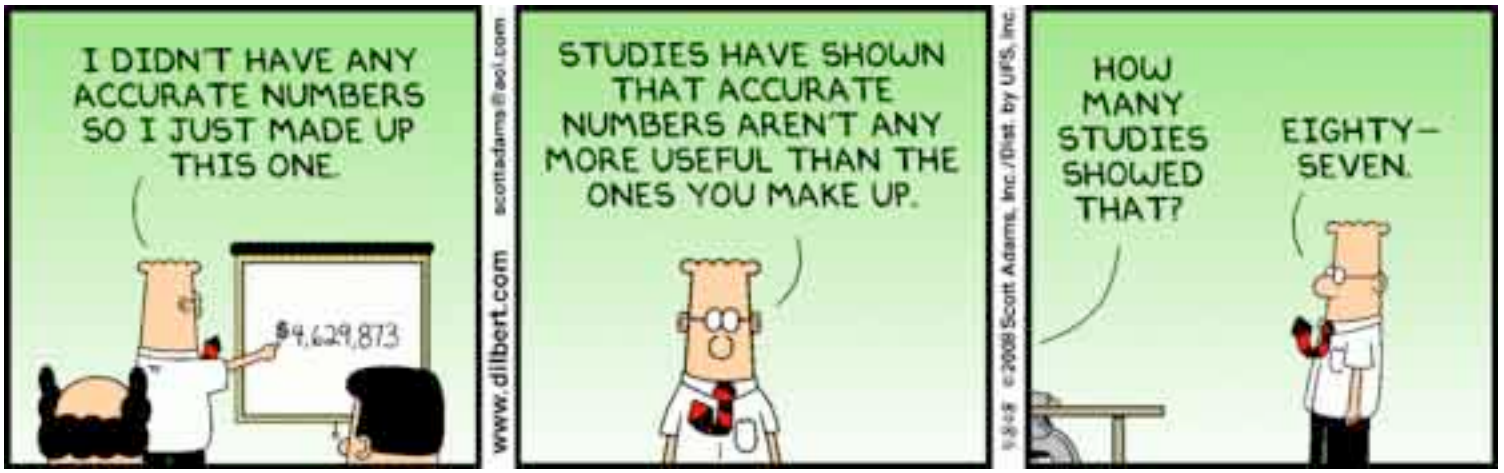


Sensitivity, Specificity, ROC

Multiple testing

Independent filtering

Wolfgang Huber (EMBL)

