Journal club

Papers to be selected by 18.00 on 17th October; please discuss it with Hubert and I before submitting pull request.

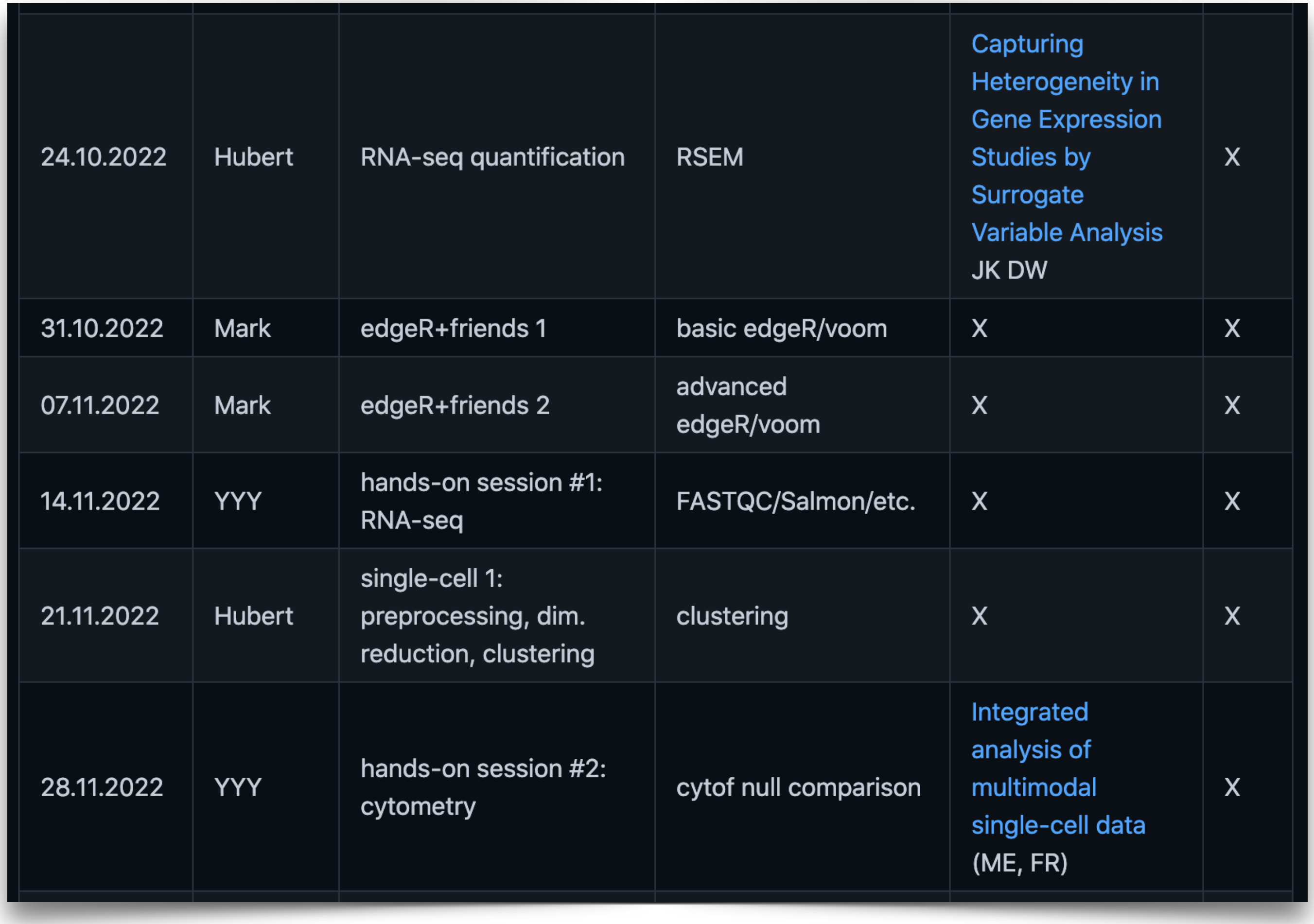
Start: Oct 24

Journal Club schedule to be finalized by 24th October

Given the number of students, groups of 2 are recommended.

Use the #journal-clubs channel (e.g., to find a group member). I will put some suggestions there.

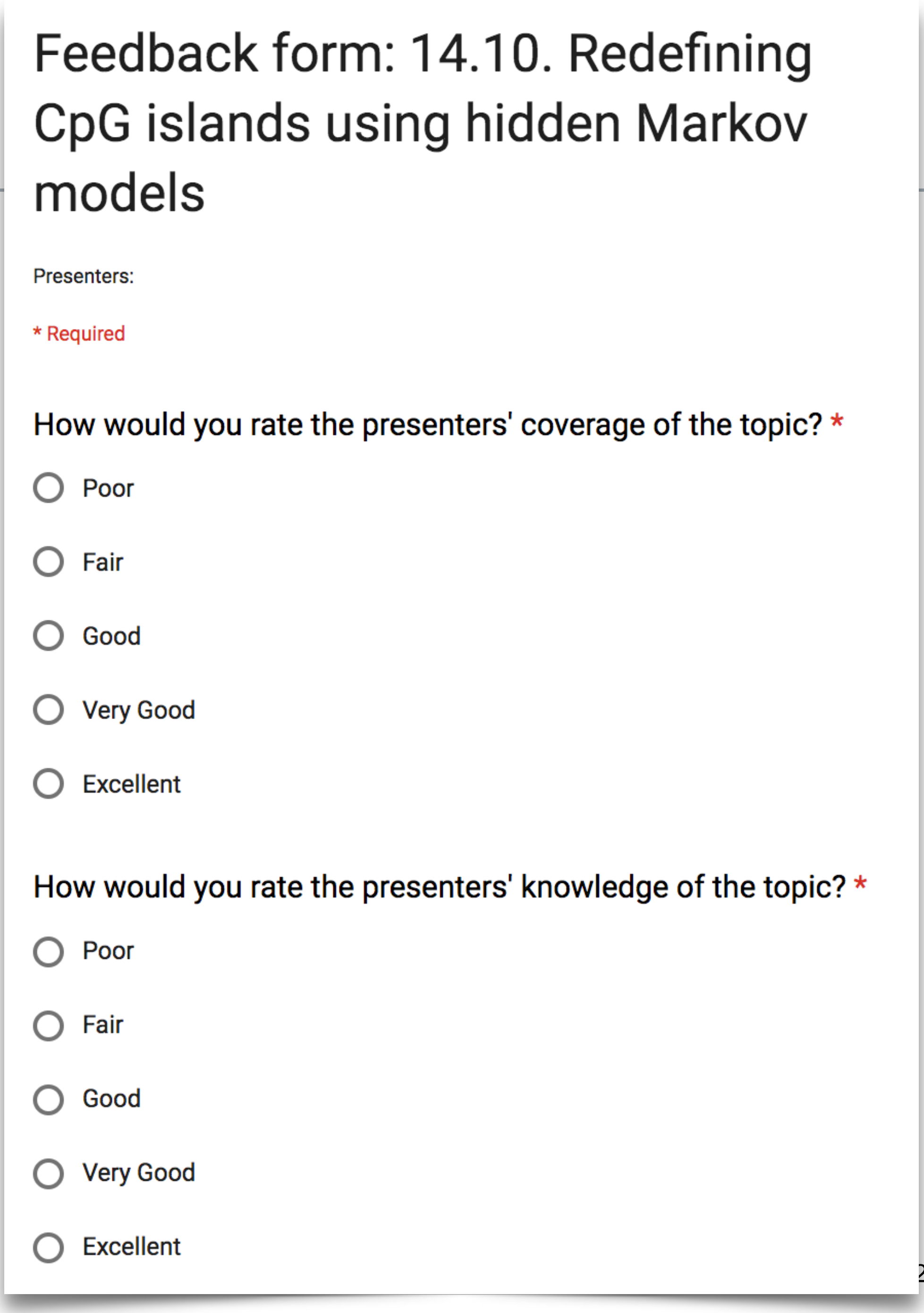
Sign up by pull request to the 'material' repo, README.md. "First come first served"



24.10.2022	Hubert	RNA-seq quantification	RSEM	Capturing Heterogeneity in Gene Expression Studies by Surrogate Variable Analysis JK DW	X
31.10.2022	Mark	edgeR+friends 1	basic edgeR/voom	X	X
07.11.2022	Mark	edgeR+friends 2	advanced edgeR/voom	X	X
14.11.2022	YYY	hands-on session #1: RNA-seq	FASTQC/Salmon/etc.	X	X
21.11.2022	Hubert	single-cell 1: preprocessing, dim. reduction, clustering	clustering	X	X
28.11.2022	YYY	hands-on session #2: cytometry	cytof null comparison	Integrated analysis of multimodal single-cell data (ME, FR)	X

Journal Club procedure

- During/after journal clubs: give the presenters some constructive feedback
- Giving feedback (via Google form) is part of your JC grade! Feedback forms must be submitted within 1 week of presentation; comments will be sent to presenters (anonymously)
- JCs will be part of the recordings



Feedback form: 14.10. Redefining CpG islands using hidden Markov models

Presenters:

