BIOST 546: Machine Learning for Biomedical Big Data

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Lecture 8: High-Dimensional Inference
Spring 2017

Recap

- More general penalized regression
  - High dimensional survival analysis
  - High dimensional Poisson regression
  - Other penalties and loss functions

Today's Class

- High dimensional hypothesis testing
  - challenges of high dimensional hypothesis testing
  - controlling family-wise error rate (FWER)
  - controlling false discovery rate (FDR)
  - significance analysis of microarrays (SAM)

Hypothesis Testing

- In this course so far, we have talked about methods for supervised and unsupervised learning in high-dimensional settings
- We now consider a seemingly much simpler topic: hypothesis testing.
- But hypothesis testing in high dimensions has its challenges!

Hypothesis Testing

Good or Bad Metabolizer?	Good	Good	Bad	Bad	Good	Bad	Good	Bad	Good	Bad
Level of Protein 1?	2	1	5	11	17	8	2	0	56	1
Level of Protein 2?	4	8	10	34	3	1	0	1	76	1
	.	.	.	.	.	.	.	.	.	.
	.	.	.	.	.	.	.	.	.	.
	.	.	.	.	.	.	.	.	.	.
Level of Protein M?	5	6	2	15	23	11	3	6	8	9

Hypothesis Testing

- We have M features, each of which is measured in n observations.
- (In this lecture only, M is number of features, rather than p.)
- We also have a response vector of length n. Could be
  - blood pressure
  - tumor size
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  - tumor size
  - survival time
  - cancer subtype
- We wish to test the null hypothesis

$$H_{0j} : j\text{th feature is not associated with the response}$$

for $j = 1, \ldots, M$.

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Regardless of the response type, we get a test statistic and a p-value for the association between the feature and the response.

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- A p-value of 0.02 means that the probability of seeing such a strong association between the response and the feature by chance, under the null hypothesis, is 0.02.
- A small p-value indicates strong evidence for association: i.e. reject the null hypothesis.

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- Physicists control Type I error at around $\alpha = 5 \times 10^{-7}$. (Then again, atoms are cheaper than patients and mice.)

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- Probability that the jth p-value will be less than 0.05, assuming the null hypothesis holds for the jth feature, is 0.05.
- But the probability that at least one of the M p-values will be less than 0.05, assuming that all of the null hypotheses hold, is much greater than 0.05.

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- Probability that the jth p-value will be less than 0.05, assuming the null hypothesis holds for the jth feature, is 0.05.
- But the probability that at least one of the M p-values will be less than 0.05, assuming that all of the null hypotheses hold, is much greater than 0.05.
- In other words, we will erroneously reject the null hypothesis if we test a lot of hypotheses and reject the null hypothesis for any p-values less than the usual threshold, like 0.05.

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- Let $\alpha = 0.05$, and assume that the tests are all independent.
  - $M = 1$: probability of falsely rejecting at least one null hypothesis is 0.05.
  - $M = 2$: probability of falsely rejecting at least one null hypothesis is 0.0975.
  - $M = 10$: probability of falsely rejecting at least one null hypothesis is 0.40.
  - $M = 200$: probability of false rejecting at least one null hypothesis is 0.9999649.

Hypothesis Testing

We will get a lot of false positives if we use 0.05 as a cut-off for rejecting the null hypothesis.

Protein	T-stat	P-Value
1	3.2	0.00137
2	-1.8	0.0718
3	5.8	6e-9
4	13.2	0
5	1.4	0.1615
6	-0.2	0.8414
.	.	.
.	.	.
.	.	.
M	4	6e-5

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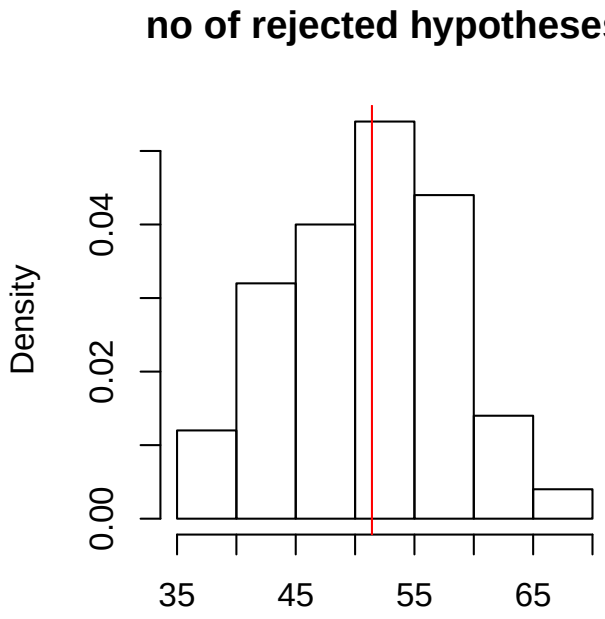

