Microarray Data Analysis

ECS 289A

Microarray Data

	Plate 1	Plate 2	…	Plate 10
Gene 1	0.013	2.14		
Gene 2	…	…		
Gene 3	…	…		
…				
…				
…				
…				
Gene 6200				

Entries:

- Ratio of the color intensities green/red (Cy3/Cy5) (spotted)

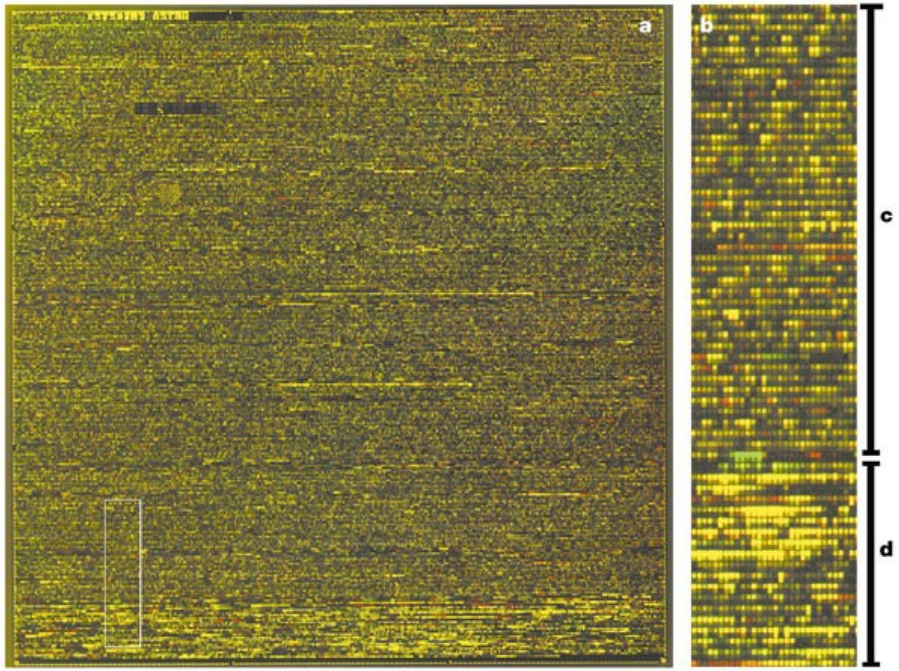
- Single color intensity (Affy)

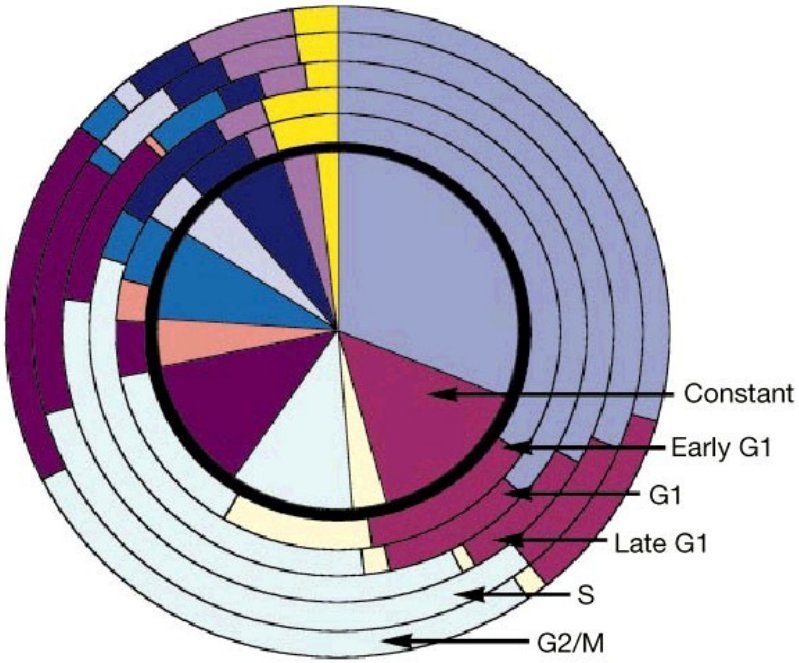
Microarray Data Properties

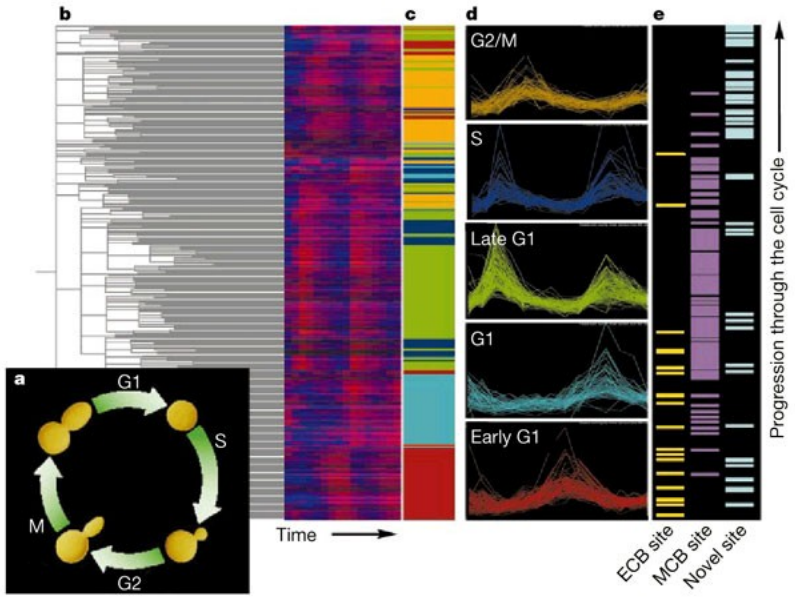
- A lot of data, but not enough!
- Many genes and few conditions (the dimensionality curse)
- Very few repeats (2, 3, 4, mainly)
- Data from different experiments difficult to compare: control conditions are different
- Inaccurate at low intensities

What Can We Do With Microarray Data?

