Week 6 notes:

- https://github.com/sta426hs2017/material/blob/master/week03_02oct2017/brainstorm_modified.md
- assignments:
  i) there should be no more pull requests, all further assignments will be done via GitHub classroom links
  ii) i am organising the marks and can release them individually
- Journal clubs start next week: some parameters
- Part 2 of the guts of limma

Journal clubs

- Starts next week!
- Aim for 20 minutes + 5 mins discussion
- Goal of audience: learn a few things about the topic + give feedback on content, clarity, etc.

23.10.2017	Mark	limma 2		
30.10.2017	Hubert	RNA-seq quantification	Assessment of batch-correction methods for scRNA-seq data with a new test metric (EC)	
06.11.2017	Mark	edgeR+friends 1	Why Most Published Research Findings Are False; Is most published research really false? (PM, SS)	Gene-level differential analysis at transcript-level resolution (CL)
13.11.2017	Charlotte	hands-on session #1: RNA-seq	X	X
20.11.2017	Mark	edgeR+friends 2	High Dimensional Classification with combined Adaptive Sparse PLS and Logistic Regression-link (TF, YY)	BSmooth: from whole genome bisulfite sequencing reads to differentially methylated regions (SO)
27.11.2017	Hubert	classification	Bayesian approach to single-cell differential expression analysis (UJ)	Guidance for RNA-seq co-expression network construction and analysis: safety in numbers (CS)
04.12.2017	Mark	single-cell	Removal of batch effects using distribution-matching residual networks (MH, SG)	DeepCpG: accurate prediction of single-cell DNA methylation states using deep learning (DR)
11.12.2017	Gosia	hands-on session #2: mass cytometry	X	X
18.12.2017	Mark	epigenomics, DNA methylation, ChIP data, gene set analysis	Linear models enable powerful differential activity analysis in massively parallel reporter assays (DP, ZY)	

Journal club expectations (from week 1 lecture)

University of Zurich[UZH]

Statistical Bioinformatics // Institute of Molecular Life Sciences

Expectations: **journal club** presentation

- 20-25 minutes (+5 minutes discussion)
- MUST:
  - ➡ be a paper about a **statistical** method in genomics
  - ➡ be approved by Mark/Hubert
- Should:
  - ➡ describe the biological context
  - ➡ describe the (new) model used
  - ➡ describe comparisons to existing methods
- Should not:
  - ➡ be one of the papers discussed in detail in lectures: limma, edgeR, DEXSeq, etc.
- (new for 2017) Expectations of observers: fill out feedback form

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

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

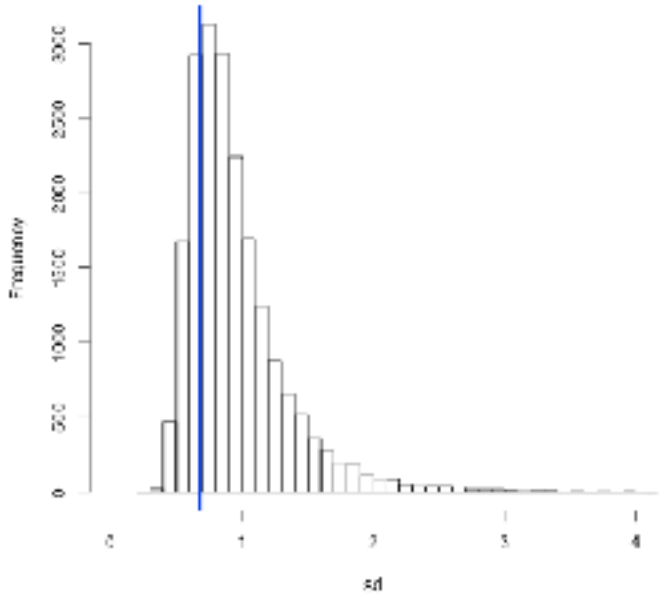
Shrink standard deviations towards common value

