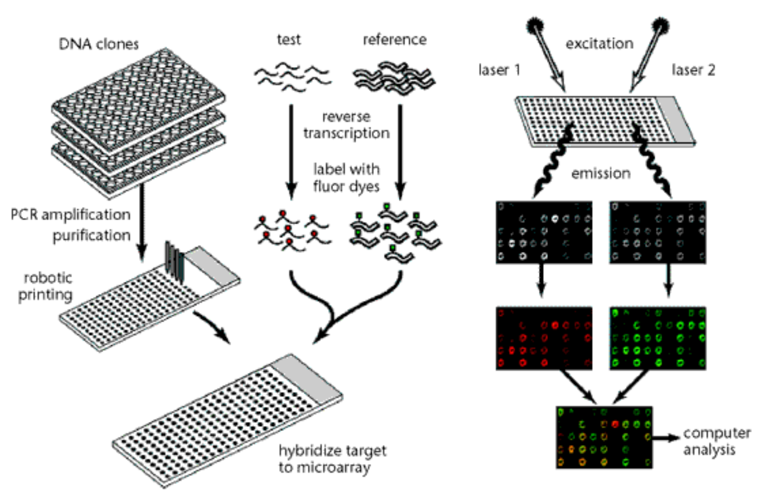
Microarrays

Thousands of simultaneously measured gene activities

Minefield I :

Microarray is not equal micorarray

Typical cDNA microarray experiment



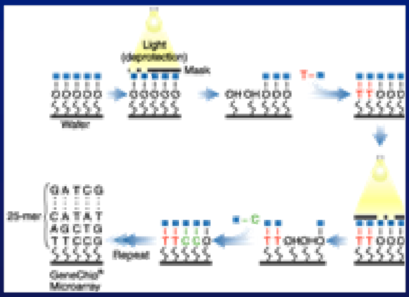
Microarray technology I

- Two-colour microarray (cDNA and spotted oligonucleotide microarrays)
  - Probes are PCR products based on a chosen cDNA library or synthesized oligonucleotides (length 50-70) optimized for specifity and binding properties >> probe design
  - Probes are mechanically spotted. To control variation of amount of printed cDNA/oligos and spot morphology, reference RNA sample is included. Thus, ratios are considered as basic units for analysing gene expression. Absolute intensities should be interpreted with care.

MOVIE 1: Array production - Galbraith lab

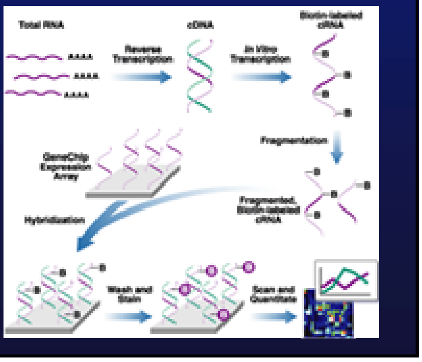
MOVIE 2: Principles – Schreiber lab

Affymetrix GeneChip technology



Production by photolitography

Hybridisation process and biotin labelíng; Fragmentation aims to destroy higher order structures of cRNA

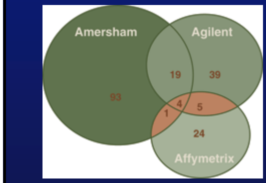


Microarray technologies II

- One-colour microarrays (Affymetrix GeneChips)
  - Measurement of hybridisation of target RNA to sets of 25-oligonucleotides (probes).
  - Probes are paired: Perfect match (PM) and mis-match (MM). PM are complementary to the gene sequence of interest. MM include a single nucleotide changed in the middle position of the oligonucleotide. MM serve for controlling of experimental variation and non-specific cross-hybridisation. Thus, MMs constitute internal references (on the probe site).
  - Average (PM-MM) delivers measure for gene expression. However, different methods to calculates summary indices exist (e.g. MAS,dchip, RMA...)

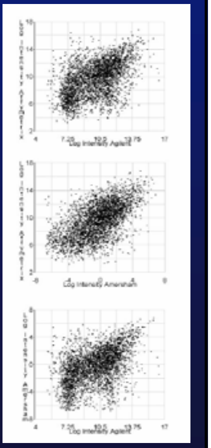
Do different microarray techniques deliver the same?

Evaluation of three commercial microarray platforms by Tan *et al.* NAR 2003



Venn diagram of diffentially expressed genes detected by different platforms

- Comparsion of expression in PANC-1 cells grown in serum-rich medium and after removal of serum
- Biological and technical replicates included
- 2009 genes with same GenBank ID present on all platforms

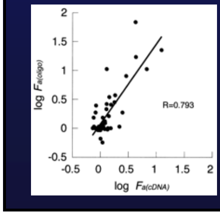


Correlations of mRNA measurements

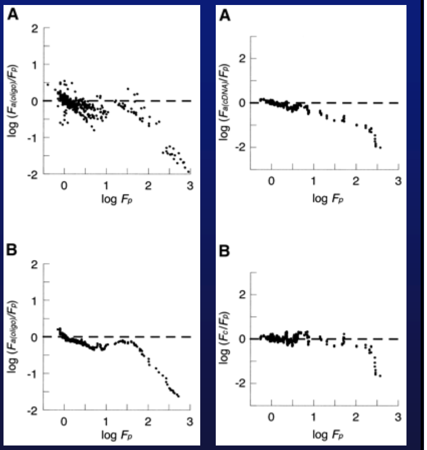
Can we trust microarrays at all?

Assessment of accuacy of Affymetrix chips and custom cDNA microarrays by Yuen et al. NAR 2002

- Analysis of gene expression in LβT2 cells after treatment with GnRh or vehicle by Affymetrix and cDNA microarrays
- Comparison with fold changes detected by qRTPCR
- Underestimation of fold changes by microarrays
- Calibration possible for cDNA microarrays: log(FC_{PCR}) = 1.8 log (FC_{cDNA})



Correlation of log fold changes

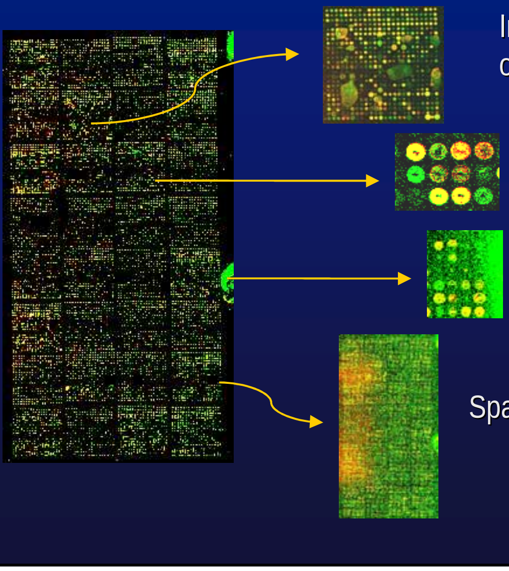


Bias of Affymetrix and cDNA microarray measurements

Minefield II :

Microarray always find something

From Images to Numbers



Impurities, overlapping spots

Donut-shaped spots, Inhomogeneous intensities

Local Background

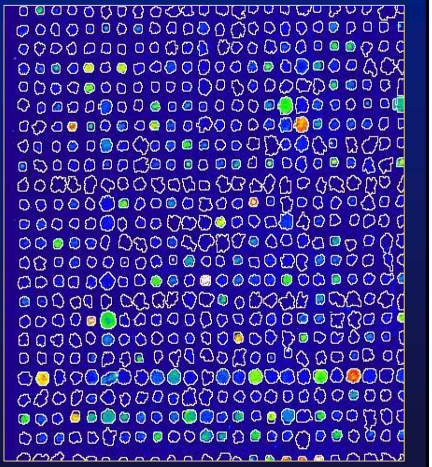
Spatial bias

Image Analysis

1. Localisation of spots: locate centres after (manual) adjustment of grid

2. Segmentation: classification of pixels either as signal or background. Different procedures to define background.

3. Signal extraction: for each spot of the array, calculates signal intensity pairs, background and quality measures.



Data acquisition

- Scans of slides are usually stored in 16-bit TIFF files. Thus, scanned intensities vary between 0 and $2^{16.}$
- Scanning of separate channels can adjusted by selection of laser power and gain of photo-multiplier.
  - Common aim: balancing of channels.
  - Common problems: avoiding of saturation of high intensity spots while increasing signal to noise ratios.
- Image processing software produces a variety of measures: Spot intensities, local background, spot morphology measures. Software vary in computational approaches of image segmentation and read-out.
- Open issues:
  - local background correction
  - derivation of ratios for spot intensities
  - flagging of spots,
  - multiple scanning procedures

Design of experiment

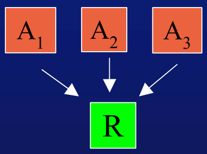
Two channel microarrays incorporate a reference sample.
Choice of reference determines follow-up analysis.

Reference design:

All samples are co-hybridised with common reference sample

. Advantage: Robust and scalable. Length of path of direct comparison equals 2.

. Disadvantage: Half of the measurements are made on reference sample which is commonly of little or no interest

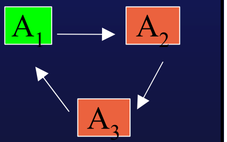


Alternative Designs:

. Dye-swap design: each comparison includes dye-swap to distinguish dye effects from differential expression (important for direct labelling method)

. Loop-design: No reference sample is involved. Increase of efficiency is, however, accompanied with a decrease of robustness.

. Latin-square design: classical design to separate effects of different experimental factors



Comparison of designs:

Yang and Speed,
Nature genetics reviews, 2002

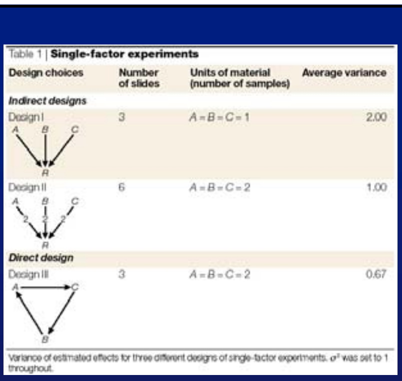


Table 1 | **Single-factor experiments**

Design choices	Number of slides	Units of material (number of samples)	Average variance
Indirect designs			
Design I	3	$A = B = C = 1$	2.00
Design II	6	$A = B = C = 2$	1.00
Direct design			
Design III	3	$A = B = C = 2$	0.67

Variance of estimated effects for three different designs of single-factor experiments. σ^2 was set to 1 throughout.

