Towards More Significant Discoveries in High Dimensional Data Analysis

Multiple Testing with Heterogeneous Multinomial Data

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January, 2016

The Main Idea

- Data: large number of attributes p, small sample size n
  - fMRI analysis, GWAS, "omics", ...
- Objective: Discover reproducible **and** interesting attributes
- Standard method:
  1. Test statistic (*p*-values / post. probs.) computed for each attribute
  2. Apply multiple testing procedure ⇒ identify *"significant"* attributes
- Problem:
  - Many *significant* attributes not interesting
  - Many interesting attributes not *significant*

Overview

1. **Motivation**
   - Rhizosphere
   - Motivating Study
   - Data Analysis
   - Problem
2. Clfdr Procedure
   - Oracle Procedure
   - Adaptive Procedure
3. Assessment
   - Application
   - Thresholding Effect
4. Remarks

Rhizosphere and Rhizobacteria

What is the rhizosphere?

- Soil near the roots of a plants (plant stomache)
- Millions of unknown species of bacteria: **rhizobacteria**

Why do we care?

- Rhizosphere composition associated with plant health / productivity
- Understand rhizosphere ⇒ manipulate rhizosphere ⇒ increase productivity

Typical Wheat Rhizosphere Studies

- Standard objective: **Who's there**?
  - Method: Rhizosphere sample(s) + RNA sequencing technology ⇒ identify abundant species of rhizobacteria
  - Called *core microbiome*
- Assumption: **"Abundance = association hypothesis"**
  - Most abundant rhizobacteria are associated with productivity

Question

Core microbiome vs. core productivity-associated microbiome

Illustration

Abundance = association hypothesis?

Study

- Objective of Anderson and Habiger (2012): Identify productivity associated microbiome
- Data collection:
  1. 5 wheat rhizosphere soil samples: Average shoot biomass (g) among wheat plants in each sample measures productivity

x_1	x_2	x_3	x_4	x_5
0.86	1.34	1.81	2.37	3.00

  2. 16s rRNA software: # DNA copies of $m = 1, 2, ..., 778$ species in each sample (abundance)

Species m	y_{1m}	y_{2m}	y_{3m}	y_{4m}	y_{5m}	Total (n_m)
1	0	1	1	0	5	7
2	9	2	0	0	3	14
⋮	⋮	⋮	⋮	⋮	⋮	⋮
778	16	10	29	18	13	81

- Refined objective: Which bacteria are associated with productivity?

Statistical Analysis

Step 1: Compute test statistics / p-values

- Models: $Y_{nm} \sim Pois(\mu_{nm})$, $log(\mu_{nm}) = \alpha_m + \beta_m x_n$
- Null hypotheses: $H_m : \beta_m = 0$
- Z-scores: $Z_m = \frac{\hat{\beta}_m}{S.E.(\hat{\beta}_m)}$
- p-Values: $P_m = \Pr(|Z_m| \geq |z_m|)$

Step 2: Define rejection threshold

- Question: Reject H_m if $P_m \leq 0.05$ or $|Z_m| \geq 1.96$?

Error Rates

Common Error Rates

Error Rate	Properties	Uses
$FDR = E\left[\frac{V}{\max\{R,1\}}\right]$	liberal	large # tests
$FWER = \Pr(V > 0)$	conservative	small # tests

- V = # false discoveries (false rejections)
- R = # discoveries (rejections)

Remark: Many other error rates

Classical FDR Procedures

- Benjamini and Hochberg (1995) procedure:
  - Implementation:
    1. Order $P_{(1)} \leq P_{(2)} \leq \ldots \leq P_{(M)}$
    2. Reject $k = \max\{m : P_{(m)} \leq \alpha m/M\}$ null hypotheses
  - Properties: $FDR \leq \pi_0 \alpha \leq \alpha$ under positive dependence
- Adaptive BH procedures: Storey et. al. (2004), Liang and Nettleton (2012), ...
  - Implementation:
    1. Estimate π_0
    2. Apply BH at $\alpha/\hat{\pi}_0$
  - Properties: $FDR \leq \alpha$[1] under weak dependence