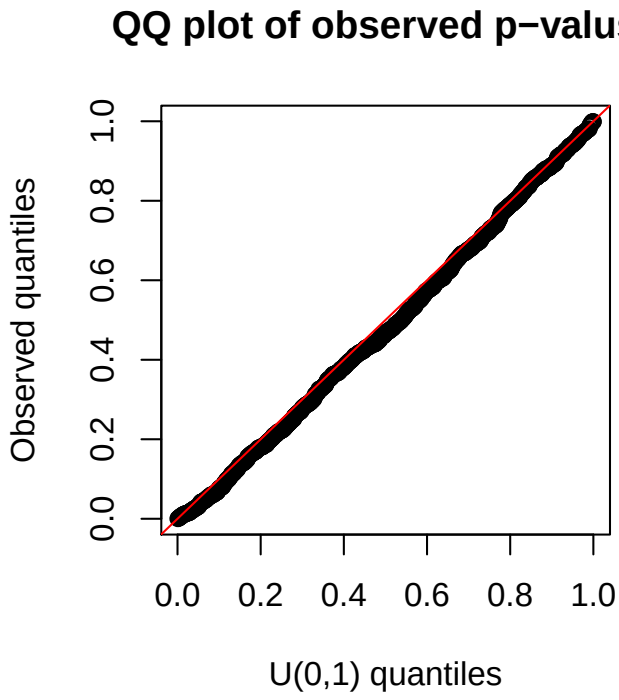

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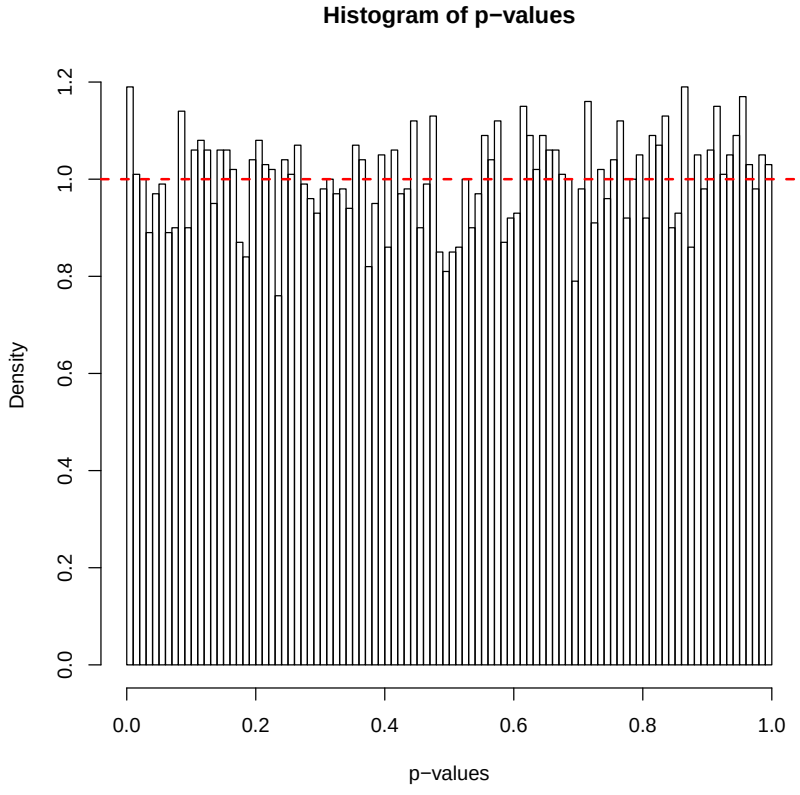