* Required

How would you rate the presenters' coverage of the topic? *

○ Poor

○ Fair

○ Good

○ Very Good

○ Excellent

How would you rate the presenters' knowledge of the topic? *

○ Poor

○ Fair

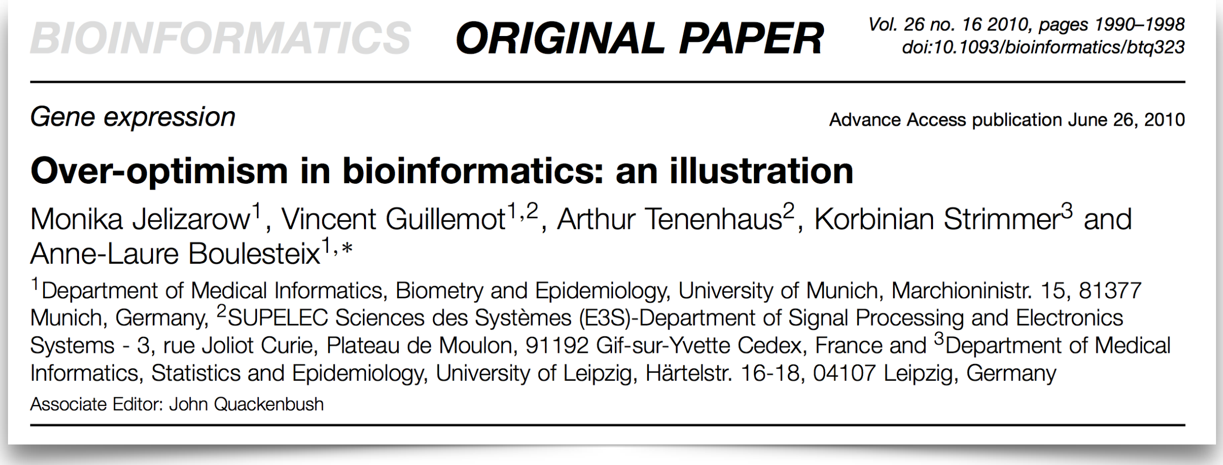
○ Good

○ Very Good

○ Excellent

From the feed: “Over-optimism” + Terry’s IMS Bulletin

We will see a lot of methods in this course – **how do we evaluate what works well in practice ?**



BIOINFORMATICS **ORIGINAL PAPER** *Vol. 26 no. 16 2010, pages 1990–1998 doi:10.1093/bioinformatics/btq323*

Gene expression Advance Access publication June 26, 2010

Over-optimism in bioinformatics: an illustration

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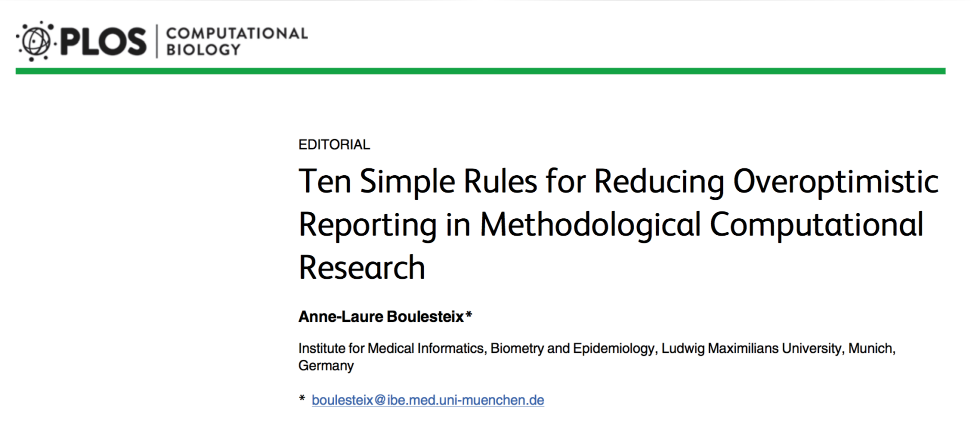
Associate Editor: John Quackenbush



PLOS | COMPUTATIONAL BIOLOGY

EDITORIAL

Ten Simple Rules for Reducing Overoptimistic Reporting in Methodological Computational Research

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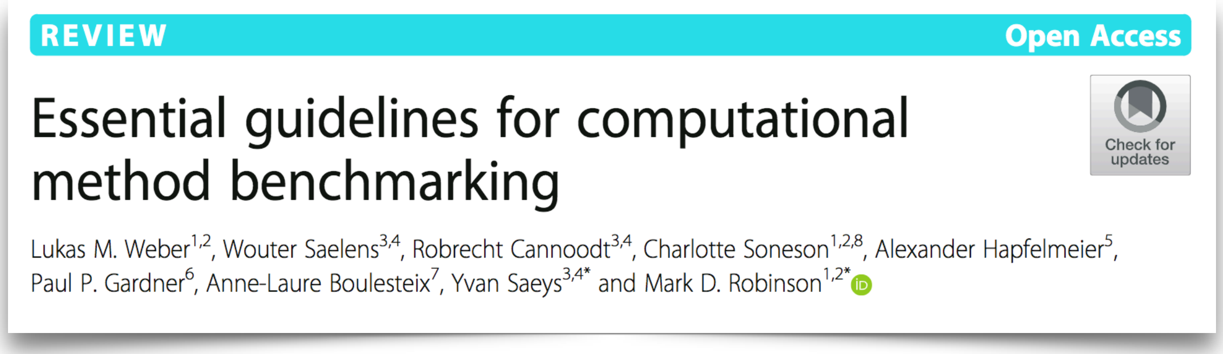
“if the improvement of a quantitative criterion such as the error rate is the main contribution of a paper, the superiority of new algorithms should always be demonstrated on independent validation data.”



REVIEW Open Access

Essential guidelines for computational method benchmarking

Lukas M. Weber[1,2], Wouter Saelens[3,4], Robrecht Cannoodt[3,4], Charlotte Soneson[1,2,8], Alexander Hapfelmeier[5], Paul P. Gardner[6], Anne-Laure Boulesteix[7], Yvan Saeys[3,4*] and Mark D. Robinson[1,2*]

In class discussion

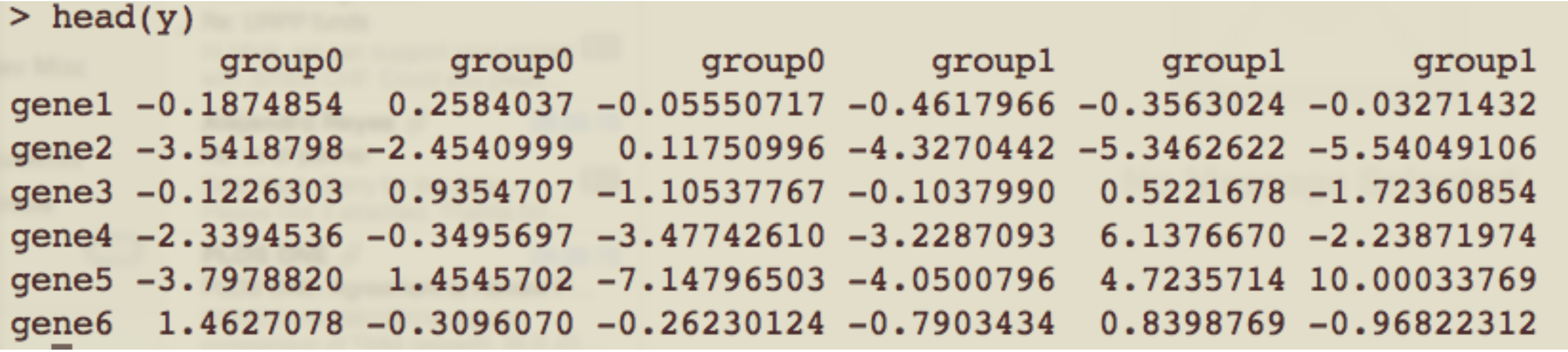
- **5 minutes**: read (the excerpt from “Terence’s Stuff”).
- **10 minutes**: Discuss with your neighbour/row and answer the following 4 questions:
  1. How do we tell what works in practice?
  2. What problems arise using simulated (synthetic) data?
  3. What problems arise using real data?
  4. What are positive/negative controls?
- **n.b. include this (method comparison) context in your Journal Club talks**

limma fundamentals

The simplistic view: Differential expression, small sample inference

- Table of data (e.g., microarray gene expression data with replicates of each of condition A, condition B)
  - rows = features (e.g., genes), columns = experimental units (samples)
- Most common problem in statistical bioinformatics: want to infer whether there is a change in the response —> a statistical test for each row of the table.

What test might you use? Why is this hard? What issues arise? How much statistical power is there [1] ?

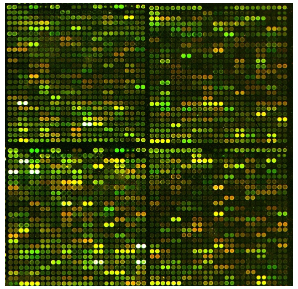


```
> head(y)
          group0     group0      group0     group1     group1      group1
gene1 -0.1874854  0.2584037 -0.05550717 -0.4617966 -0.3563024 -0.03271432
gene2 -3.5418798 -2.4540999  0.11750996 -4.3270442 -5.3462622 -5.54049106
gene3 -0.1226303  0.9354707 -1.10537767 -0.1037990  0.5221678 -1.72360854
gene4 -2.3394536 -0.3495697 -3.47742610 -3.2287093  6.1376670 -2.23871974
gene5 -3.7978820  1.4545702 -7.14796503 -4.0500796  4.7235714 10.00033769
gene6  1.4627078 -0.3096070 -0.26230124 -0.7903434  0.8398769 -0.96822312
```