Statistics 101

←bias accuracy→

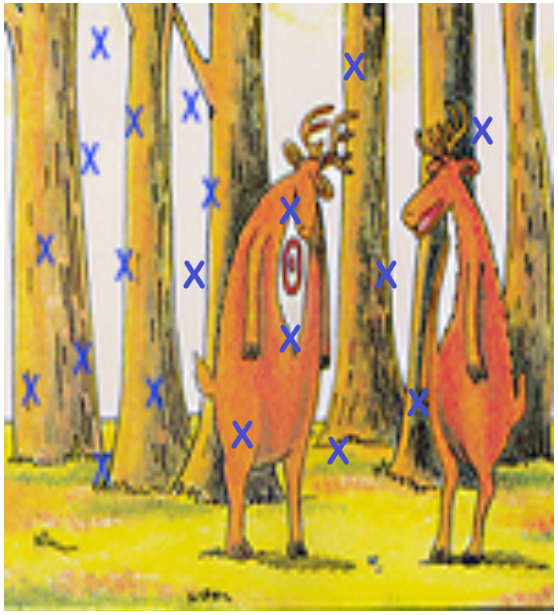
← precision dispersion→

Basic dogma of data analysis

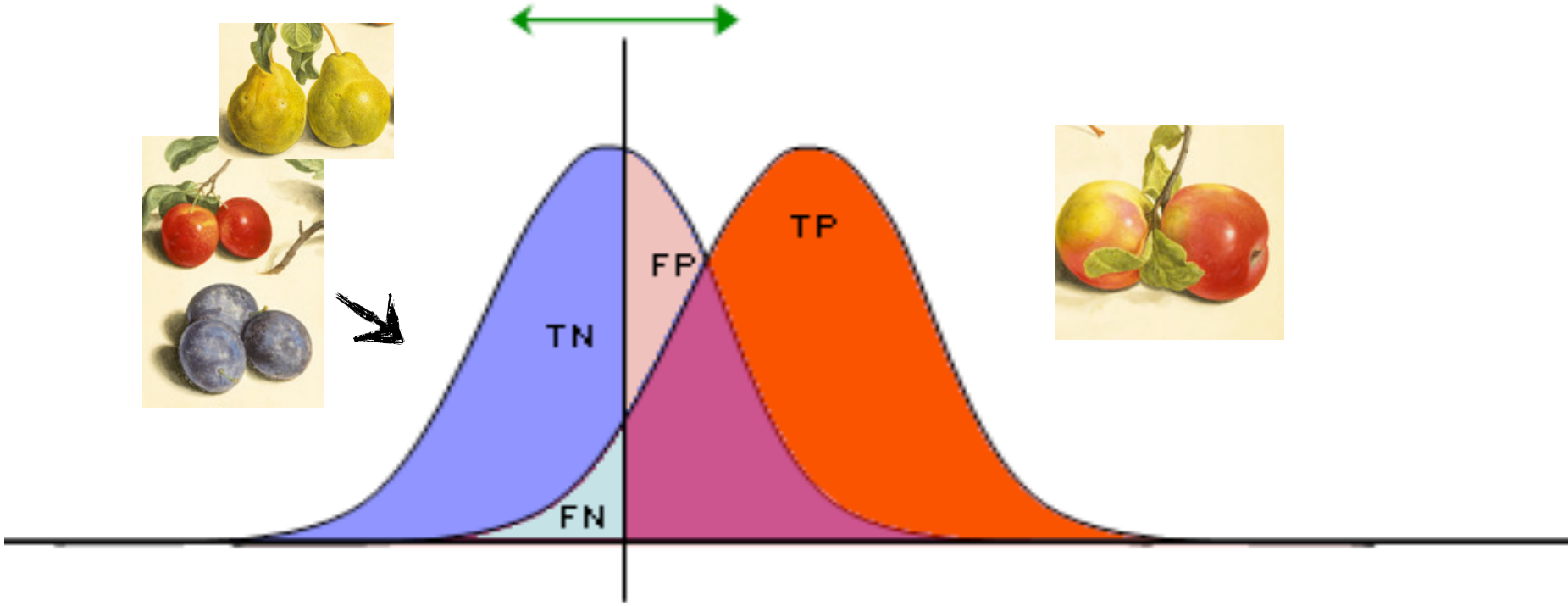
Can always increase sensitivity on the cost of specificity, or vice versa, the art is to

- optimize both

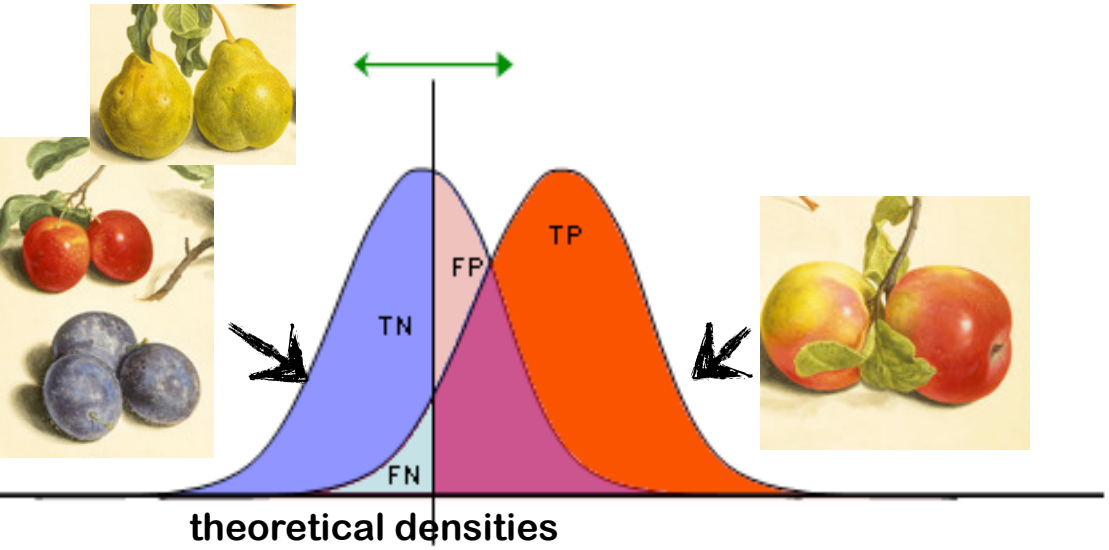
- find the best trade-off



Problem: detecting apples from other fruit



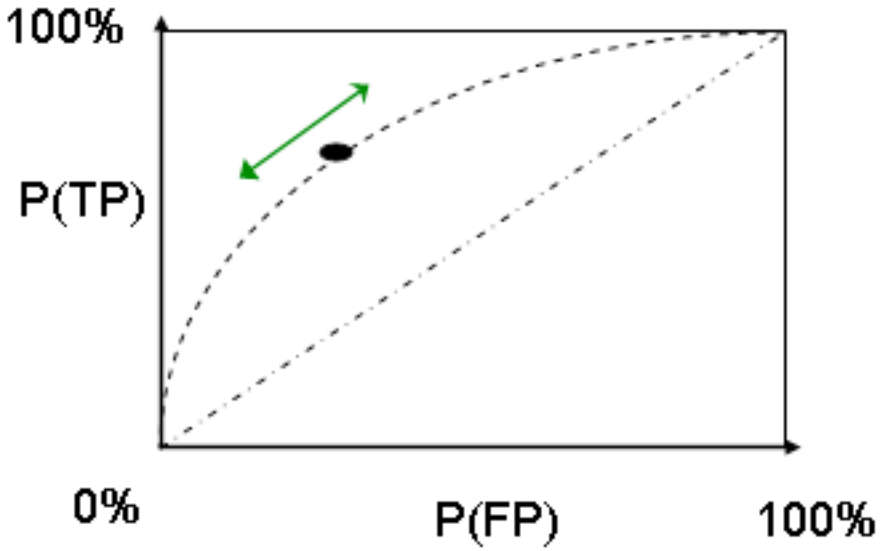
The apple detection assay and the receiver operating characteristic curve