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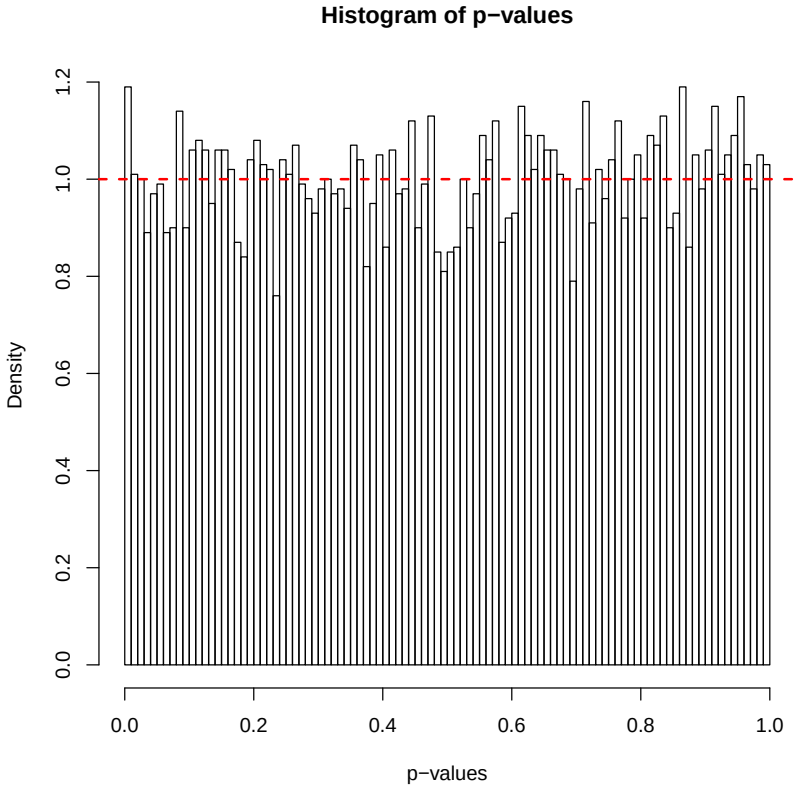
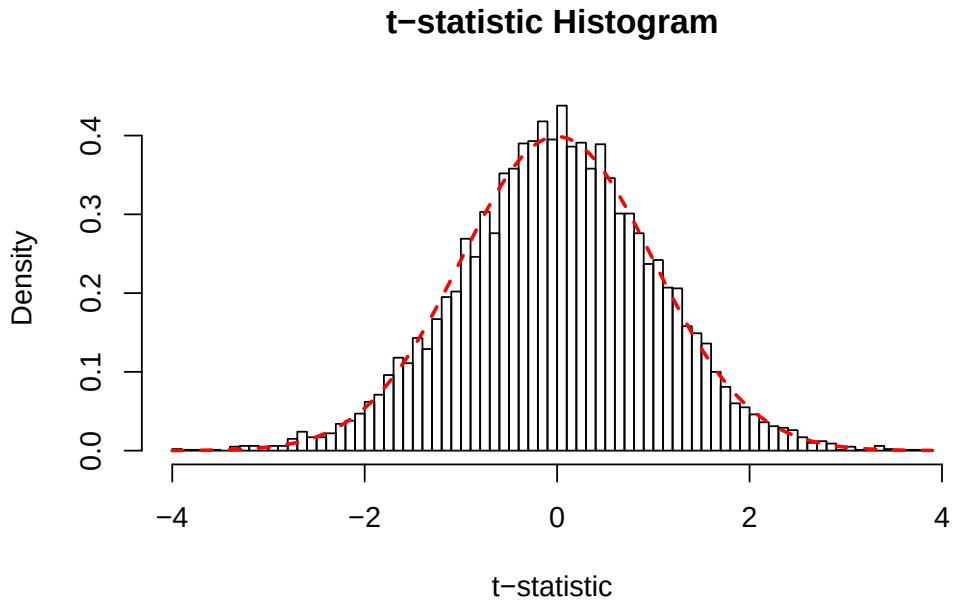
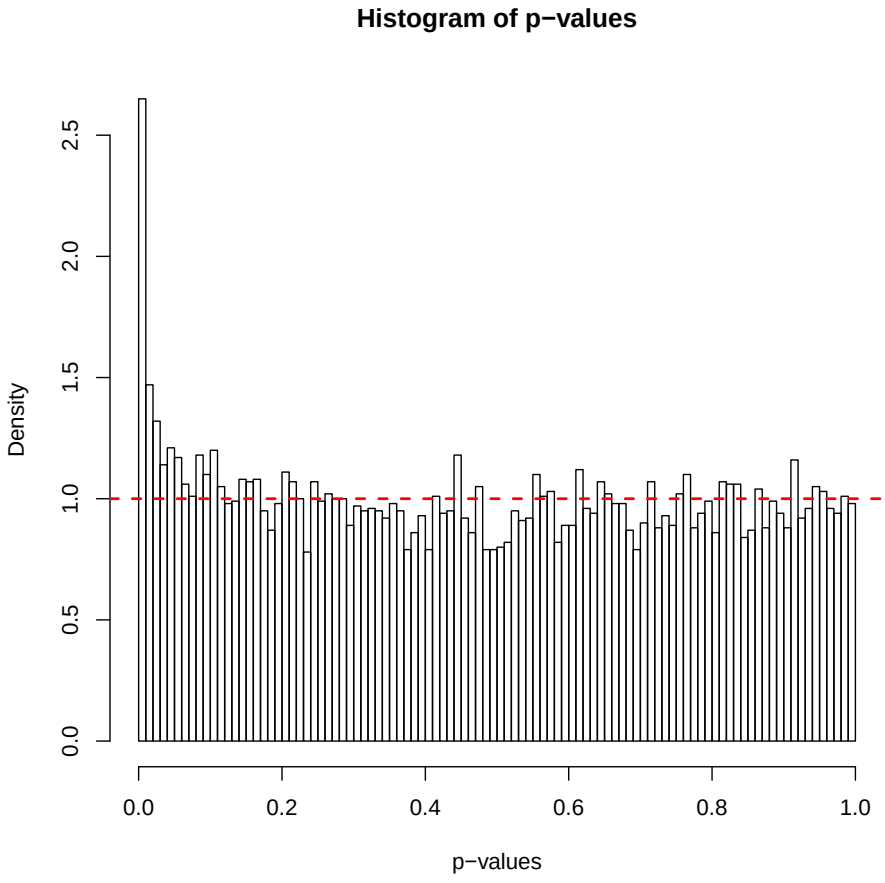




Example 2: Not All Null Hypotheses Hold



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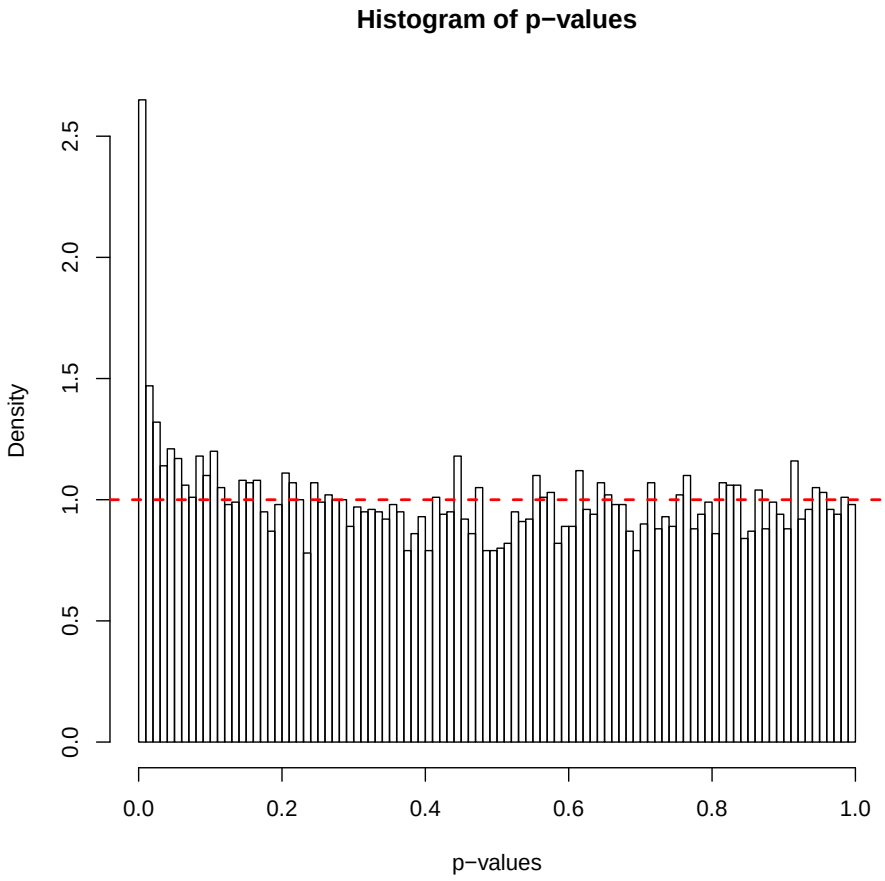