[1] http://www.stat.ubc.ca/~rollin/stats/ssize/n2.html

Microarray expression measures

Two-colour



$$\overset{\text{array}}{} y_{\underset{\text{probe or gene}}{g}\overset{}{a}} = \log_2(R/G)$$

array

probe or gene

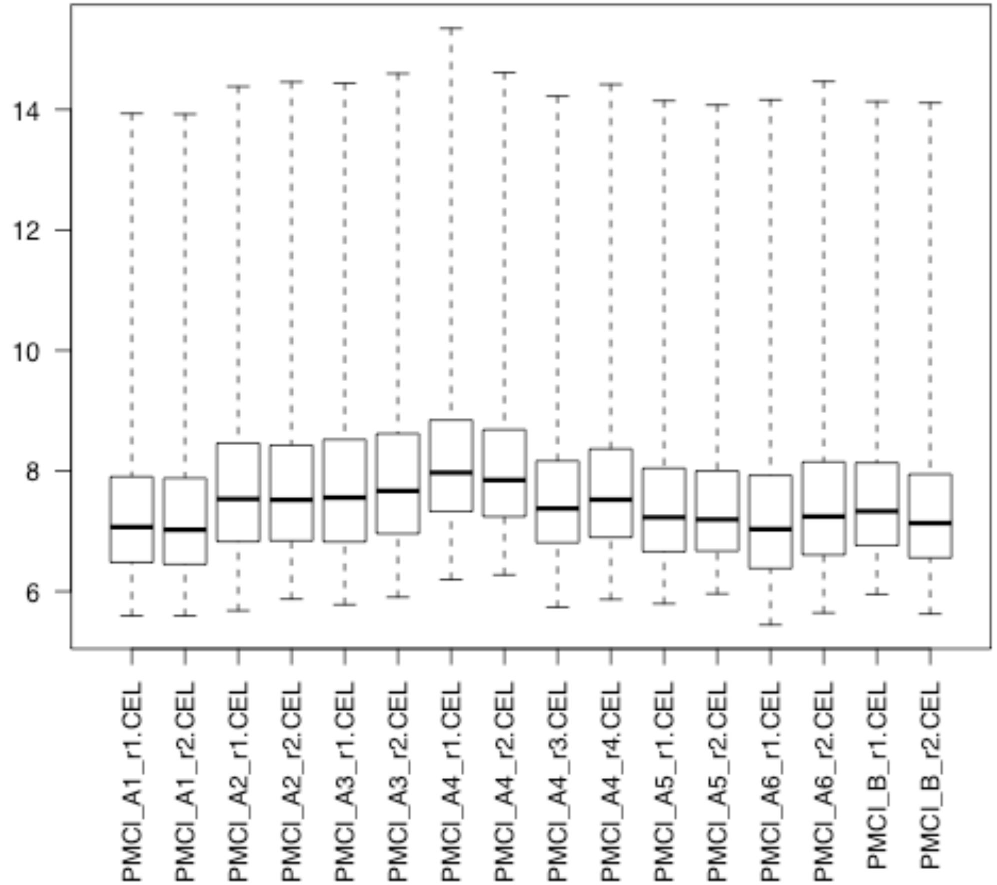
Affymetrix

y_{ga} = log-intensity (summarized over probes)

Illumina

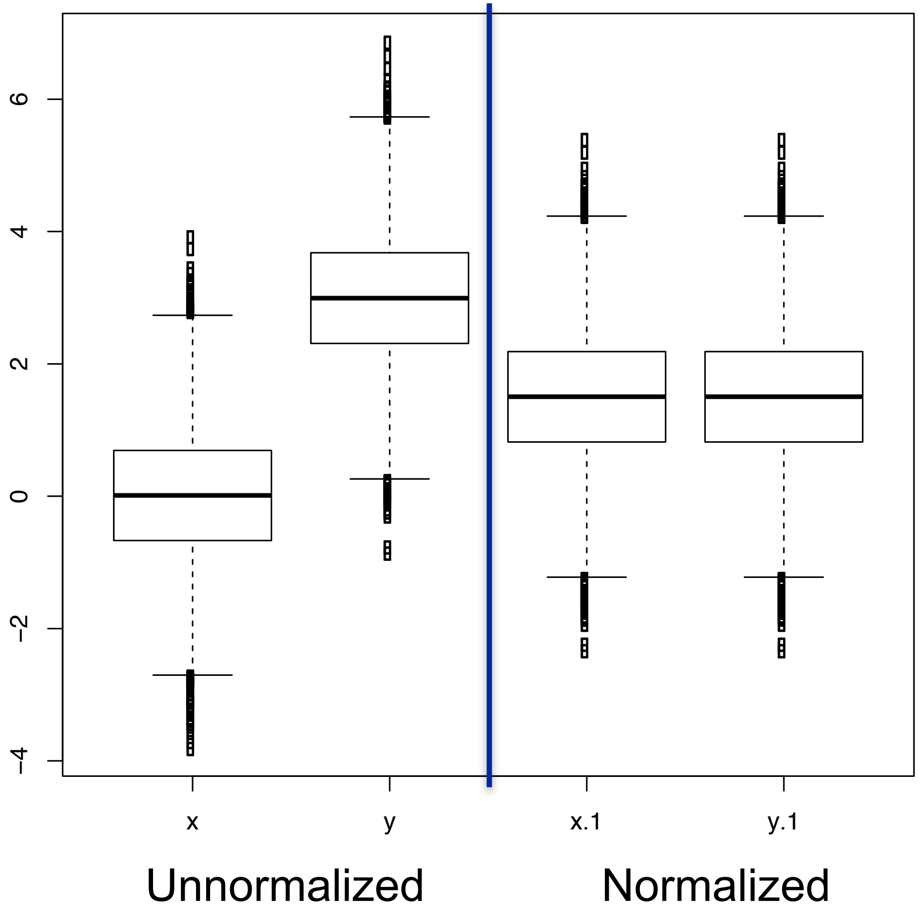
y_{ga} = log-intensity (summarized over beads)

Normalization: one-colour



Similarly for single channel data, adjustments need to be made for all samples to be comparable.

Quantile normalization



```
x <- rnorm(10000, mean=0, sd=1)
y <- rnorm(10000, mean=3)
z <- cbind(x,y)

# create "reference" distribution
s <- apply(z,2,sort)
sm <- rowMeans(s)

# impose ref. distribution by ranks
r <- apply(z,2,rank)
n <- apply(r,2,function(u) sm[u])

boxplot( data.frame(x=x,y=y,n) )

#> library(limma)
#> zn <- normalizeQuantiles(z)
#> all(zn==n)
#[1] TRUE
```

Preprocessing: additive + multiplicative error model

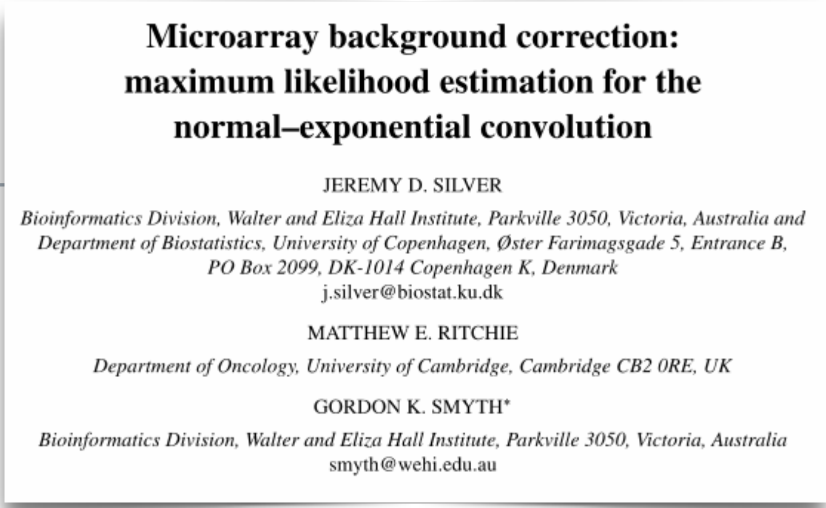
Observe intensity for one probe on one array

Intensity = background + signal

$$I = \underset{\substack{\uparrow \\ \text{additive} \\ \text{errors}}}{B} + \underset{\substack{\uparrow \\ \text{multiplicative errors}}}{S}$$

This idea underlies variance stabilizing transformations vsn (two colour data) and vst (for Illumina data)

normexp convolution model



Microarray background correction: maximum likelihood estimation for the normal–exponential convolution

JEREMY D. SILVER

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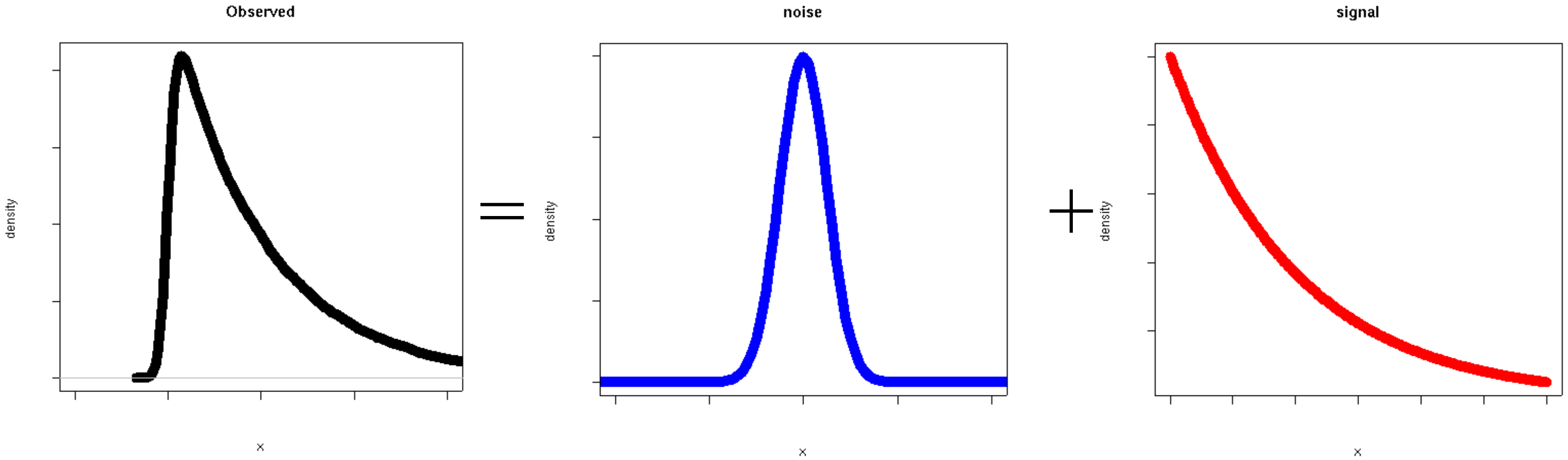
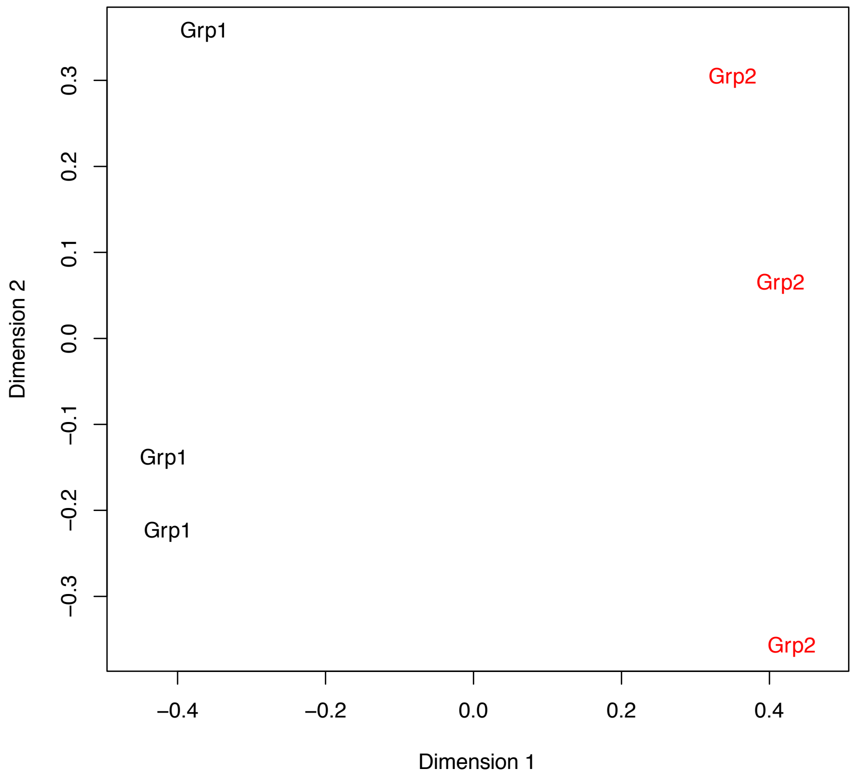
Intensity = Background + Signal