	apples	other fruit
apple detection assay	TP	FP
	FN	TN
	P	N

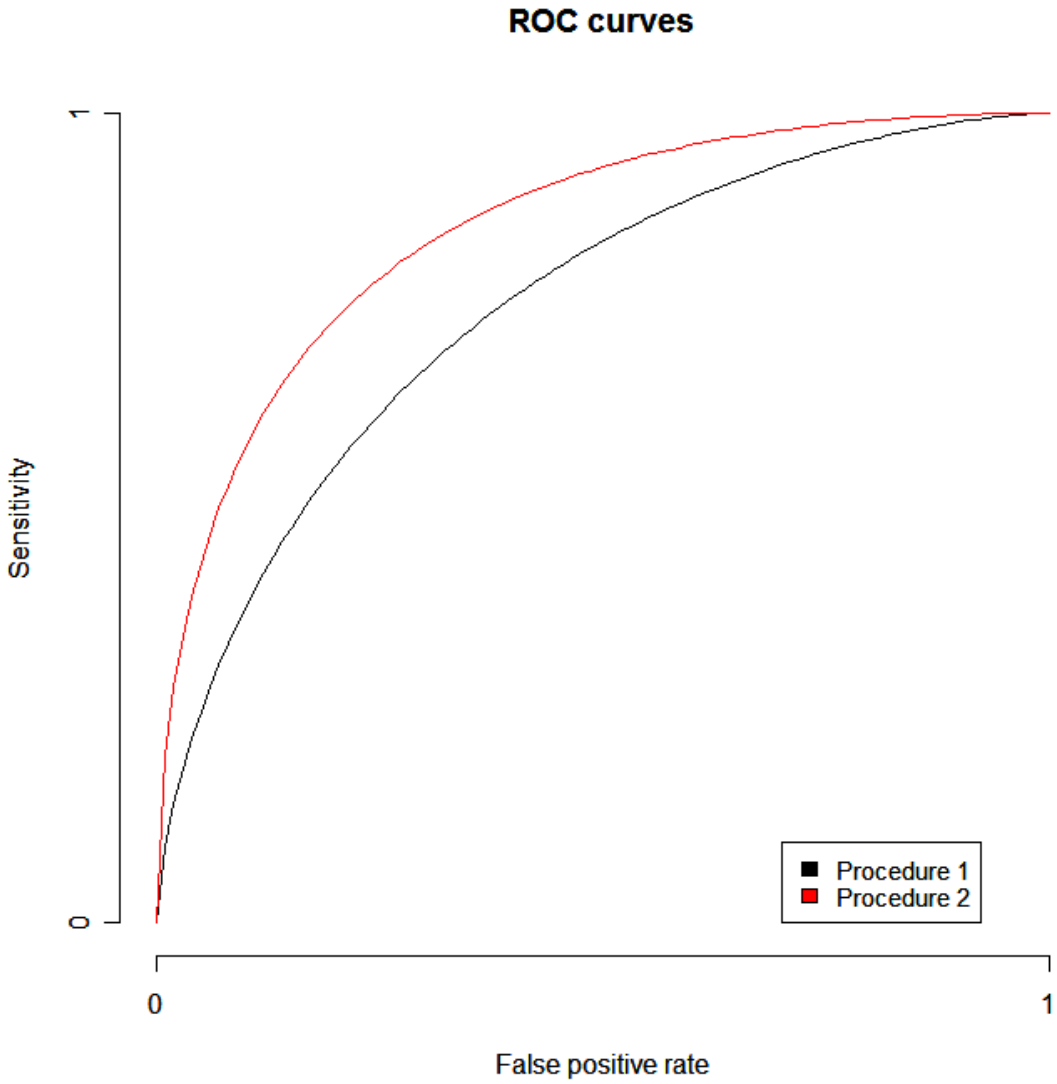
empirical results

Sensitivity: Probability that a detected object is really an apple. Can be estimated by TP / P.