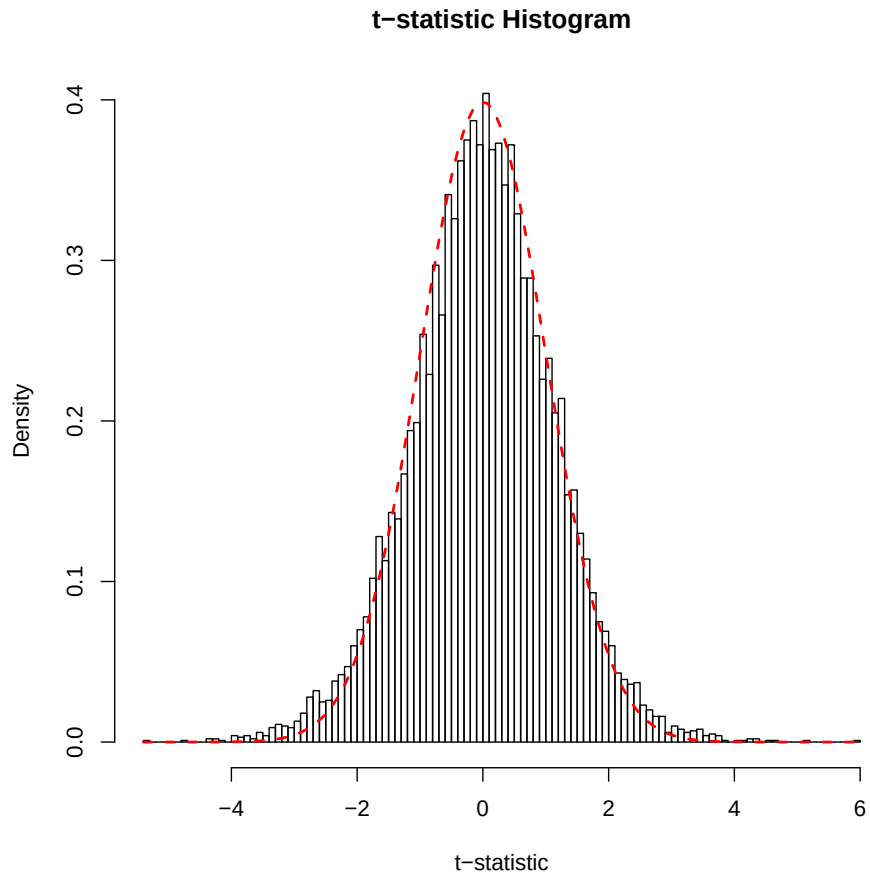

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- Rather than rejecting the jth null hypothesis is less than 0.05, we reject the jth null hypothesis if the jth p-value is less than $0.05/M$.
- More generally, to control the overall Type I error – probability of falsely rejecting the null hypothesis – at level α, we must reject the jth null hypothesis if its p-value is below α/M.

Why does the Bonferroni correction control Type I Error?

Consider test statistics T_j for tests of M features (actually T_j here is the absolute value of the test statistic, so we reject test j if T_j is large)

$$
\begin{aligned}
P(\max T_j > t^*) &= P(\text{at least one } T_j > t^*) \\
&= P(\{T_1 > t^*\} OR \{T_2 > t^*\} OR \ldots OR \{T_M > t^*\}) \\
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we can find t^* such that

$$M \times P(\max T_j > t^*) \leq 0.05 \Leftrightarrow P(\max T_j > t^*) \leq 0.05/M$$

$\dagger$ considering the setting that there is no effect!

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- Let A denote the event that at least one null hypothesis is falsely rejected, and let A_j be the event that the jth null hypothesis is falsely rejected. So

$$P(A) = P(\bigcup_{j=1}^{M} A_j) \leq \sum_{j=1}^{M} P(A_j).$$

  This means that if we keep $P(A_j)$ below α/M, then $P(A)$ will be below α.

- And keeping $P(A_j)$ below α/M means that we reject the jth null hypothesis if the p-value is below α/M.

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- This is very conservative! We will hardly ever reject the null hypothesis.
- Slightly less conservative alternatives to Bonferroni, that also control overall Type I error, are also available (but not discussed here).

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- In other words: willing to have some false positives, in exchange for more true positives.
- A new notion of error control: false discovery rate.
- Proposed in 1995, but still a very active area of research!

Possible Outcomes from M Hypothesis Tests

	Called Not Significant	Called Significant	Total
H_0 True	U	V	M_0
H_0 False	T	S	M_1
Total	$M - R$	R	M

- V is number of false positives.
- T is number of false negatives.
- Type I error is $E(V)/M_0$.
- Type II error is $E(T)/M_1$.
- The power is $1 - E(T)/M_1$.
- The Bonferroni correction guarantees that $P(V \geq 1) \leq \alpha$.

