Mixture modeling of the distribution of *p*-values from t-tests

4/9/2009

A Typical Microarray Data Set

Gene ID	Treatment 1					Treatment 2					p-value
1	4835.8	4578.2	4856.3	4483.7	4275.3	4170.7	3836.9	3901.8	4218.4	4094.0	p_1
2	153.9	161.0	139.7	173.0	160.1	180.1	265.1	201.2	130.8	130.7	p_2
3	3546.5	3622.7	3364.3	3433.6	2757.2	3346.9	2723.8	2892.0	3021.3	2452.7	p_3
4	711.3	717.3	776.6	787.5	750.3	910.2	813.3	687.9	811.1	695.6	p_4
5	126.3	178.2	114.5	158.7	157.3	231.7	147.0	102.8	157.6	146.8	p_5
6	4161.8	4622.9	3795.7	4501.2	4265.8	3931.3	3327.6	3726.7	4003.0	3906.8	p_6
7	419.3	555.3	509.6	515.5	488.9	426.6	425.8	500.8	347.8	580.3	p_7
8	2420.7	2616.1	2768.7	2663.7	2264.6	2379.7	2196.2	2491.3	2710.0	2759.1	p_8
9	321.5	540.6	471.9	348.2	356.6	382.5	375.9	481.5	260.6	515.7	p_9
10	1061.4	949.4	1236.8	1034.7	976.8	1059.8	903.6	1060.3	960.1	1134.5	p_{10}
11	1293.3	1147.7	1173.8	1173.9	1274.2	1062.8	1172.1	1113.0	1432.1	1012.4	p_{11}
12	336.1	413.5	425.2	462.8	412.2	391.7	388.1	363.7	310.8	404.6	p_{12}
13	5718.1	4105.5	5620.9	6786.8	7823.0	1297.8	1303.8	1318.8	1189.2	1171.5	p_{13}
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
22690	249.6	283.6	271.0	246.9	252.7	214.2	217.9	266.6	193.7	413.2	p_{22690}

We want to test $H_{i0} : \mu_{i1} = \mu_{i2}$ for gene i=1,...,m

Test statistic for gene i: $t_i = \dfrac{\bar{X}_{i1} - \bar{X}_{i2}}{\sqrt{s_i^2\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$

$|t_i| \sim |t|$ where

$$t \sim t(n_1 + n_2 - 2, \text{ncp} = \delta_i)$$

$$\delta_i = \frac{|\mu_{i1} - \mu_{i2}|}{\sqrt{\sigma_i^2\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

Equivalently Expressed (EE) and Differentially Expressed (DE) Genes

- A certain proportion, say π_0, of the tested genes have expression distributions that are the same for both treatments. (EE genes)
- For other genes, the mean expression level differs between treatments. (DE genes)
- For DE genes, the degree of differential expression, summarized by the non-centrality parameter

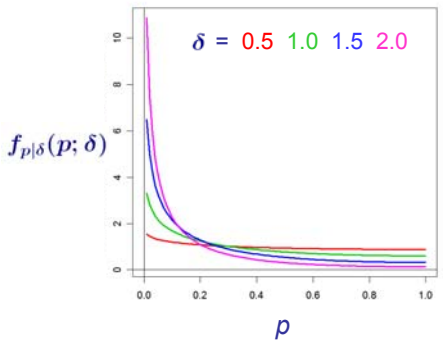
$$\delta_i = \frac{|\mu_{i1} - \mu_{i2}|}{\sqrt{\sigma_i^2\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}},$$

varies from gene to gene.

Objectives

- Estimate π_0= proportion of non-centrality parameters that are zero (i.e., proportion of genes that are EE)
- Estimate $g(\delta)$= density that approximates the true distribution of nonzero non-centrality parameters.
- Estimate false discovery rates (FDR)
- Estimate falsely interesting discovery rates (FIDR)
- Perform power and sample size calculations for future experiments

Conditional Densities of *p*-values Given δ



The Marginal Distribution of the *t*-test *p*-value

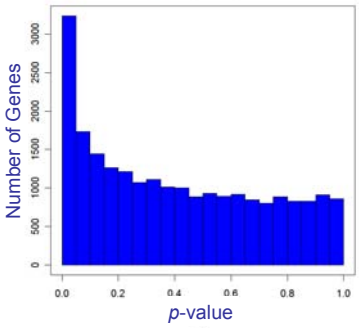
Suppose that each non-centrality parameter δ is 0 with probability π_0 and a draw from a continuous distribution $g(\delta)$ with probability $(1-\pi_0)$