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

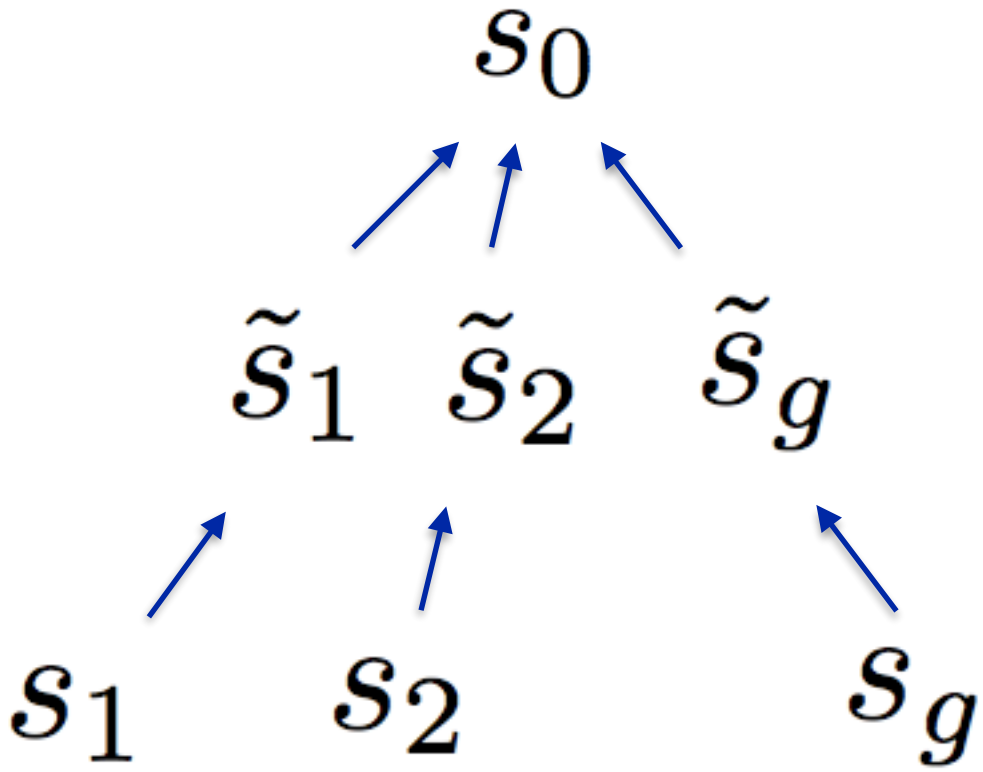
d = degrees of freedom

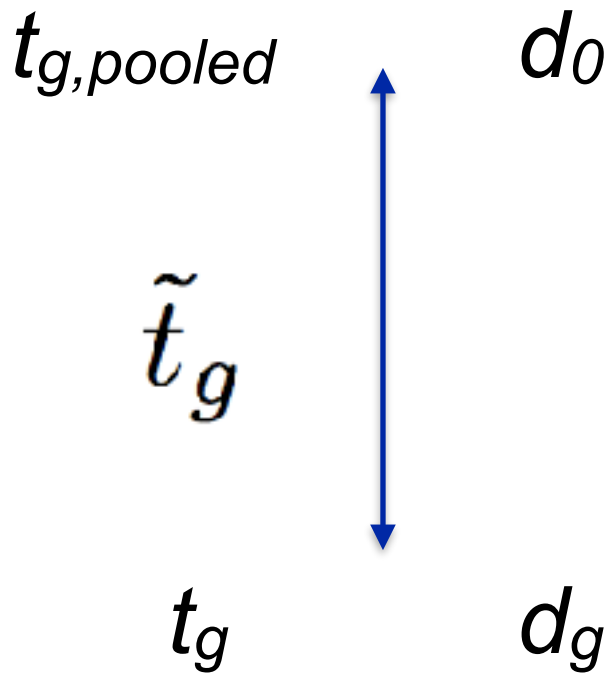
Moderated t-statistics

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



Shrinkage of standard deviations





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

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

Linear Models

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

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

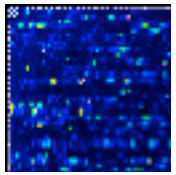
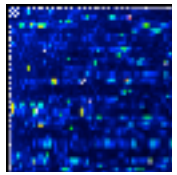
vector of observed data

design matrix

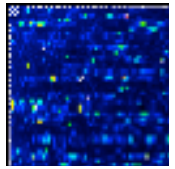
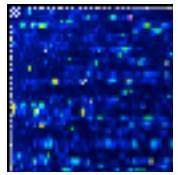
Vector of parameters to estimate

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

$E[y_1]=E[y_2]=\beta_1$ $E[y_3]=E[y_4]=\beta_1+\beta_2$

What layers to add today

- 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

In-class Exercise:

where does the t-distribution come from?