$N(\mu,\sigma^2)$ Exponential(α)



Quality assessments / spot checks

Multidimensional scaling plot



```
sd <- 0.3*sqrt(4/rchisq(1000,df=4))
x <- matrix(rnorm(1000*6,sd=sd),1000,6)
x[1:50,4:6] <- x[1:50,4:6] + 2

mds <- plotMDS(x)

> round(mds$distance.matrix,3)
      [,1]  [,2]  [,3]  [,4] [,5] [,6]
[1,] 0.000 0.000 0.000 0.000 0.00    0
[2,] 0.835 0.000 0.000 0.000 0.00    0
[3,] 0.850 0.793 0.000 0.000 0.00    0
[4,] 1.089 1.068 1.058 0.000 0.00    0
[5,] 1.050 1.058 1.072 0.863 0.00    0
[6,] 0.991 1.047 1.046 0.865 0.85    0
```

"To consult the statistician after an experiment is finished is often merely to ask him[her] to conduct a post mortem examination. He[She] can perhaps say what the experiment died of." R. A. Fisher

Motivation for exploratory data analysis: Case Study

(from Stefano, a former M.Sc. student in my Institute)

He is studying gene expression in fruitfly and is interested in transcriptional responses following "heat shock".

Basic schematic of experiment:

CTL	t0		t12		
TRT		t4	t12	t24	t72

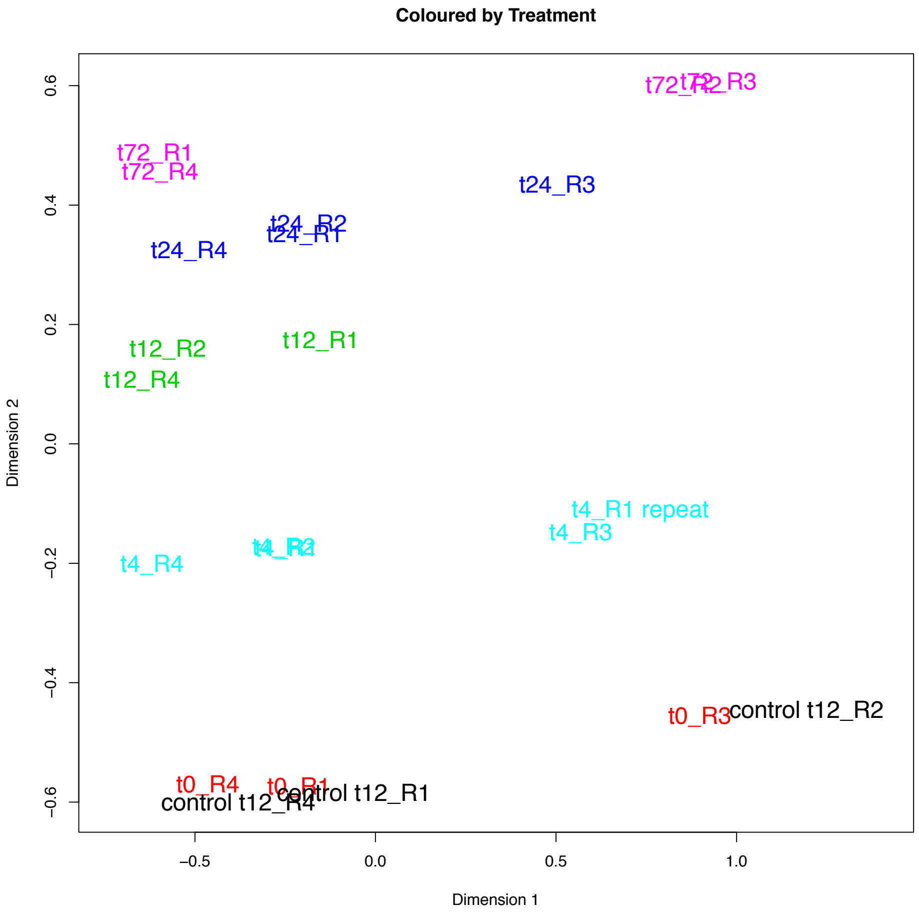
↑ Change to lower temperature.

~4 replicates for each condition

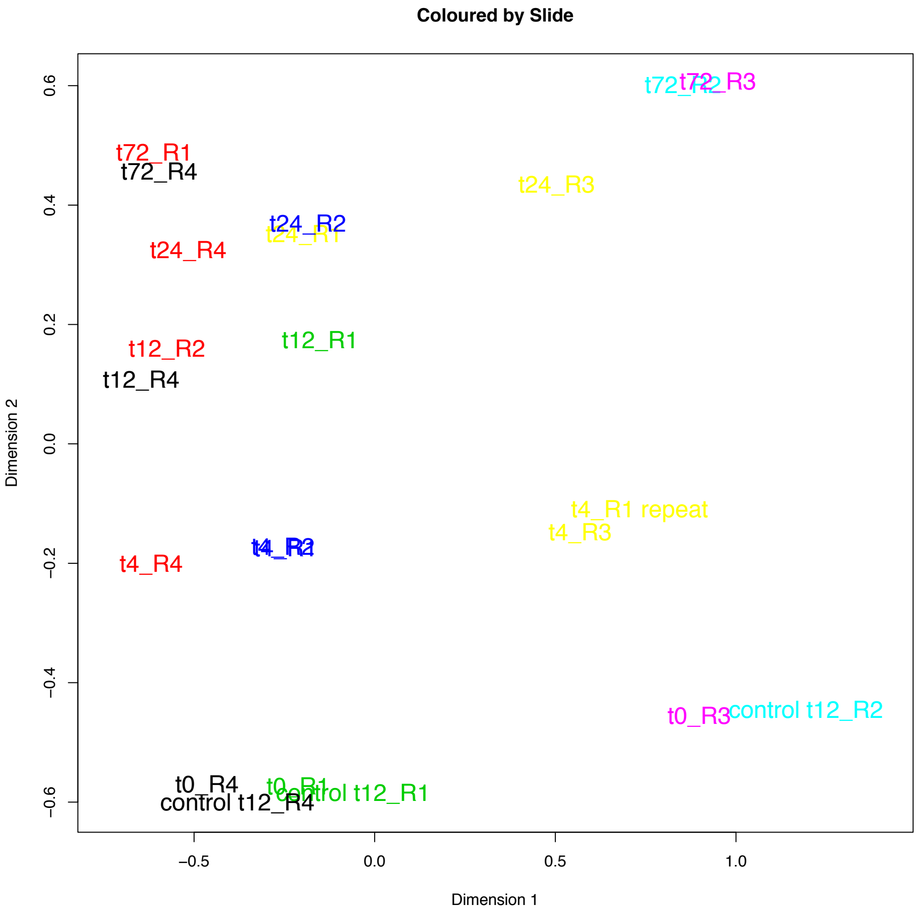
http://en.wikipedia.org/wiki/Multidimensional_scaling

```
library(limma)
plotMDS(d)  # ‘d’ is a matrix
```

Take a close look at where the replicates are to each other relative to the X- and Y-axes



Coloured by Treatment



Coloured by Slide

22 samples x ~20,000 genes

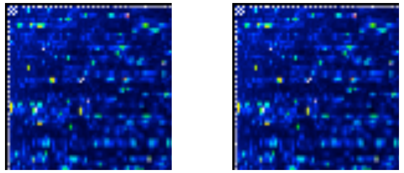
reduced to 22 samples x 2 dimensions

Limma concept: borrowing information across genes

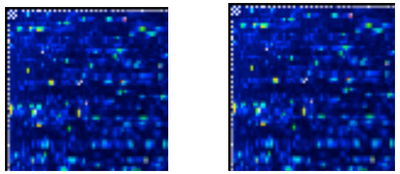
- Small data sets: few samples, generally under-powered for 1 gene
- Curse of dimensionality: many tests, need to adjust for multiple testing (= loss of power)
- Benefit of parallelism: same model is fit for every gene. Can borrow information from one gene to another
  - Hard: assume parameters are constant across genes
  - Soft: smooth genewise parameters towards a common value in a graduated way, e.g., Bayes, empirical Bayes, Stein shrinkage …

A very common experiment (1-colour)

Mutant x 2



WT x 2



Gene X

Which genes are differentially expressed?