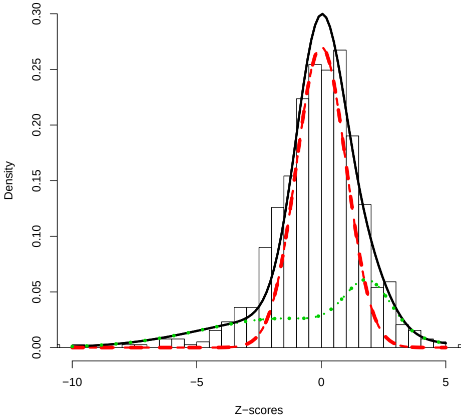
[1] $FDR = \alpha$ for any π_0 under least favorable p-Value configuration - Habiger (2014)

Local FDR / Bayesian Procedures

- Local FDR (lFDR) - Efron (2010)
  - Mixture model: $Z_m \sim f(z) = \pi_0 f_0(z) + (1 - \pi_0) f_1(z)$
  - Local FDR: $lFDR(z) = \frac{\pi_0 f(z)}{f(z)} = \Pr(H_m \text{ true } | Z_m = z)$
  - Local FDR statistics: $lFDR_m = lFDR(Z_m)$
  - Adaptive: $\hat{\pi}_0, \hat{f}_1 \rightarrow \widehat{lFDR}_m$
- Adaptive lFDR procedure - Sun and Cai (2007)
  1. Order $\widehat{lFDR}_{(1)} \leq \widehat{lFDR}_{(2)} \leq ... \leq \widehat{lFDR}_{(M)}$
  2. Reject $k = \max\left\{m : \sum_{i=1}^{m} \widehat{lFDR}_{(i)} \leq \alpha m\right\}$ null hypotheses
- Properties:
  - $FDR \rightarrow \alpha$
  - Asymptotically "optimal"

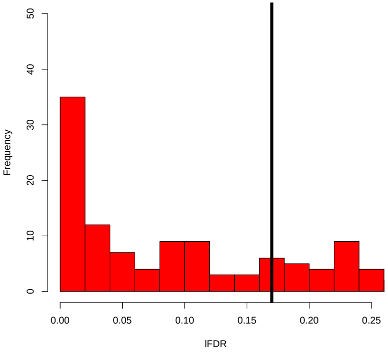
Estimated Mixture Model

$$f(z) = 0.67\phi(z; 0, 1) + 0.33 f_1(z)$$



lFDR Procedure: $\alpha = 0.05$

$$lFDR_m = lFDR(z_m) = \frac{0.67\phi(z_m)}{f(z_m)}$$



85 discoveries ⇒ **productivity-associated microbiome**?

"Significant"

Question: Which species is discovered?

m	Y_{1m}/n_m	Y_{2m}/n_m	Y_{3m}/n_m	Y_{4m}/n_m	Y_{5m}/n_m	$\hat{\beta}_m$	n_m	$\widehat{lFDR}_m$	Discover
1	0.36	0.50	0.00	0.07	0.07	?	?	?	?
2	0.15	0.13	0.28	0.25	0.19	?	?	?	?
Null	0.20	0.20	0.20	0.20	0.20	0	—	1	x

"Significant"

Question: Which species is discovered?

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1	0.36	0.50	0.00	0.07	0.07	**-1.09**	?	?	?
2	0.15	0.13	0.28	0.25	0.19	**0.19**	?	?	?
Null	0.20	0.20	0.20	0.20	0.20	0	—	1	x

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1	0.36	0.50	0.00	0.07	0.07	-1.09	**11**	?	?
2	0.15	0.13	0.28	0.25	0.19	0.19	**911**	?	?
Null	0.20	0.20	0.20	0.20	0.20	0	—	1	x

"Significant"