Define before experiment what differences (contrasts) should be determined to make best use out of (usually) limited number of arrays

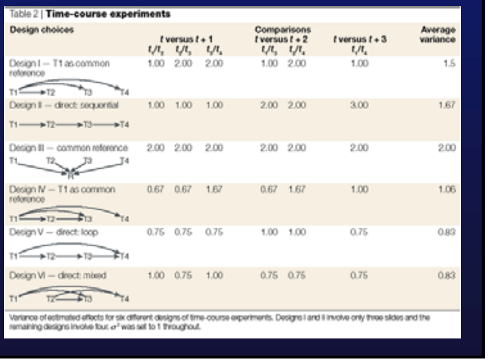


Table 2 | **Time-course experiments**

Design choices	Comparisons						Average variance
	t versus *t* + 1			*t* versus *t* + 2		*t* versus *t* + 3	
	t_1/t_2	t_2/t_3	t_3/t_4	t_1/t_3	t_2/t_4	t_1/t_4	
Design I – T1 as common reference	1.00	2.00	2.00	1.00	2.00	1.00	1.5
Design II – direct: sequential	1.00	1.00	1.00	2.00	2.00	3.00	1.67
Design III – common reference	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Design IV – T1 as common reference	0.67	0.67	1.67	0.67	1.67	1.00	1.06
Design V – direct: loop	0.75	0.75	0.75	1.00	1.00	0.75	0.83
Design VI – direct: mixed	1.00	0.75	1.00	0.75	0.75	0.75	0.83

Variance of estimated effects for six different designs of time-course experiments. Designs I and II involve only three slides and the remaining designs involve four. σ^2 was set to 1 throughout.

Sources of variation in gene expression measurements using microarrays

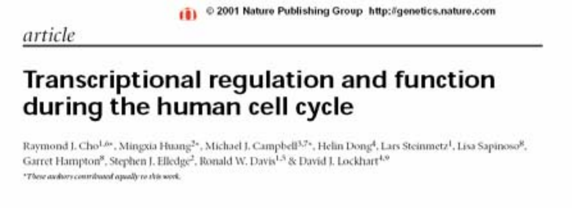
- Microarray platform
- Manufacturing or spotting process
  - Manufacturing batch
  - Amplification by PCR and purification
  - Amount of cDNA spotted, morphology of spot and binding of cDNA to substrate
- mRNA extraction and preparation
  - Protocol of mRNA extraction and amplification
  - Labelling of mRNA
- Hybridisation
  - Hybridisation conditions such as temperature, humidity, hyb-buffer,...
- Scanning
  - Type of scanner
  - Scanning intensity and PMT settings
- Imaging
  - Software
  - Flagging, background correction,...

Planning an microarray experiment

Essentials:

- Technical replicates assess variability induced by experimental procedures.
- Biological replicates (assess generality of results).
- Number of replicates depends on desired sensitivity and sensibility of measurements and research goal.
- Randomisation to avoid confounding of experimental factors. Blocking to reduce number of experimental factors.
- Control spots assess reproducibility within and between array, background intensity, cross-hybridisation and/or sensitivity of measurement. They can consists of empty spots or hybridisation-buffer, genomic DNA, foreign DNA, house-holding genes of foreign (non-cross-hybridising) cDNA.
- Validation of results is crucial
  - by other experimental techniques (e.g. Northern, RT-PCR)
  - By comparison with independent experiments.

What can go wrong:



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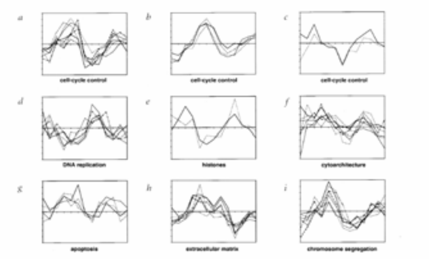
article

Transcriptional regulation and function during the human cell cycle

Raymond J. Cho[1,6*], Mingxia Huang[2*], Michael J. Campbell[3,7*], Helin Dong[4], Lars Steinmetz[1], Lisa Sapinoso[8], Garret Hampton[8], Stephen J. Elledge[2], Ronald W. Davis[1,5] & David J. Lockhart[4,9]

*These authors contributed equally to this work.

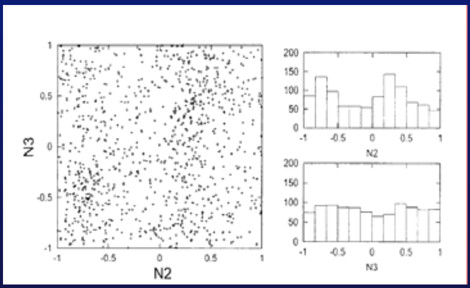
- Measurement of gene expression in human fibroblasts
- Duplicate experiments spanning two cell cycle; measurements were subsequently averaged
- Synchronization by double thymidine-block
- Idenfication of 700 periodically expressed genes (300 uncharacterized ESTs)



Analysis of cell-cycle-specific gene expression in human cells as determined by microarrays and double-thymidine block synchronization

Kerby Shedden* and Stephen Cooper†‡

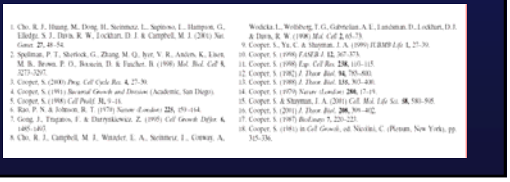
*Department of Statistics, University of Michigan, Ann Arbor, MI 48109-1285; and †Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI 48109-0620



Check of reproducibility: Comparison of results of the two replicated cell-cycle experiments

>> No significant correlation of cyclicity
Failure of synchronization?

Peak times in both experiments



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15. Cooper, S. & Shayman, J. A. (2001) *Cell. Mol. Life Sci.* **58**, 580–595.
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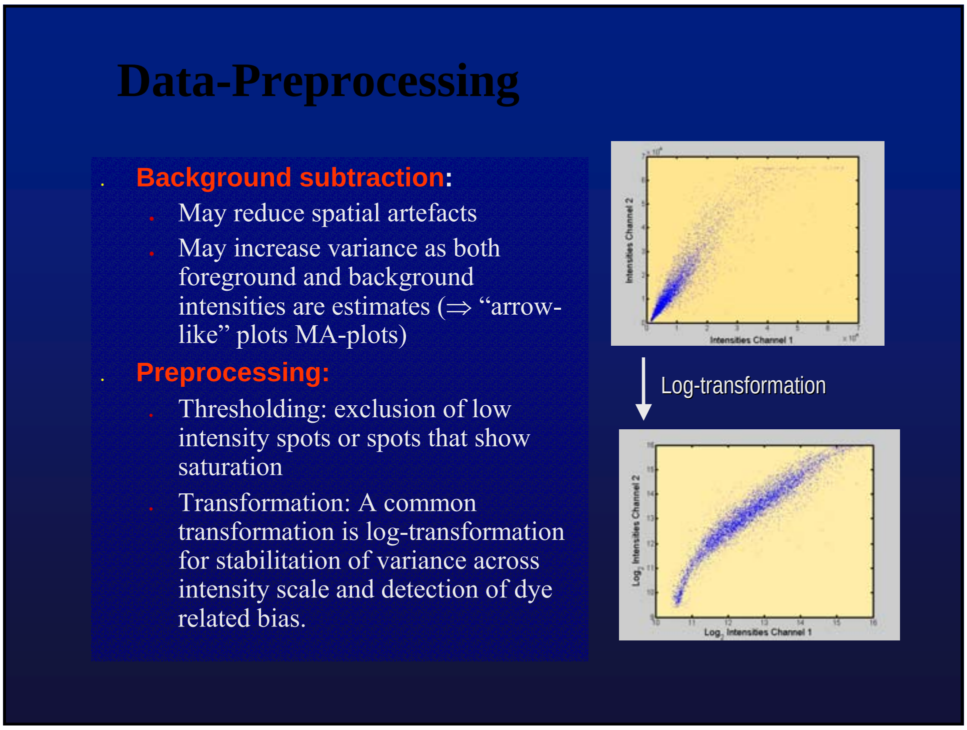
18. Cooper, S. (1981) in *Cell Growth*, ed. Nicolini, C. (Plenum, New York), pp. 315–336.

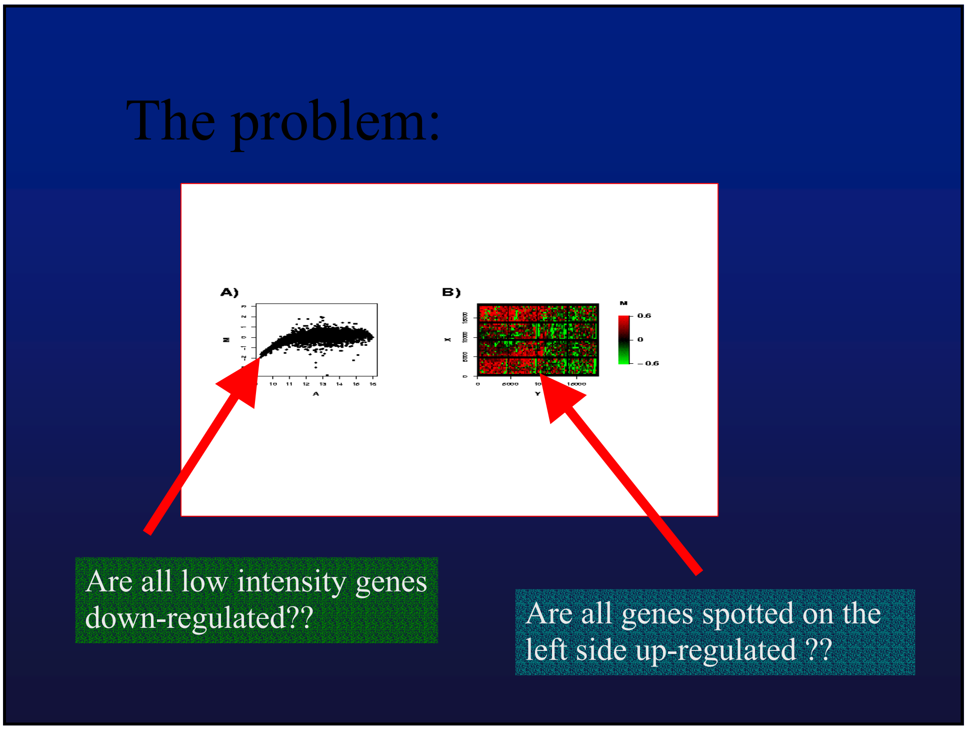
Minefield III :
Not everything is gold that shines



Data-Preprocessing

- **Background subtraction:**
  - May reduce spatial artefacts
  - May increase variance as both foreground and background intensities are estimates (⇒ “arrow-like” plots MA-plots)
- **Preprocessing:**
  - Thresholding: exclusion of low intensity spots or spots that show saturation
  - Transformation: A common transformation is log-transformation for stabilitation of variance across intensity scale and detection of dye related bias.