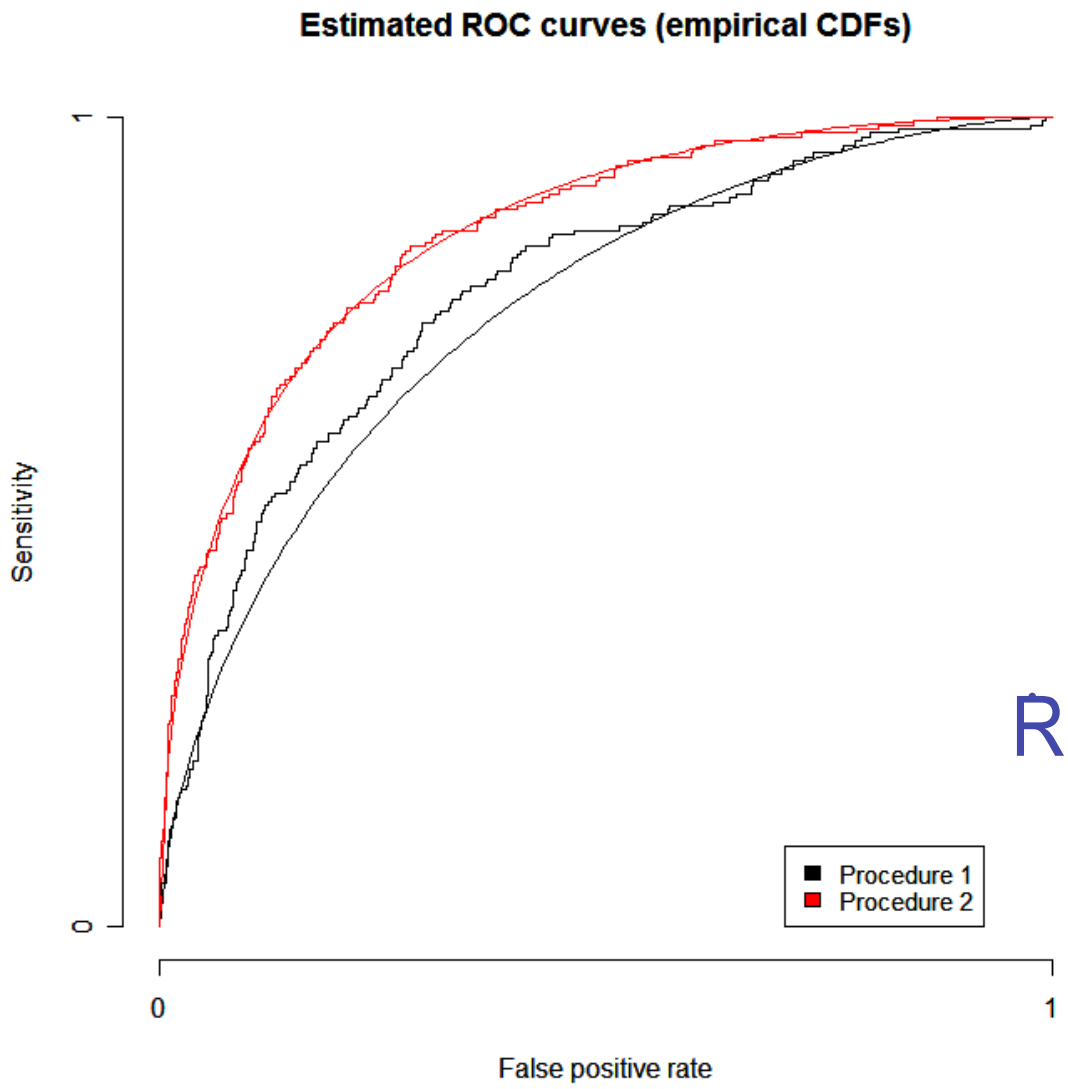


Specificity: Probability that a non-detected object is really not an apple. Estimated by TN / N.

ROC curves for method comparison



Empirical estimation of ROC curves



$$\hat{F}(t) = \frac{1}{m}\sum_{i=1}^{m} 1\{X_i \le t\}$$

$$t\}$$

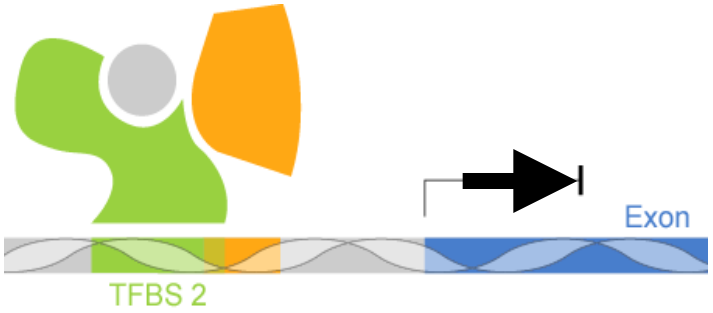
$$RO \qquad t)\Big)\Big\}_{t\in i}$$

This assumes that we know the "ground truth". Can we still do it if we don't?

Example: identification of transcription factor binding sites

H_0 = {regions *with no* binding site}

H_1 = {regions *with* a binding site}



True positives?

Small numbers of known sites for most factors.

