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 Update Talk

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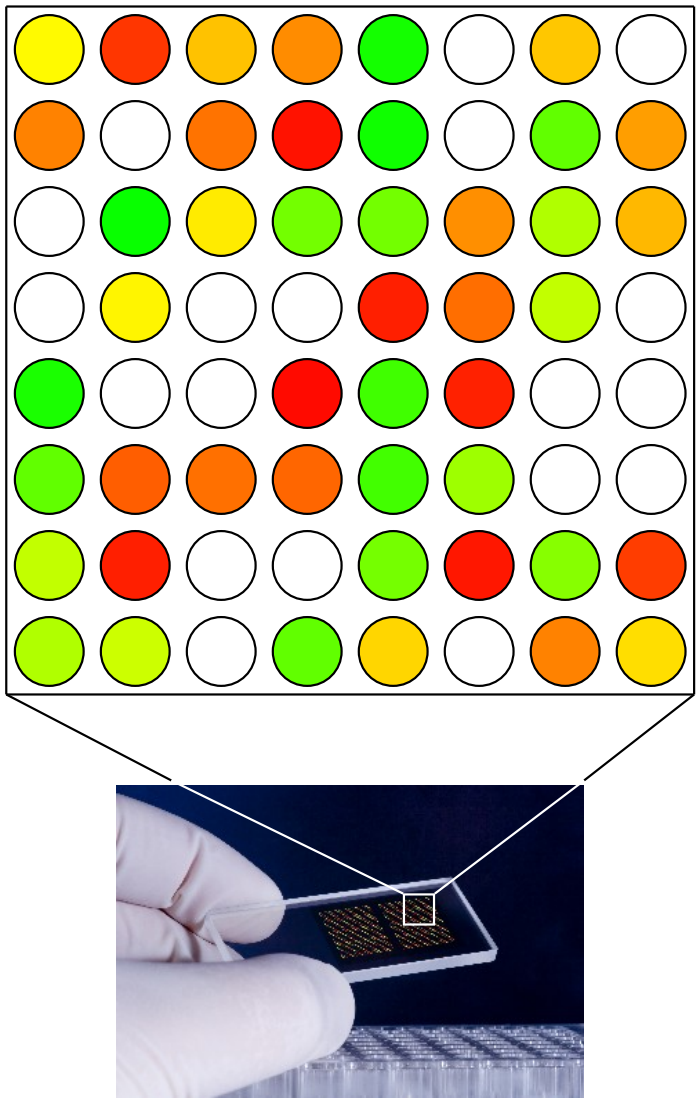
Microarray Data



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

Assumptions

Sample of n microarrays:

- Response vector $\mathbf{y}_g = (y_{g1}, \ldots, y_{gn})^T$ for each gene $g = 1, \ldots, G$
- Assume the linear model

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

  for known design matrix X and weight matrix W_g
- Assume estimates have distributions

$$\hat{\boldsymbol{\beta}}_g|\boldsymbol{\beta}_g, \sigma_g^2 \sim N(\boldsymbol{\beta}_g, V_g\sigma_g^2) \quad \text{and} \quad s_g^2|\sigma_g^2 \sim \frac{\sigma_g^2}{d_g}\chi_{d_g}^2$$

  independent across all genes

Differential Expression

Under $H_0 : \beta_{gj} = 0$, we have

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

Problem: Since n is often low, test statistics have high variance, leading to many false positives

Solution: Share variance information across all genes to improve estimates for σ_g^2

Bayesian Estimation of σ_g^2

Assume prior distribution on σ_g^{-2}:

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

with hyperparameters s_0^2 and d_0

Through conjugacy, we get posterior distribution:

$$\sigma_g^{-2} | s_g^2 \sim \frac{1}{d_g s_g^2 + d_0 s_0^2} \chi^2_{d_g + d_0}$$

Bayesian Estimation of σ_g^2

Now estimate σ_g^2 using the posterior mean

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

From this, we get the **moderated** t-statistic

$$\tilde{t}_{gj} = \frac{\hat{\beta}_{gj}}{\tilde{s}_g \sqrt{v_{gj}}}$$

(Note: $\tilde{t} \to t$ as $d_0 \to 0$, and $\tilde{t} \to c\hat{\beta}$ as $d_0 \to \infty$)