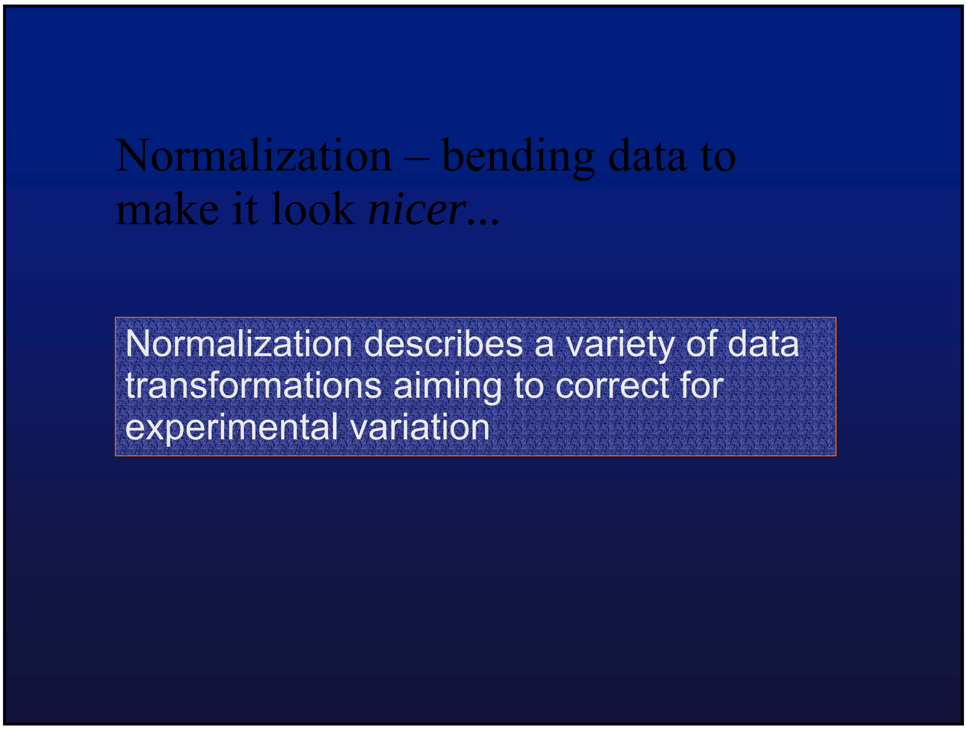
Log-transformation



The problem:

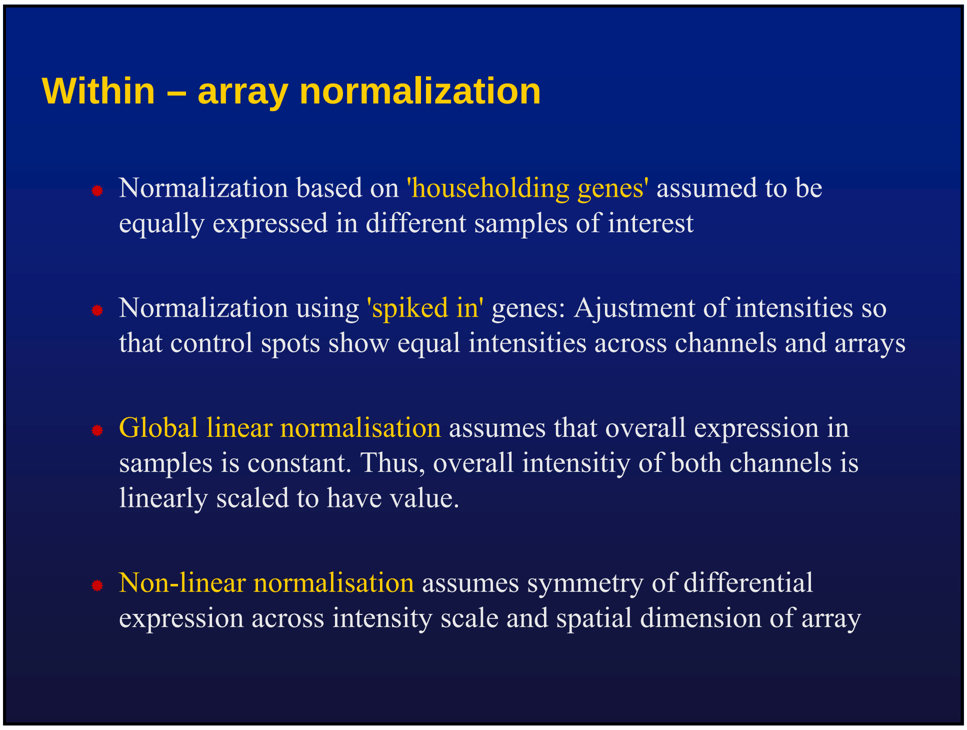
Are all low intensity genes down-regulated??

Are all genes spotted on the left side up-regulated ??



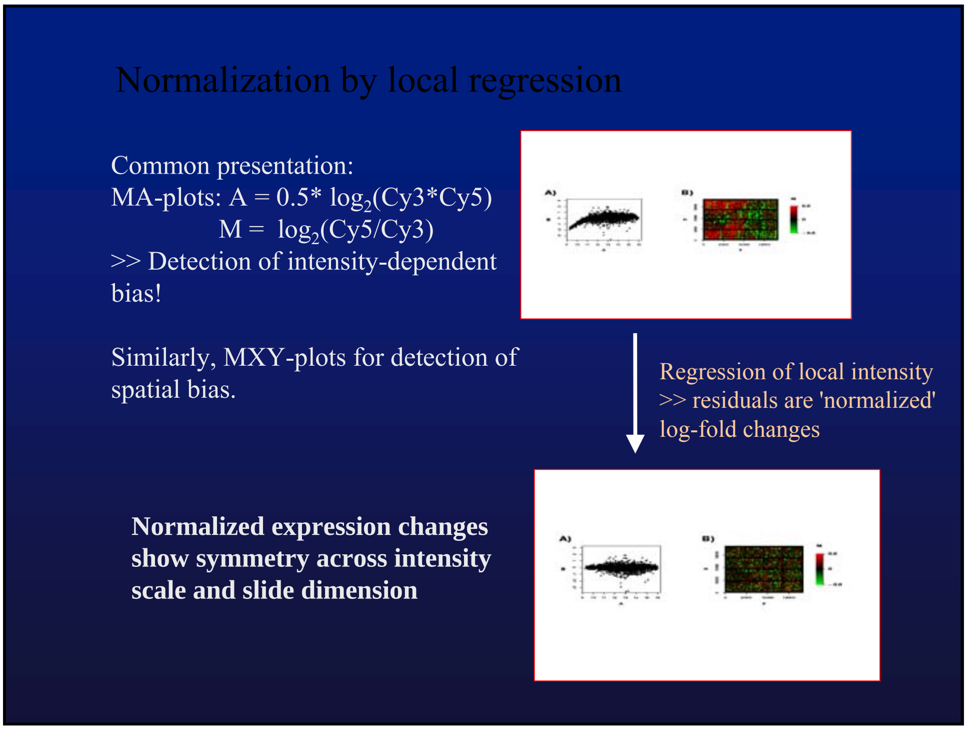
Normalization – bending data to make it look *nicer*...

Normalization describes a variety of data transformations aiming to correct for experimental variation



Within – array normalization

- Normalization based on 'householding genes' assumed to be equally expressed in different samples of interest
- Normalization using 'spiked in' genes: Ajustment of intensities so that control spots show equal intensities across channels and arrays
- Global linear normalisation assumes that overall expression in samples is constant. Thus, overall intensitiy of both channels is linearly scaled to have value.
- Non-linear normalisation assumes symmetry of differential expression across intensity scale and spatial dimension of array



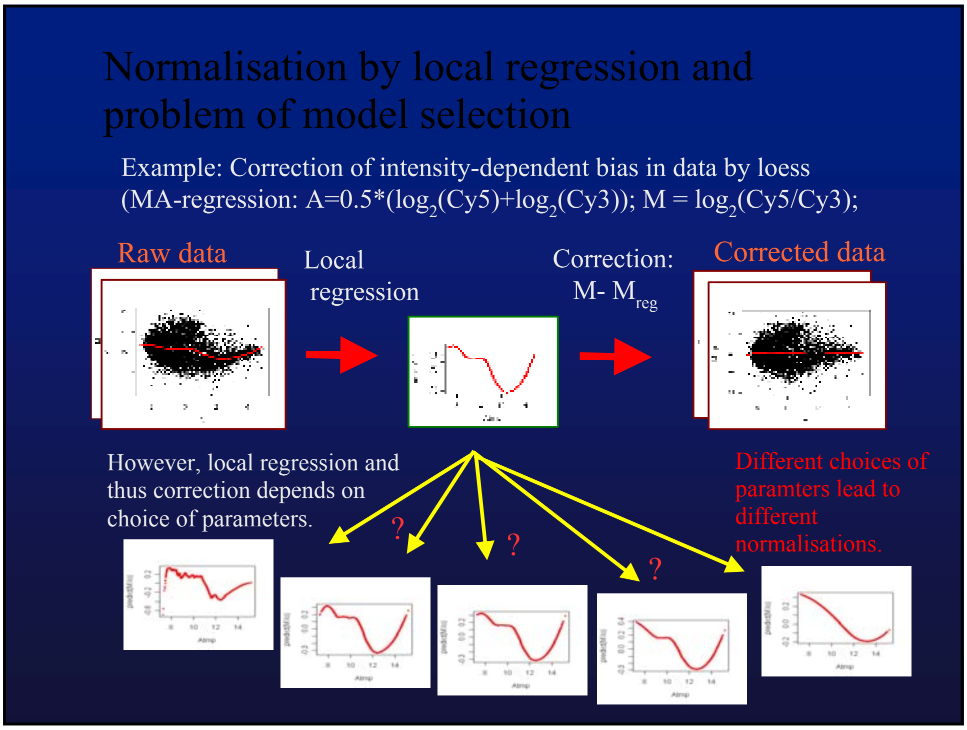
Normalization by local regression

Common presentation:
MA-plots: A = 0.5* $\log_2$(Cy3*Cy5)
M = $\log_2$(Cy5/Cy3)
>> Detection of intensity-dependent bias!

Similarly, MXY-plots for detection of spatial bias.