Then the marginal density of the *t*-test *p*-value is given by

$$f_p(p) = \pi_0 + (1-\pi_0)\int_0^\infty f_{p|\delta}(p;\delta)g(\delta)d\delta$$

7

Histogram of *p*-values from Two-Sample *t*-Tests



8

Approximate *g* with a Linear Spline Function

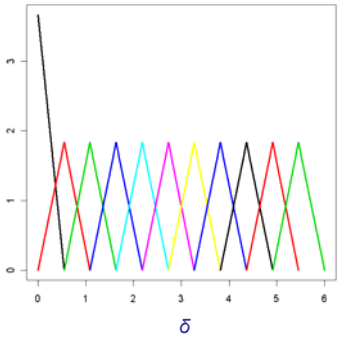
$$g(\delta) \approx g(\delta,\beta) = \sum_{k=1}^{K-1} \beta_k B_k(\delta)$$

where $B_1(\delta),\ldots,B_{K-1}(\delta)$ are B-splines normalized to be densities

and $\beta_1,\ldots,\beta_{K-1} \geq 0 \quad \sum_{k=1}^{K-1}\beta_k = 1$.

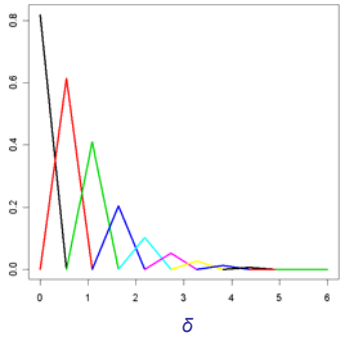
9

The B-Splines



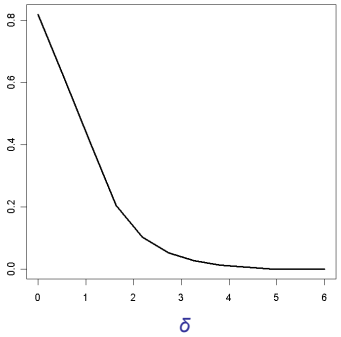
10

Weighted B-Splines



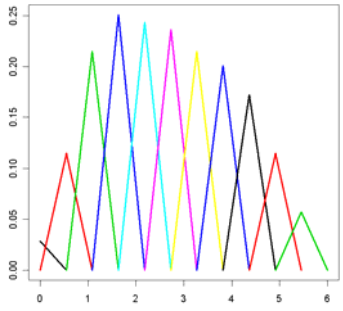
11

Linear Spline Estimate of *g*



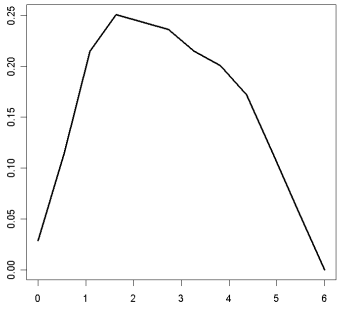
12

Weighted B-Splines



13

Linear Spline Estimate of g



14

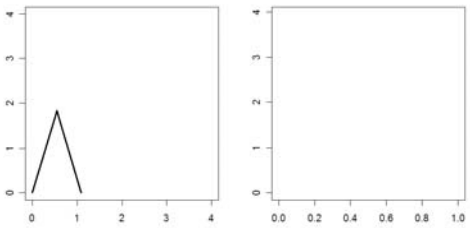
Approximating the Marginal Density of *p*-values

$$
\begin{aligned}
f_p(p) &= \pi_0 + (1-\pi_0)\int_0^\infty f_{p|\delta}(p;\delta)g(\delta)d\delta \\
&\approx \pi_0 + (1-\pi_0)\int_0^\infty f_{p|\delta}(p;\delta)g(\delta,\beta)d\delta \\
&= \pi_0 + (1-\pi_0)\int_0^\infty f_{p|\delta}(p;\delta)\sum_{k=1}^{K-1}\beta_k B_k(\delta)d\delta \\
&= \pi_0 \cdot 1 + \sum_{k=1}^{K-1}(1-\pi_0)\beta_k \int_{\mathcal{S}_k} f_{p|\delta}(p;\delta)B_k(\delta)d\delta \\
&\equiv \theta_1 z_1(p) + \sum_{k=1}^{K-1}\theta_{k+1}z_{k+1}(p) \\
&= \sum_{k=1}^{K}\theta_k z_k(p) \equiv f_p(p;\theta)
\end{aligned}
$$

