Linear Models and Empirical Bayes Methods for Assessing Differential Expression in Microarray Experiments

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(as interpreted by Aaron J. Baraff)

STAT 572 Intro Talk

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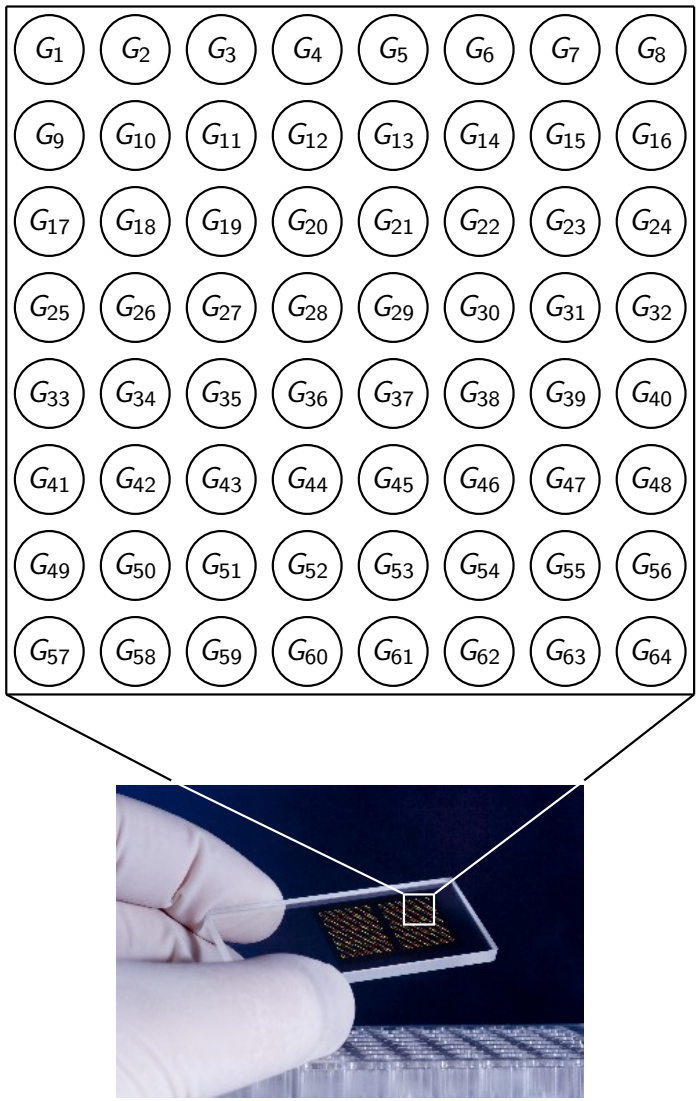
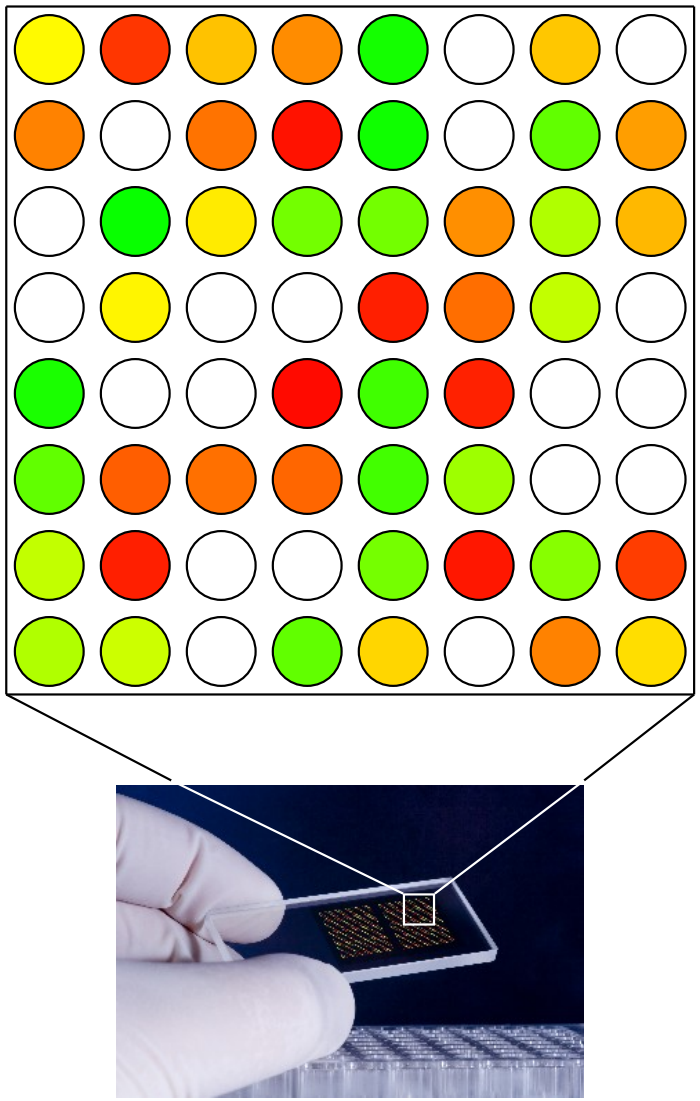
Microarray Data