Regression of local intensity
>> residuals are 'normalized' log-fold changes

Normalized expression changes show symmetry across intensity scale and slide dimension



Normalisation by local regression and problem of model selection

Example: Correction of intensity-dependent bias in data by loess (MA-regression: A=0.5*($\log_2$(Cy5)+$\log_2$(Cy3)); M = $\log_2$(Cy5/Cy3);

Raw data

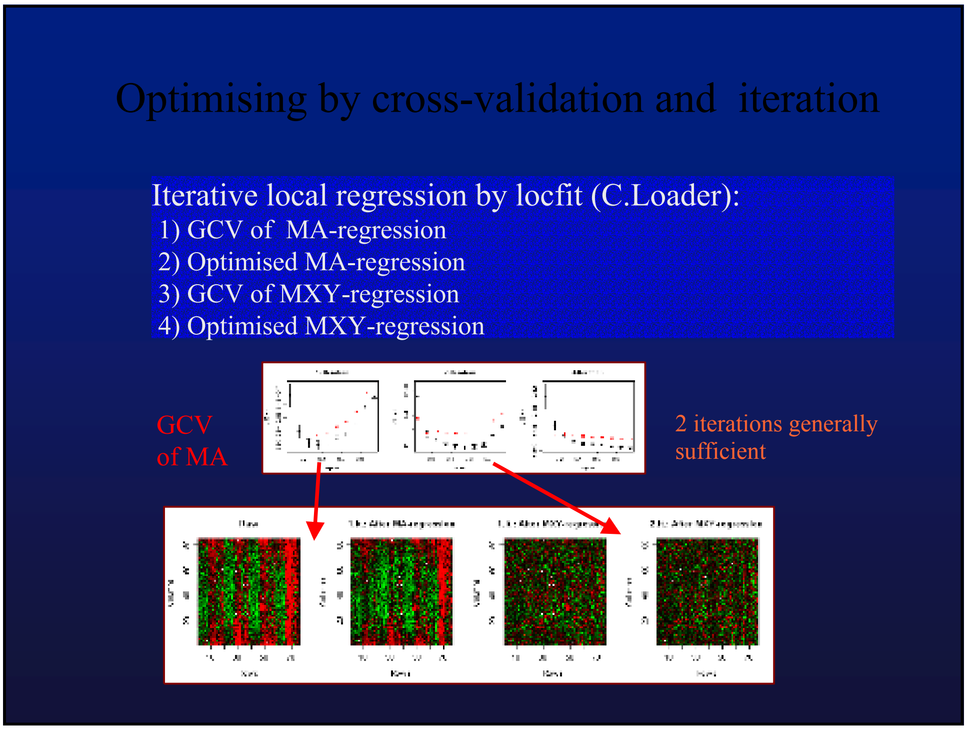
Local regression

Correction: M- M_{reg}

Corrected data

However, local regression and thus correction depends on choice of parameters.

Different choices of paramters lead to different normalisations.



Optimising by cross-validation and iteration

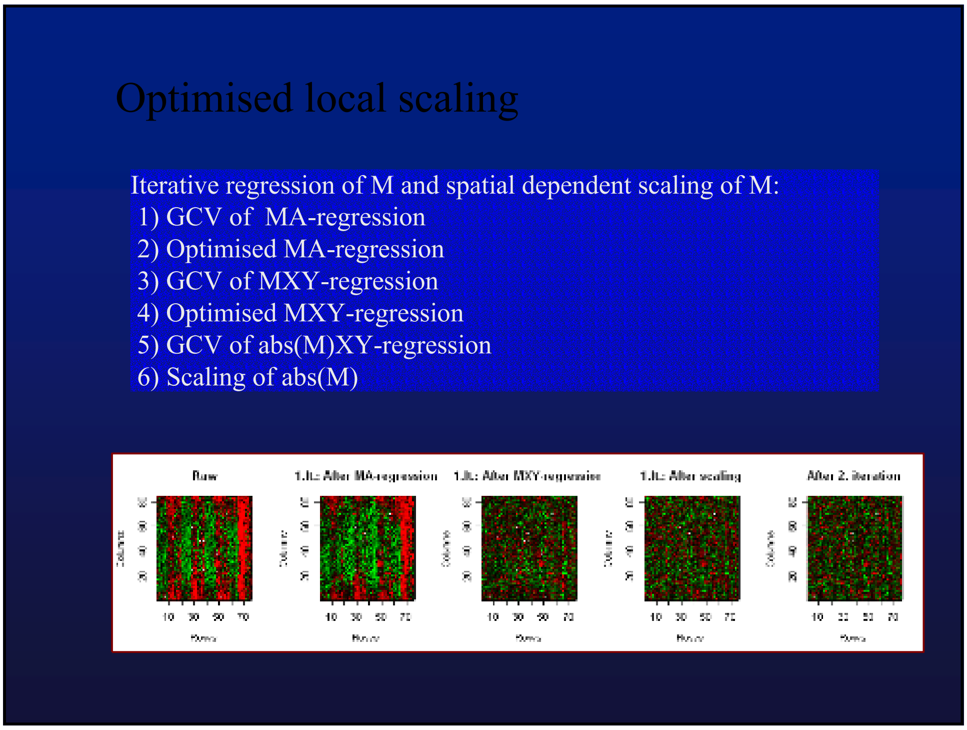
Iterative local regression by locfit (C.Loader):

1) GCV of MA-regression
2) Optimised MA-regression
3) GCV of MXY-regression
4) Optimised MXY-regression

GCV
of MA

2 iterations generally
sufficient

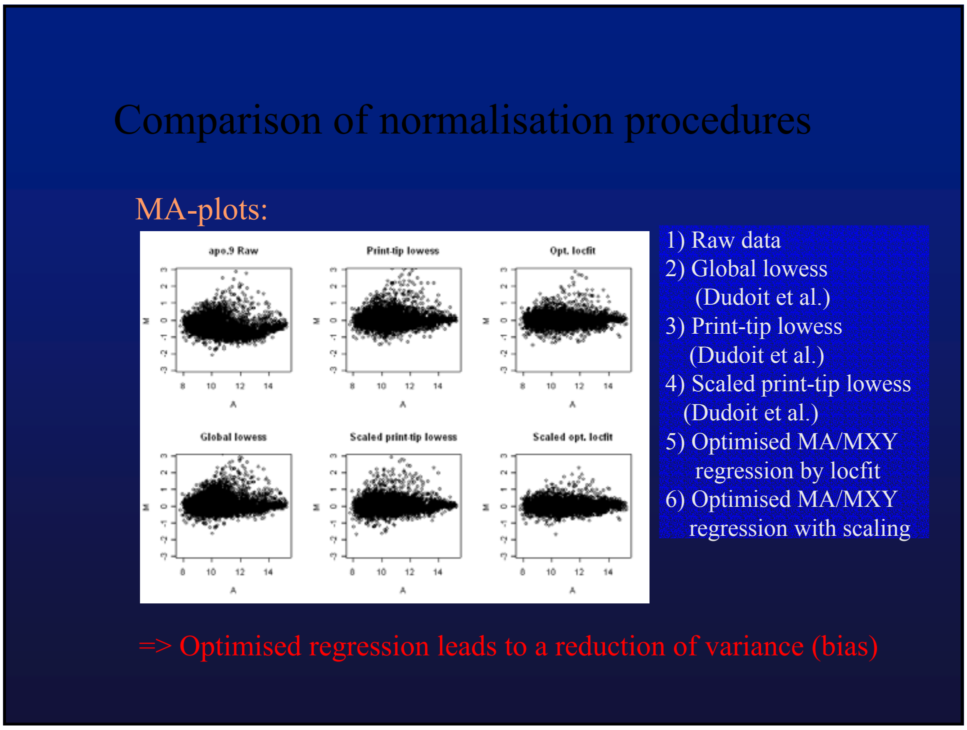
Raw
1.It.: After MA-regression
1.It.: After MXY-regressio
2.It.: After MXY-regression
Column
Row



Optimised local scaling

Iterative regression of M and spatial dependent scaling of M:

1) GCV of MA-regression
2) Optimised MA-regression
3) GCV of MXY-regression
4) Optimised MXY-regression
5) GCV of abs(M)XY-regression
6) Scaling of abs(M)

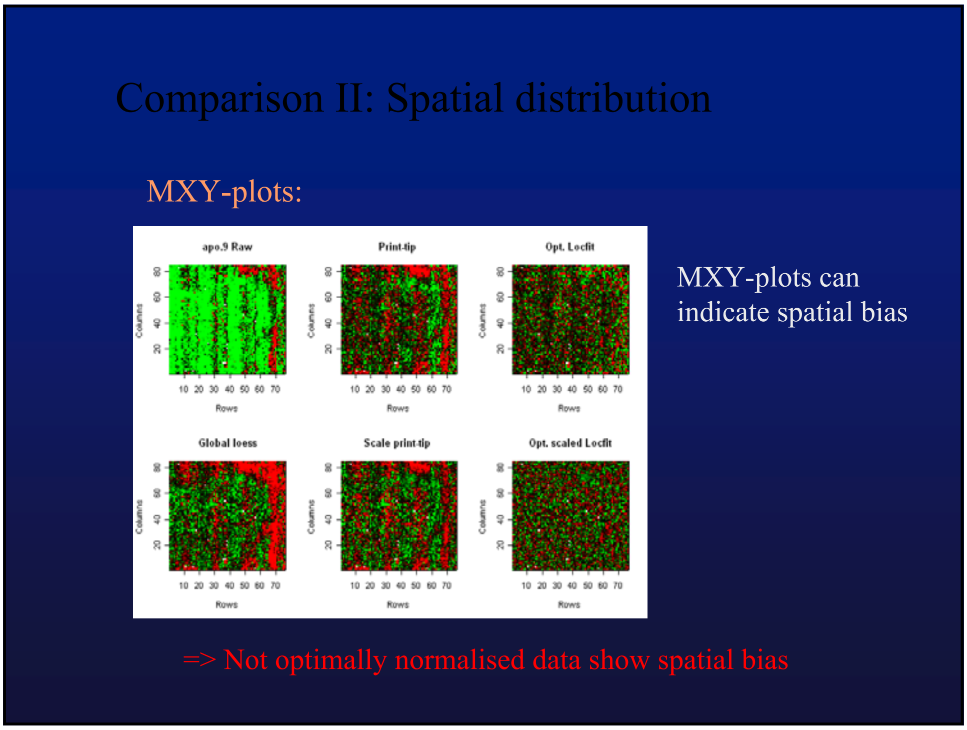


Comparison of normalisation procedures

MA-plots:

1) Raw data
2) Global lowess (Dudoit et al.)
3) Print-tip lowess (Dudoit et al.)
4) Scaled print-tip lowess (Dudoit et al.)
5) Optimised MA/MXY regression by locfit
6) Optimised MA/MXY regression with scaling

=> Optimised regression leads to a reduction of variance (bias)

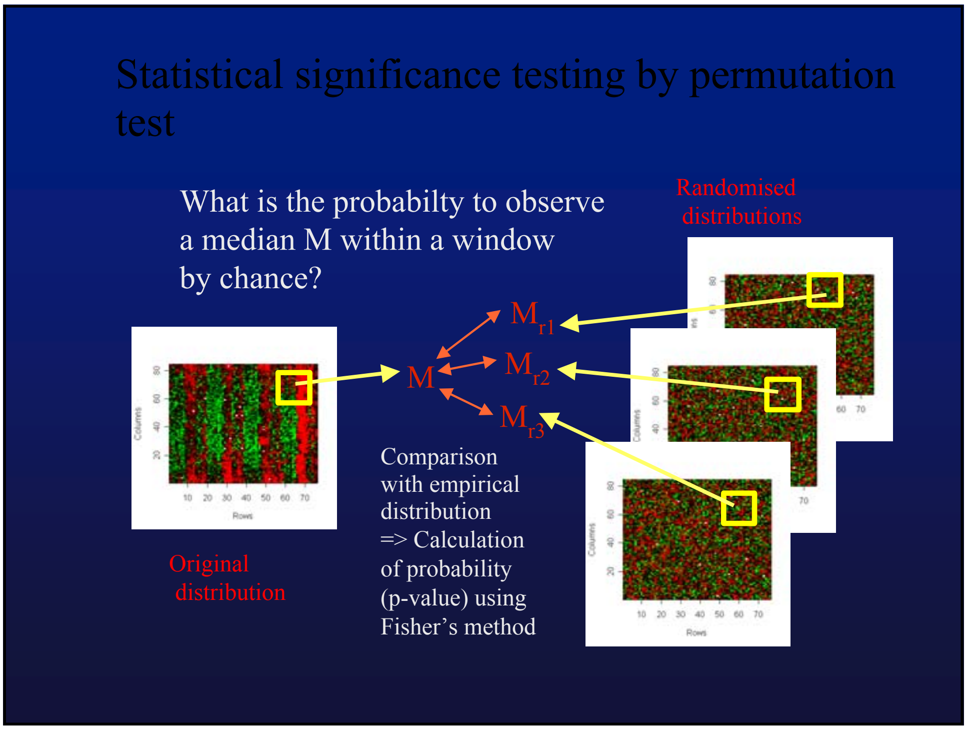


Comparison II: Spatial distribution

MXY-plots:

MXY-plots can indicate spatial bias

=> Not optimally normalised data show spatial bias



Statistical significance testing by permutation test

What is the probabilty to observe a median M within a window by chance?