15

$$z_3(p) = \int_{\mathcal{S}_2} f_{p|\delta}(p;\delta)B_2(\delta)d\delta$$

$B_2(\delta)$ $\qquad$ $f_{p|\delta}(p;\delta)$

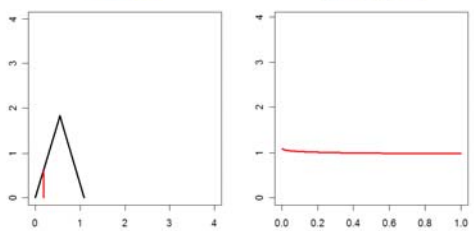


δ $\qquad$ *p*-value

16

$$z_3(p) = \int_{\mathcal{S}_2} f_{p|\delta}(p;\delta)B_2(\delta)d\delta$$

$B_2(\delta)$ $\qquad$ $f_{p|\delta}(p;\delta)$

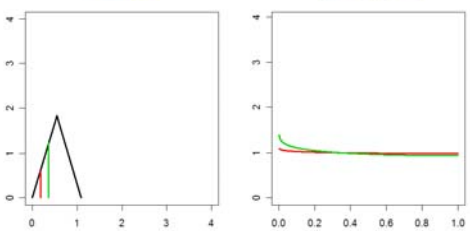


δ $\qquad$ *p*-value

17

$$z_3(p) = \int_{\mathcal{S}_2} f_{p|\delta}(p;\delta)B_2(\delta)d\delta$$

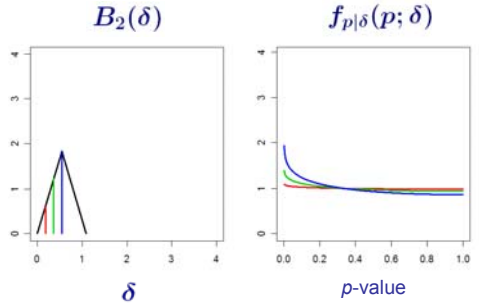
$B_2(\delta)$ $\qquad$ $f_{p|\delta}(p;\delta)$