- Fishing Expeditions vs. Hypotheses: differentially expressed genes
- Part/Whole Genome Hypotheses: cell/tissue classification
- Gene Expression vs. Gene Function: guilt by association (co-regulation)
- Transcription Regulation
- Fingerprinting
- Genome analysis
- Gene Circuitry







- Signal transduction
- Cellular biognesis
- Intracellular transport
- Transport facilitation
- Protein destination
- Protein synthesis
- Transcription
- Cell growth, division, DNA synthesis
- Energy
- Metabolism
- Cellular organization

Lochart and Winzeler 2000

How Do We Do Those Things?

- Single Gene Differential Expression
- Similarity in Expression Patterns of Genes and Experiments (Classification)
- Co-regulation of Genes: function and pathways (Clustering)
- Network Inference (Modeling)

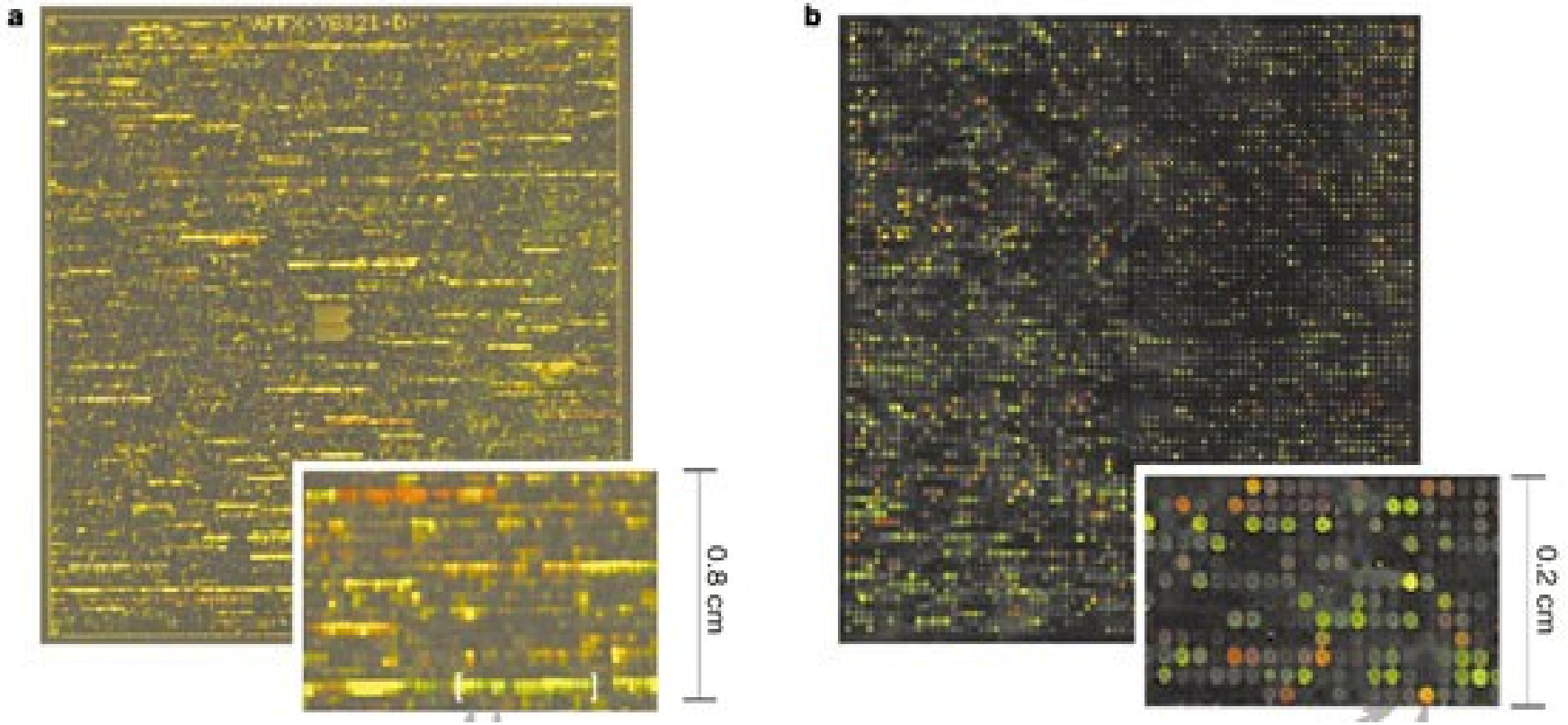
Microarray Data Analysis I

1. Experimental Design
2. Normalization and Transformation
3. Identification of differentially expressed genes
   - Fold test
   - T-test
   - Correction for multiple testing

Microarray Data Analysis II: Discovery

1. Classification
2. Clustering
3. Local Pattern Discovery
4. Projection Methods
   - PCA
   - SVD

1. Choice of Technology

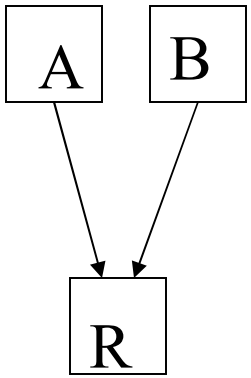


a) Oligonucleotide and b) Spotted Arrays

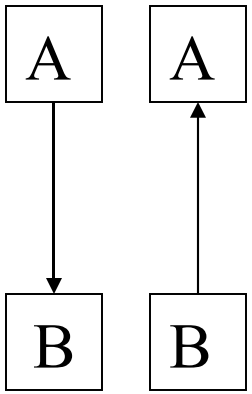
Lochart and Winzeler 2000

- Array Design
  - Affy
  - cDNA libraries and probes
- Controls
  - Affy: PM-MM, and others
  - Negative controls