Randomised distributions

M_{r1}

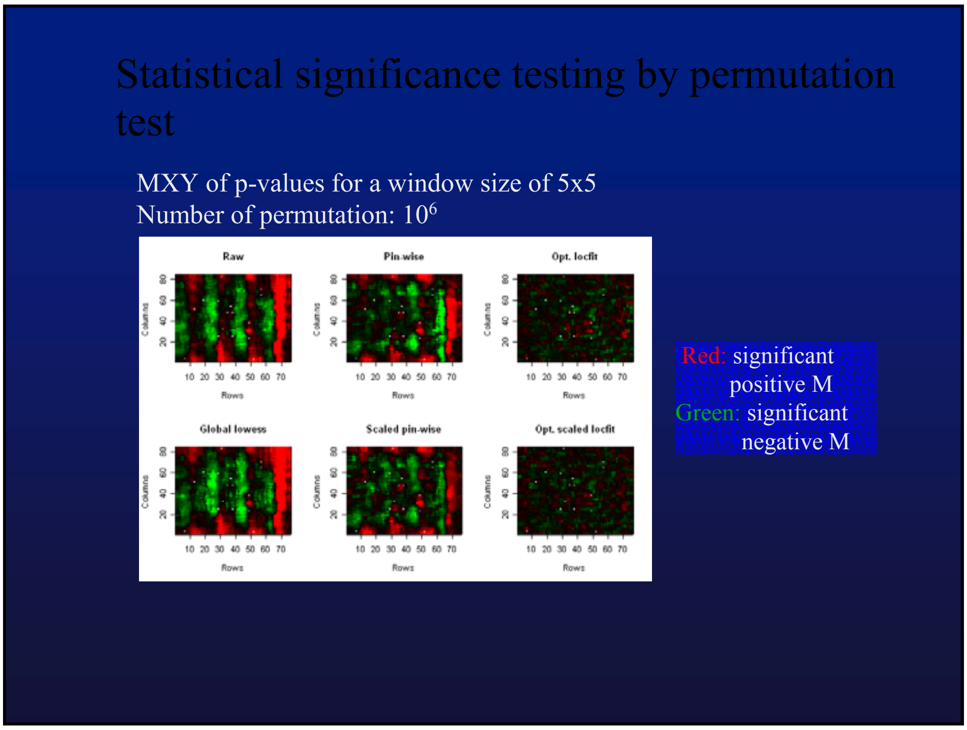
M

M_{r2}

M_{r3}

Original distribution

Comparison with empirical distribution => Calculation of probability (p-value) using Fisher’s method



Statistical significance testing by permutation test

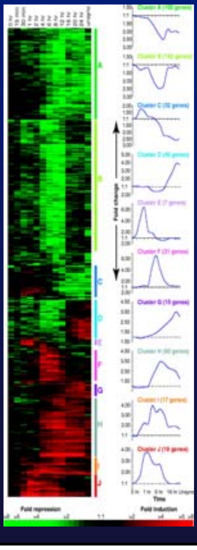
MXY of p-values for a window size of 5x5

Number of permutation: 10^6

Red: significant positive M

Green: significant negative M

"Ultimately, the challenge is not to make "statistically nice" data, but to recover something that is a reflection of reality, and this manuscript suffers from a failure to actually validate the results arising from the application of these normalization methods."

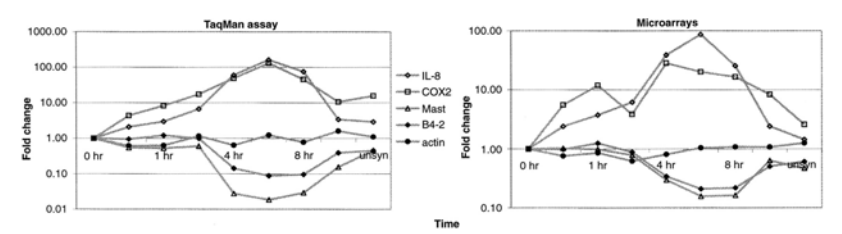


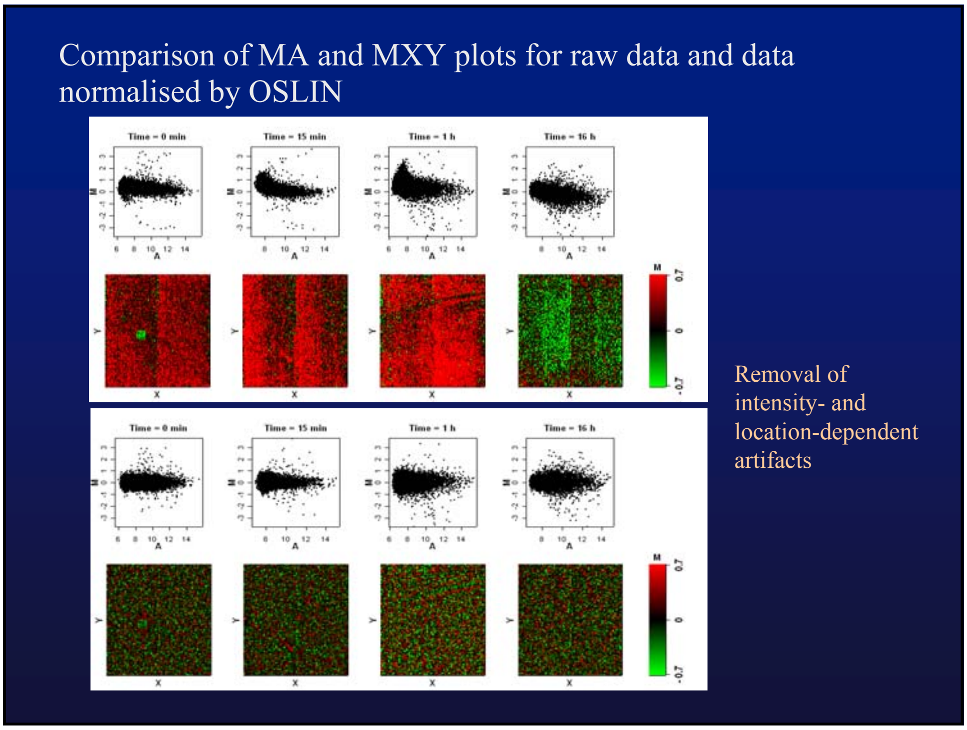
Case study: The transcriptional program in the response of human fibroblasts to serum by Iyer et al., Science, 1999

- Monitoring of temporal changes in gene expression in after addition of fetal bovine serum with cDNA arrays representing 8600 genes
- Detection of distinct clusters relating to cell cycle progression and wound repair

Verification of microarray measurements by RTPCR

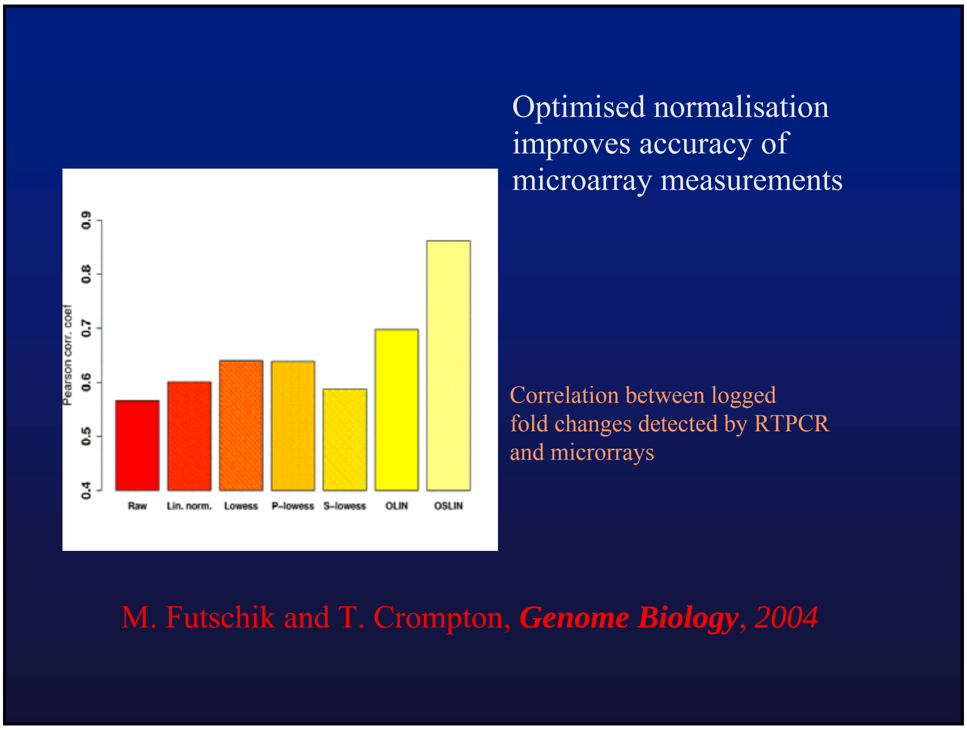
- Correlation of logged fold changes (note underestimation by microarray)
- Derivation for COX2 because of "localized area of low intensity on array scan"





Comparison of MA and MXY plots for raw data and data normalised by OSLIN

Removal of intensity- and location-dependent artifacts



Optimised normalisation improves accuracy of microarray measurements

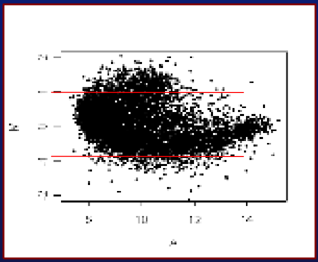
Correlation between logged fold changes detected by RTPCR and microrrays