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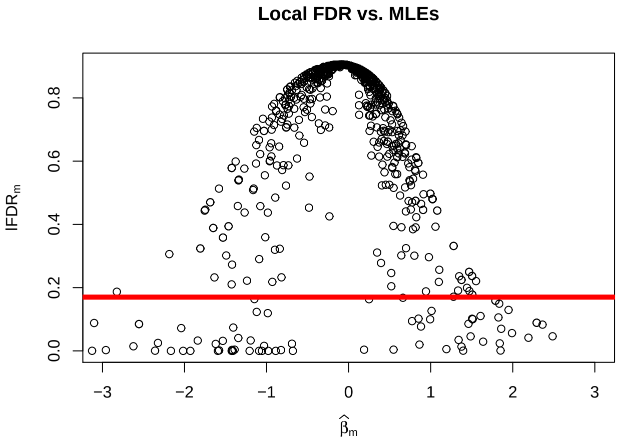
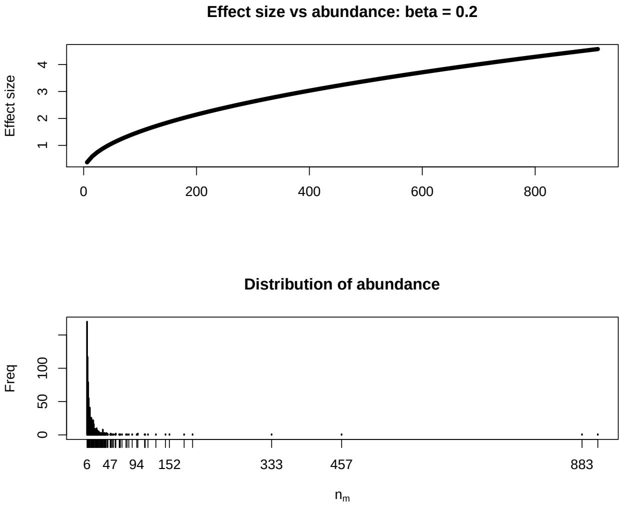
m	Y_{1m}/n_m	Y_{2m}/n_m	Y_{3m}/n_m	Y_{4m}/n_m	Y_{5m}/n_m	$\hat{\beta}_m$	n_m	$\widehat{lFDR}_m$	Discover
1	0.36	0.50	0.00	0.07	0.07	-1.09	11	0.29	**x**
2	0.15	0.13	0.28	0.25	0.19	0.19	911	0.003	✓
Null	0.20	0.20	0.20	0.20	0.20	0	—	1	x

Illustration



Negligible Associations Detected if Abundant Enough



Consequence

	Abundant	Rare
Strong association	✓	o
Weak association	✓	o

✓ = discovered as "associated with productivity"

- **Abundance = association hypothesis** RETAINED!

Illustration

"Statistics show that Fred is associated with productivity"

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Multinomial Mixture Model

- Under log-linear model $\boldsymbol{Y}_m | N_m = n_m \sim Multinomial(n_m, \boldsymbol{p}(\beta_m))$
  - $p_n(\beta_m) = \frac{\exp\{\beta_m x_n\}}{\sum_{n=1}^{N} \exp\{\beta_m x_n\}}$
  - pmf notation: $p(\boldsymbol{y}_m | n_m; \beta_m)$
- Prior $\Pr(\beta_m = \gamma_k) = \pi_k$ for $k = 0, 1, ..., K$
  - Null prior: Take $\gamma_0 = 0 \Rightarrow \Pr(\beta_m = 0) = \Pr(H_m \text{ true }) = \pi_0$
- Mixture of Multinomial pmfs:

$$p(\boldsymbol{y}_m | n_m; \boldsymbol{\gamma}, \boldsymbol{\pi}) = \pi_0 p(\boldsymbol{y}_m | n_m; 0) + \pi_1 p(\boldsymbol{y}_m | n_m; \gamma_1) + ... + \pi_K p(\boldsymbol{y}_m | n_m; \gamma_K)$$

Oracle clFDR Procedure

1. Compute clFDRs :

$$clFDR_m \equiv \frac{\pi_0 p(\boldsymbol{y}_m | n_m; \gamma_0)}{p(\boldsymbol{y}_m | n_m; \boldsymbol{\gamma}, \boldsymbol{\pi})} = \Pr(\beta_m = 0 | \boldsymbol{y}_m, n_m; \boldsymbol{\gamma}, \boldsymbol{\pi})$$

2. Rank clFDRs: $clFDR_{(1)} \leq clFDR_{(2)} \leq \ldots \leq clFDR_{(M)}$

3. Reject k nulls with smallest clFDR:

$$k = \max\left\{ m : \sum_{i=1}^{m} clFDR_{(i)} \leq \alpha m \right\}$$

FDR control

Theorem

If each $(\boldsymbol{Y}_m, \beta_m)$ is generated according to the Multinomial mixture model, then the clFDR procedure has FDR $\leq \alpha$ regardless of $(n_1, n_2, ..., n_M)$.