Marginal Distributions

Under $H_0 : \beta_{gj} = 0$, we have

$$\begin{aligned} p(\tilde{t}_{gj}, s_g^2 | \beta_{gj} = 0) &= \tilde{s}_g v_g p(\hat{\beta}_{gj}, s_g^2 | \beta_{gj} = 0) \\ &= \tilde{s}_g v_g \int p(\hat{\beta}_{gj} | \sigma_g^{-2}, \beta_{gj} = 0) p(s_g^2 | \sigma_g^{-2}) \pi(\sigma_g^{-2}) d\sigma_g^{-2} \\ &= [\text{pdf for } t_{d_g + d_0}] \times [\text{pdf for } s_0^2 F_{d_g, d_0}] \end{aligned}$$

Therefore,

$$\tilde{t}_{gj} \sim t_{d_g + d_0} \quad \text{and} \quad s_g^2 \sim s_0^2 F_{d_g, d_0}$$

and they are independent

Estimation of Hyperparameters

Want to use s_g^2 across all genes to estimate s_0^2 and d_0

Let $z_g = \log s_g^2$ (Fisher's z):

- $\mathrm{E}(z_g) = \log s_0^2 + \psi(d_g/2) - \psi(d_0/2) + \log(d_0/d_g)$
- $\mathrm{Var}(z_g) = \psi'(d_g/2) + \psi'(d_0/2)$

Method of moments! Solve:

$$\psi'(d_0/2) = \frac{1}{G}\sum_{g=1}^{G}[(z_g - \bar{z})^2 - \psi'(d_g/2)]$$

$$\log s_0^2 = \frac{1}{G}\sum_{g=1}^{G}[z_g - \psi(d_g/2) + \psi(d_0/2) - \log(d_0/d_g)]$$

Simulation Study - Setup

Data sets simulated under the assumed model:

$$\begin{aligned}
\hat{\beta}_g | \beta_g, \sigma_g^2 &\sim N(\beta_g, v_g \sigma_g^2) \\
s_g^2 | \sigma_g^2 &\sim \sigma_g^2 \chi_{d_g}^2 / d_g \\
\beta_{gj} | \sigma_g^2, \beta_g \neq 0 &\sim N(0, v_0 \sigma_g^2) \\
\sigma_g^{-2} &\sim \chi_{d_0}^2 / (d_0 s_0^2)
\end{aligned}$$

Using the parameters:

- $G = 15{,}000$ (300 differentially expressed)
- $d_g = 4$, $v_g = 1/3$, $v_0 = 2$, $s_0^2 = 4$
- $d_0 = 1, 4, 1000$, more to less variable

Simulation Study - Setup

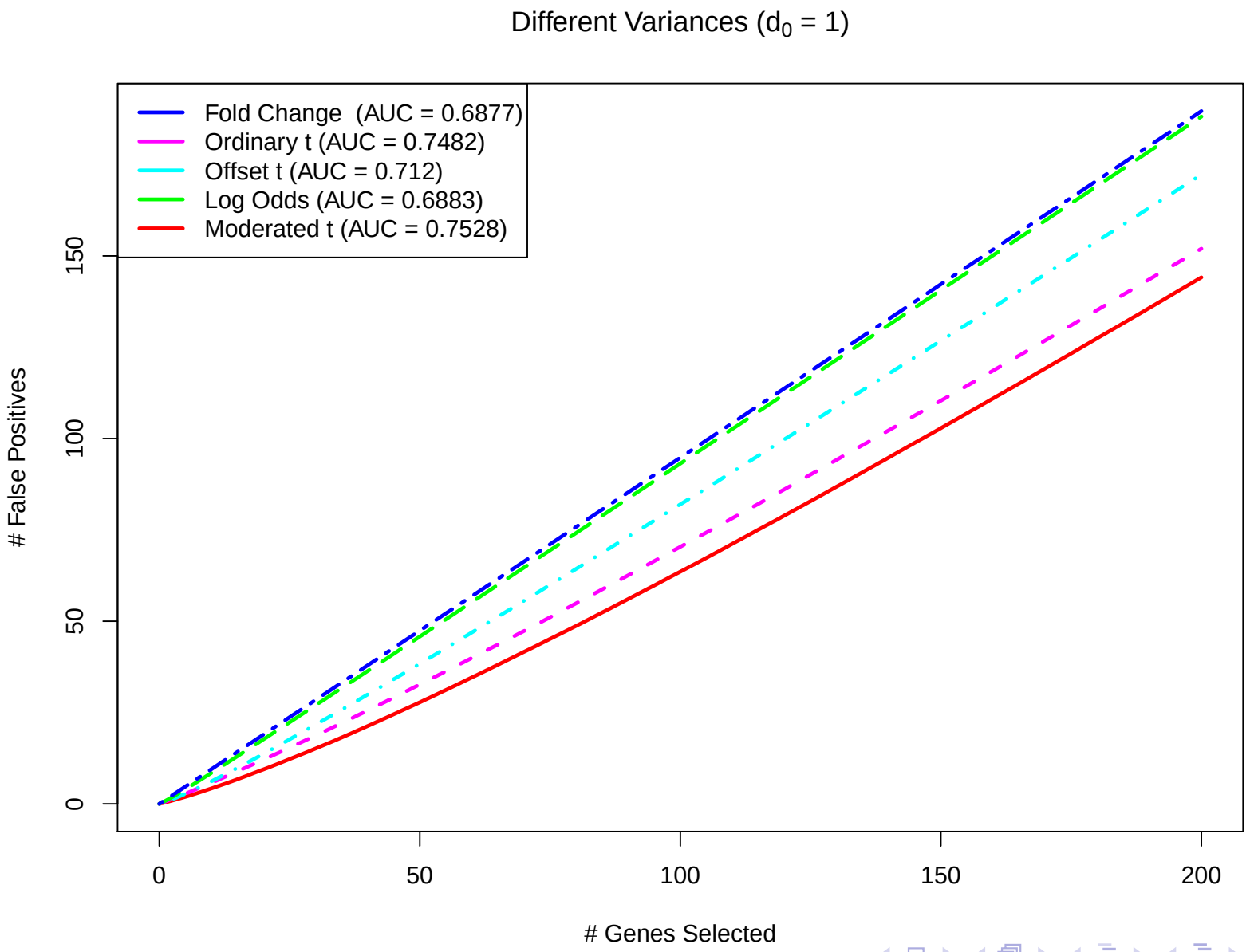
The following statistics were compared:

1. **Fold Change**: $M_g = \hat{\beta}_g$
2. **Ordinary t** - Student (1908): $t_g = \frac{\hat{\beta}_g}{s_g \sqrt{v_g}}$
3. **Offset t** - Efron et al (2001): $t_g^* = \frac{\hat{\beta}_g}{(s_g + s_{0.9})\sqrt{v_g}}$
4. **Log Odds** - Lönnstedt and Speed (2002):

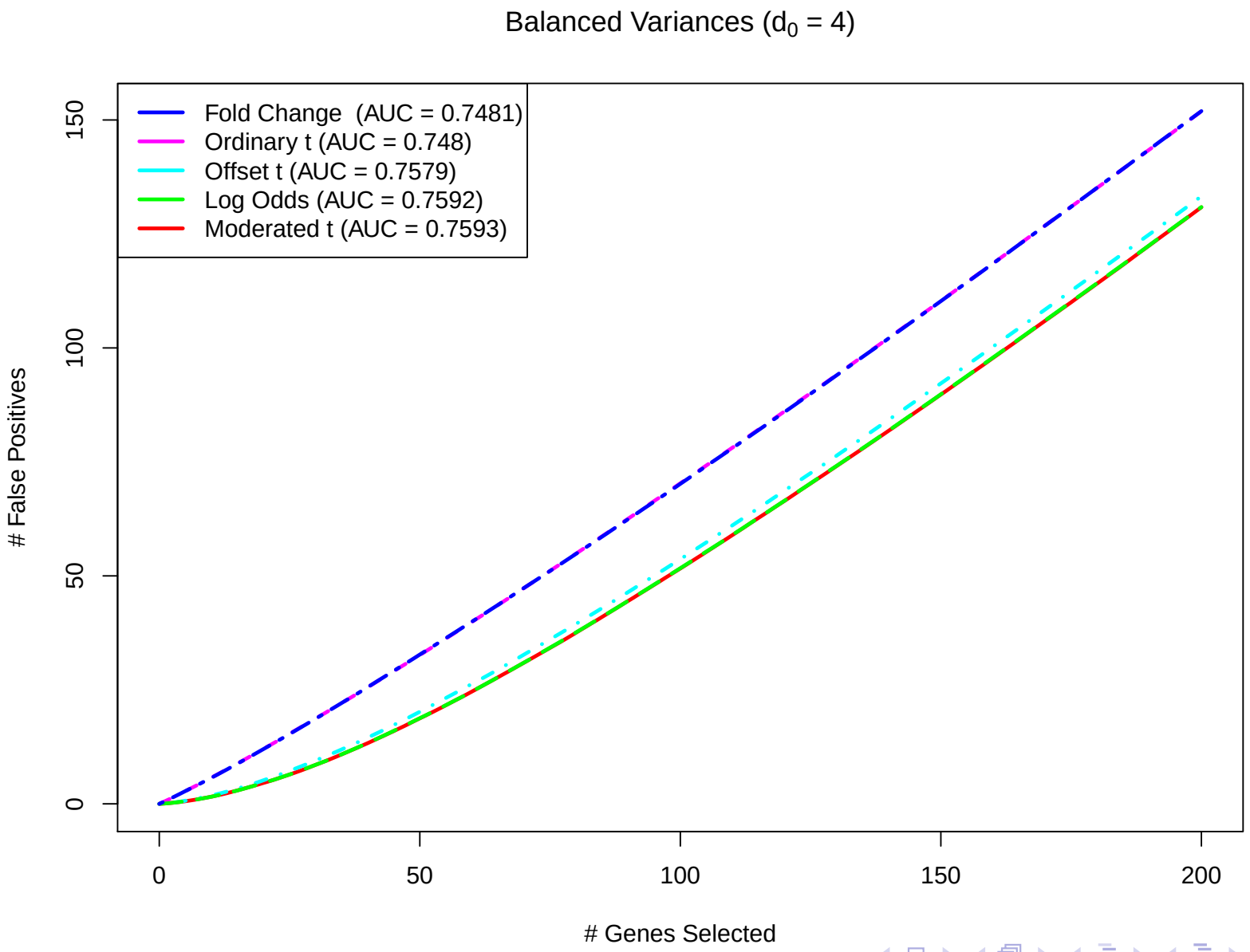
$$B_g = \log \frac{P(\beta_g \neq 0 | \hat{\beta}_g, s_1^2, \ldots, s_G^2)}{P(\beta_g = 0 | \hat{\beta}_g, s_1^2, \ldots, s_G^2)}$$