False Discovery Rate

$$FDR = E\left(\frac{\text{number of null hypotheses falsely rejected}}{\text{number of null hypotheses rejected}}\right).$$

For instance, in a gene expression experiment,

$$FDR = E\left(\frac{\text{number of genes incorrectly declared significant}}{\text{number of genes declared significant}}\right).$$

In other words, this is the fraction of discoveries that are false positives.

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- For instance, in a GWAS, if we reject the null hypothesis of no association for SNPs with $FDR \leq 0.2$, then we expect no more than 20% of those SNPs to be false positives.

Benjamini-Hochberg Algorithm for FDR Control

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4. Alternatively, define

$$L = \max \left\{ j : p_{(j)} < \alpha \frac{j}{M} \right\}.$$

5. Reject the jth null hypothesis if $p_j \leq p_{(L)}$, the Benjamini-Hochberg rejection threshold.

FDR vs Bonferroni Control

- Benjamini & Hochberg:
  - Find the maximum order statistic (k) such that

$$p(k) \leq \frac{\alpha k}{M}$$

  - Reject all tests j with $p_j < p(k)$.
- Bonferroni:
  - Reject test j if

$$p_j \leq \frac{\alpha}{M}$$

Example in R: All Null Hypotheses Hold

```
x <- matrix(rnorm(1000*50),ncol=50)
y <- sample(c(0,1),50,rep=TRUE)
ps <- NULL
for(i in 1:1000) ps <- c(ps,
 t.test(x[i,y==0],x[i,y==1])$p.value)
cat("Around 5% of p-values are below 0.05:",
mean(ps<.05),fill=TRUE)
fdrs.bh <- p.adjust(ps, method="BH")
plot(ps,fdrs.bh)
plot(fdrs.bh)
```

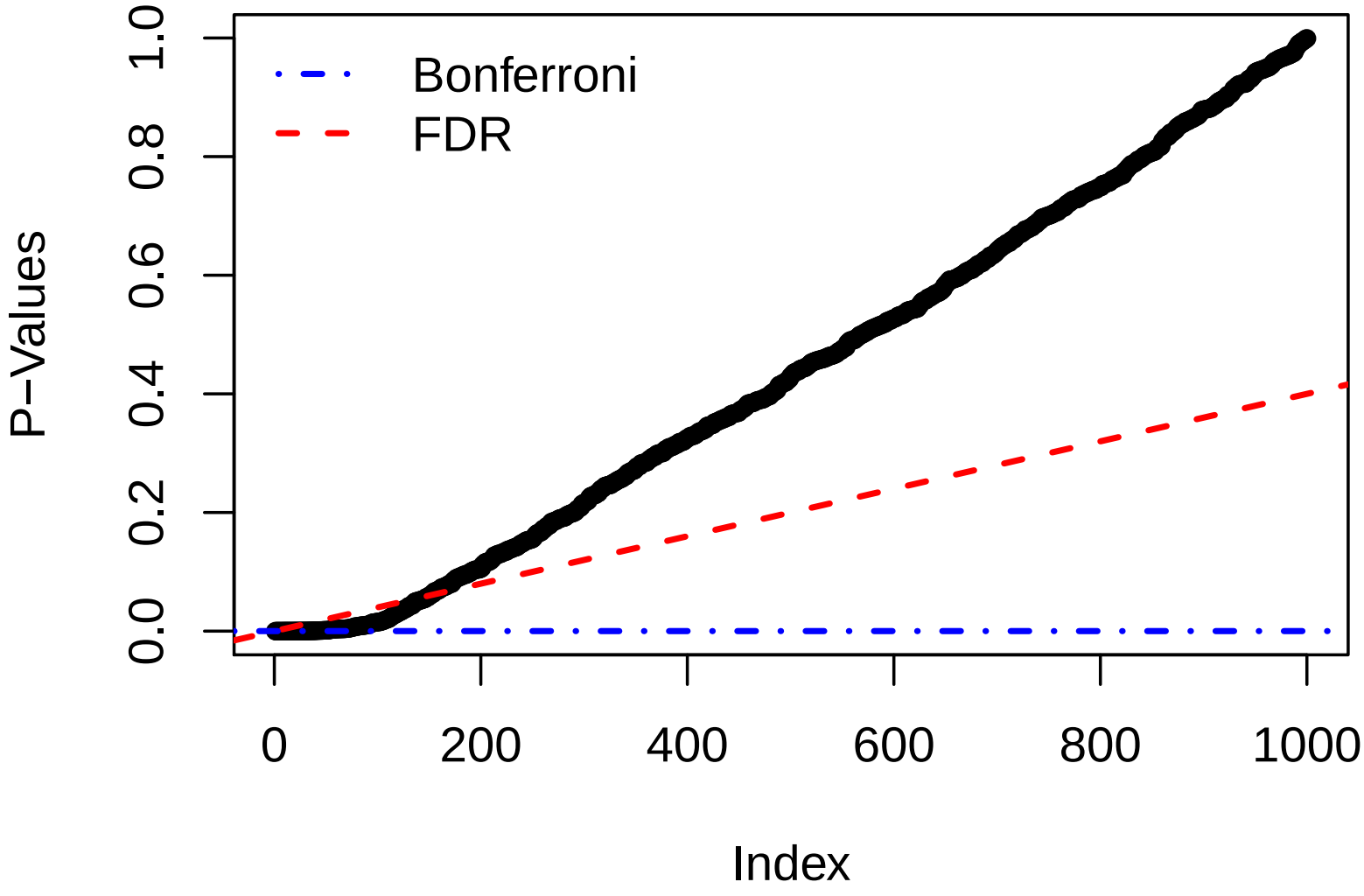
Example in R: Not All Null Hypotheses Hold

```
x <- matrix(rnorm(1000*50),ncol=50)
y <- sample(c(0,1),50,rep=TRUE)
x[1:100,y==0] <- x[1:100,y==0] + 1
ps <- NULL
for(i in 1:1000) ps <- c(ps,
t.test(x[i,y==0],x[i,y==1])$p.value)
cat("Way more than 5% of p-values are below 0.05:",
mean(ps<.05),fill=TRUE)
fdrs.bh <- p.adjust(ps, method="BH")
plot(ps,fdrs.bh)
plot(fdrs.bh)
```