Problem: $\boldsymbol{\pi}, \boldsymbol{\gamma}$ unknown.

Idea

- Adaptive procedure plugs in **consistent** estimates for $\boldsymbol{\pi}$ and $\boldsymbol{\gamma}$
- Maximum likelihood estimation:
  - Under conditional independence get log likelihood

$$l(\boldsymbol{\gamma}, \boldsymbol{\pi}) = \sum_{m=1}^{M} \log \left(\sum_{k=0}^{K} \pi_k p(\boldsymbol{y}_m | n_m; \gamma_k) \right).$$

  - Use EM algorithm to get MLE

EM Algorithm

- E step: $\hat{z}_{km} = \frac{\pi_k^{old} p(\boldsymbol{y}_m | n_m; \gamma_k^{old})}{\sum_{k=0}^{K} \pi_k^{old} p(\boldsymbol{y}_m | n_m; \gamma_k^{old})}$.
- M step: Maximize $Q(\gamma, \boldsymbol{\pi})$ s.t. $\sum_k \pi_k = 1$

$$Q(\gamma, \boldsymbol{\pi}) \equiv \sum_{m=1}^{M} \sum_{k=0}^{K} \hat{z}_{km} \log(\pi_k p(\boldsymbol{y}_m | n_m; \gamma_k))$$

$$= \sum_{m=1}^{M} \sum_{k=0}^{K} \hat{z}_{km} \log(\pi_k) + \sum_{m=1}^{M} \sum_{k=0}^{K} \hat{z}_{km} \log p(\boldsymbol{y}_m | n_m; \gamma_k)$$

  - 1st quantity + contraint $\Rightarrow \hat{\pi}_k^{new} = \frac{1}{M} \sum_m \hat{z}_{km}$
  - 2nd quantity + tweeked optim() $\Rightarrow \hat{\gamma}_k^{new}$

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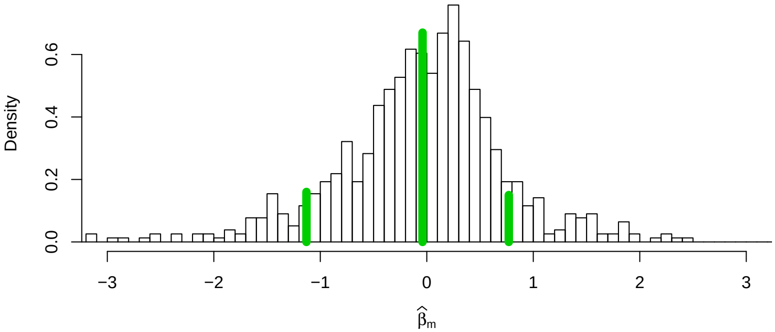
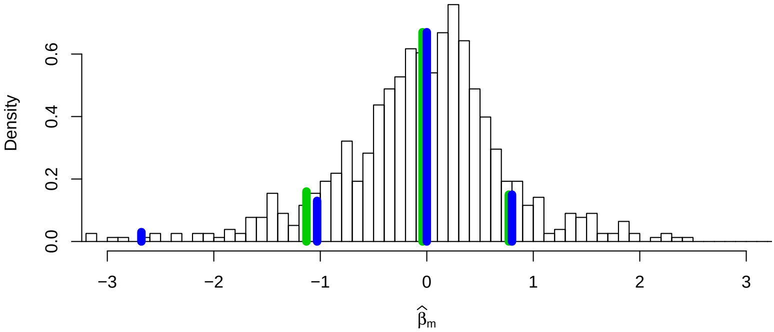
Model 1 and Results