10-15 minutes: discuss with your neighbour, use the resources provided and/or search the web to explain .. where does the t-distribution originate from?

Unexpected mathematics: Why do degrees of freedom add?

The construction of the classical t-statistic:

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

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

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

Stated another way → Exercise (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

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

Prior

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

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

Posterior

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

Optional exercise
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?

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

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

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

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

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

Unexpected mathematics: Why do degrees of freedom add?

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

The integrand is

$$\begin{aligned}
& \frac{1}{(2\pi v\sigma^2)^{1/2}} \exp\left(-\frac{\hat{\beta}^2}{2v\sigma^2}\right) \\
\times & \left(\frac{d}{2\sigma^2}\right)^{d/2} \frac{s^{2(d/2-1)}}{\Gamma(d/2)} \exp\left(-\frac{ds^2}{2\sigma^2}\right) \\
\times & \left(\frac{d_0 s_0^2}{2}\right)^{d_0/2} \frac{\sigma^{-2(d_0/2-1)}}{\Gamma(d_0/2)} \exp\left(-\sigma^{-2}\frac{d_0 s_0^2}{2}\right) \\
= & \frac{(d_0 s_0^2/2)^{d_0/2}(d/2)^{d/2} s^{2(d/2-1)}}{(2\pi v)^{1/2}\Gamma(d_0/2)\Gamma(d/2)} \\
& \sigma^{-2(1/2+d_0/2+d/2-1)} \exp\left\{-\sigma^{-2}\left(\frac{\hat{\beta}^2}{2v} + \frac{ds^2}{2} + \frac{d_0 s_0^2}{2}\right)\right\}
\end{aligned}$$

Unexpected mathematics: Why do degrees of freedom add?

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

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

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

σ^{-2} is chi-squared (or gamma)

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

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

Unexpected mathematics: Why do degrees of freedom add?

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

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

Unexpected mathematics: Why do degrees of freedom add?

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

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

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

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

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

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

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

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

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

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

and

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

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

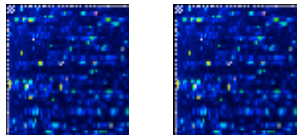
Obtain a linear model for each gene g

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

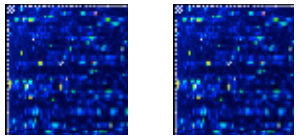
$$\text{var}(\underset{\sim}{y}_g) = W_g^{-1}\sigma_g^2$$

Analysis of Variance → Linear model

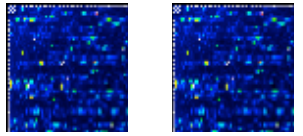
WT x 2



Cond A x 2



Cond B x 2



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

α_1 = wt log-expression

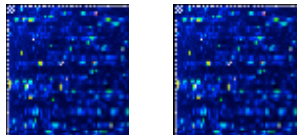
α_2 = Cond A - wt

α_3 = Cond B - wt

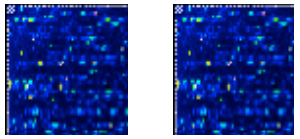
$E[y_1]=E[y_2]=\alpha_1$ $E[y_3]=E[y_4]=\alpha_1+\alpha_2$ $E[y_5]=E[y_6]=\alpha_1+\alpha_3$

Analysis of Variance → Linear model, alternative parameterization

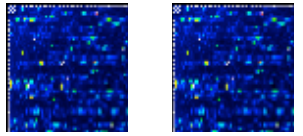
WT x 2



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

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

Linear Model Estimates – `lmFit()`

Obtain a linear model for each gene g

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

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

Estimate:

coefficients $\hat{\alpha}_{gj}$

standard deviations s_g

standard errors $\text{se}(\hat{\beta}_{gj})^2 = c_{gj}s_g^2$

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 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