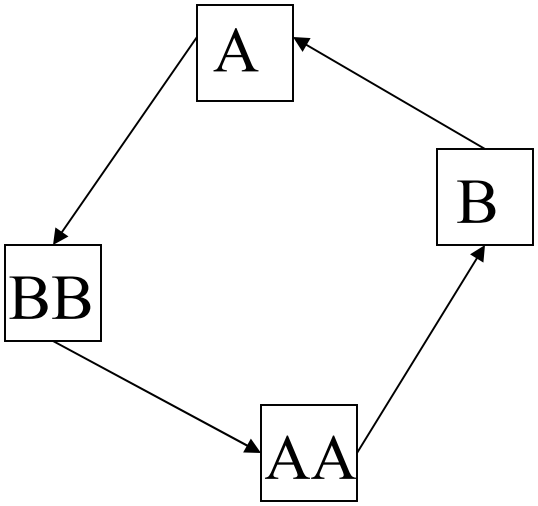
2. Experimental Design
(two-color DNA microarrays)



Reference



Direct
(with dye swap)

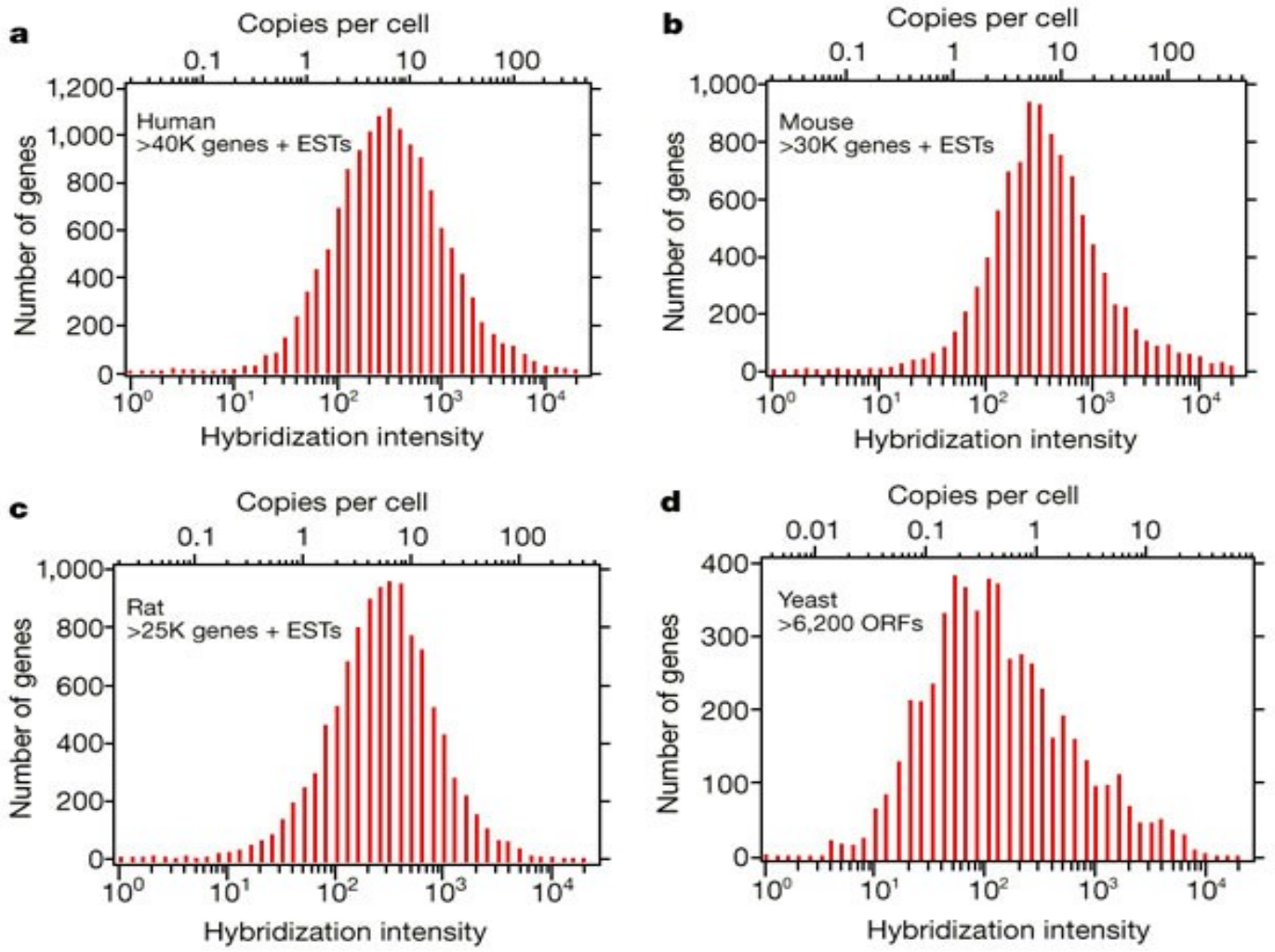


Loop

Types of Microarray Data Experiments

- Control vs. Test
- Time-wise
  - Snapshots (each experiment is different conditions)
  - Time-Course Experiments (each experiment is a time-point)
- Gene-knockout (perturbation experiments)

Distribution of Observed Values



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Distribution of Observed Values is ~ log-normal

log (Color Intensity) or *log R/G* is a good estimator of differential expression