$n_1 = n_2 = 2$ Affymetrix arrays

~30,000 probe-sets

Ordinary t-tests (1-colour)

$$t_g = \frac{\overline{y}_{\mathrm{mu}} - \overline{y}_{\mathrm{wt}}}{s_g \, c}$$

give very high false discovery rates

$$c = \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$$

Residual df = 2

t-tests with common variance

$$t_{g,\text{pooled}} = \frac{\overline{y}_{\text{mu}} - \overline{y}_{\text{wt}}}{s_0 \; c}$$

with residual standard deviation s_0 pooled across genes

More stable, but ignores gene-specific variability

$$c = \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$$

A better compromise

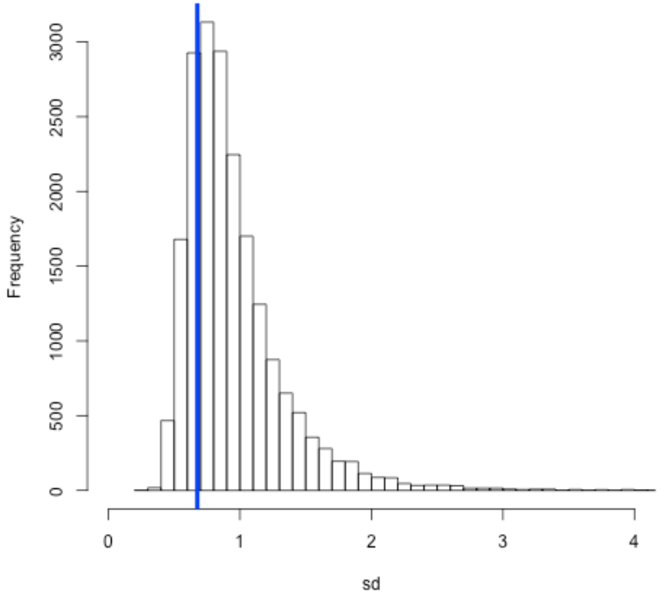
Shrink standard deviations towards common value

$$\tilde{s}_g^2 = \frac{d_0 s_0^2 + d_g s_g^2}{d_0 + d_g}$$

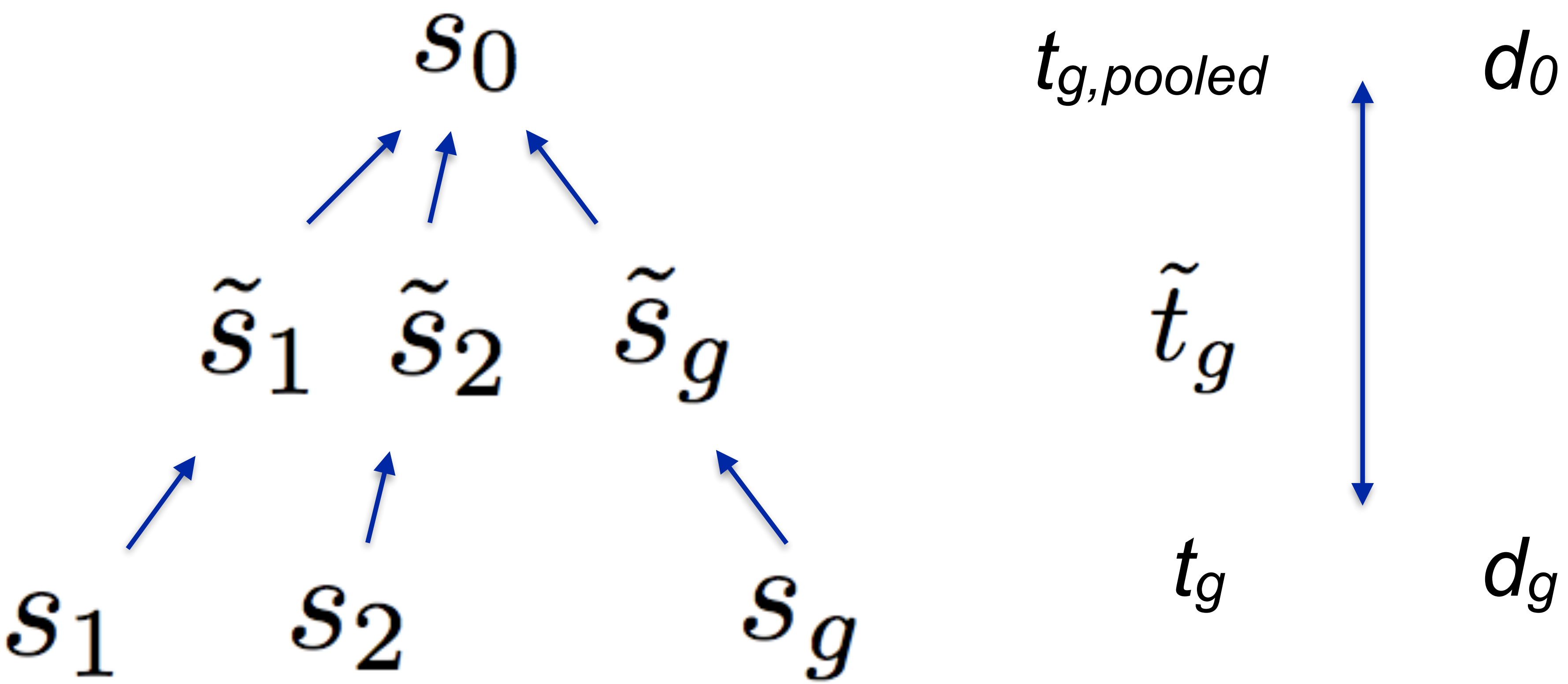
d = degrees of freedom

Moderated t-statistics

$$\tilde{t}_g = \frac{\overline{y}_{\mathrm{mu}} - \overline{y}_{\mathrm{wt}}}{\tilde{s}_g\, u}$$



Shrinkage of standard deviations



The **data decides** whether $\tilde{t}_g$ should be closer to $t_{g,pooled}$ or t_g

Why does it work?

- We learn what is the typical variability level by looking at all genes, but allow some flexibility from this for individual genes
- Adaptive – data (through hyperparameter estimates, d_0 and s_0) suggests how much to “squeeze”/„moderate“ toward common value

Hierarchical model for variances

Data

$$s_g^2 \sim \sigma_g^2 \frac{\chi^2_{d_g}}{d_g}$$

Prior

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

Posterior

$$E\left(\frac{1}{\sigma_g^2} \mid s_g^2\right) = \frac{d_0 + d_g}{s_0^2 d_0 + s_g^2 d_g}$$

Posterior Statistics

Posterior variance estimators

$$\tilde{s}_g^2 = \frac{s_0^2 d_0 + s_g^2 d_g}{d_0 + d_g}$$

Moderated t-statistics

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

Baldi & Long 2001, Wright & Simon 2003, Smyth 2004

Exact distribution for moderated t

An unexpected piece of mathematics shows that, under the null hypothesis,

$$\tilde{t}_g \sim t_{d_0+d_g}$$

The degrees of freedom add!

The Bayes prior in effect adds d_0 extra arrays for estimating the variance.

Wright and Simon 2003, Smyth 2004

Aside: Marginal Distributions to calculate

Under usual likelihood model, s_g is independent of the estimated coefficients.

Under the hierarchical model, s_g is independent of the moderated t-statistics instead

$$s_g^2 \sim s_0^2 F_{d,d_0}$$

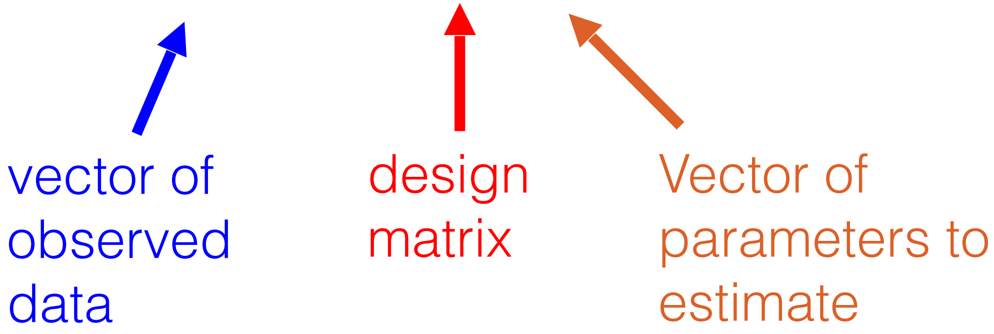
Multiple testing and adjusted p-values

- Each statistical test has an associated false positive rate
- Traditional method in statistics is to control family wise error rate, e.g., by Bonferroni.
- Controlling the false discovery rate (FDR) is more **appropriate** in microarray studies
- Benjamini and Hochberg method controls expected FDR for independent or weakly dependent test statistics. Simulation studies support use for genomic data.
- All methods can be implemented in terms of adjusted p-values.

Linear Models

- In general, need to specify:
  - Dependent variable
  - Explanatory variables (experimental design, covariates, etc.)
- More generally:

$$y = X\beta + \epsilon$$



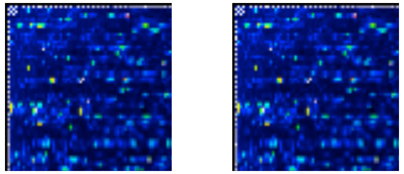
vector of observed data

design matrix

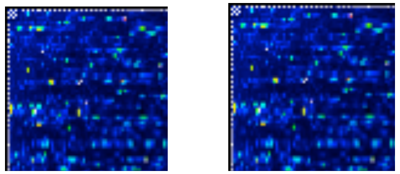
Vector of parameters to estimate

Design → Linear models

WT x 2



Mutant x 2



$$\begin{bmatrix} y_1 \\ y_2 \\ y_3 \\ y_4 \end{bmatrix} = \begin{bmatrix} 1 & 0 \\ 1 & 0 \\ 1 & 1 \\ 1 & 1 \end{bmatrix} \begin{bmatrix} \beta_1 \\ \beta_2 \end{bmatrix}$$

β_1 = wt log-expression

β_2 = mutant – wt

$E[y_1]=E[y_2]=\beta_1$

$E[y_3]=E[y_4]= \beta_1+ \beta_2$

Layers to add ..