Even the real sites are not active under all conditions.

True negatives?

Non-canonical / unexpected locations can hold real sites.

True ROC curve

$$H_0 = \{\text{regions } \textit{with no} \text{ binding site}\}$$

$$H_1 = \{\text{regions } \textit{with} \text{ a binding site}\}$$

Test statistic	Set	Distribution
$X_1,\ldots,X_m$	H_0	**F**
$Y_1,\ldots,Y_n$	H_1	**G**

"Pseudo-ROC" curve

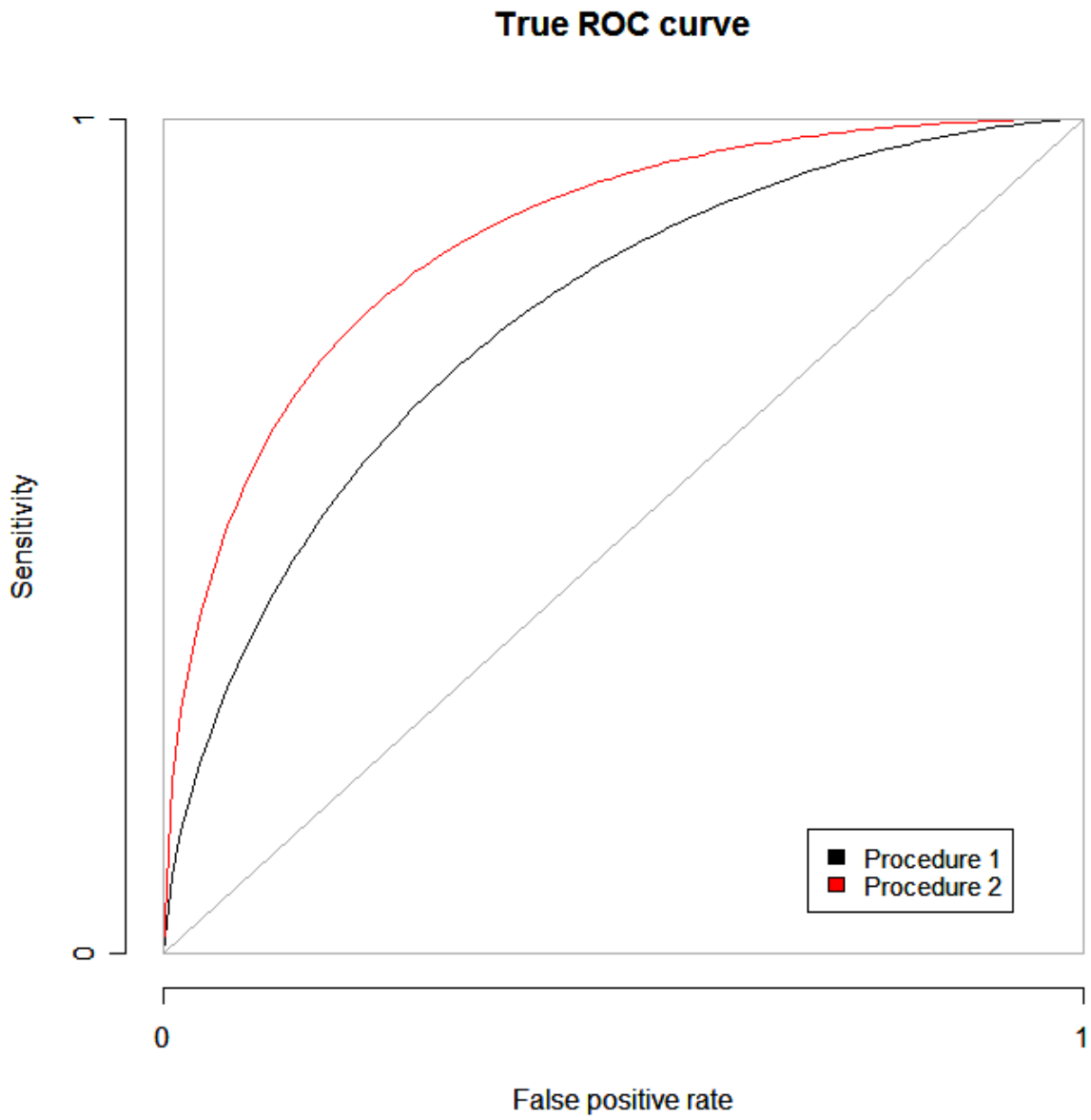
$S_0 = \{\text{regions } \textit{less} \text{ likely to have a binding site}\}$