But one can do better by properly accounting for all systematic sources of error

Log-ing the Data

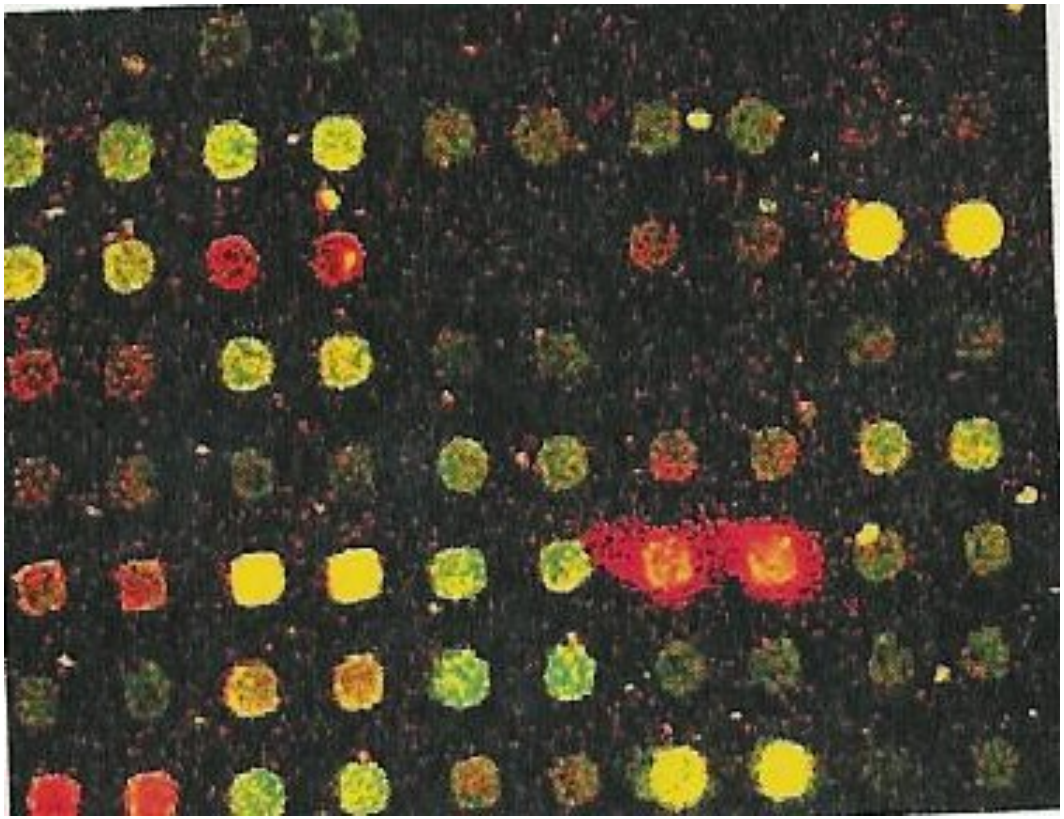
- It should always be done unless a more sophisticated analysis will follow
- Good for two symmetry between up- and down-regulated genes
- Simple comparison across experimental designs

3. Data Acquisition and Visualization

- Image quantification (spot reading)
- Dynamic Range and spatial effects
- Scatterplots
- Systematic sources of error

1. Data Visualization

Image quantification (spot reading)



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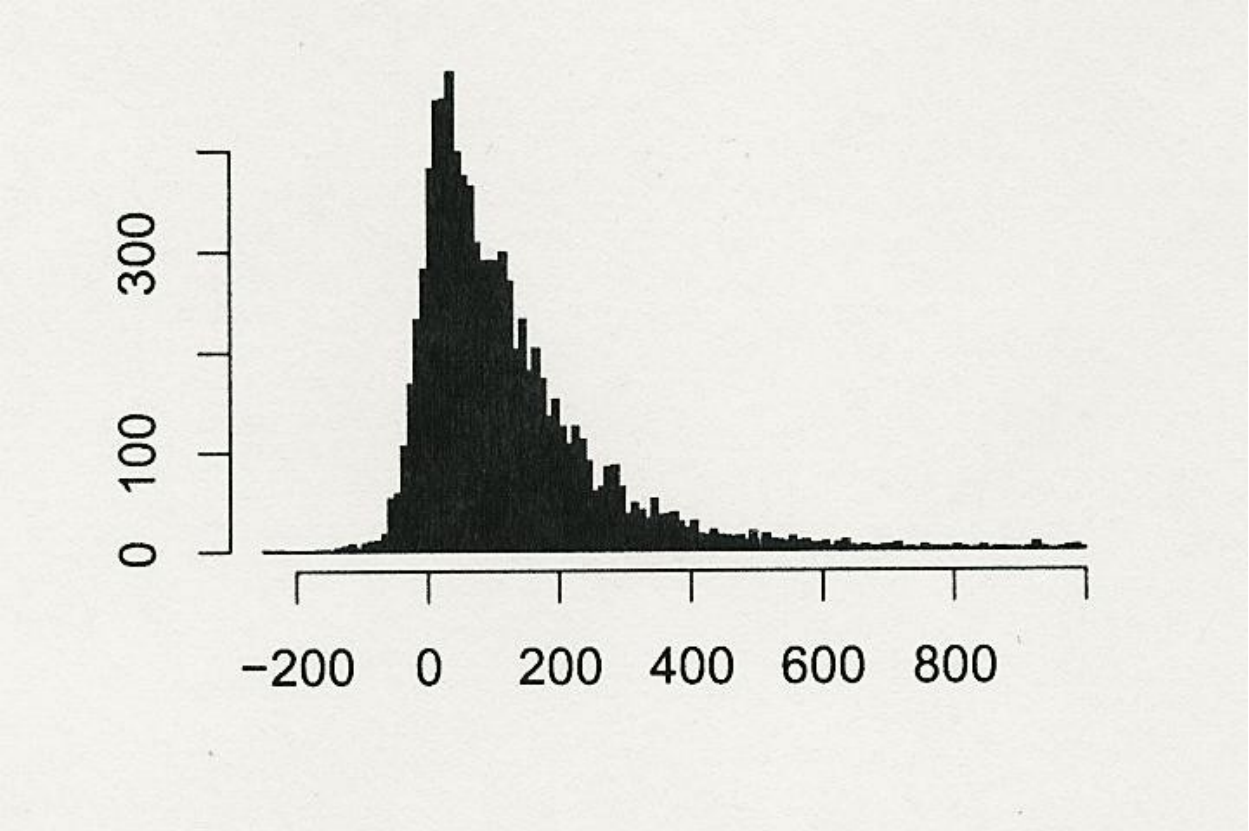
Dynamic Range