- Where does the moderated variance come from?
- Why the degrees of freedom add: $d_0 + d$
- empirical Bayes: how to estimate the hyperparameters (d_0 and s_0)
- Design matrices + contrast matrices in practice

Unexpected mathematics: Why do degrees of freedom add?

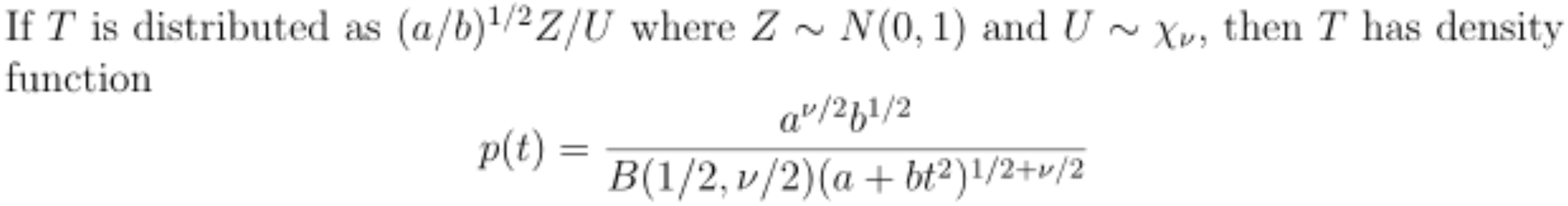
The construction of the classical t-statistic:

$$Z = \left(\overline{X}_n - \mu\right)\frac{\sqrt{n}}{\sigma}$$

$$V = (n-1)\frac{S_n^2}{\sigma^2}$$

$$T \equiv \frac{Z}{\sqrt{V/\nu}} = \left(\overline{X}_n - \mu\right)\frac{\sqrt{n}}{S_n},$$

Bonus Exercise Part a (optional): what are a, b above?



If T is distributed as $(a/b)^{1/2}Z/U$ where $Z \sim N(0,1)$ and $U \sim \chi_\nu$, then T has density function

$$p(t) = \frac{a^{\nu/2}b^{1/2}}{B(1/2, \nu/2)(a + bt^2)^{1/2+\nu/2}}$$

http://en.wikipedia.org/wiki/Bayesian_inference

Optional exercise: Derive the posterior

Data

$$\boxed{s_g^2 \sim \sigma_g^2 \frac{\chi_{d_g}^2}{d_g}}$$

Prior

$$\boxed{\frac{1}{\sigma_g^2} \sim s_0^2 \frac{\chi_{d_0}^2}{d_0}}$$

$$p(\theta|x) = \frac{\boxed{f(x|\theta)}\boxed{p(\theta)}}{\int f(x|\theta)p(\theta)d\theta}$$

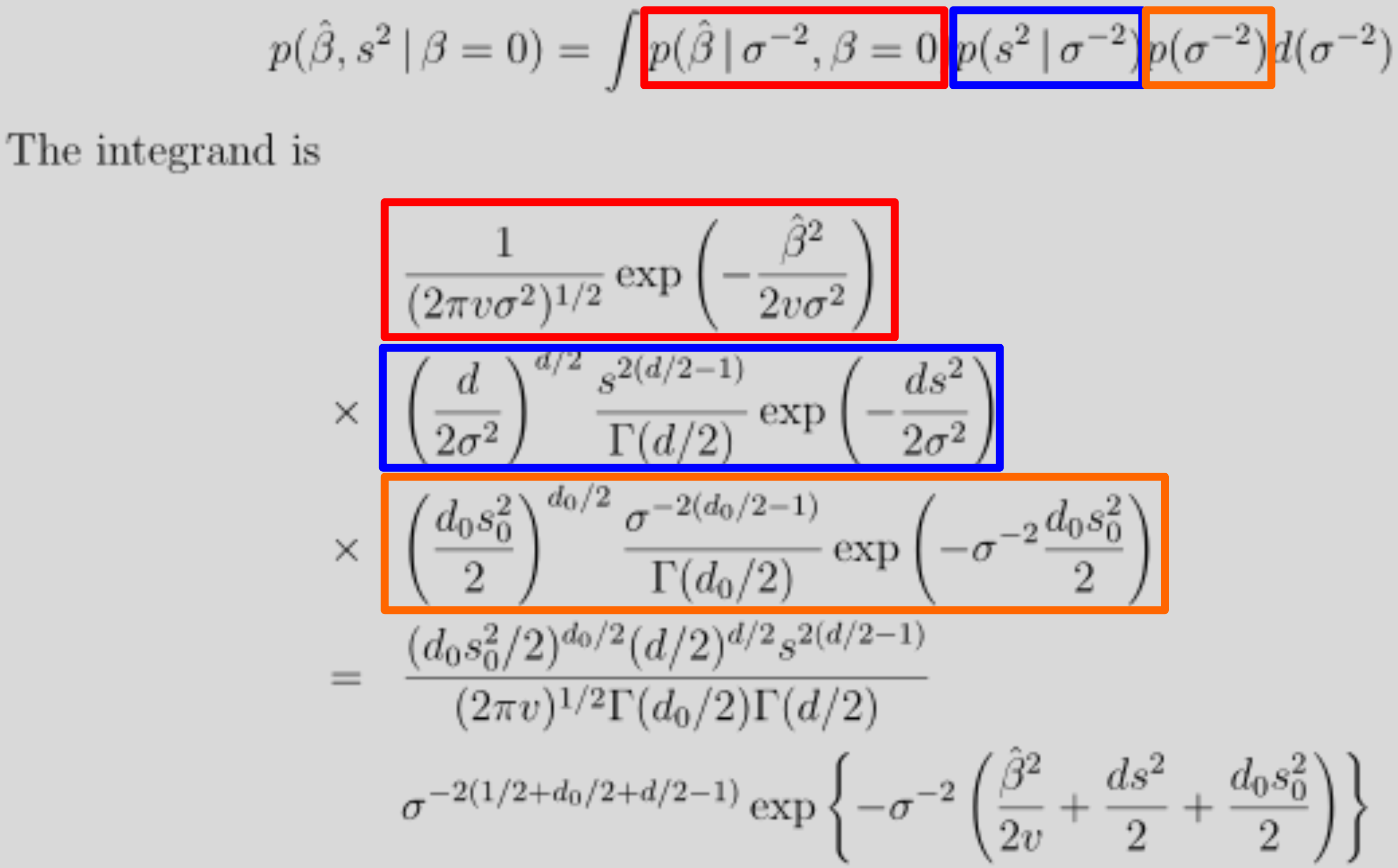
Posterior

$$E\left(\frac{1}{\sigma_g^2} \mid s_g^2\right) = \frac{d_0 + d_g}{s_0^2 d_0 + s_g^2 d_g}$$

Bonus exercise Part b

Sketch: i) Let $x=s^2$, $\theta=\sigma^{-2}$; ii) Using the functional form of chi-squared distribution, calculate only the numerator (since denominator does not contain θ); iii) collect terms and see if you can identify the distribution and the parameters of it; iv) What is the mean of this distribution?

Unexpected mathematics: Why do degrees of freedom add?



$$p(\hat{\beta}, s^2 \,|\, \beta = 0) = \int \boxed{p(\hat{\beta} \,|\, \sigma^{-2}, \beta = 0)} \boxed{p(s^2 \,|\, \sigma^{-2})} \boxed{p(\sigma^{-2})} d(\sigma^{-2})$$

The integrand is

$$\boxed{\frac{1}{(2\pi v \sigma^2)^{1/2}} \exp\left(-\frac{\hat{\beta}^2}{2v\sigma^2}\right)}$$

$$\times \boxed{\left(\frac{d}{2\sigma^2}\right)^{d/2} \frac{s^{2(d/2-1)}}{\Gamma(d/2)} \exp\left(-\frac{ds^2}{2\sigma^2}\right)}$$

$$\times \boxed{\left(\frac{d_0 s_0^2}{2}\right)^{d_0/2} \frac{\sigma^{-2(d_0/2-1)}}{\Gamma(d_0/2)} \exp\left(-\sigma^{-2}\frac{d_0 s_0^2}{2}\right)}$$

$$= \frac{(d_0 s_0^2/2)^{d_0/2}(d/2)^{d/2} s^{2(d/2-1)}}{(2\pi v)^{1/2}\Gamma(d_0/2)\Gamma(d/2)}$$

$$\sigma^{-2(1/2+d_0/2+d/2-1)} \exp\left\{-\sigma^{-2}\left(\frac{\hat{\beta}^2}{2v} + \frac{ds^2}{2} + \frac{d_0 s_0^2}{2}\right)\right\}$$

Unexpected mathematics: Why do degrees of freedom add?

$$p(\hat\beta, s^2 \mid \beta = 0) = \int p(\hat\beta \mid \sigma^{-2}, \beta = 0) p(s^2 \mid \sigma^{-2}) p(\sigma^{-2}) d(\sigma^{-2})$$

$$= \frac{(d_0 s_0^2/2)^{d_0/2}(d/2)^{d/2} s^{2(d/2-1)}}{(2\pi v)^{1/2}\Gamma(d_0/2)\Gamma(d/2)}$$

$$\boxed{\sigma^{-2(1/2+d_0/2+d/2-1)} \exp\left\{-\sigma^{-2}\left(\frac{\hat\beta^2}{2v} + \frac{ds^2}{2} + \frac{d_0 s_0^2}{2}\right)\right\}}$$

↗

σ^{-2} is chi-squared (or gamma)

$$f(x;\, k) = \begin{cases} \frac{x^{(k/2)-1} e^{-x/2}}{2^{k/2}\Gamma\left(\frac{k}{2}\right)}, & x \geq 0; \\ 0, & \text{otherwise.} \end{cases}$$

http://en.wikipedia.org/wiki/Chi-squared_distribution

Unexpected mathematics: Why do degrees of freedom add?