Measure expression level across large numbers of genes simultaneously

- Genes express by producing mRNA $\rightarrow$ translated into proteins
- $\sim$20,000 protein-coding genes in humans
- Microarray chip contains cDNA for a different gene at each spot
- Sample cDNA hybridizes with cDNA on chip

Microarray Data



Two-color:

- cDNA from two samples dyed red and green
- Response is log-ratio of intensity

$$y_g = \log_2 \frac{R_g}{G_g}$$

- Relative expressions only (fold changes)

Single-channel:

- cDNA from a single dyed sample
- Absolute expressions

Microarray Data

Issues:

- **Expensive!** - sample sizes are low, number of genes is high

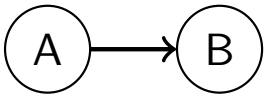
$$n << p$$

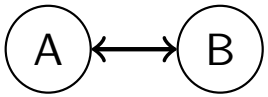
- Multiple comparisons
  - control for false discovery rate (FDR), e.g. Benjamini and Hochberg (1995, 2000)
  - often assumes independence between genes

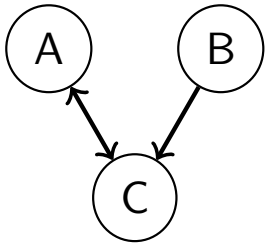
- For two-color microarrays, experimental design is more complicated

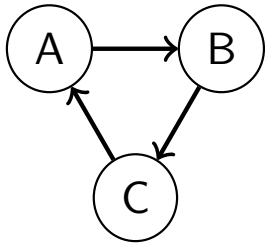
Microarray Data

Design:









Matrix:

$$X = \begin{pmatrix} 1 \end{pmatrix} \qquad X = \begin{pmatrix} 1 \\ -1 \end{pmatrix} \qquad X = \begin{pmatrix} -1 & 0 \\ 1 & 0 \\ -1 & -1 \end{pmatrix} \qquad X = \begin{pmatrix} 1 & 0 \\ 0 & 1 \\ -1 & -1 \end{pmatrix}$$

Coefficients:

$$\boldsymbol{\alpha} = \begin{pmatrix} B - A \end{pmatrix} \qquad \boldsymbol{\alpha} = \begin{pmatrix} B - A \end{pmatrix} \qquad \boldsymbol{\alpha} = \begin{pmatrix} A - C \\ B - A \end{pmatrix} \qquad \boldsymbol{\alpha} = \begin{pmatrix} B - A \\ C - B \end{pmatrix}$$

Assumptions

Sample of n microarrays:

- Response vector $\mathbf{y}_g = (y_{g1}, \ldots, y_{gn})^T$ for each gene g
- Assume

$$\mathrm{E}(\mathbf{y}_g) = X\boldsymbol{\alpha}_g, \quad \text{and} \quad \mathrm{Var}(\mathbf{y}_g) = W_g\sigma_g^2$$

  for known design matrix X and weight matrix W_g
- Usually interested in contrasts of coefficients

$$\boldsymbol{\beta}_g = C^T\boldsymbol{\alpha}_g$$

  for known contrast matrix C

Assumptions

Fitting the model gives:

- Coefficient estimators $\hat{\boldsymbol{\alpha}}_g$ for $\boldsymbol{\alpha}_g$
- Contrast estimators $\hat{\boldsymbol{\beta}}_g = C^T \hat{\boldsymbol{\alpha}}_g$ for $\boldsymbol{\beta}_g$
- Variance estimators s_g^2 for σ_g^2

(Note: no assumption that $\mathbf{y}_g$ is normal or model is fit by OLS)

Assumptions

Assume:

- covariance matrices

$$\text{Var}(\hat{\boldsymbol{\alpha}}_g) = V_g \sigma_g^2 \quad \text{and} \quad \text{Var}(\hat{\boldsymbol{\beta}}_g) = C^T V_g C \sigma_g^2$$

- distributions

$$\hat{\beta}_{gi} | \beta_{gj}, \sigma_g^2 \sim N(\beta_{gj}, v_{gj} \sigma_g^2) \quad \text{and} \quad s_g^2 | \sigma_g^2 \sim \frac{\sigma_g^2}{d_g} \chi^2_{d_g}$$

all independent, where v_{gj} is the jth diagonal element of $C^T V_g C$

Differential Expression

The problem:

- Would like to test

$$H_0 : \beta_{gj} = 0 \quad \text{vs} \quad H_1 : \beta_{gj} \neq 0$$

- **Too many genes!** - multiple comparison methods assume independence across genes
- Instead, think of p-values as statistics used to rank genes

Differential Expression

Previous methods for ranking genes:

- Fold changes - use $|\hat{\beta}_{gj}|$ directly
- t-statistics

$$|t_{gj}| = \frac{|\hat{\beta}_{gj}|}{s_g \sqrt{v_{gj}}}$$

  Problem: s_g small $\rightarrow |t_{gj}|$ large
- Offset t-statistics - inflate s_g
  - Tusher et al (2001) - minimize coefficient of variation
  - Efron et al (2001) - percentile of sample variances
- Odds ratios - Lönnstedt and Speed (2002) - empirical Bayes methods to estimate odds of differential expression - replicated experiments only
- Other methods dependent on specific designs

Differential Expression

Goal: Extend empirical Bayes method from Lönnstedt and Speed (2002) to more general experiments

Priors:

- Variance

$$\frac{1}{\sigma_g^2} \sim \frac{1}{d_0 s_0^2} \chi^2_{d_0}$$

- Differential expression

$$P(\beta_{gj} \neq 0) = p_j$$

- Fold change

$$\beta_{gj} | \sigma_g^2, \beta_{gj} \neq 0 \sim N(0, v_{0j} \sigma_g^2)$$

Differential Expression

After a bunch of calculgebra that I haven't done yet, we get posterior mean

$$\tilde{s}_g^2 = \frac{1}{\mathrm{E}(\sigma_g^2 | s_g^2)} = \frac{d_0 s_0^2 + d_g s_g^2}{d_0 + d_g},$$

and **moderated** t-statistic

$$\tilde{t}_{gj} = \frac{\hat{\beta}_{gj}}{\tilde{s}_g \sqrt{v_{gj}}} \sim t_{d_0 + d_g}, \quad \text{under } H_0.$$

Since this is an **empirical** Bayes method, estimate hyperparameters s_0^2 and d_0 from the data.

Results

Simulation study:

- All parameters and hyperparameters held constant except $d_0 = 1, 10, 1000$
- Moderated t has fewer false positives than other methods
- Rigging the game?

Swirl data:

- Mutation in known gene in zebrafish
- Degrees of freedom for t increases from 4 to 7.17
- Ranking more sensible than other methods

Discussion

Modern relevance:

- Method included in `R` package `limma` as part of Bioconductor
- Later papers extended the idea of sharing variance - Cui et al (2005) uses a James-Stein-type shrinkage estimator
- Applications to other *-omics* data with similar high-dimensional problems
- Digital gene expression (DGE) starting to overtake microarrays
  - observed as count data
  - modeled with overdispersed Poisson
  - empirical Bayes used to share data about overdispersion parameter across genes

Discussion

What next?

- Do the mathy stuff
  - calculation of posterior and marginal distributions
  - estimation of hyperparameters
- Data normalization
- Perform simulations
- Develop critique
  - paper makes a lot of unrealistic assumptions about distribution and independence of σ_g^2
  - imperfect method for imperfect data?

The End

Any questions?