5. **Moderated t** - My paper!: $t_g = \frac{\hat{\beta}_g}{\tilde{s}_g \sqrt{v_g}}$

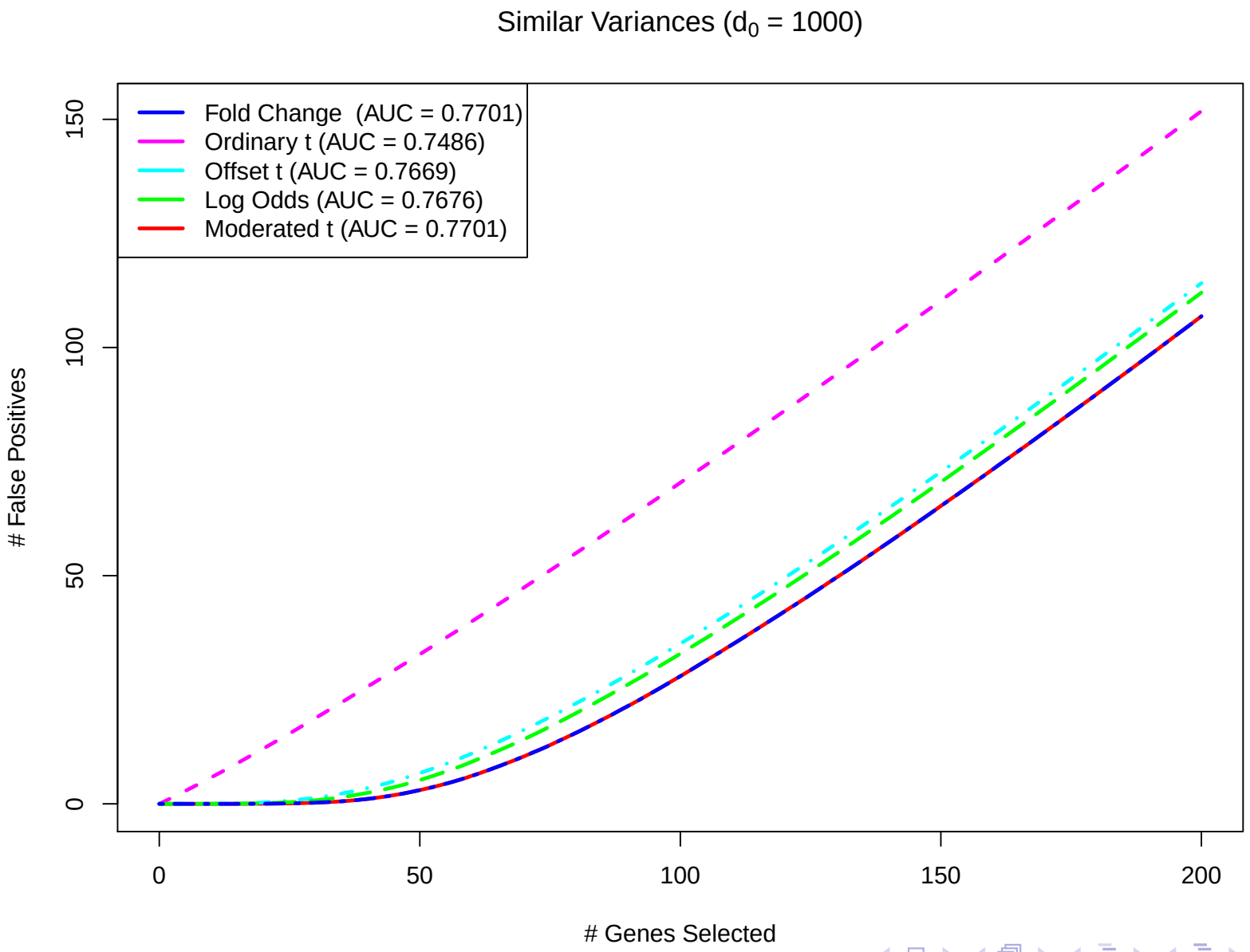
Simulation Study - Results



Simulation Study - Results