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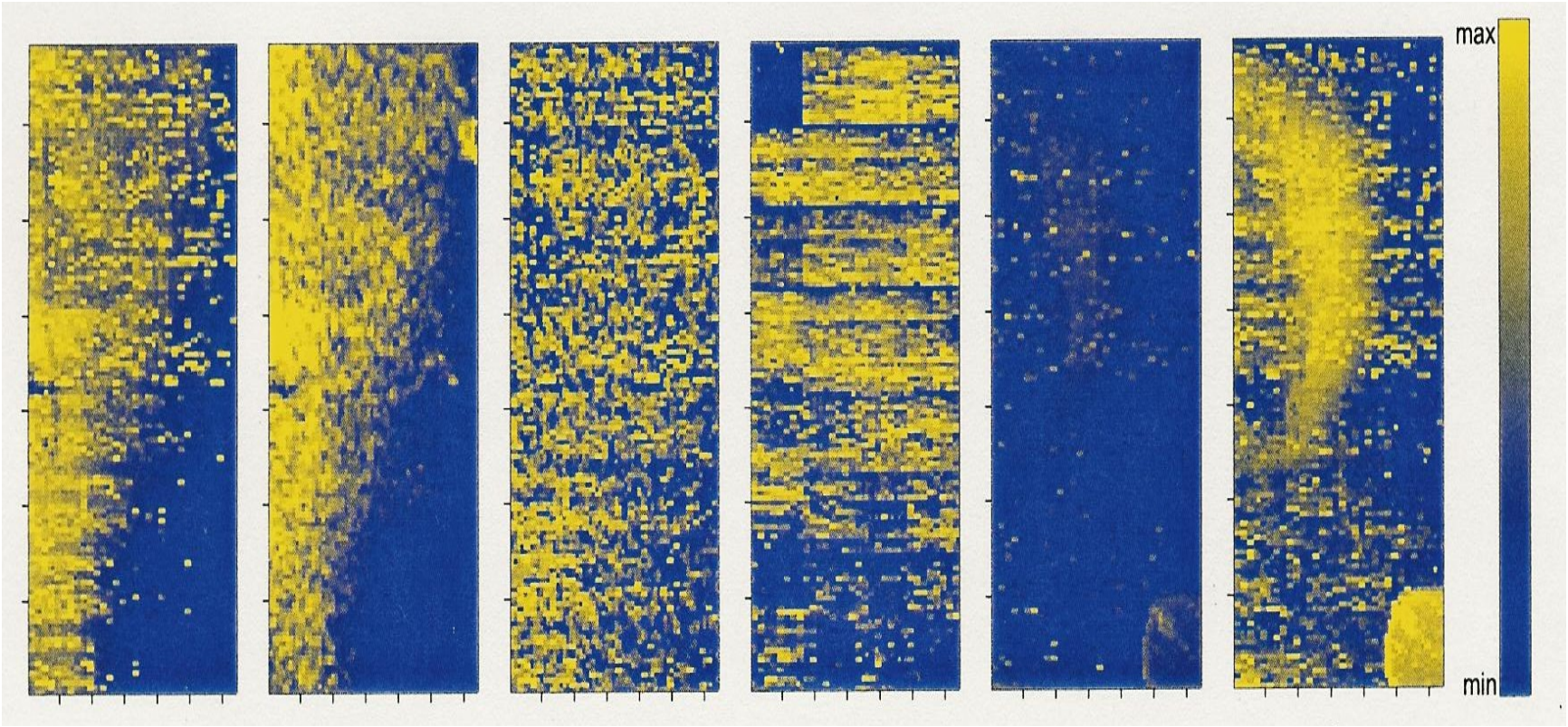
Some values are negative after background subtraction

Spatial Effects



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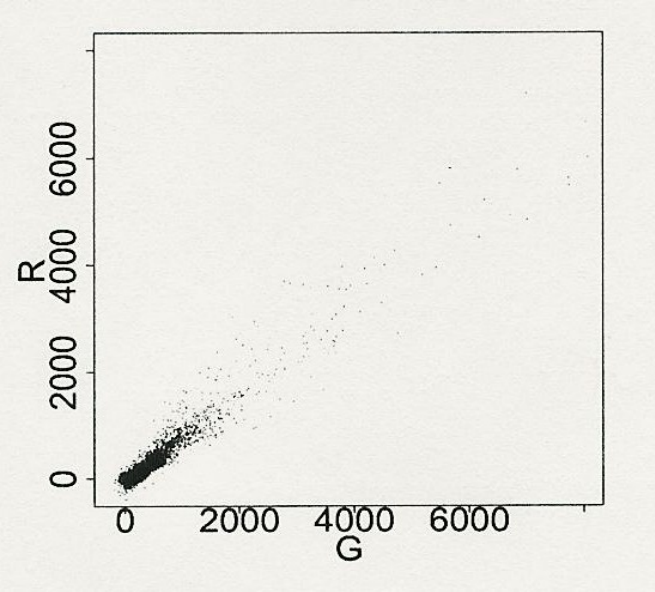
Checking the Data: Scatterplots