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

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., $H_0 : \beta_g = 0$. 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
```

Affymetrix + RMA + IRLS

Other statistical aspects that are useful to know w.r.t. microarray data

Affymetrix probe design

Early platforms (11 or 20 probes in a set), 25bp probes, 3' biased

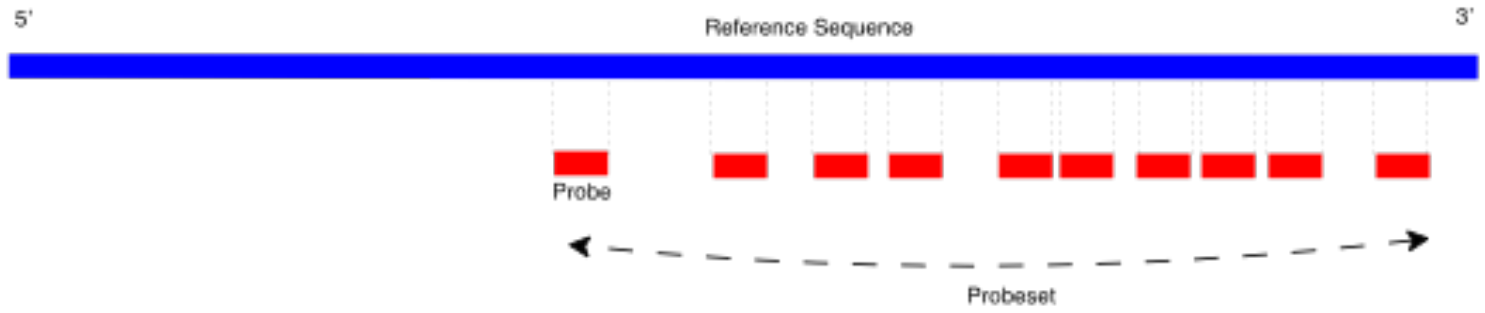


Figure 1.1: Multiple probes interrogating the sequence for a particular gene make up probesets.

Figure 1.2: Pefect Match and Mismatch Probes.

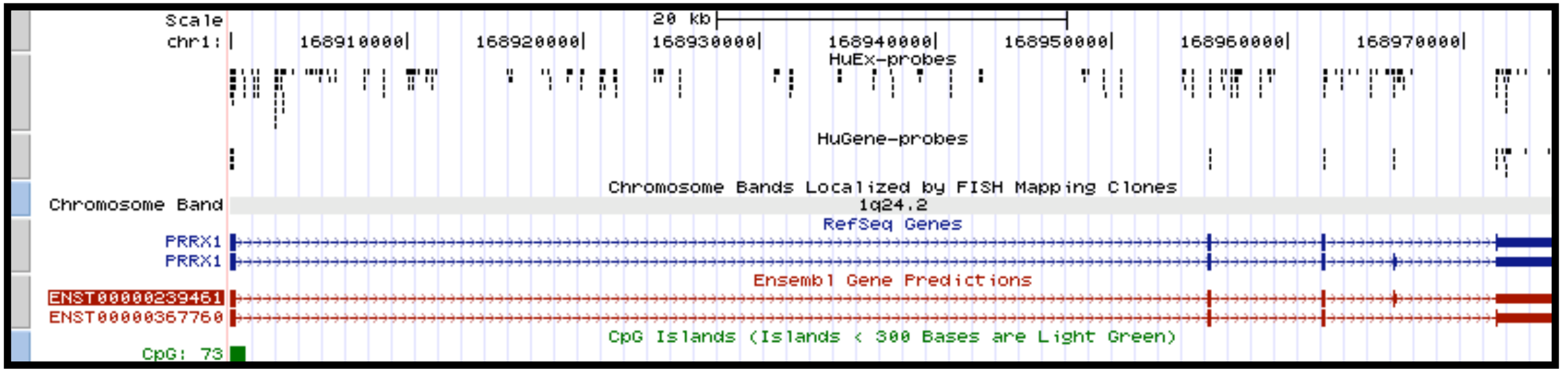
Ben Bolstad's PhD thesis

Latest Affymetrix design: “whole transcript” arrays