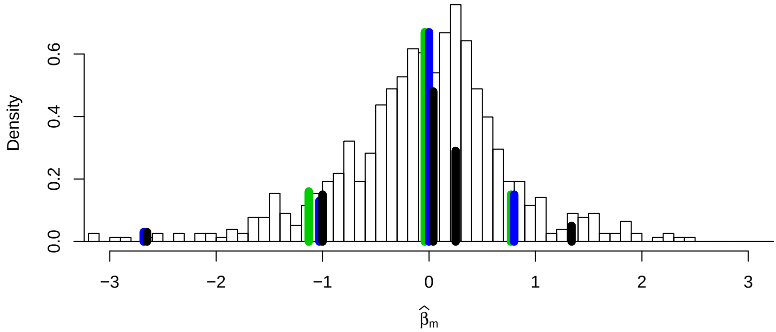
K	$\hat{\pi}_0$	$(\hat{\pi}_1, \hat{\gamma}_1)$	$(\hat{\pi}_2, \hat{\gamma}_2)$	$(\hat{\pi}_3, \hat{\gamma}_3)$	$(\hat{\pi}_4, \hat{\gamma}_3)$	AIC	BIC	Disc.
2	0.69	(0.16, -1.13)	(0.15,0.78)	NA	NA	1222	1224	99
3								
4								

Model 2 and Results



K	$\hat{\pi}_0$	$(\hat{\pi}_1, \hat{\gamma}_1)$	$(\hat{\pi}_2, \hat{\gamma}_2)$	$(\hat{\pi}_3, \hat{\gamma}_3)$	$(\hat{\pi}_4, \hat{\gamma}_3)$	AIC	BIC	Disc.
2	0.69	(0.16, -1.13)	(0.15,0.78)	NA	NA	1222	1224	99
3	0.69	(0.03, -2.68)	(0.13, -1.03)	(0.15, 0.79)	NA	1214	1217	97
4								

Model 3 and Results



K	$\hat{\pi}_0$	$(\hat{\pi}_1, \hat{\gamma}_1)$	$(\hat{\pi}_2, \hat{\gamma}_2)$	$(\hat{\pi}_3, \hat{\gamma}_3)$	$(\hat{\pi}_4, \hat{\gamma}_3)$	AIC	BIC	Disc.
2	0.69	(0.16, -1.13)	(0.15,0.78)	NA	NA	1222	1224	99
3	0.69	(0.03, -2.68)	(0.13, -1.03)	(0.15, 0.79)	NA	1214	1217	97
4	0.48	(0.03, -2.68)	(0.15, -1.03)	(0.29, 0.25)	(0.05,1.34)	1211	1215	114

"Significant"

Question: Now which species is discovered?

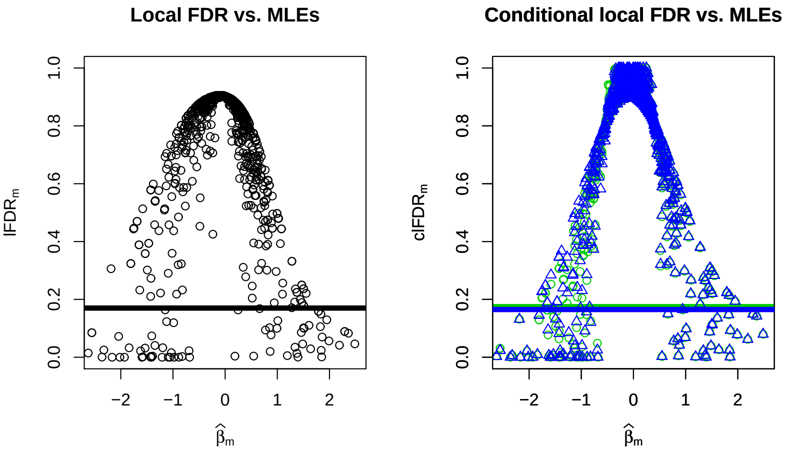
Local FDR procedure

m	Y_{1m}/n_m	Y_{2m}/n_m	Y_{3m}/n_m	Y_{4m}/n_m	Y_{5m}/n_m	$\hat{\beta}_m$	n_m	$\widehat{lFDR}_m$	Disc.
1	0.36	0.50	0.00	0.07	0.07	-1.09	11	0.29	**x**
2	0.15	0.13	0.28	0.25	0.19	0.19	911	0.003	✓
Null	0.20	0.20	0.20	0.20	0.20	0	—	1	x