Example, Continued

```
cat("Number of Tests with FDR below 0.4:",
sum(fdrs.bh<0.4), fill=TRUE)
cat("Compute the BH FDR Directly:",
max(which(sort(ps,decreasing=FALSE) < .4*(1:1000)/1000)), fill=TRU
plot(sort(ps,decreasing=FALSE),ylab="P-Values")
abline(a=0, b=0.4/1000,col="red")
```

Output From R



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- In real data examples, genes, proteins etc work together, and are correlated
- How would methods of multiple testing adjustment work if tests are correlated?

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- Benjamini & Hochberg can handle positive dependence: think of this as all genes being positively correlated with each other
- However, in practice genes may have both positive (inducers) and negative (inhibitors) correlations with each other, so this assumption may not hold
- This is still an active area of research

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- In R, `p.adjust(ps, method="BY")`

A Real Data Example

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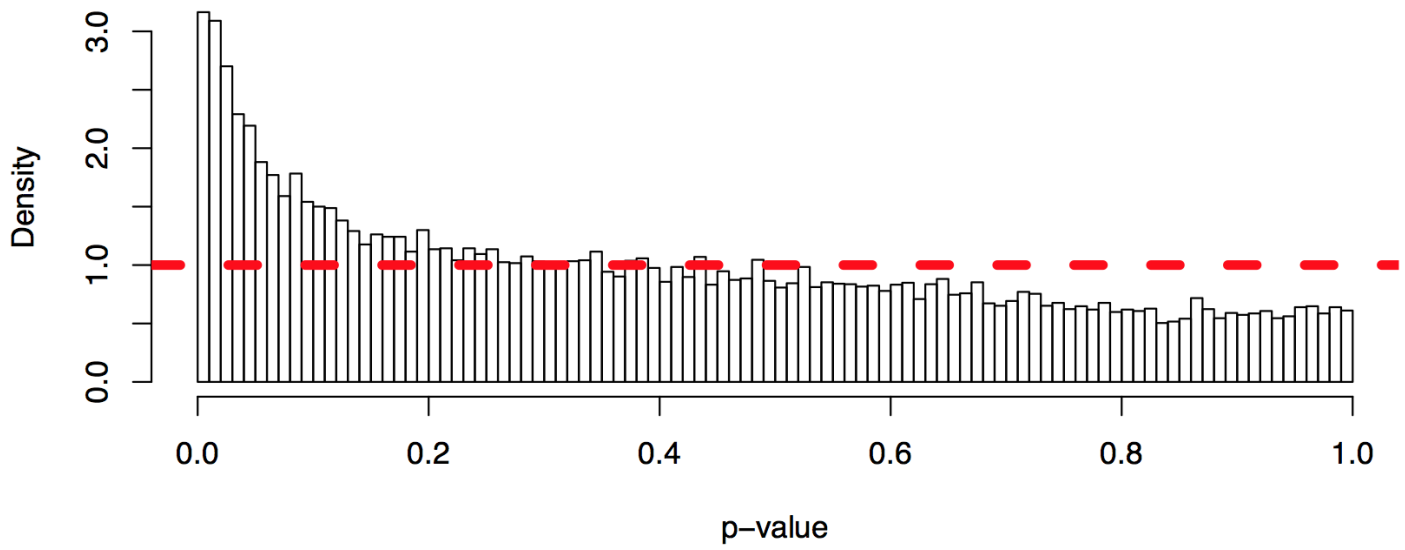
Let's look at the p-values