Simulation Study - Results



Simulation Study - Redux

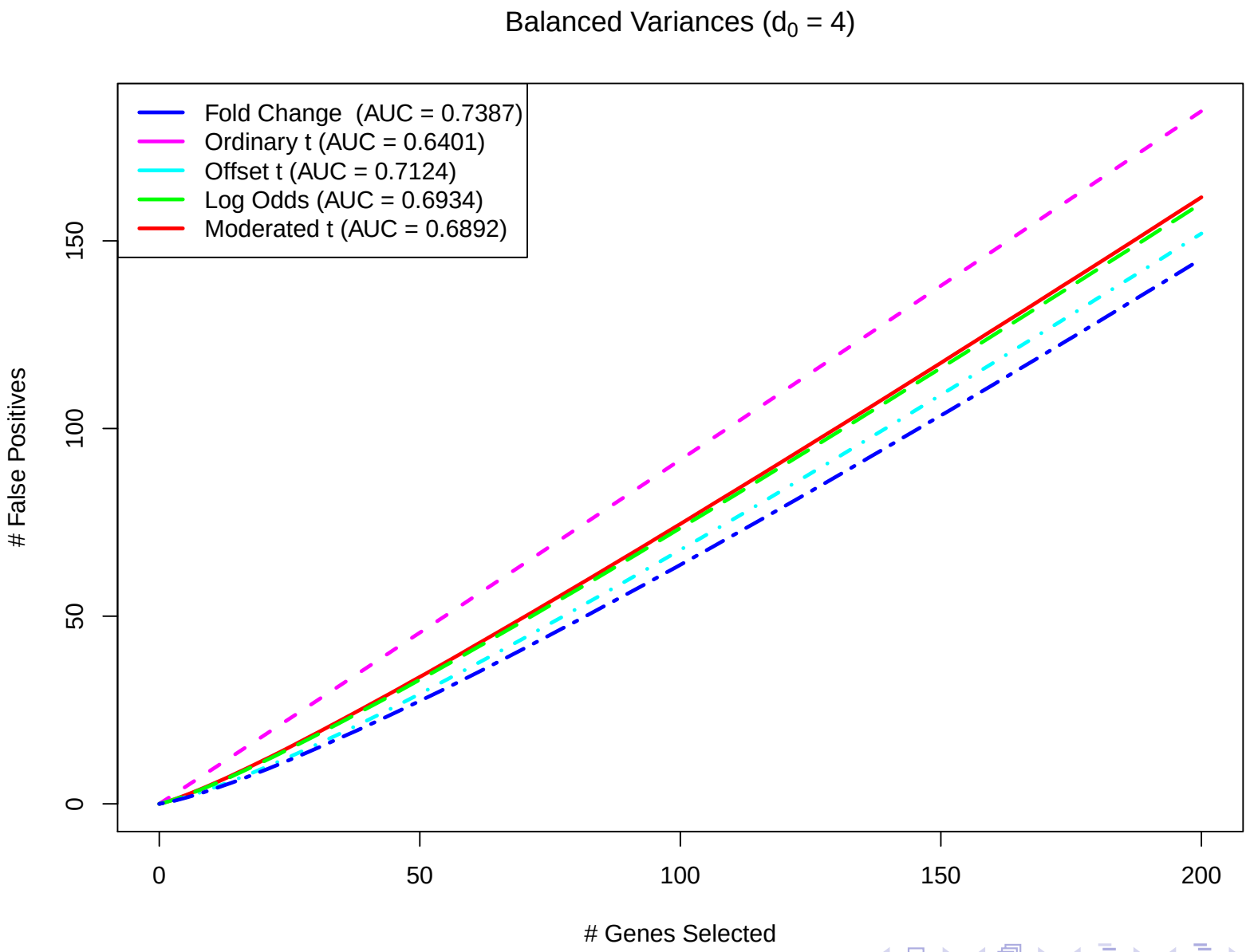
Unfair to simulate data from the assumed hierarchical model?