M. Futschik and T. Crompton, ***Genome Biology***, *2004*

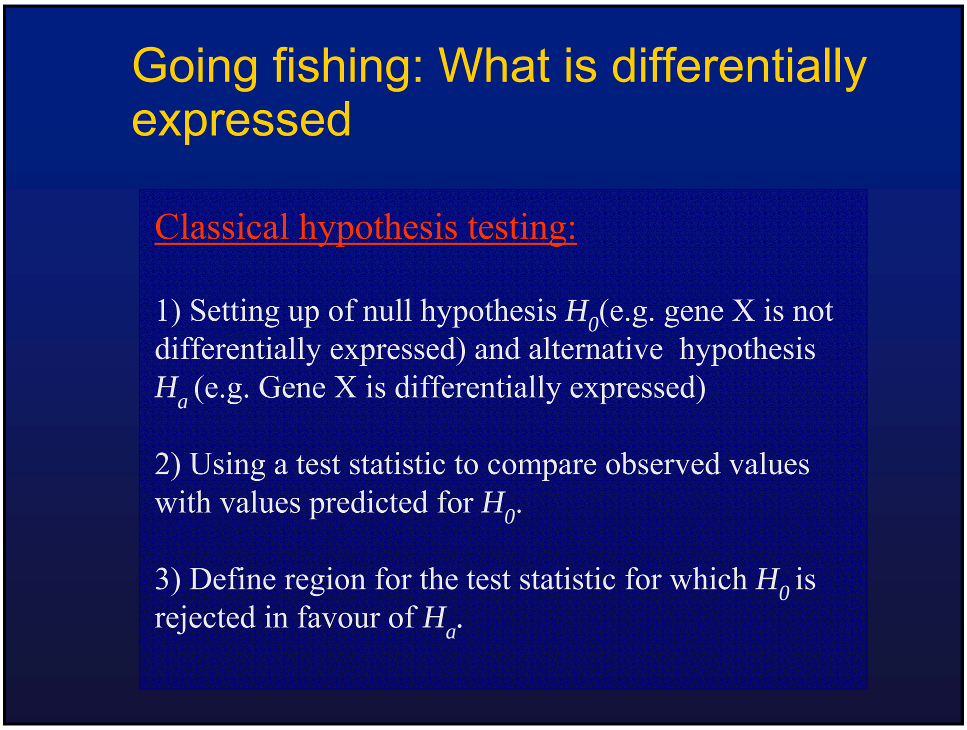
Minefield IV :
Choosing the right sieve

Detection of differential expression

- What makes differential expression differential expression? What is noise?
- Foldchanges are commonly used to quantify differenitial expression but can be misleading (intensity-dependent).
- Basic challange: Large number of (dependent/correlated) variables compared to small number of replicates (if any).



Can you spot the interesting spots?



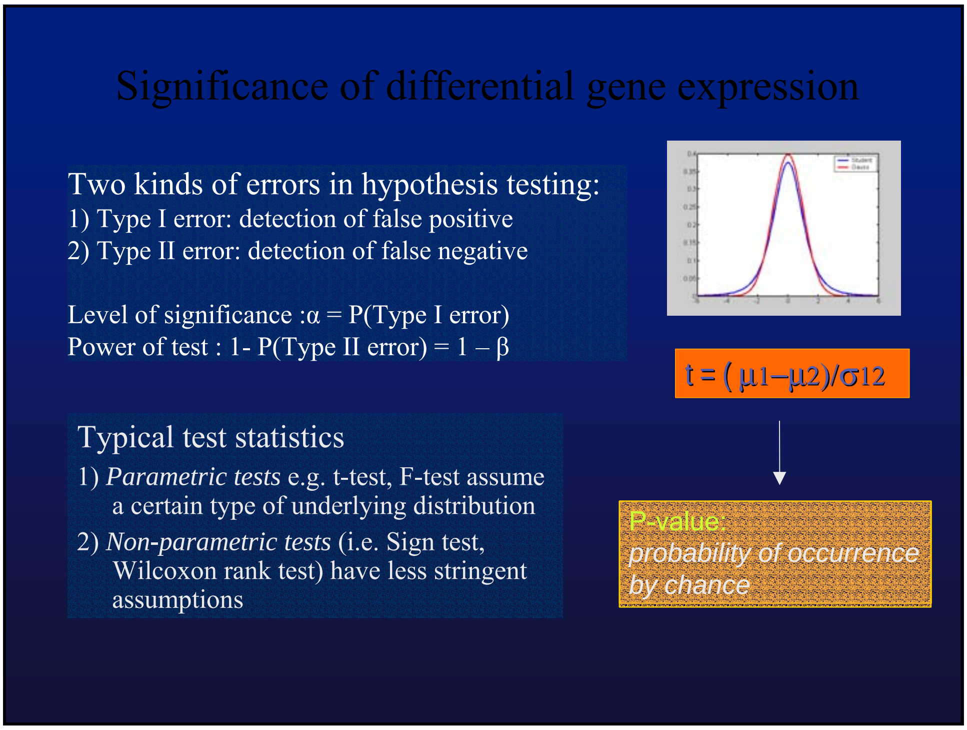
Going fishing: What is differentially expressed

Classical hypothesis testing:

1) Setting up of null hypothesis H_0(e.g. gene X is not differentially expressed) and alternative hypothesis H_a (e.g. Gene X is differentially expressed)

2) Using a test statistic to compare observed values with values predicted for H_0.

3) Define region for the test statistic for which H_0 is rejected in favour of H_a.



Significance of differential gene expression

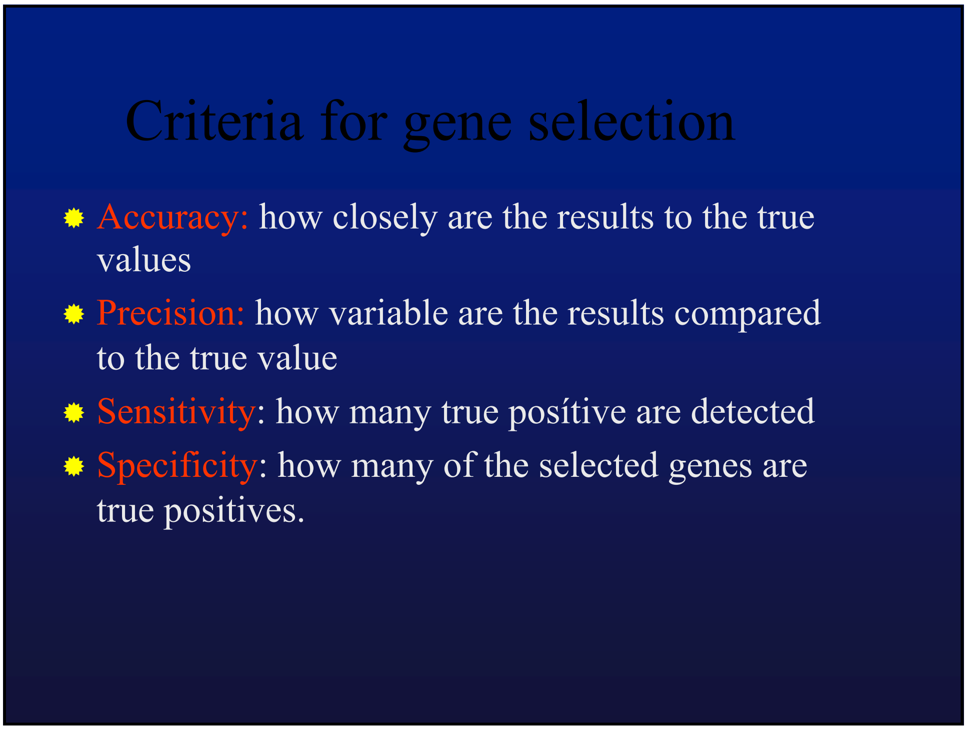
Two kinds of errors in hypothesis testing:
1) Type I error: detection of false positive
2) Type II error: detection of false negative

Level of significance :α = P(Type I error)
Power of test : 1- P(Type II error) = 1 – β

Typical test statistics
1) *Parametric tests* e.g. t-test, F-test assume a certain type of underlying distribution
2) *Non-parametric tests* (i.e. Sign test, Wilcoxon rank test) have less stringent assumptions

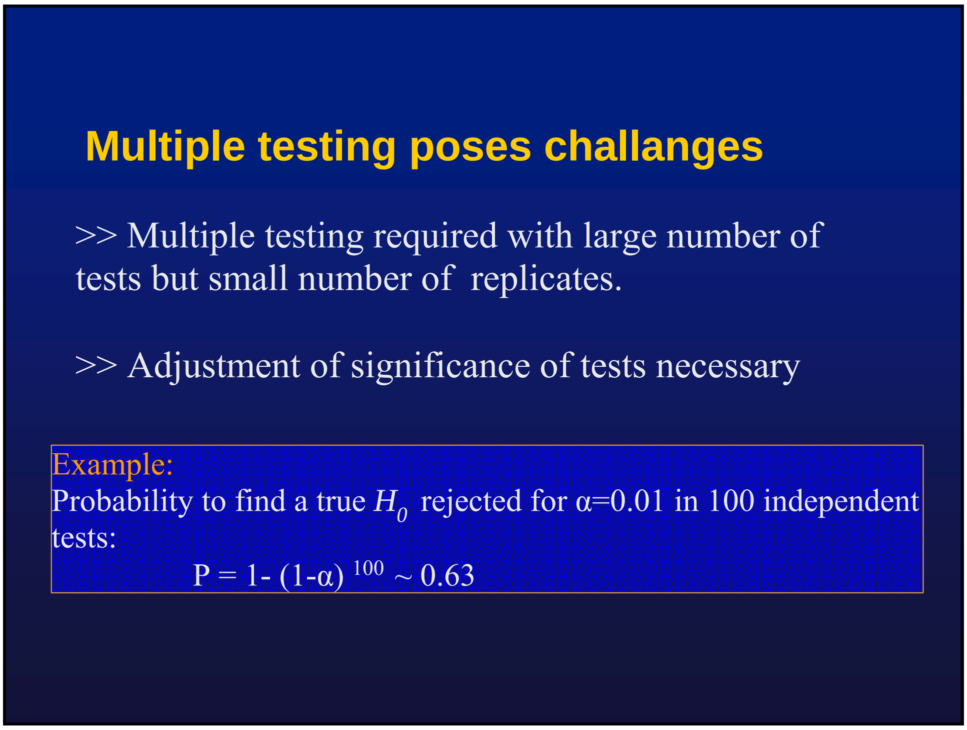
$t = (\mu1–\mu2)/\sigma12$

P-value:
probability of occurrence by chance



Criteria for gene selection

- Accuracy: how closely are the results to the true values
- Precision: how variable are the results compared to the true value
- Sensitivity: how many true posítive are detected
- Specificity: how many of the selected genes are true positives.