δ $\qquad$ *p*-value

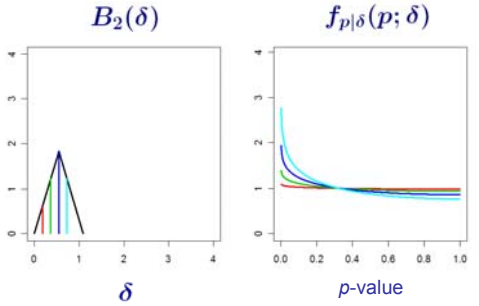
18

$$z_3(p) = \int_{\mathcal{S}_2} f_{p|\delta}(p;\delta) B_2(\delta) d\delta$$



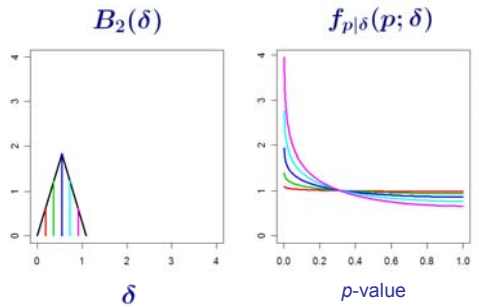
19

$$z_3(p) = \int_{\mathcal{S}_2} f_{p|\delta}(p;\delta) B_2(\delta) d\delta$$



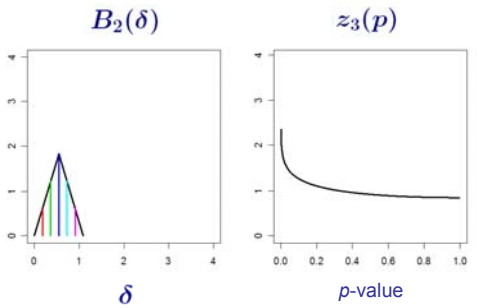
20

$$z_3(p) = \int_{\mathcal{S}_2} f_{p|\delta}(p;\delta) B_2(\delta) d\delta$$



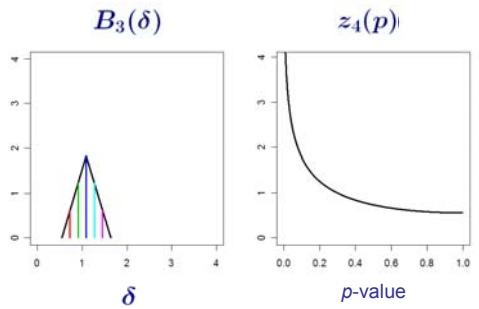
21

$$z_3(p) = \int_{\mathcal{S}_2} f_{p|\delta}(p;\delta) B_2(\delta) d\delta$$



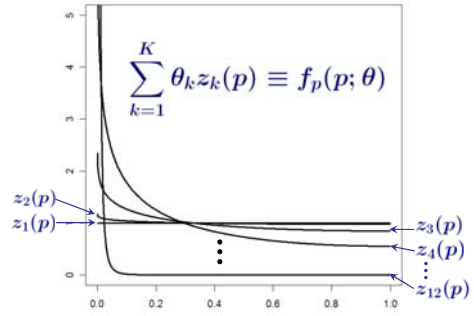
22

$$z_4(p) = \int_{\mathcal{S}_3} f_{p|\delta}(p;\delta) B_3(\delta) d\delta$$



23

We seek a convex combination of the z functions that fits our empirical *p*-value distribution well.



24

Find $\boldsymbol{\theta}$ that minimizes

Number of Histogram Bins (e.g., 2000)

Observed Density Height for i[th] Bin

Smoothing Parameter

$$SS(\theta, \lambda) = \sum_{i=1}^{N_{bin}} w_i \{y_i - f_p(c_i; \theta)\}^2 + \lambda Q(\theta)$$

Weight Assigned to i[th] Bin

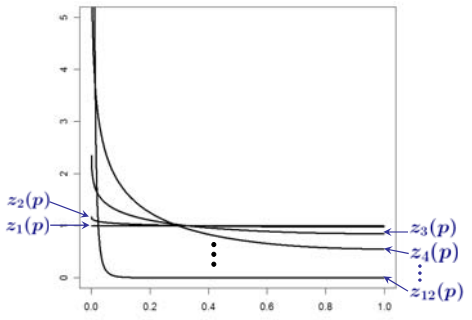
Semiparametric Approximation of Density Height for the i[th] Bin

Penalty for lack of smoothness in *g*

Solved via quadratic programming.

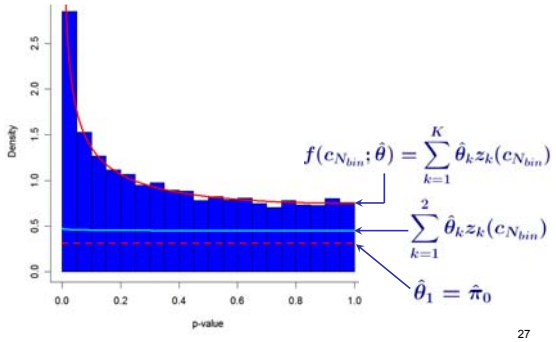
25

Solution to the penalized least squares problem yields a convex combination of z functions as a density estimate.



26

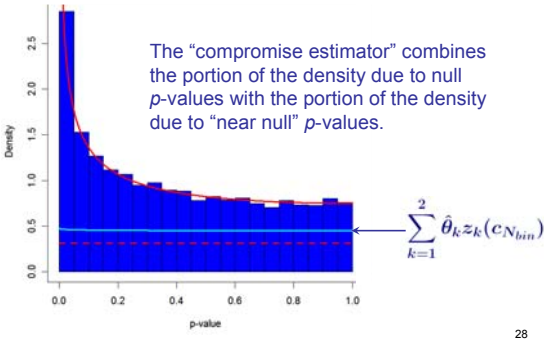
Two-Sample *t*-test *p*-values with Estimated Density