Conditional local FDR procedure

m	Y_{1m}/n_m	Y_{2m}/n_m	Y_{3m}/n_m	Y_{4m}/n_m	Y_{5m}/n_m	$\hat{\beta}_m$	n_m	$\widehat{clFDR}_m$	Disc.
1	0.36	0.50	0.00	0.07	0.07	-1.09	11	0.10, 0.12	✓
2	0.15	0.13	0.28	0.25	0.19	0.19	911	1, 1	x

Illustration: lFDR vs clFDR



Setting for Theoretical Study

- Model: $\Pr(\beta_m = 0) = \pi_0$, $\Pr(\beta_m = \gamma_1) = (1 - \pi_0)$, $\gamma_1 > 0$
- Z-score: $Z_m = \frac{T_m - E[T_m|\beta_m=0, N_m=n_m]}{\sqrt{Var(T_m|\beta_m=0, N_m=n_m)}}$
- Conditional lFDR procedure
  - $f(z|N_m = n) = \pi_0\phi(z;0,1) + (1-\pi_0)\phi(z;\mu(\gamma_1, n), \sigma^2(\gamma_1))$
  - $clFDR(z, n) = \pi_0\phi(z;0,1)/f(z|N_m = n)$
  - $[clFDR(z, n) \leq \lambda] = [z \geq a(n)]$
- lFDR procedure
  - $f(z) = \pi_0\phi(z;0,1) + (1-\pi_0)\sum_{n\in\mathcal{N}} \phi(z;\mu(\gamma_1, n), \sigma^2(n))p(n)$
  - $lFDR(z) = \pi_0\phi(z;0,1)/f(z)$
  - $[lFDR(z) \leq \lambda] = [z \geq b]$

Thresholding Effect

Theorem

Under $f(z|N_m = n)$, the rejection threshold $a(n)$ is increasing in n whenever

$$\mu(n,\gamma_1)^2 > 2\log\left(\sigma(\gamma_1)\frac{\pi_0(1-\lambda)}{(1-\pi_0)\lambda}\right). \quad (1)$$

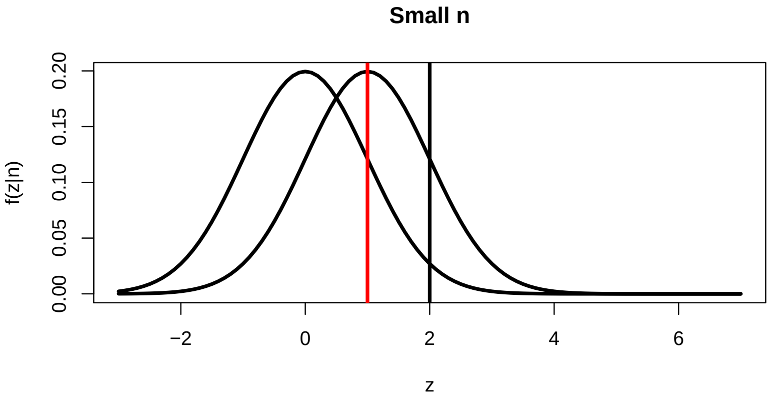
for any $\gamma_1 > 0, \lambda > 0$ and $\pi_0 \in (0,1)$.