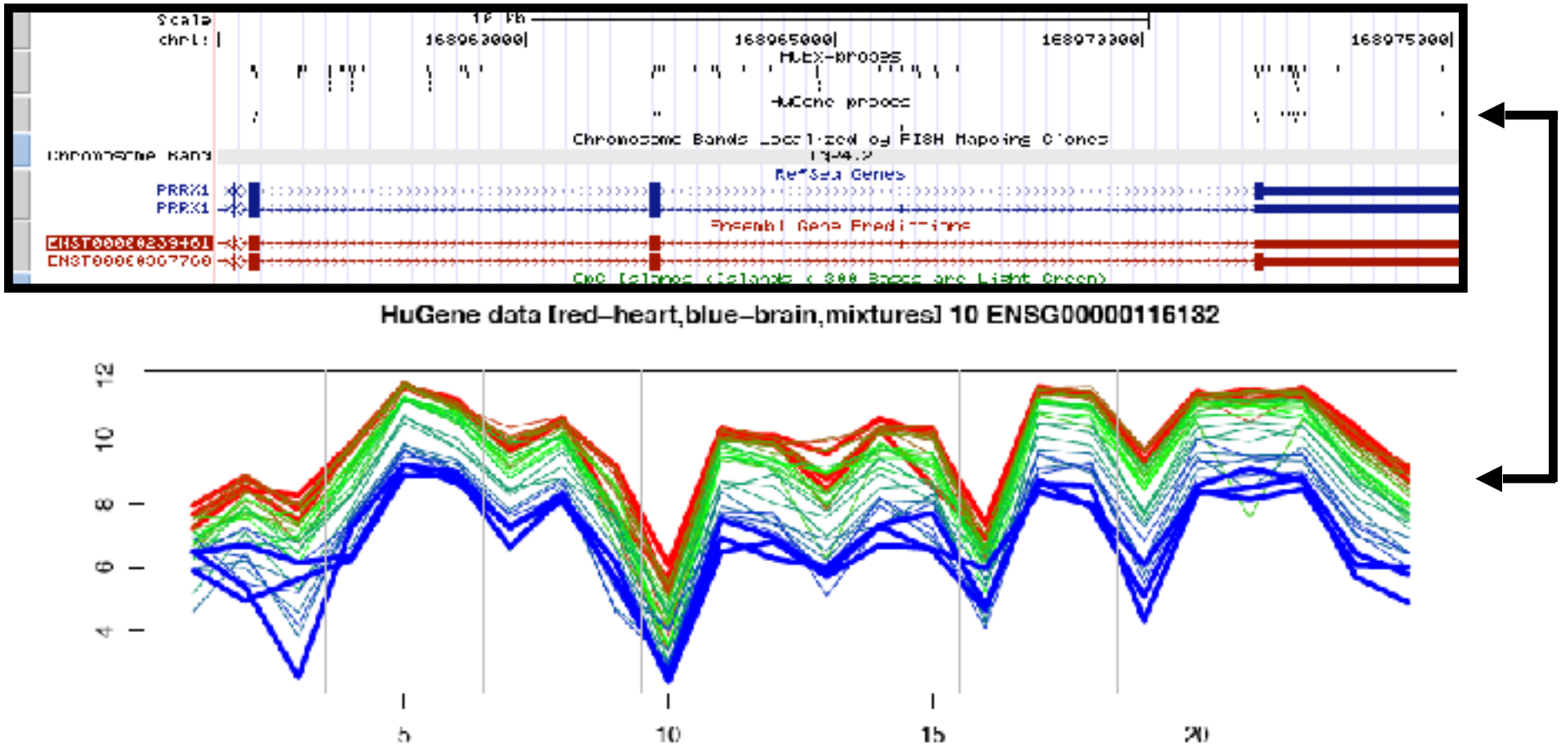
Still 25 base pair probes, multiple probes per transcript (“probesets”)

No more mismatch probes.

- HuExon: *Human **Exon** 1.0 ST* (~40 probes per gene, 4 probes per “exon”, annotated and predicted transcripts)
- HuGene: *Human **Gene** 1.0 ST* (~25 probes per gene, annotated genes only)



The nature of Affymetrix Probe Level Data



- Data for one gene that is differentially expressed between heart (red is 100% heart) and brain (blue is 100% brain).
- 11 mixtures x 3 replicates = 33 samples (33 lines)
- Note the parallelism: probes have different affinities

"Summarization": Going from probesets to summarized expression level