Everything the same except:

$$\hat{\beta}_g | \beta_g, \sigma_g^2 \sim N(\beta_g, v_g(1 + |\beta_g|)\sigma_g^2)$$
$$s_g^2 | \sigma_g^2 \sim (1 + |\beta_g|)\sigma_g^2 \chi_{d_g}^2 / d_g$$

Residual variance proportional to fold change

Simulation Study - Redux



Swirl Data

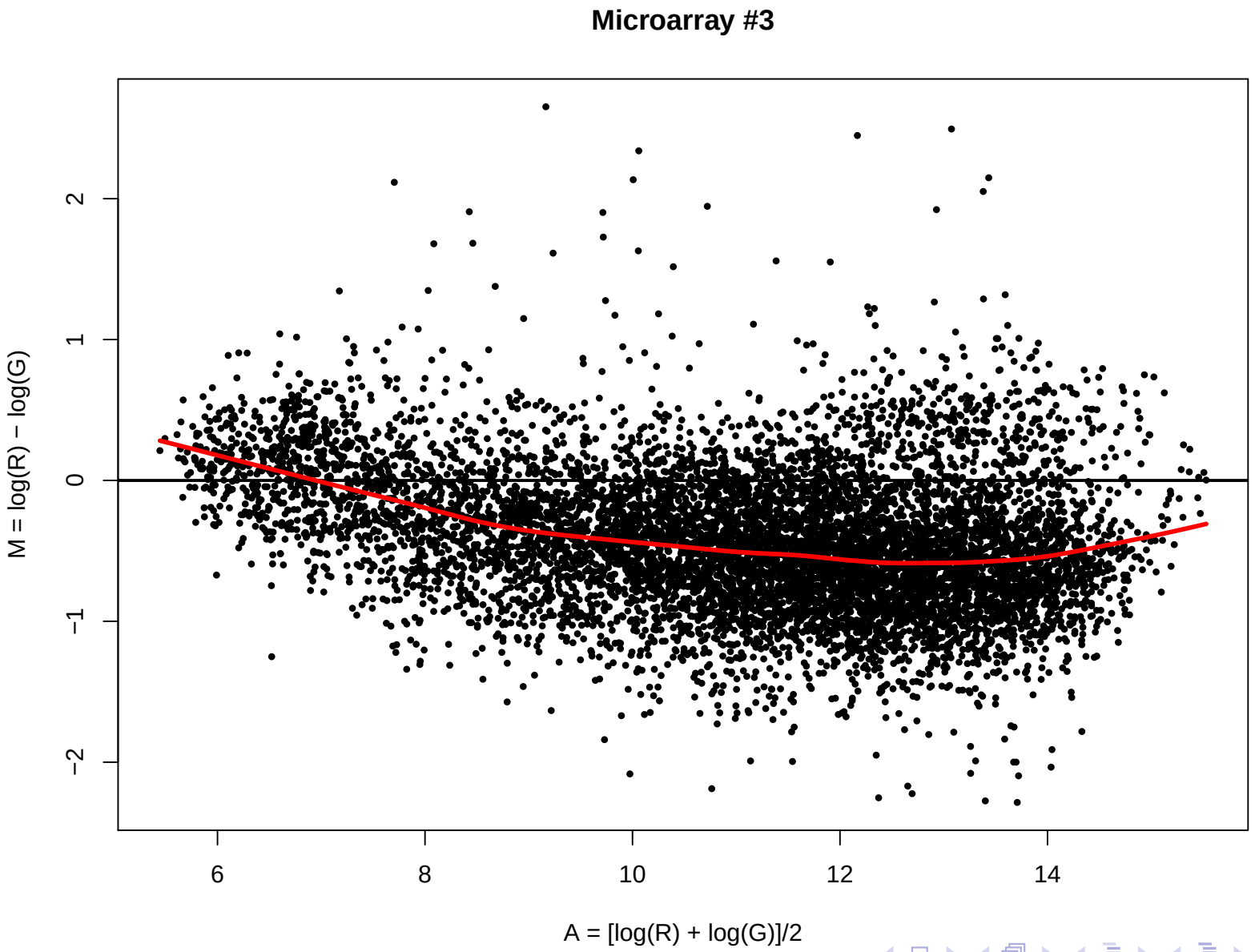
Example data:

- Swirl - mutation in BMP2 gene of zebrafish affecting dorsal/ventral body axis
- 4 microarrays - 2 dye-swap pairs

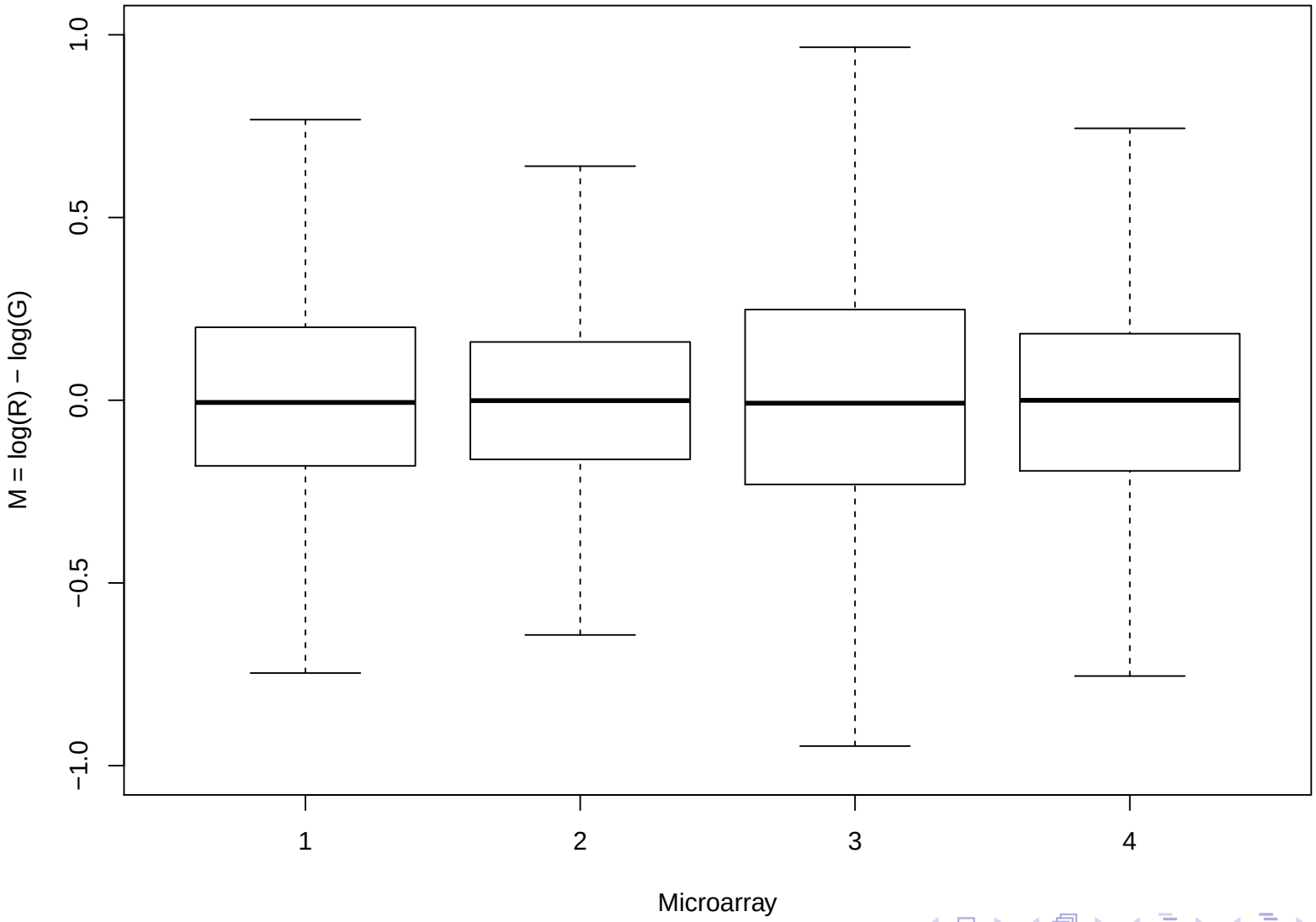
$$X = (1, -1, 1, -1)^T$$

- 8448 spots (genes) on array
- Raw data must be normalized first

Normalization - Within



Normalization - Between



Swirl Results

Prior degrees of freedom $d_0 = 4.17$

- total df increases from 3 to 7.17
- 54% reduction in variance of t under H_0

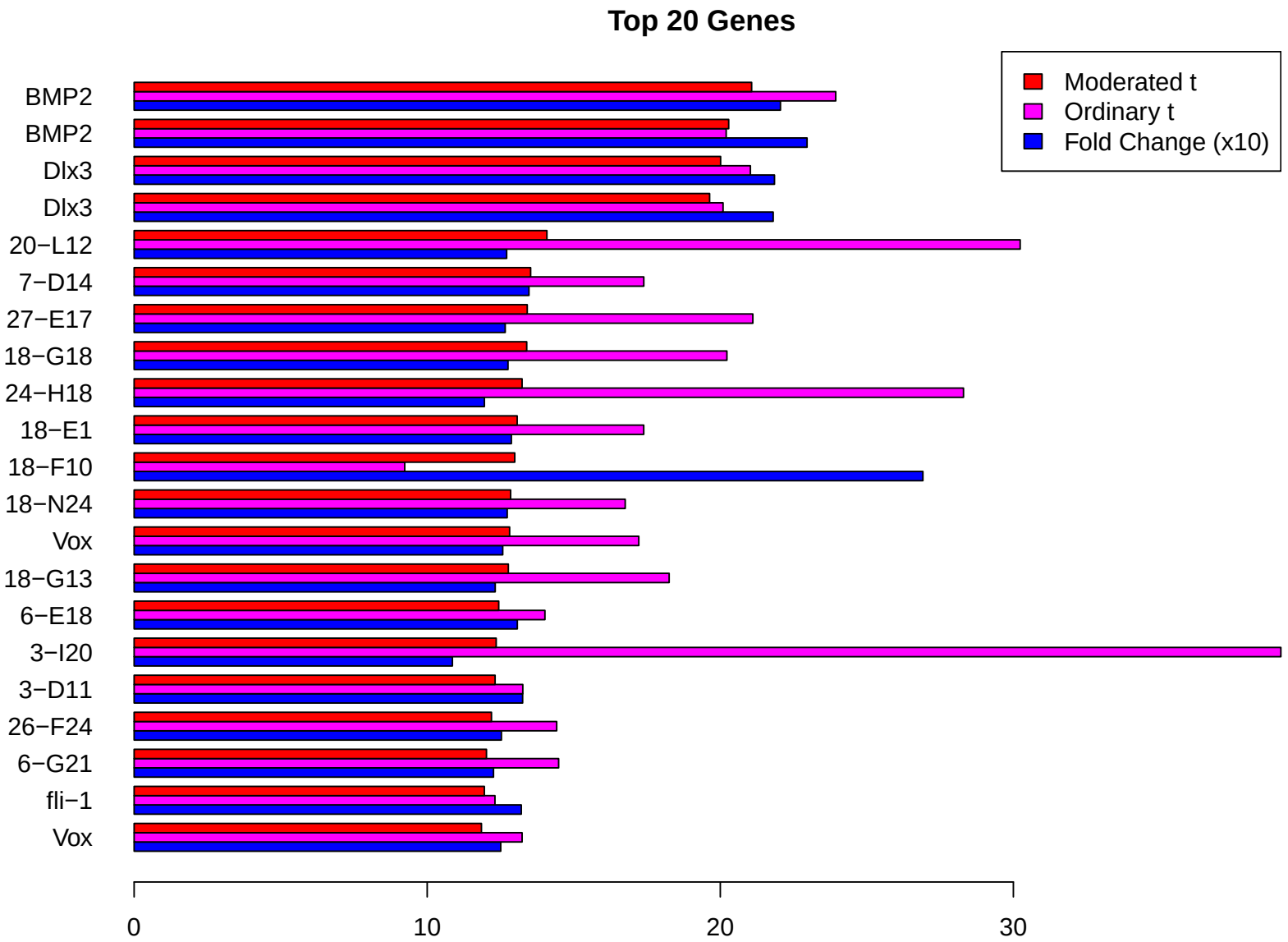
Prior variance $s_0^2 = 0.0509$

- less then mean, greater than median

Moderated t finds BMP2 and Dix3 (a known target) more clearly than other statistics

(These match the paper exactly! Yay!)

Swirl Results



Conclusions

- Need for sharing variance information across genes
- Empirical Bayes uses data to estimate hyperparameters
- Simulations showed method works well under model assumptions
  - best when variances are balanced
  - robustness issues when model is violated
- Swirl data also gives favorable results
  - small sample and balanced variances
- Classification problem, not inference?

Questions

Questions for you:

- Drop microarray design?
- Drop normalization?
- Any more ideas for breaking the method?

Questions for me?