A Real Data Example

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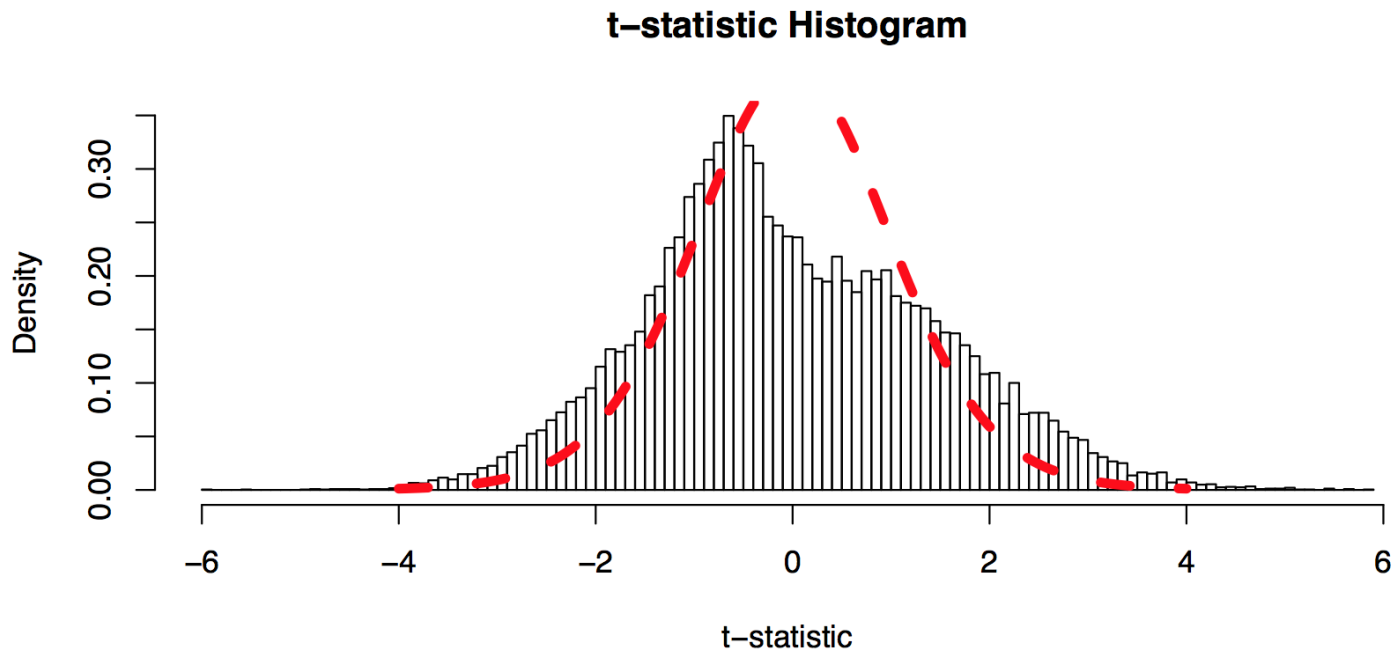
And, test statistics



What is going on??

A Real Data Example

- With dependence correction, 0 rejections for FDR control < 1
- Unclear how significant findings are...
  - Report K (10, 100, ...) most significant effects
  - Give FDR estimates from both “BH" and “BY"
  - Small sample-size and strange histogram shape add extra skepticism
  - We also need to check for batch effects and other potentially damaging factors

Example: Radiation Treatment Sensitivity

- Microarray data set on sensitivity of cancer patients to ionizing radiation treatment.
- Citation: Rieger, Hong et al, PNAS, 2004
- $M = 12,625$ genes, $n = 58$ samples: 44 samples with a normal reaction, 14 patients with severe reaction to radiation.

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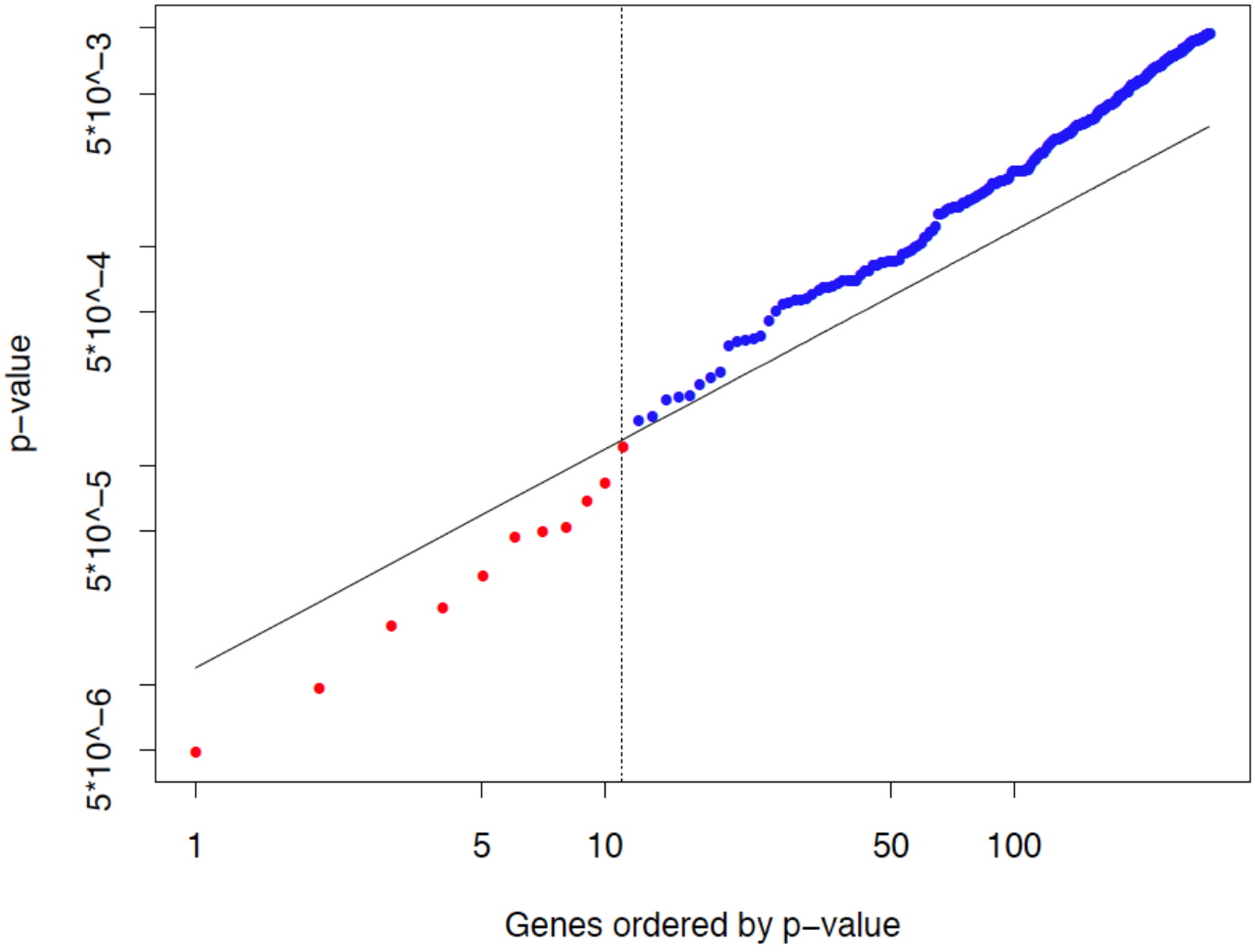
- $M = 12,625$ genes, $n = 58$ samples: 44 samples with a normal reaction, 14 patients with severe reaction to radiation.
- Compute two-sample t-statistic for each gene's association with response to radiation.
- NO genes are declared significant after Bonferroni correction at level $\alpha = 0.05$.
- 11 genes had FDR below $\alpha = 0.15$.
- For each gene we can get a q-value, which is like a p-value for that gene, but quantifies the FDR associated with that gene.