27

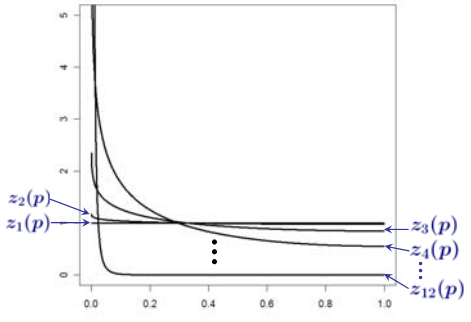
Two-Sample *t*-test *p*-values with Estimated Density

The "compromise estimator" combines the portion of the density due to null *p*-values with the portion of the density due to "near null" *p*-values.



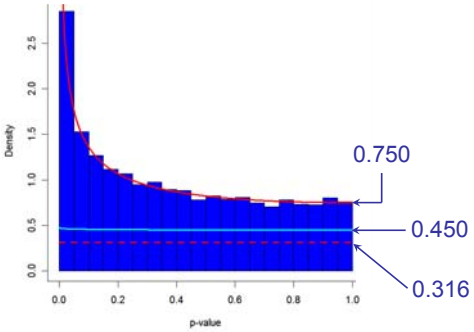
28

Note that the similarity of z_1 and z_2.



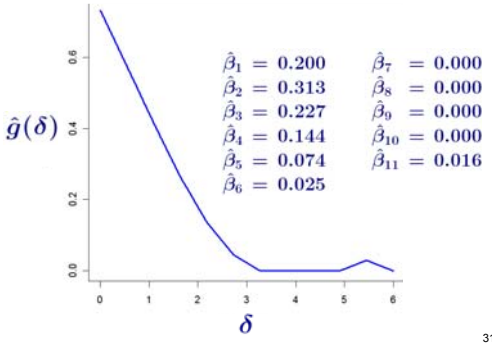
29

Two-Sample *t*-test *p*-values with Estimated Density



30

Estimated Density of Nonzero Non-Centrality Parameters



31

Other Quantities of Interest

Posterior Probability of Differential Expression
PPDE(p) = P(DE|p-value=p)

False Discovery Rate
FDR(c) = P(EE|p≤c)

True Positive Rate
TPR(c) = P(DE|p≤c)
= 1 – FDR(c)

True Negative Rate
TNR(c) = P(EE|p>c)

Expected Discovery Rate
EDR(c) = P(p≤c|DE)

Gadbury et al. (2004). *Stat. Meth. in Med. Res.* **13**, 325-338 discuss last three quantities in power and sample size context.

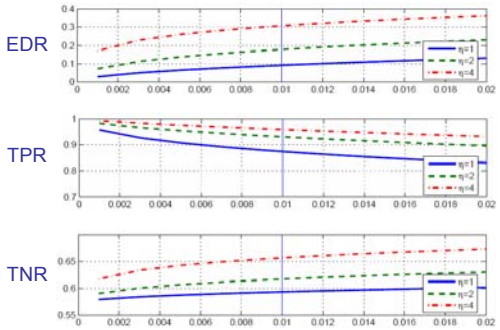
32

Power-Sample Size Calculations

- If we have estimates of π_0 and g(δ) from a previous experiment, we can examine how our ability to discover differentially expressed genes will vary with sample size.
- Suppose the within-treatment sample sizes for a new experiment differ from the previous experiment by a factor of η.
- If δ denotes the NCP for a gene in the previous experiment, then the NCP for the same gene in the new experiment will be $\sqrt{\eta}\delta$.
- We can see how quantities of interest vary with η to guide samples size selection in the new experiment.

33

Power-Sample Size Calculations



p-value threshold for significance

34

"Interesting Discovery" Rates

$$\mathrm{FIDR}(c) = P(\delta < \delta^* | p \le c)$$

researcher-determined threshold that defines "interesting discovery"

$$\mathrm{EIDR}(c) = P(p \le c | \delta \ge \delta^*)$$

35

Main Reference

Ruppert, D., Nettleton, D., Hwang, J.T.G. (2007). Exploring the information in *p*-values for the analysis and planning of multiple-test experiments. *Biometrics.* **63** 483-495.

36