- Visual Aids for Data Calibration
- Plotting Red vs Green Expression



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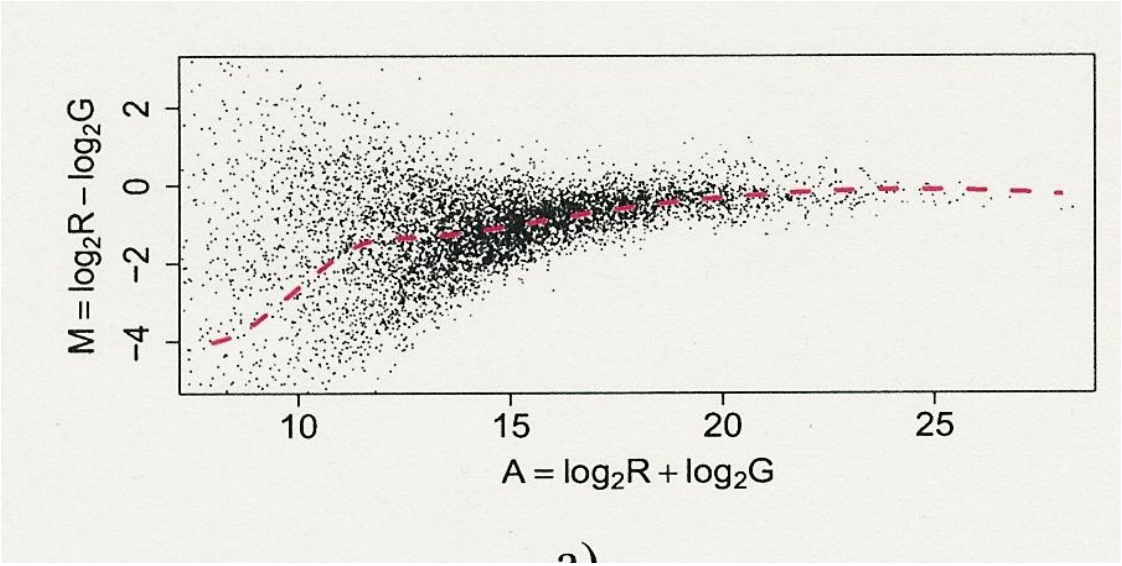
Advanced Scatterplots: MA Plots

- Plotting Average vs. Differential Expression
  - A = log R+log G
  - M = log R - log G
  - Emphasize differences rather than similarities



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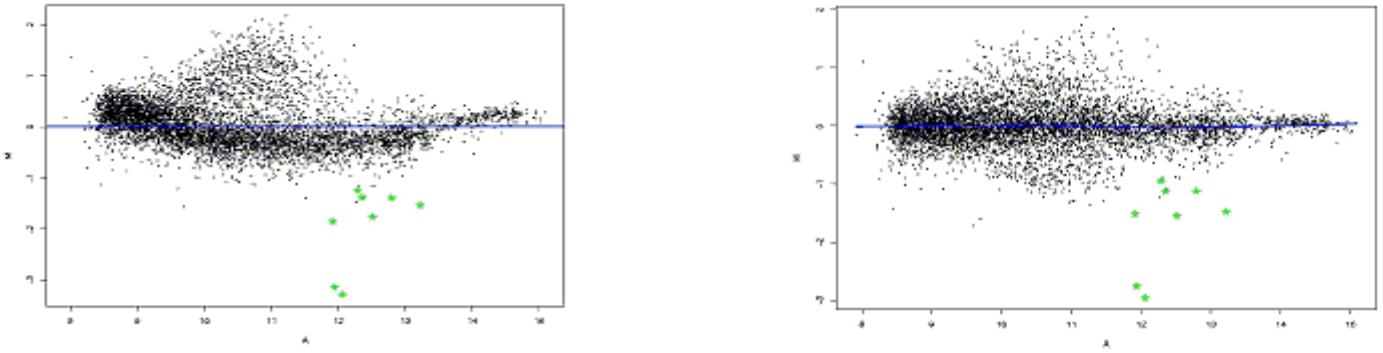
- Mean and Variance have to be corrected in general

Sources of Error

- Spotting errors (tips, robot arm etc.)
- Imbalance in Red/Green Intensities
- PCR yield variance
- Preparation protocols (RNA degrading)
- Scanner and image analysis

4. Data Normalization

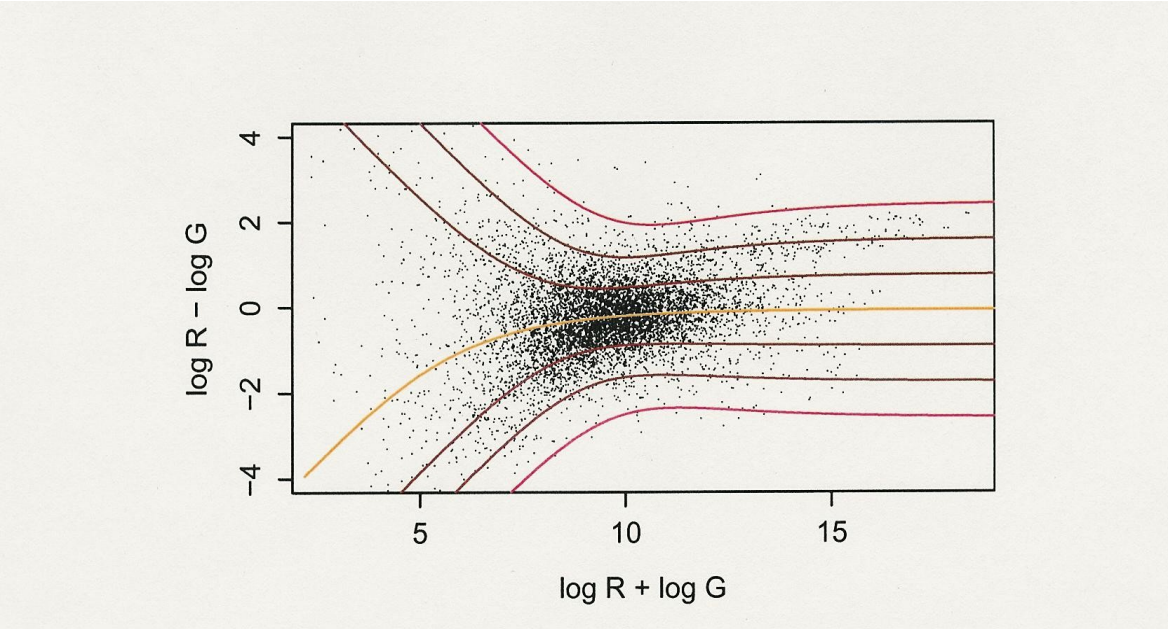
- Identification and removal of systematic sources of variation (adjusting the mean)
- To allow within slide and between slide data comparison



Before and after lowess normalization of log data (locally weighted linear regression method)

5. Variance Stabilization

- Stochastic processes cause the variance of the log-values to be different at different intensities



A Simple, Realistic Model for Reducing Systematic Error

Y = Measured intensity, x = True abundance

$$Y = a + bx + \varepsilon$$

a is an additive factor, corresponding to systemic effects stemming from the experimental medium and does not result from x

b is a gain factor resulting from the relationships between the abundance, x, and the rest of the experiment, i.e. color, detector gain, hybridization, etc.