$S_1 = \{\text{regions } \textit{more} \text{ likely to have a binding site}\}$

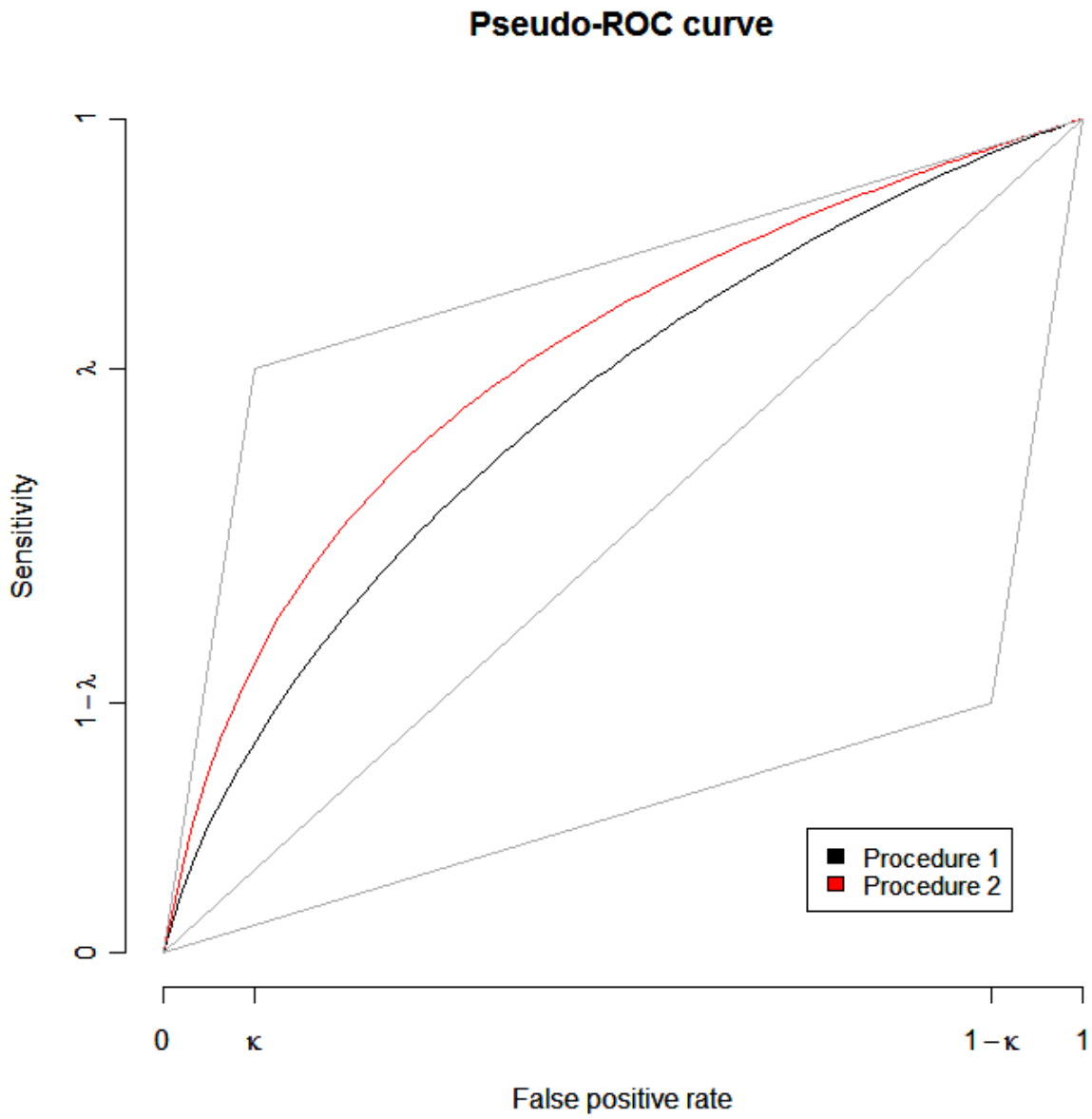
Test statistic	Set	Distribution
$X_1,\ldots,X_m$	H_0	**F**
$Y_1,\ldots,Y_n$	H_1	**G**
$X'_1, \cdots, X'_m$	S_0	$(1-\kappa)F + \kappa G$
$Y'_1, \cdots, Y'_m$	S_1	$(1-\lambda)F + \lambda G$

If $\kappa = 0$ and $\lambda = 1$, test data are correctly classified.

Correctly classified test data



Contaminated test data



Linear transform

$$\mathrm{ROC}' = \left\{\left(1-F'(t),1-G'(t)\right): t \in ¡ \right\}$$

$$= \left\{\left(1-(1-\kappa)F(t)-\kappa G(t),1-(1-\lambda)F(T)-\lambda G(t)\right): t \in ¡ \right\}$$

$$= \left\{\left(1-F(t),1-G(t)\right)\begin{pmatrix} 1-\kappa & 1-\lambda \\ \kappa & \lambda \end{pmatrix}: t \in ¡ \right\}$$

$$= \left\{(p,q)\begin{pmatrix} 1-\kappa & 1-\lambda \\ \kappa & \lambda \end{pmatrix}: (p,q) \in \mathrm{ROC}\right\}$$

Comparing two methods

$$\mathrm{ROC}' = \left\{ (p,q) \begin{pmatrix} 1-\kappa & 1-\lambda \\ \kappa & \lambda \end{pmatrix} : (p,q) \in \mathrm{ROC} \right\}$$

The transformation depends on the contamination fractions only, not F_1 and G_1, or F_2 and G_2.