Important points:

- Eq. (1) satisfied for all large enough n: $\mu(n,\gamma_1) \nearrow n$
- Safeguard against $\gamma_1 \approx 0$ and large n
- No such safeguard for lFDR procedure

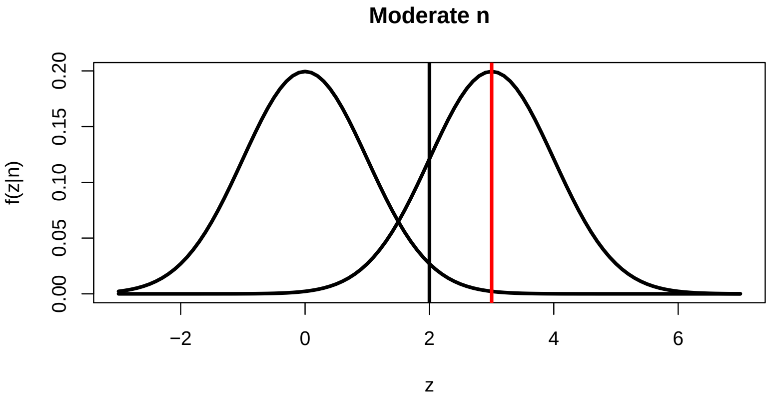
Thresholding Illustration

lFDR threshold vs. clFDR threshold: fixed γ_1



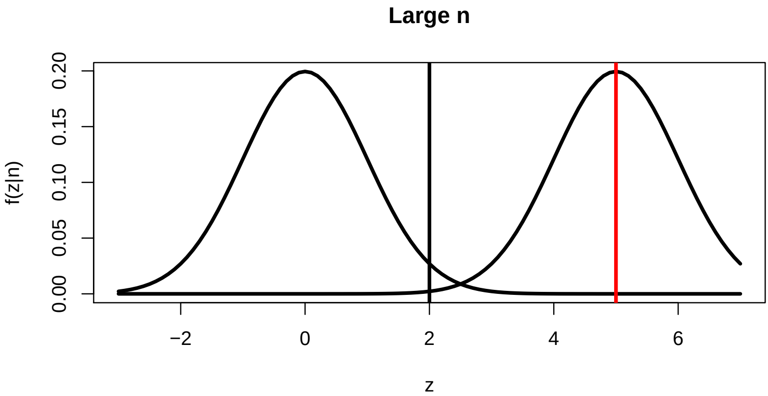
Thresholding Illustration

lFDR threshold vs. clFDR threshold: fixed γ_1



Thresholding Illustration

lFDR threshold vs. clFDR threshold: fixed γ_1



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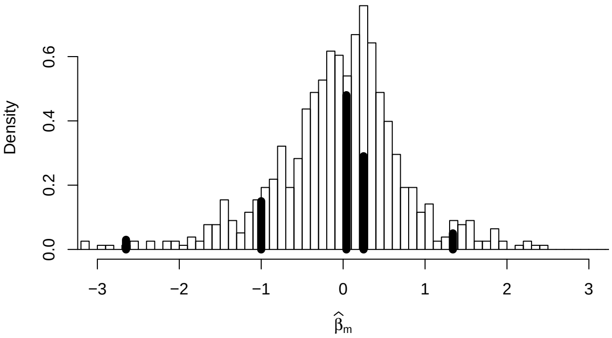
What We Did

- **Standard objective: Maximize # discoveries** s.t. FDR controlled
  - Method: Rank attributes according to $lFDR_m$
  - Problem: $lFDR_m \to 0$ as $n_m \to \infty$ if $\beta_m \neq 0$
    - **Statistical significance does not imply practical significance**

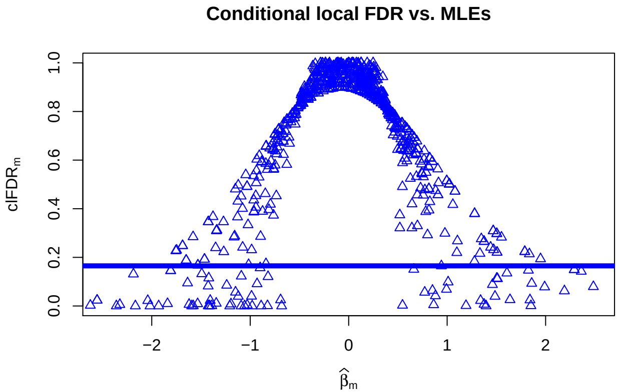
- **Better objective: Maximize # interesting discoveries** s.t. FDR controlled
  - Method: Given n_m, rank attributes according to $clFDR_m$
  - Solution: $clFDR_m \to 1$ as $n_m \to \infty$ if $\beta_m \in \mathcal{N}(0)$
    - **Statistical significance does imply practical significance**