ε is a normally distributed random error

Realistic Assumptions in the Model Yield Better Normalization

Y = Measured intensity, x = True abundance

$$Y = a + bx + \varepsilon$$

$$b = e^{\eta}$$

$$\eta = N(0,\sigma_{\eta}), \varepsilon = N(0,\sigma_{\varepsilon})$$

- The driving idea behind the model is to capture the variation of the variance at low intensities
- The normalcy assumptions are good approximations of real data

Fitting the Data

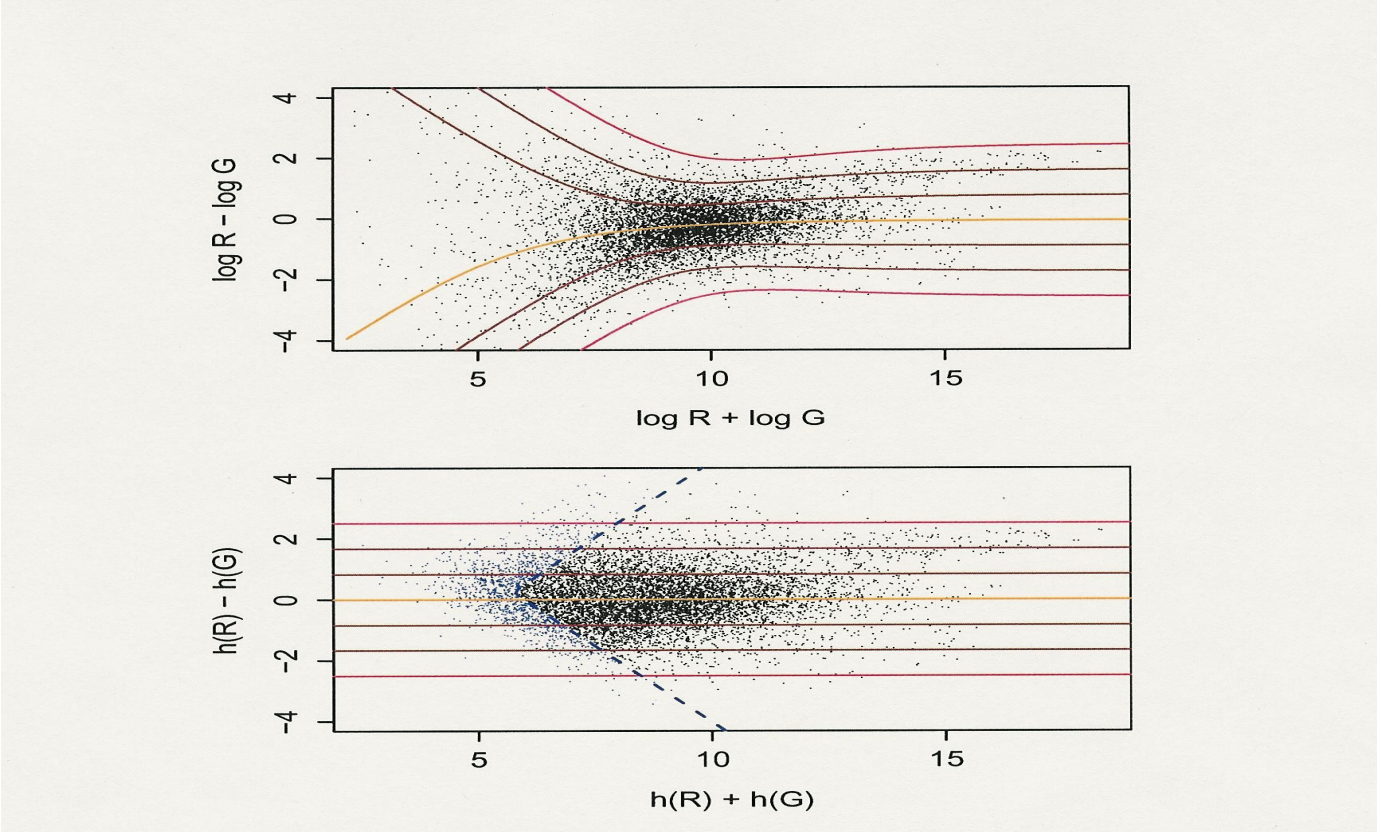
- Estimating the parameters of the model
- a, b, etc.
- Possible approaches:
  - least squares fit
  - Regression analysis

Consequences of the model

- *log Yr/Yg* is no longer the best estimator for *log* x_r/x_g.
- The appropriate measure of differential expression becomes

$$\Delta h = ar\sinh\left(\frac{\sigma_\varepsilon}{\sigma_\eta}\cdot\frac{Yr - a}{b}\right) - ar\sinh\left(\frac{\sigma_\varepsilon}{\sigma_\eta}\cdot\frac{Yg - a}{b}\right)$$

This estimator has a constant variance across the range of intensities



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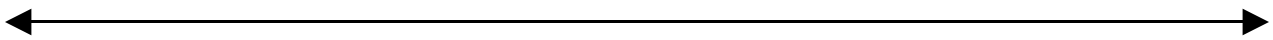
6. Identification of Differentially Expressed Genes in Replicated Microarray Experiments

Which genes are expressed differentially in different experiments?

	1,1	1,2	2,1	2,2
Gene 1	1	0	0	1
Gene 2	1	1	0	0



False Negatives
(wrongly not identified)

False Positives
(wrongly identified)

6.1. Statistical Tests

- Simple Fold Test
- Student t-test
- Wilcoxon rank sum

Simple Fold Accounting

- A gene is differentially expressed up (down) if *log R/G > 2 (< 0.5)*

- Not good for low and high intensities (because the distribution of log-expression values has tails!)