$$p(\hat{\beta}, s^2 \mid \beta = 0) = \int p(\hat{\beta} \mid \sigma^{-2}, \beta = 0) p(s^2 \mid \sigma^{-2}) p(\sigma^{-2}) d(\sigma^{-2})$$

$$\begin{aligned} & p(\hat{\beta}, s^2 \mid \beta = 0) \\ & \quad = \frac{(1/2v)^{1/2}(d_0 s_0^2/2)^{d_0/2}(d/2)^{d/2} s^{2(d/2-1)}}{D(1/2, d_0/2, d/2)} \left(\frac{\hat{\beta}^2/v + d_0 s_0^2 + d s^2}{2}\right)^{-(1+d_0+d)/2} \end{aligned}$$

Unexpected mathematics: Why do degrees of freedom add?

$$p(\hat{\beta}, s^2 \,|\, \beta = 0) = \frac{(1/2v)^{1/2}(d_0 s_0^2/2)^{d_0/2}(d/2)^{d/2} s^{2(d/2-1)}}{D(1/2, d_0/2, d/2)} \left(\frac{\hat{\beta}^2/v + d_0 s_0^2 + d s^2}{2}\right)^{-(1+d_0+d)/2}$$

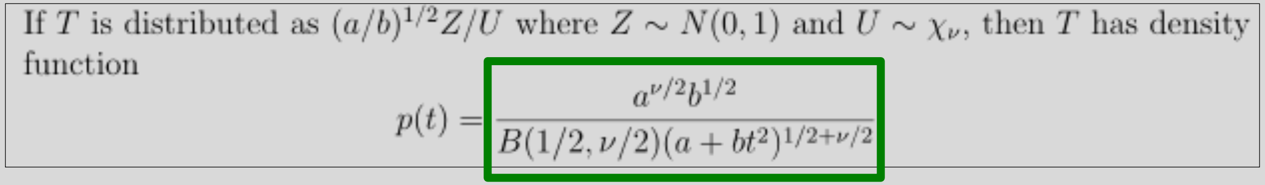
The null joint distribution of $\tilde{t}$ and s^2 is

$$p(\tilde{t}, s^2 \,|\, \beta = 0) = \tilde{s} v^{1/2} \boxed{p(\hat{\beta}, s^2 \,|\, \beta = 0)}$$

http://en.wikipedia.org/wiki/Random_variable#Distribution_functions_of_random_variables

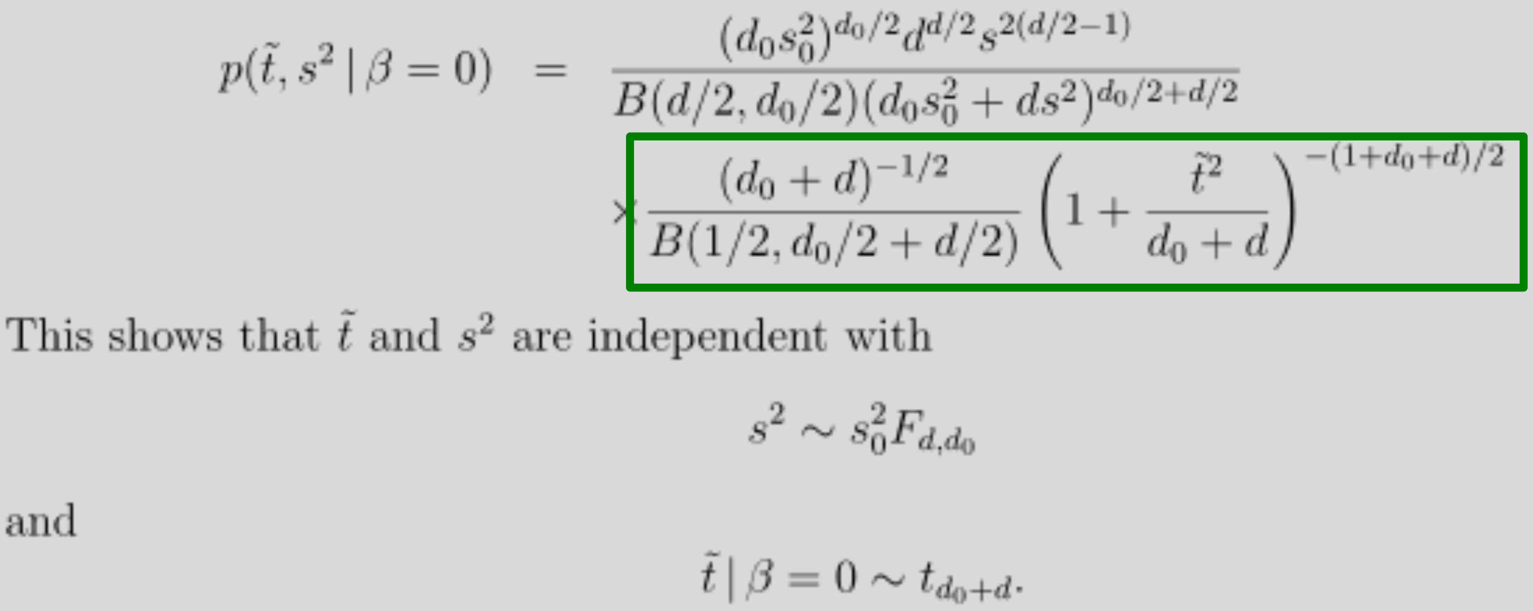
$$f_Y(y) = f_X(g^{-1}(y)) \left| \frac{d g^{-1}(y)}{dy} \right|$$

Unexpected mathematics: Why do degrees of freedom add?



If T is distributed as $(a/b)^{1/2}Z/U$ where $Z \sim N(0,1)$ and $U \sim \chi_\nu$, then T has density function

$$p(t) = \boxed{\frac{a^{\nu/2}b^{1/2}}{B(1/2,\nu/2)(a+bt^2)^{1/2+\nu/2}}}$$



$$p(\tilde{t}, s^2 \,|\, \beta = 0) \;=\; \frac{(d_0 s_0^2)^{d_0/2} d^{d/2} s^{2(d/2-1)}}{B(d/2, d_0/2)(d_0 s_0^2 + d s^2)^{d_0/2+d/2}} \times \boxed{\frac{(d_0+d)^{-1/2}}{B(1/2, d_0/2+d/2)} \left(1 + \frac{\tilde{t}^2}{d_0+d}\right)^{-(1+d_0+d)/2}}$$

This shows that $\tilde{t}$ and s^2 are independent with

$$s^2 \sim s_0^2 F_{d,d_0}$$

and

$$\tilde{t} \,|\, \beta = 0 \sim t_{d_0+d}.$$

Linear Models

- In general, need to specify:
  - Dependent variable
  - Explanatory variables (experimental design, covariates, etc.)
- More generally:

$$y = X\alpha + \epsilon$$

vector of observed data

design matrix

Vector of parameters to estimate

Obtain a linear model for each gene g

$$E(\underset{\sim}{y}_g) = X\underset{\sim}{\alpha}_g$$

$$\mathrm{var}(\underset{\sim}{y}_g) = W_g^{-1}\sigma_g^2$$

Contrasts -- `contrasts.fit()`

A *contrast* is any linear combination of the coefficients α_j which we want to test equal to zero.

Define contrasts

$$\underset{\sim}{\beta}_g = C^T \underset{\sim}{\alpha}_g$$

were C is the contrast matrix.

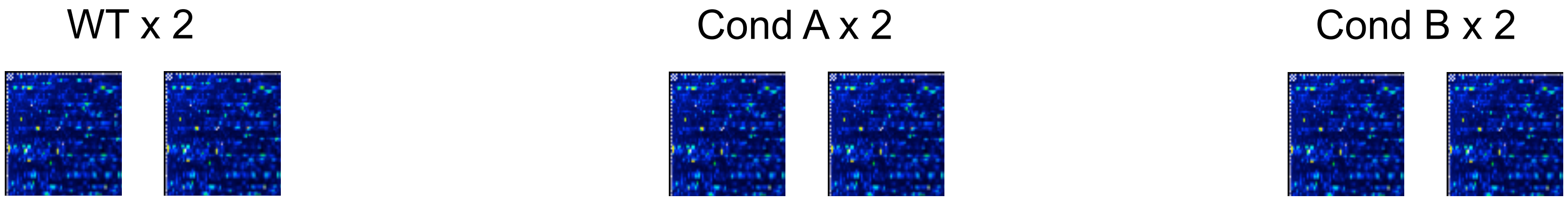
Want to test

$$H_0 : \beta_{gj} = 0$$

vs

$$H_a : \beta_{gj} \neq 0$$

Analysis of Variance → Linear model



$$\begin{bmatrix} y_1 \\ y_2 \\ y_3 \\ y_4 \\ y_5 \\ y_6 \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 \\ 1 & 0 & 0 \\ 1 & 1 & 0 \\ 1 & 1 & 0 \\ 1 & 0 & 1 \\ 1 & 0 & 1 \end{bmatrix} \begin{bmatrix} \alpha_1 \\ \alpha_2 \\ \alpha_3 \end{bmatrix} + \begin{bmatrix} \epsilon_1 \\ \epsilon_2 \\ \epsilon_3 \\ \epsilon_4 \\ \epsilon_5 \\ \epsilon_6 \end{bmatrix}$$

α_1 = wt log-expression

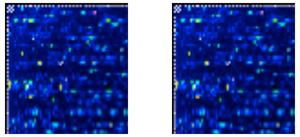
α_2 = Cond A - wt

α_3 = Cond B - wt

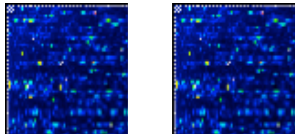
$E[y_1]=E[y_2]=\alpha_1$ $E[y_3]=E[y_4]=\alpha_1+ \alpha_2$ $E[y_5]=E[y_6]= \alpha_1+ \alpha_3$