Multiple testing poses challanges

>> Multiple testing required with large number of tests but small number of replicates.

>> Adjustment of significance of tests necessary

Example:
Probability to find a true H_0 rejected for $\alpha=0.01$ in 100 independent tests:

$$P = 1- (1-\alpha)^{100} \sim 0.63$$

Compound error measures:

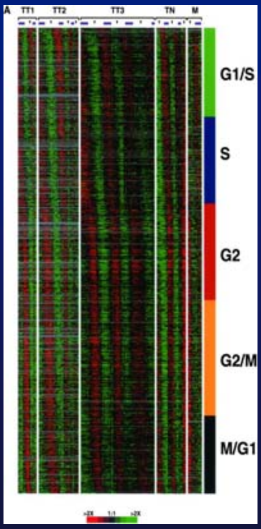
Per comparison error rate: PCER= E[V]/N
Familiywise error rate: FWER=P(V≥1)
False discovery rate: FDR= E[V/R]

N: total number of tests
V: number of reject true H_0 (FP)
R: number of rejected *H* (TP+FP)

Aim to control the error rate:

1) by p-value adjustment (step-down procedures: Bonferroni, Holm, Westfall-Young, ...)

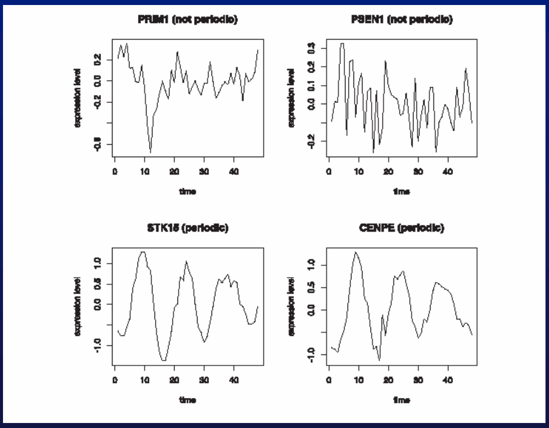
2) by direct comparison with a background distribution (commonly generated by random permuation)



Identification of periodically expressed genes in human cell cycle by Whitfield *et al.*, 2002, *Molecular Biology of the Cell*

- Expression in HeLa cells were monitored using cDNA arrays
- Several synchronization protocols were used to detect artifacts
- 800 genes were detected as periodically expressed by spectral analysis.

A second look reveals doubtful classifications



All genes were identified as periodically expressed by Whitfield *et al.*

Wichert et al., *Bioinformatics*, 2004

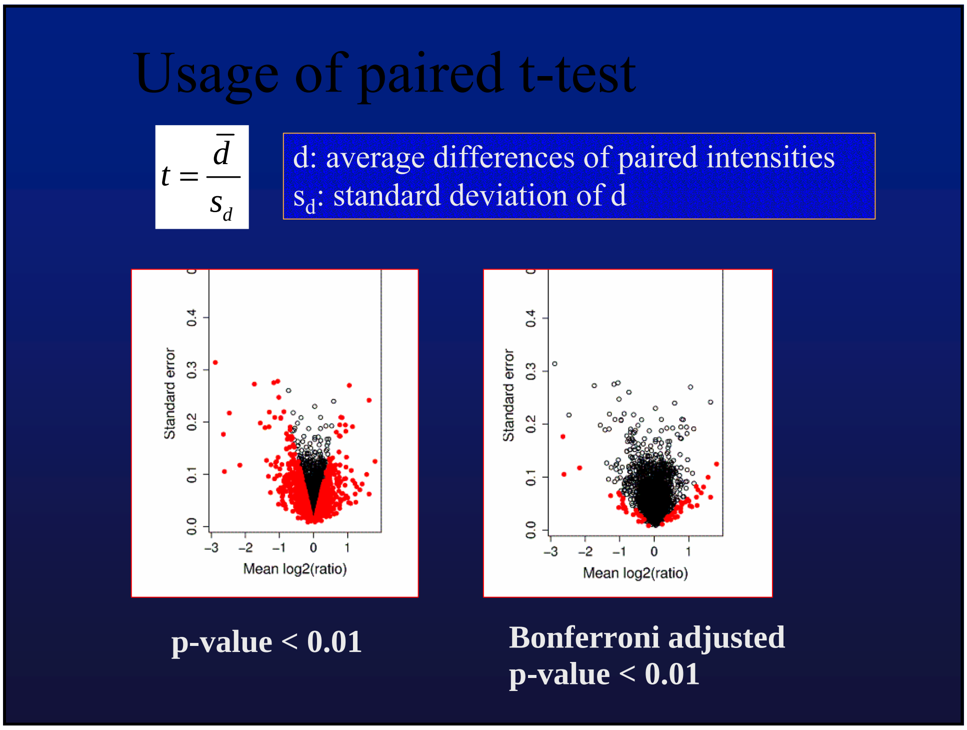
Constistency of replications

Case study: SW480/620 cell line comparison
SW480: derived from primary tumour
SW 620: derived from lymphnode metastisis of same patient

⇒ Model for cancer progression

Experimental design:

- 4 independent hybridisations (technical replicates)#
- 4000 genes



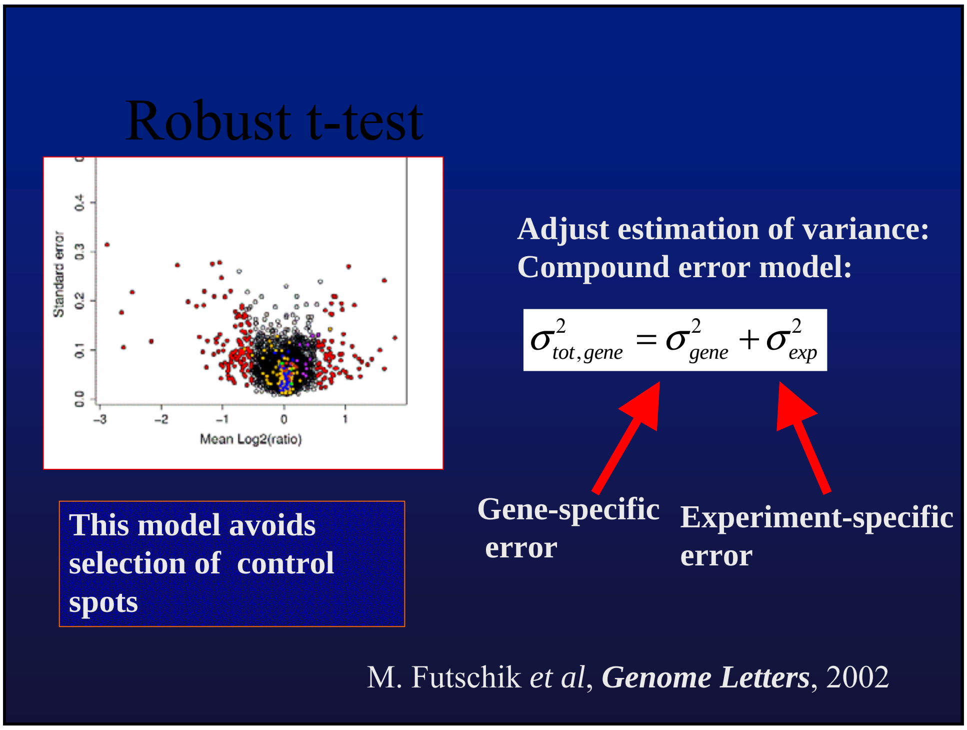
Usage of paired t-test

$$t = \frac{\bar{d}}{s_d}$$

d: average differences of paired intensities
s_d: standard deviation of d

p-value < 0.01

Bonferroni adjusted p-value < 0.01



Robust t-test

Adjust estimation of variance: Compound error model:

$$\sigma^2_{tot,gene} = \sigma^2_{gene} + \sigma^2_{exp}$$

Gene-specific error

Experiment-specific error

This model avoids selection of control spots

M. Futschik *et al*, ***Genome Letters***, 2002

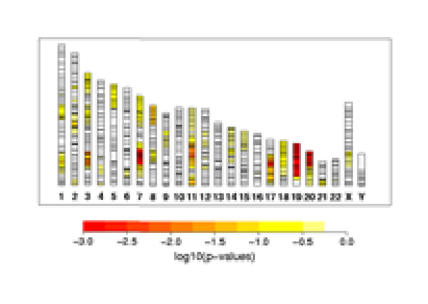
Mapping of gene expression to chromosomal location

Significance analysis
of chromosomal location of differential
gene expression (SW620 vs SW480)

The p-value for finding at least *k* from a total of *s* significant differentially expressed genes within a cytoband window is

$$P = 1 - \sum_{i=0}^{k-1} \frac{\binom{s}{i}\binom{g-s}{n-i}}{\binom{g}{s}}$$

where *g* is the total number of genes with cytoband location and *n* the total number of genes within the cytoband window.

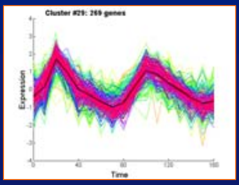


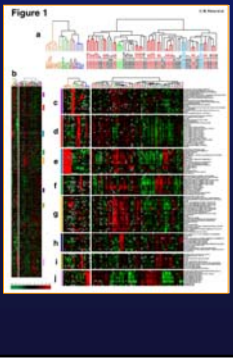
M. Futschik *et al*, ***Genome Letters***, 2002

Minefield V :
There is more than just nuggets and soil

Clustering: Birds of a feather flock together

- Clustering of genes
  - Co-expression indicates co-regulation: functional annotation
  - Clustering of time series
- Clustering of array:
  - finding new subclasses in sample-space
- Two-way clustering:
  - Parallel clustering of samples and genes





Hard clustering vs. soft clustering

Hard clustering:

- Based on classical set theory
- Assigns a gene to exactly one cluster
- No differentiation how well gene is represented by cluster centroid
- Examples: hierachical clustering, k-means, SOMs, ...

Soft clustering:

- Can assign a gene to several cluster
- Differentiate grade of representation (cluster membership)
- Example: Fuzzy c-means, HMMs, ...

K-means clustering

- Partitional clustering splits the data in *k* partitions with a given integer *k*.
- Partition can represented by a partition matrix *U* that contains the membership values μ_{ij} of each object *i* for each cluster *j*.
- For clustering methods, which is based on classical set theory, clusters are mutually exclusive. This leads to the so called *hard partitioning* of the data.

Hard partions are defined as

$$M_{hc} = \left\{ U_{ij} \in R^{k \times N} \;\middle|\; \begin{array}{l} \mu_{ij} \in \{0,1\} \forall i,j \\ \sum_{i=1}^{k} \mu_{ij} = 1 \forall j \\ 0 < \sum_{j=1}^{N} \mu_{ij} < N \forall i \end{array} \right\}$$

k is the number of clusters and N is the number of data objects.