Student-t test

Null Hypotheses Rejection:

- H_j = mean expression levels are equal for control and treatment for gene j, $j=1,\ldots,k$
- Let $x_1^c,\ldots,x_{nc}^c$ and $x_1^t,\ldots,x_{nt}^t$ be the normalized expression levels of n_c and n_t samples, respectively, in the control and test groups
- t-test for gene j

$$t_j = \frac{x_t - x_c}{\sqrt{\frac{\sigma^2_t}{n_t} + \frac{\sigma^2_c}{n_c}}}$$

where $\bar{x}$ is the average and σ the standard deviation

Alternatives for Student-t for Small Number of Replicates

- Regularized t-statistic
  - Estimate additional observations based on the overall data
- Full Bayesian Approaches

6.2. p-values

- H_j is rejected if the significance of the t-test score is high, i.e. the probability of it happening at random is low (based on the Student-t distribution)
- Probability of happening at random:

  $\alpha > 5\%$

  Rejection probability:

  $\alpha < 0.5\ \%$

Correction for Multiple Hypotheses

- Even at small α, say 0.5, when testing 1000 genes for differential expression we get 5 hits at random: high amount of false positives
- FWER, FDR
- Correcting for testing k hypothesis:
  - FWEER, FDR, Bonferoni

    Bonferoni correction:

    $p = \min(k*p_t , 1)$

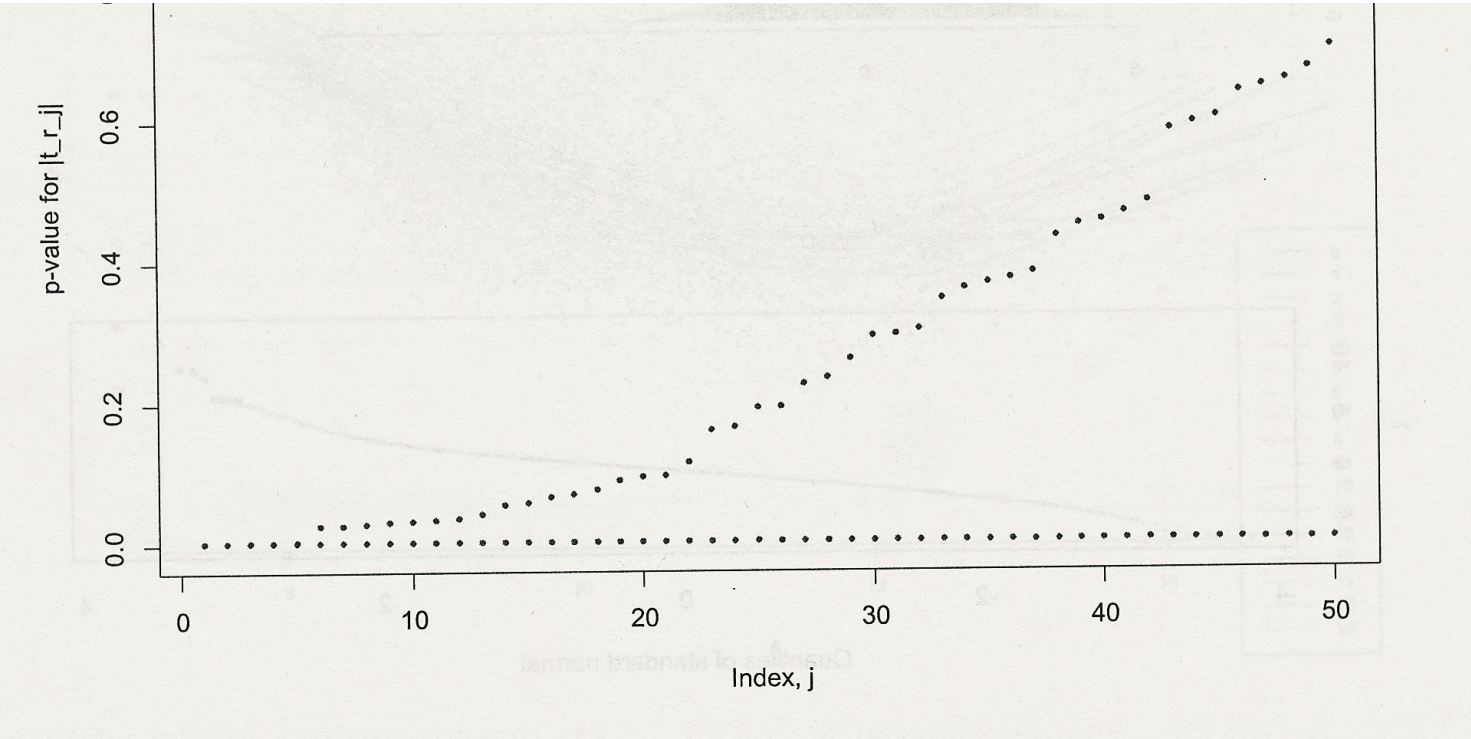
Bonferoni doesn't work well for microarray data!!!

Alternatives to Bonferoni

Bonferoni is a very conservative correction, resulting in too many false negatives

- Westfall and Young step-down adjusted p-values
  - Not as conservative, but computationally intensive
- FDR (ex. SAM) most promissing

Adjusted vs. Unadjusted p-values



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7. Microarray Data Standard

- Beyond systematic errors, microarray data from every experiment is different:
  - Environment
  - Experiment design
  - Data processing
- A Microarray Data standard is needed: MIAME: the minimal set of information about a microarray experiment

Microarray Standard (MAIME)

- Environmental Conditions
- Control Conditions
- Test Conditions
- Data
- Data Processing (if any)

8. Verification and Validation

- Verify results in the lab
- Validation: check for enrichment
  - Functional categories
  - GO annotation