MAS 4.0

$$AvDiff = \frac{1}{|A|} \sum_{j \subset A} (PM_j - MM_j)$$

MAS 5.0

$$CT_j = \begin{cases} MM_j, & \text{if } MM_j < PM_j \\ \text{less than } PM_j, & \text{if } MM_j \geq PM_j \end{cases}$$

$$signal = TukeyBiweight\{log(PM_j - CT_j)\}$$

dChip (MBEI)

$$PM_{ij} - MM_{ij} = \theta_i \cdot \phi_j + \varepsilon_{ij}, \qquad \varepsilon_{ij} \sim N(0, \sigma^2)$$

θ_i expression index
ϕ_j probe-specific affinity
ε_{ij} noise component

RMA, GCRMA

Robust multichip analysis (RMA)

Exploration, normalization, and summaries of high density oligonucleotide array probe level data

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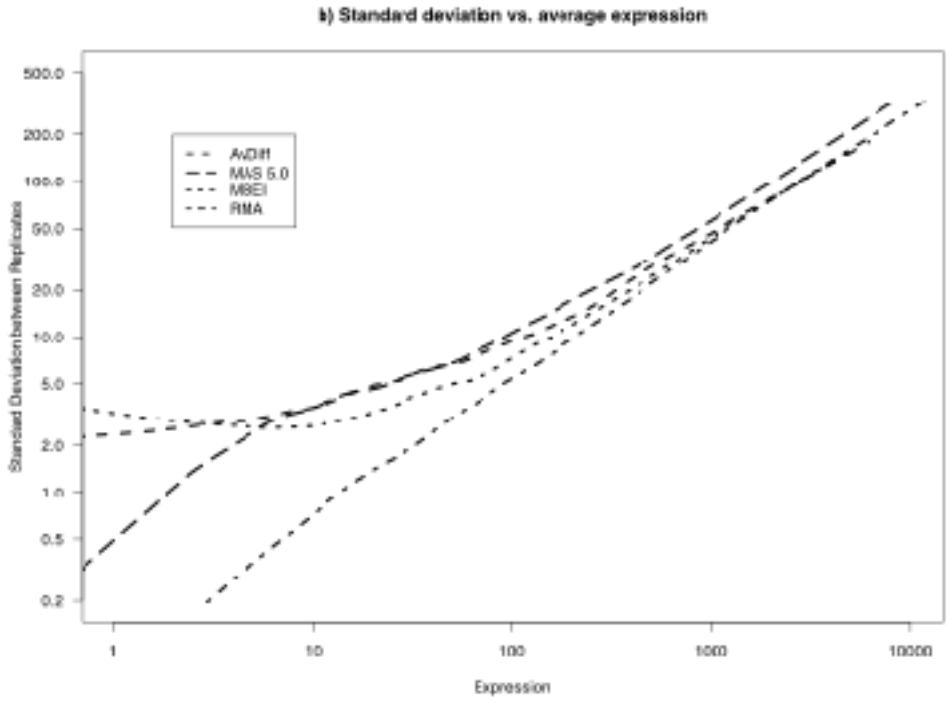