Analysis of Variance → Linear model, alternative parameterization

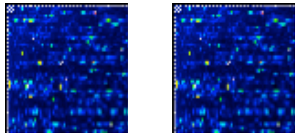
WT x 2



Cond A x 2



Cond B x 2



$$\begin{bmatrix} y_1 \\ y_2 \\ y_3 \\ y_4 \\ y_5 \\ y_6 \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 \\ 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} \alpha_1 \\ \alpha_2 \\ \alpha_3 \end{bmatrix} + \begin{bmatrix} \epsilon_1 \\ \epsilon_2 \\ \epsilon_3 \\ \epsilon_4 \\ \epsilon_5 \\ \epsilon_6 \end{bmatrix}$$

α_1 = wt log-expression

α_2 = Cond A log-expression

α_3 = Cond B log-expression

$E[y_1]=E[y_2]=\alpha_1$ $E[y_3]=E[y_4]=\alpha_2$ $E[y_5]=E[y_6]= \alpha_3$

An example use of design and contrast matrices

design matrix

$$\begin{bmatrix} y_1 \\ y_2 \\ y_3 \\ y_4 \\ y_5 \\ y_6 \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 \\ 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} \alpha_1 \\ \alpha_2 \\ \alpha_3 \end{bmatrix} + \begin{bmatrix} \epsilon_1 \\ \epsilon_2 \\ \epsilon_3 \\ \epsilon_4 \\ \epsilon_5 \\ \epsilon_6 \end{bmatrix}$$

$E[y_1]=E[y_2]=\alpha_1$

$E[y_3]=E[y_4]=\alpha_2$

$E[y_5]=E[y_6]=\alpha_3$

contrast matrix

$$\beta = C\alpha = \begin{bmatrix} -1 & 1 & 0 \\ 0 & -1 & 1 \end{bmatrix} \begin{bmatrix} \alpha_1 \\ \alpha_2 \\ \alpha_3 \end{bmatrix} = \begin{bmatrix} \alpha_2 - \alpha_1 \\ \alpha_3 - \alpha_2 \end{bmatrix}$$

Contrasts -- `contrasts.fit()`

A *contrast* is any linear combination of the coefficients α_j that we want to test equal to zero.

Define contrasts

$$\underset{\sim}{\beta}_g = C^T \underset{\sim}{\alpha}_g$$

were C is the contrast matrix.

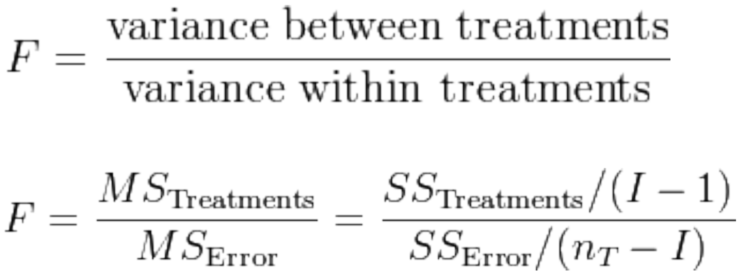
Want to test

$$H_0 : \beta_{gj} = 0$$

vs

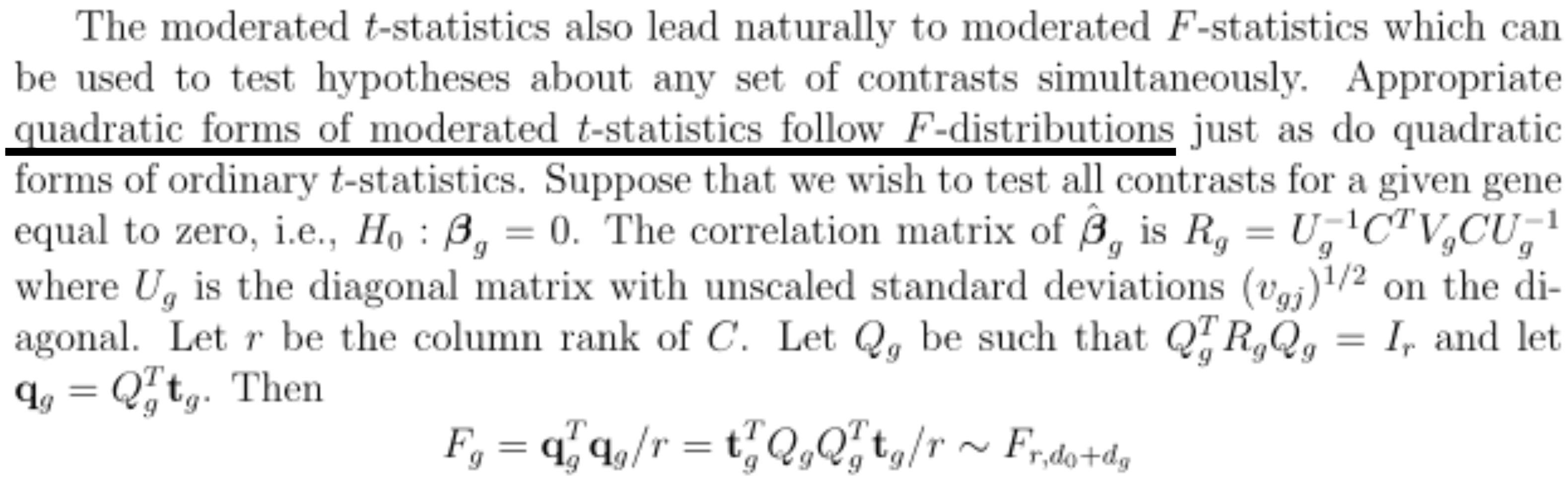
$$H_a : \beta_{gj} \neq 0$$

Limma / Analysis of Variance



$$F = \frac{\text{variance between treatments}}{\text{variance within treatments}}$$

$$F = \frac{MS_{\text{Treatments}}}{MS_{\text{Error}}} = \frac{SS_{\text{Treatments}}/(I-1)}{SS_{\text{Error}}/(n_T - I)}$$



The moderated t-statistics also lead naturally to moderated F-statistics which can be used to test hypotheses about any set of contrasts simultaneously. Appropriate quadratic forms of moderated t-statistics follow F-distributions just as do quadratic forms of ordinary t-statistics. Suppose that we wish to test all contrasts for a given gene equal to zero, i.e., $H_0 : \boldsymbol{\beta}_g = 0$. The correlation matrix of $\hat{\boldsymbol{\beta}}_g$ is $R_g = U_g^{-1} C^T V_g C U_g^{-1}$ where U_g is the diagonal matrix with unscaled standard deviations $(v_{gj})^{1/2}$ on the diagonal. Let r be the column rank of C. Let Q_g be such that $Q_g^T R_g Q_g = I_r$ and let $\mathbf{q}_g = Q_g^T \mathbf{t}_g$. Then

$$F_g = \mathbf{q}_g^T \mathbf{q}_g / r = \mathbf{t}_g^T Q_g Q_g^T \mathbf{t}_g / r \sim F_{r, d_0 + d_g}$$

Aside: Marginal Distributions to calculate

Fun fact: Under usual likelihood model, s_g is independent of the estimated coefficients.

Under the hierarchical model, s_g is independent of the moderated t-statistics instead

$$s_g^2 \sim s_0^2 F_{d,d_0}$$

Thus, the set of s_g can be used to estimated d_0 and s_0

Section 6.2 limma paper: other tricks, such as Fisher's z distribution to estimate d_0 and s_0

Relate to limma objects

$$\begin{bmatrix} y_1\\ y_2\\ y_3\\ y_4\\ y_5\\ y_6 \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0\\ 1 & 0 & 0\\ 0 & 1 & 0\\ 0 & 1 & 0\\ 0 & 0 & 1\\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} \alpha_1\\ \alpha_2\\ \alpha_3 \end{bmatrix} + \begin{bmatrix} \epsilon_1\\ \epsilon_2\\ \epsilon_3\\ \epsilon_4\\ \epsilon_5\\ \epsilon_6 \end{bmatrix}$$

$E[y_1]=E[y_2]=\alpha_1$

$E[y_3]=E[y_4]=\alpha_2$

$E[y_5]=E[y_6]=\alpha_3$

$$\beta = C\alpha = \begin{bmatrix} -1 & 1 & 0\\ 0 & -1 & 1 \end{bmatrix} \begin{bmatrix} \alpha_1\\ \alpha_2\\ \alpha_3 \end{bmatrix} = \begin{bmatrix} \alpha_2 - \alpha_1\\ \alpha_3 - \alpha_2 \end{bmatrix}$$

```
> design
  alpha1 alpha2 alpha3
1      1      0      0
2      1      0      0
3      0      1      0
4      0      1      0
5      0      0      1
6      0      0      1
> cont.matrix <- makeContrasts(beta1="alpha2-alpha1",
                beta2="alpha3-alpha2",levels=design)
> cont.matrix
        Contrasts
Levels   beta1 beta2
  alpha1    -1     0
  alpha2     1    -1
  alpha3     0     1

fit <- lmFit(y,design)

fit.c  <- contrasts.fit(fit, cont.matrix)
fit.c  <- eBayes(fit.c)

> head(round(y,2),3)
       [,1]   [,2]  [,3]  [,4]   [,5]   [,6]
[1,]  -1.62   1.49  2.50  1.57  -0.71   0.38
[2,]  -4.50  -4.95 -3.66 -7.83  -1.59   6.94
[3,] -10.17 -21.90 14.03  3.66 -12.21 -15.26

> head(round(fit$coef,2),3)
     alpha1 alpha2 alpha3
[1,]  -0.07   2.03  -0.16
[2,]  -4.73  -5.75   2.67
[3,] -16.04   8.85 -13.74

> head(round(fit.c$coef,2),3)
      Contrasts
       beta1  beta2
  [1,]  2.10  -2.20
  [2,] -1.02   8.42
  [3,] 24.89 -22.59
```

Bonus exercise

- Part a: t-distribution
- Part b: derive posterior mean of limma variance model

- Submit together with Exercise 4 (same repo)
- Optional!
- Counts as a separate exercise (marked out of 3)