Example: Radiation Treatment Sensitivity



Permutation Approach to FDR Estimation

- Another way to estimate FDRs: by permutation.
- Easy and natural – unlike Benjamini-Hochberg, can explain it pretty simply to a non-statistician.
- Does not require computing p-values, which can be helpful in settings where p-values are (1) difficult to compute, or (2) unreliable.
- Also known as “plug-in estimate for FDR".

Permutation Approach

1. Compute $t_1, \ldots, t_M$, the test statistic for each of the M features.
2. Create K permutations of the responses, and for each permutation from $k = 1, \ldots, K$, and for each feature $j = 1, \ldots, M$, compute $t_j^1, \ldots, t_j^K$.
3. For a range of values of the cut-point C, let

$$R_{obs} = \sum_{j=1}^{M} 1_{(|t_j| > C)},$$

and

$$\widehat{E(V)} = \frac{1}{K} \sum_{j=1}^{M} \sum_{k=1}^{K} 1_{(|t_j^k| > C)}.$$

4. Estimate the FDR by $\widehat{FDR} = \widehat{E(V)}/R_{obs}$.

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- We estimate $E(V)$ by permuting the response and calculating the number of test statistics that exceed the threshold, in the absence of any real relationship between the features and the response.
- Actually, $\widehat{E(V)}$ estimates $(M/M_0)E(V)$, but since $M > M_0$, this over-estimate is conservative.

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- How to get p-value for this new test statistic? Who knows!
- But estimating FDR using a permutation approach is pretty straightforward.

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- We might want to follow up on genes whose FDR is below 10% or 20% in a gene expression experiment.
- In general, the FDR threshold that we use will depend on the data set as well as the number of genes with small FDRs... as well as the number of rejected null hypotheses that we can afford to follow up on in the lab!

P-Values Using Permutations

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- We could also compute p-values using a permutation-type approach: the p-value for the jth feature is given by

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$$p_j = \frac{1}{K} \sum_{k=1}^{K} 1_{(|t_{j'}^k| > |t_j|)}.$$

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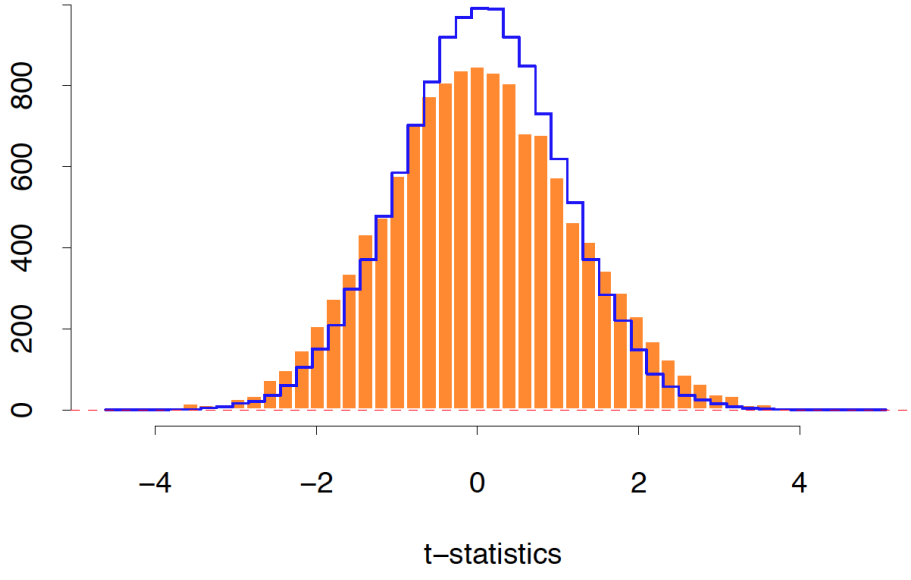
- Example on radiation sensitivity data: orange shows true t-statistics, and blue shows t-statistics for permuted data.



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- In some cases, (most) differentially expressed genes change in the positive (or negative) direction. In such cases, it may be better to use different cut-points for negative and positive test statistics
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- uses a QQ-plot to find test statistics that are far from expected
- the cutoff value is determined by finding a band of length Δ around the expected test-statistics
- is implemented in the `R`-package `samr`: `SAM(x,y=NULL,...)`

SAM