Biostatistics 2003

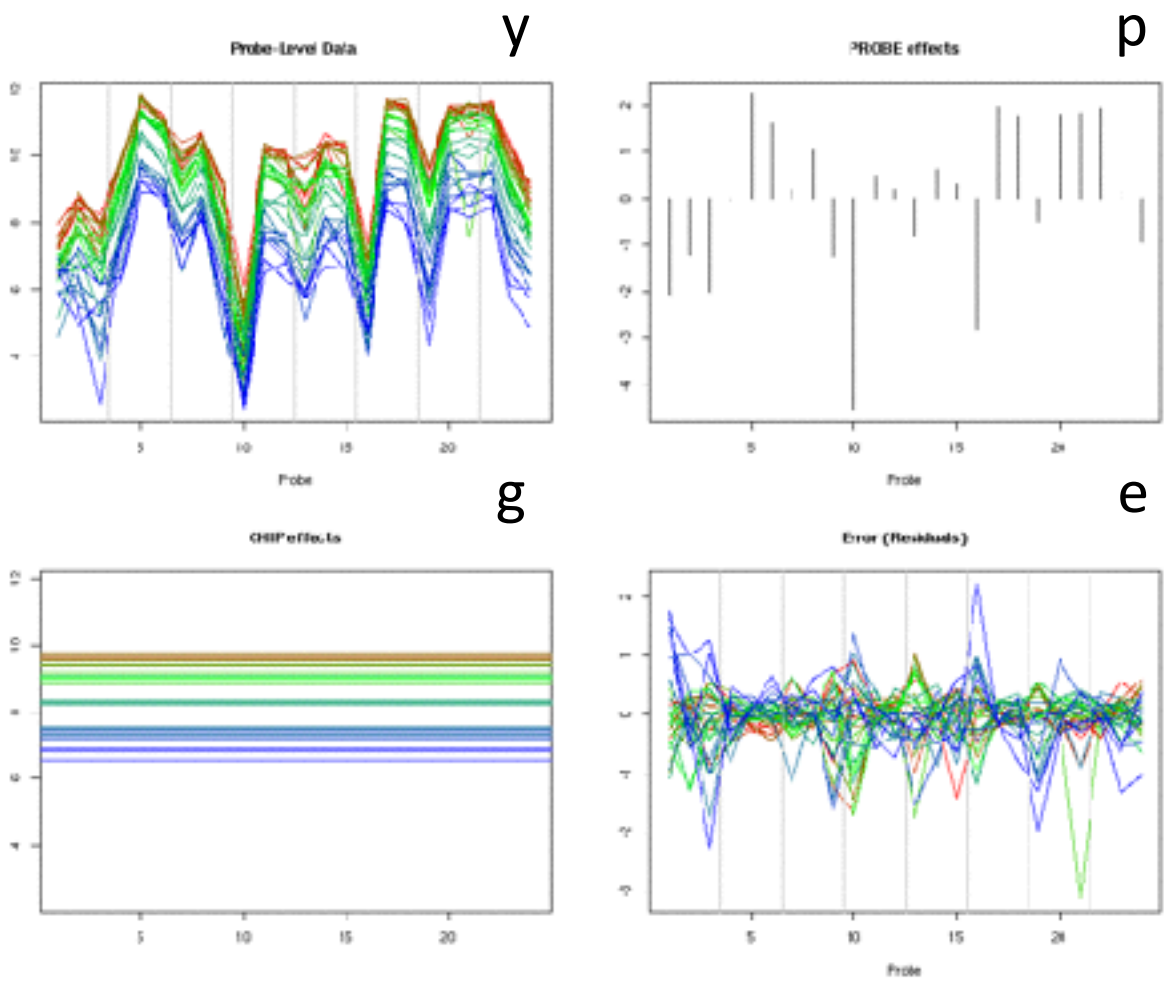
Encompasses 3 steps

- background correction
- normalization
- probe level model fit ("summarization")

s.d. between replicates



Linear model decomposes the probe-level data into PROBE effects and CHIP effects



Linear model:

$y_{ik} = g_i + p_k + e_{ik}$

Robust Multichip Analysis (RMA) uses this model.
Irizarry et al. 2003, Biostatistics

Parameters are estimated robustly, meaning a small number of outliers have minimal effect

Tissue mixture dataset

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

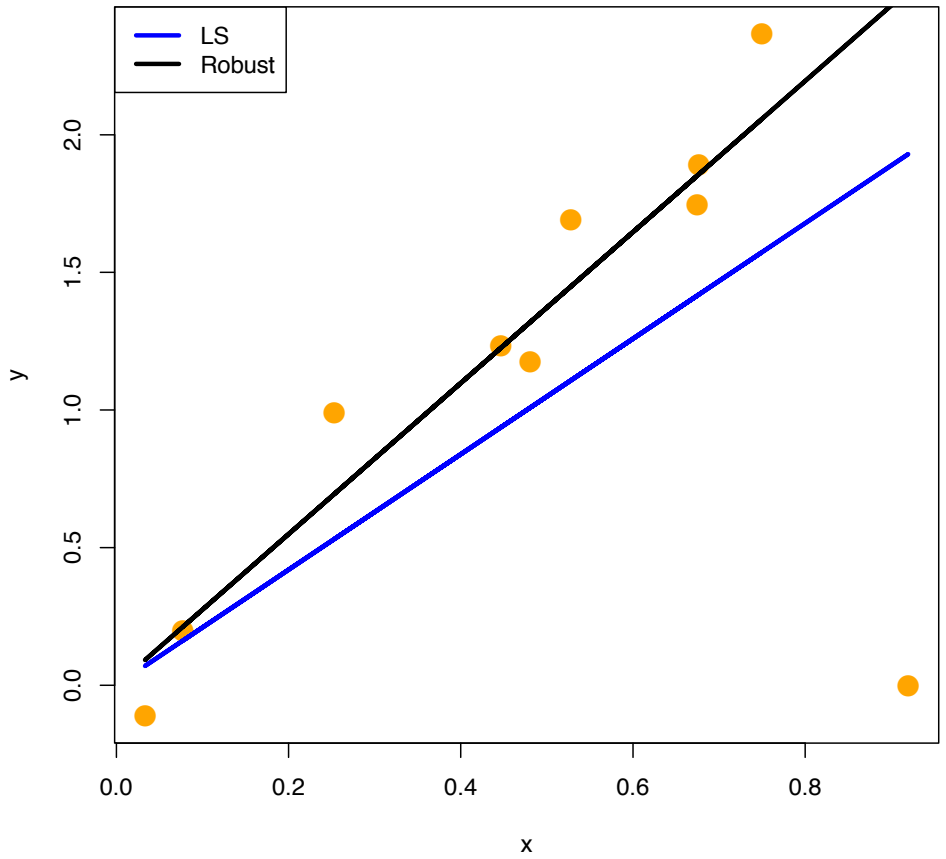
Robust regression – motivating example