Assuming $\kappa < \lambda$, the linear transform preserves the ordering of curves and of the area under them (AUC).

The area between (and under) the curves is compressed — more severely as $\lambda \to 0$ or $\kappa \to 1$.

Assumption! With classification variable $C \in \{0,1\}$, X is independent of C_X, and Y, of C_Y.

Summary

If, for both procedures being compared,

- correctly and incorrectly classified true positives have the same statistical properties, and
- correctly and incorrectly classified true negatives have the same statistical properties, then

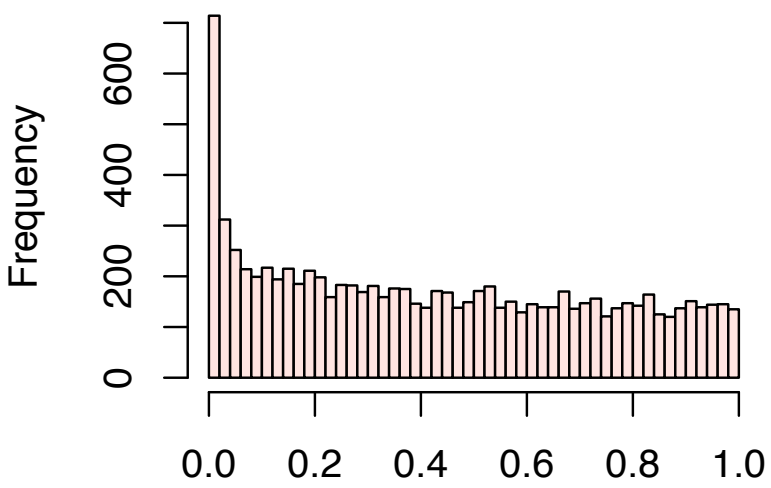
the pseudo-ROC and true ROC select the same procedure as superior.

Multiple testing

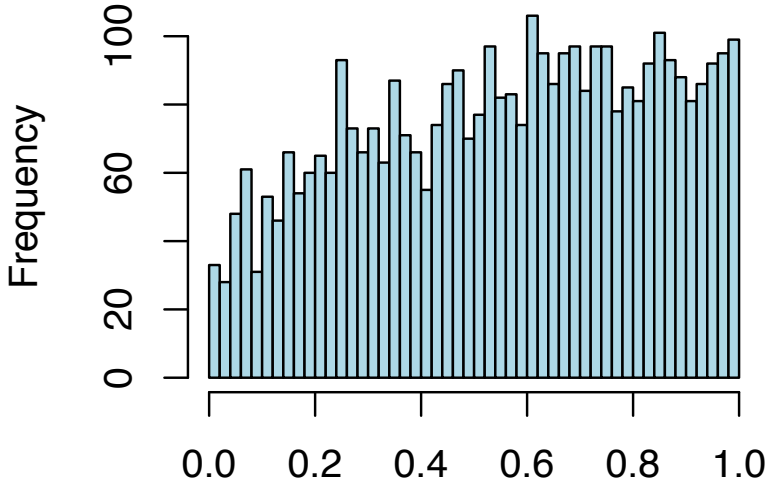
Many data analysis approaches in genomics rely on item-by-item (i.e. multiple) testing:

Microarray or RNA-Seq expression profiles of “normal” vs “perturbed” samples: gene-by-gene
ChIP-chip: locus-by-locus
RNAi and chemical compound screens
Genome-wide association studies: marker-by-marker
QTL analysis: marker-by-marker and trait-by-trait