Future work 1

Empirical vs. theoretical null: Efron (2004) and Bickel (2012)

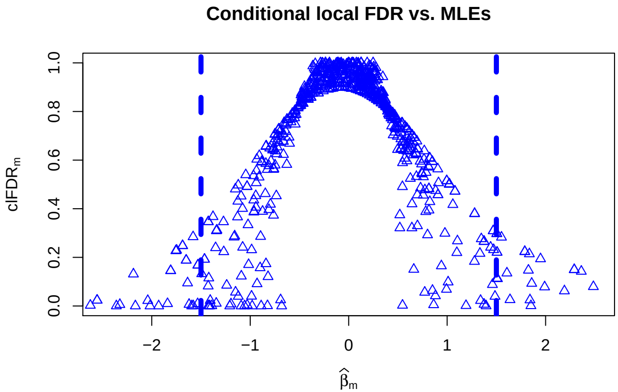


Future Work 2



Should we use this rejection region?

Future Work 2



Should we use this rejection region?

Future Work 3

A general procedure:

1. Rank attributes using any measure of practical significance
   - $\hat{\beta}$, SSR, AIC, R^2, IMCR . . .
2. Choose threshold
   - Compute $Q_m = \Pr(H_m \text{ true}|\text{data})$
   - Let $\mathcal{R} \subseteq \{1, 2, ..., M\}$ index any arbitrary set of discoveries, say the set of R most practically significant attributes. If $\sum_{m \in \mathcal{R}} Q_m \leq \alpha|\mathcal{R}|$ then $FDR \leq \alpha$

Development:

- Parameter estimation effect?
- Dependence?
- FDR?
- Measures of practical significance?

Some References

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Overtime: The classical approach

Weighted Adaptive BH Procedure

1. Compute weights $w_m = w(n_m)$
2. Get weighted p-value: $Q_m = P_m / w_m$
3. Estimate π_0:

$$\hat{\pi}_0 = \frac{\sum_m I(Q_m \geq \lambda) + 1}{1 - \lambda}$$

4. Apply BH procedure to Q_ms at level $\alpha / \hat{\pi}_0$

Finite Sample Results

Theorem

If P_ms ind under H_ms and independent of other P_ms

$$FDR \leq \alpha \bar{w}_0 \frac{1-\lambda}{1-\lambda \bar{w}_0}$$

for $\bar{w}_0$ mean weight among true H_ms.

Corollaries for *FDR* control

- $\bar{w}_0 \leq 1$
- $\boldsymbol{w} = \mathbf{1}$ - Storey et. al (2004)
- Take $\alpha^* = \alpha \frac{1}{w_{(M)}} \frac{1-\lambda w_{(M)}}{1-\lambda}$

Asymptotic Results

Under weak dependence, as $M \to \infty$...

Theorem

The weighted adaptive BH procedure almost surely dominates its unadaptive counterpart in that it uses a larger rejection threshold.

Theorem

The weighted adaptive procedure has FDP $\leq \alpha$ almost surely if $\lim_{M\to} \bar{w}_0 = \mu_0 \leq 1$. Equality is achieved under least favorable configuration if $\mu_0 = 1$ (α-exhaustive).

Corollaries for FDP control:

- optimal weights for random effects model
- weights positively correlated with optimal weights
- $w_m \overset{i.i.d.}{\sim} E[W_m] = 1$
- Storey et. al. (2004) is α exhaustive

Weights

- For any fixed γ_ks + technical details $\Rightarrow w_m = \frac{Mt_m}{\sum_m t_m}$ where

$$t_m = 2\bar{\Phi}\left(0.5\bar{\Phi}^{-1}(\alpha/4)\left[\frac{\sqrt{n_m}}{\sqrt{n_.}/M} + \frac{\sqrt{n_.}/M}{\sqrt{n_m}}\right]\right)$$

- Main point: w_m is decreasing in n_m for all large enough n_m