```
library(MASS)

n <- 10
x <- runif(n)
y <- 3*x + rnorm(n,sd=.2)
y[which.max(y)] <- 0  # add in outlier

f <- lm(y~0+x)
fr <- rlm(y~0+x)

plot(x,y,pch=19,col="orange",cex=2)
lines(x,predict(fr),lwd=3)
lines(x,predict(f),lwd=3,col="blue")
legend("topleft",c("LS","Robust"),
       lwd=3,lty=1,col=c("blue","black"))
```



OLS = ordinary least squares

The OLS estimator is … optimal in the class of linear unbiased estimators when the errors are homoscedastic and serially uncorrelated … OLS provides minimum-variance mean-unbiased estimation when the errors have finite variances.

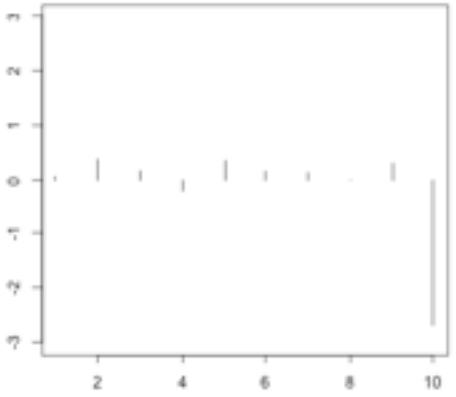
i.e., OLS has good properties, when the data is “nice”.

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

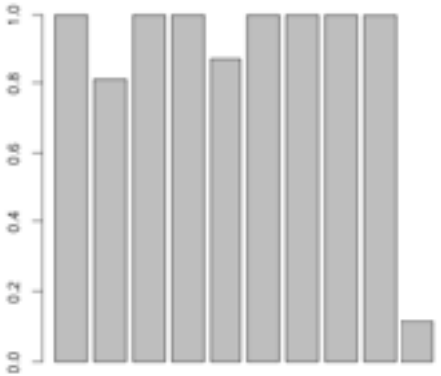
Replace:
$\arg\min_\beta \sum_{i=1}^{n} (y_i - f_i(\beta))^2$
with:
$\arg\min_\beta \sum_{i-1}^{n} w_i(\beta)(y_i - f_i(\beta))^2$

Robust regression – mechanics of iteratively reweighted least squares

Residuals



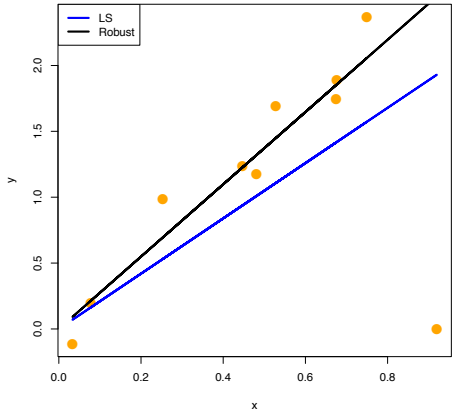
Weights



Sketch of IRLS:

Calculate initial estimates of parameters

Repeat until very little change:
- Calculate residuals
- Using standardized residuals, weight observations
- Re-estimate parameters




```
# this construction only works for the
# 1-parameter no-intercept linear model
tukey <- function(r,k=1.345) {
  abs(r) < k + k/abs(r)*(abs(r)>k)
}

w <- 1
niter <- 2
b <- sum(w*y*x)/sum(w*x^2)

for(i in 1:niter) {
  r <- y-b*x
  w <- tukey( r/mad(r) )
  b <- sum(w*y*x)/sum(w*x^2)
}


par(mfrow=c(2,1))
plot(r,type="h",ylim=c(-3,3))
barplot(w)
```

More details – weight functions (as function of standardized residuals)