Partitional clustering is frequently based on the optimisation of a given objective function. If the data is given as a set of *N* dimensional vectors, a common objective function is the square error function:

$$E = \sum_i \sum_j d(\mathbf{x}_i, \mathbf{c}_j)^2$$

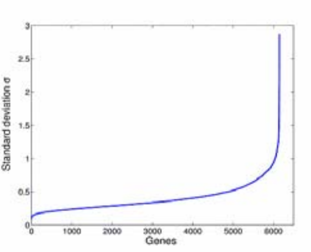
where *d* is the distance metric and c_j is the centre of clusters.

Hard clustering is sensitive to noise

Example data set:

Yeast cell cylce data by Cho et al.

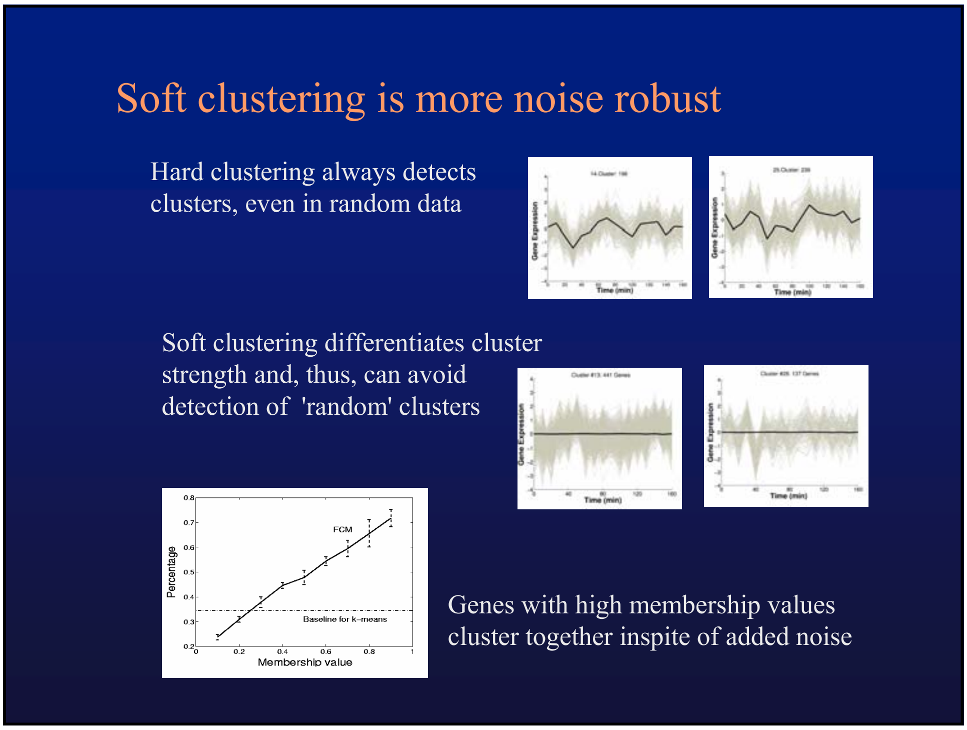
Standard deviation of expression



Standard procedure is pre-filtering of genes based on variation due to noise sensitvity of hard clustering. However, no obvious threshold exists!
(Heyer et al.: ca. 4000 genes, Tavazoe et al.: 3000 genes, Tamayo et al.: 823 genes)

=> Risk of essential losing information

=> Need of noise robust clustering method

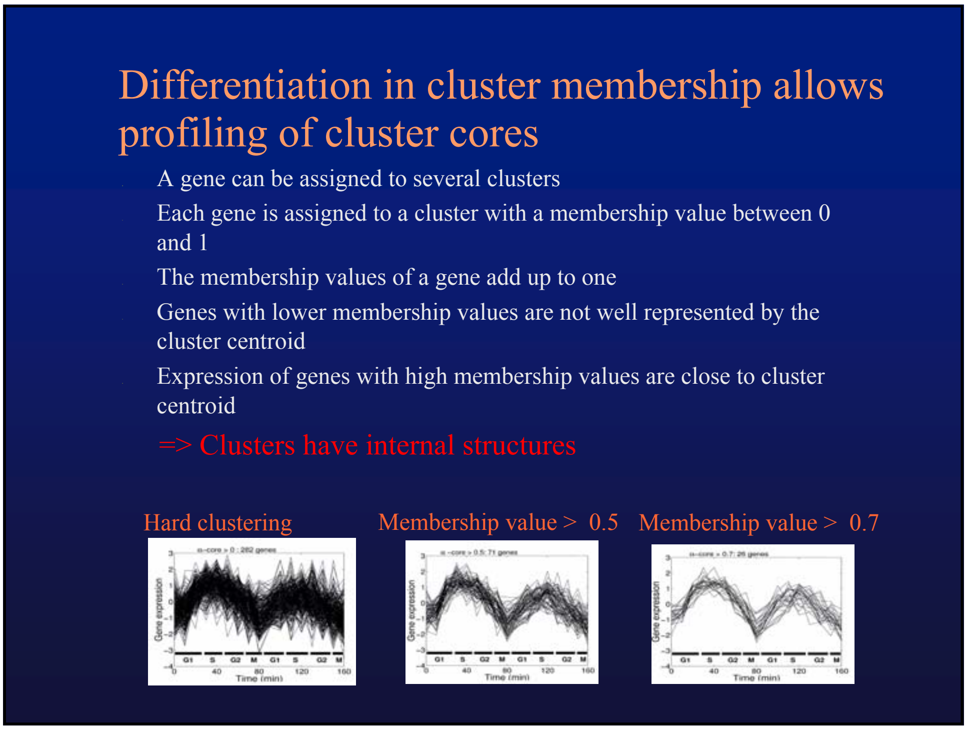


Soft clustering is more noise robust

Hard clustering always detects clusters, even in random data

Soft clustering differentiates cluster strength and, thus, can avoid detection of 'random' clusters

Genes with high membership values cluster together inspite of added noise



Differentiation in cluster membership allows profiling of cluster cores

- A gene can be assigned to several clusters
- Each gene is assigned to a cluster with a membership value between 0 and 1
- The membership values of a gene add up to one
- Genes with lower membership values are not well represented by the cluster centroid
- Expression of genes with high membership values are close to cluster centroid

=> Clusters have internal structures

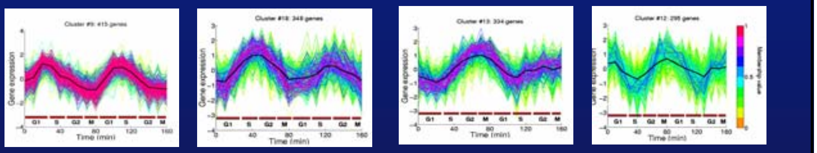
Hard clustering

Membership value > 0.5

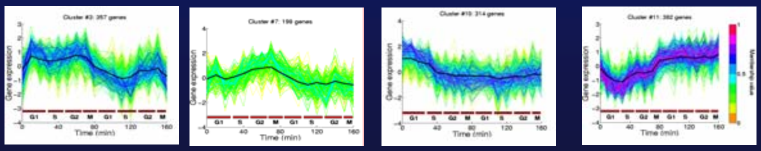
Membership value > 0.7

Periodic and aperiodic clusters

Periodic clusters of yeast cell cycle:



Aperiodic clusters:



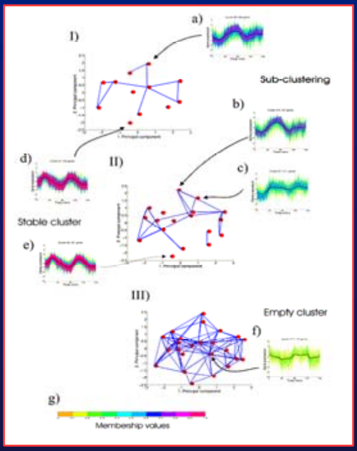
=> Aperiodic clusters were generally weaker than periodic clusters

Global clustering structure

Non-linear 2D-projection by Sammon's Mapping

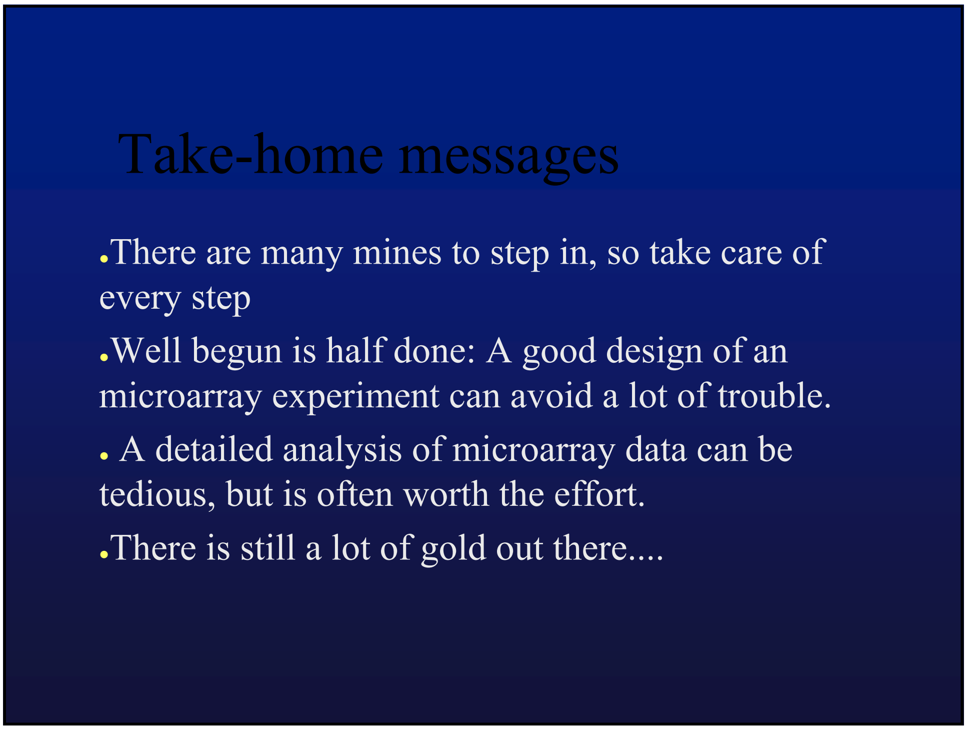
c-means clustering allows definition of overlap of clusters i.e. how many genes are shared by two clusters. This enables to define a similarity measure between clusters.
Global clustering structures can be visualised by graphs i.e. edges representing overlap.

=> Sub-clustering reveals sub-structures



Increasing number of clusters

M. Futschik and B. Carlisle, Noise robust, soft clustering of gene expression data *(submitted)*



Take-home messages

- There are many mines to step in, so take care of every step
- Well begun is half done: A good design of an microarray experiment can avoid a lot of trouble.
- A detailed analysis of microarray data can be tedious, but is often worth the effort.
- There is still a lot of gold out there....

Thanks!

This talk, the OLIN software and further information can be found at http://itb.biologie.hu-berlin.de/~futschik