Diagnostic plot: the histogram of p-values

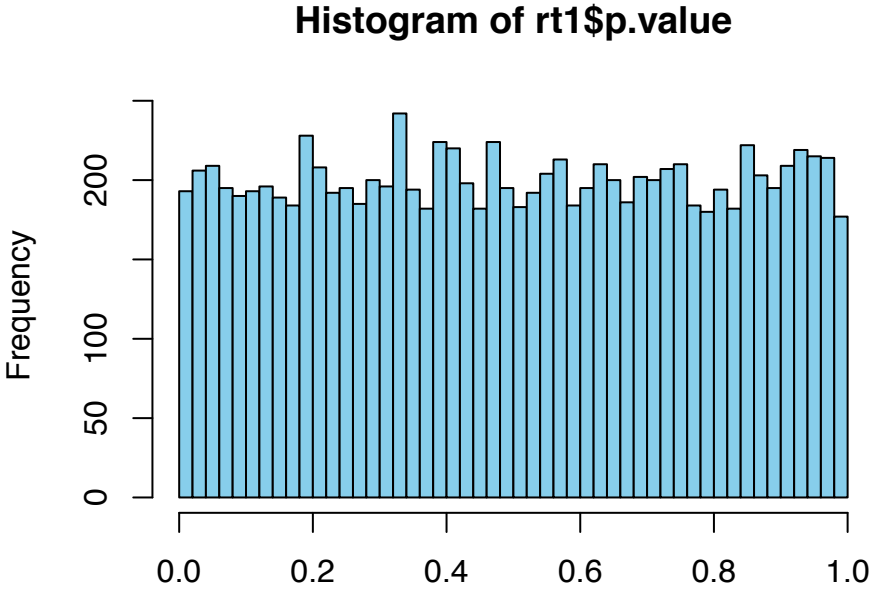


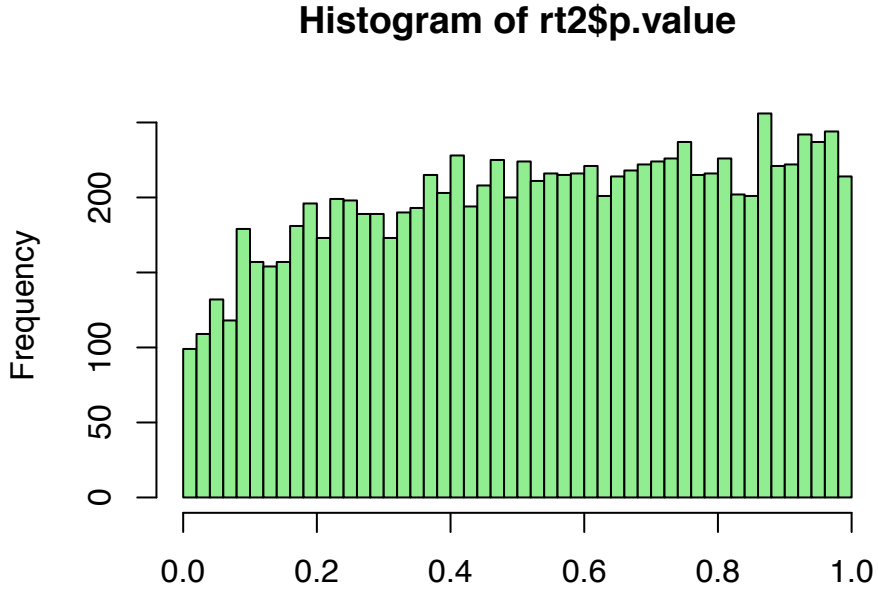
Observed p-values are a mix of samples from
- **a uniform distribution (from true nulls) and**
- **from distributions concentrated at 0 (from true alternatives)**



Depletion of small p can indicate the presence of confounding hidden variables ("batch effect")

Batch effects or “latent variables”






```
n = 10000
m = 20
x = matrix(rnorm(n*m), nrow=n, ncol=m)
fac = factor(c(rep(0, 10), rep(1, 10)))
rt1 = rowttests(x, fac)

x[, 6:15] = x[, 6:15]+1
rt2 = rowttests(x, fac)
```

`sva` package; Leek JT, Storey JD. Capturing heterogeneity in gene expression studies by surrogate variable analysis. PLoS Genet. 2007

Stegle O, Parts L, Durbin R, Winn J. A Bayesian framework to account for complex non-genetic factors in gene expression levels greatly increases power in eQTL studies. PLoS Comput Biol. 2010.

Multiple testing

Classical hypothesis test:

null hypothesis H_0, alternative H_1

test statistic $X \mapsto t(X) \in R$

$\alpha = P(t(X) \in \Gamma_{rej} \mid H_0)$ type I error (false positive)

$\beta = P(t(X) \notin \Gamma_{rej} \mid H_1)$ type II error (false negative)

When n tests are performed, what is the extent of type I errors, and how can it be controlled?

E.g.: 20,000 tests at α=0.05, all with H_0 true: expect 1,000 false positives

Experiment-wide type I error rates

	Not rejected	Rejected	Total
True null hypotheses	U	V	m_0
False null hypotheses	T	S	m_1
Total	m – R	R	m

Family-wise error rate: P(V > 0), the probability of one or more false positives. For large m_0, this is difficult to keep small.

False discovery rate: E[V / max{R,1}], the expected fraction of false positives among all discoveries.

FWER: The Bonferroni correction

Suppose we conduct a hypothesis test for each gene $g = 1, \ldots, m$, producing

an observed test statistic: T_g

an unadjusted p–value: p_g.

Bonferroni adjusted p–values:

$$\tilde{p}_g = \min\bigl(mp_g, 1\bigr).$$

Selecting all genes with $\tilde{p}_g \leq \alpha$ controls the FWER at level α, that is, $Pr(V > 0) \leq \alpha$.

Controlling the FDR (Benjamini/Hochberg)

❍ FDR: the expected proportion of false positives among the significant genes.

❍ Ordered unadjusted p–values: $p_{r_1} \leq p_{r_2} \leq \ldots \leq p_{r_m}$.

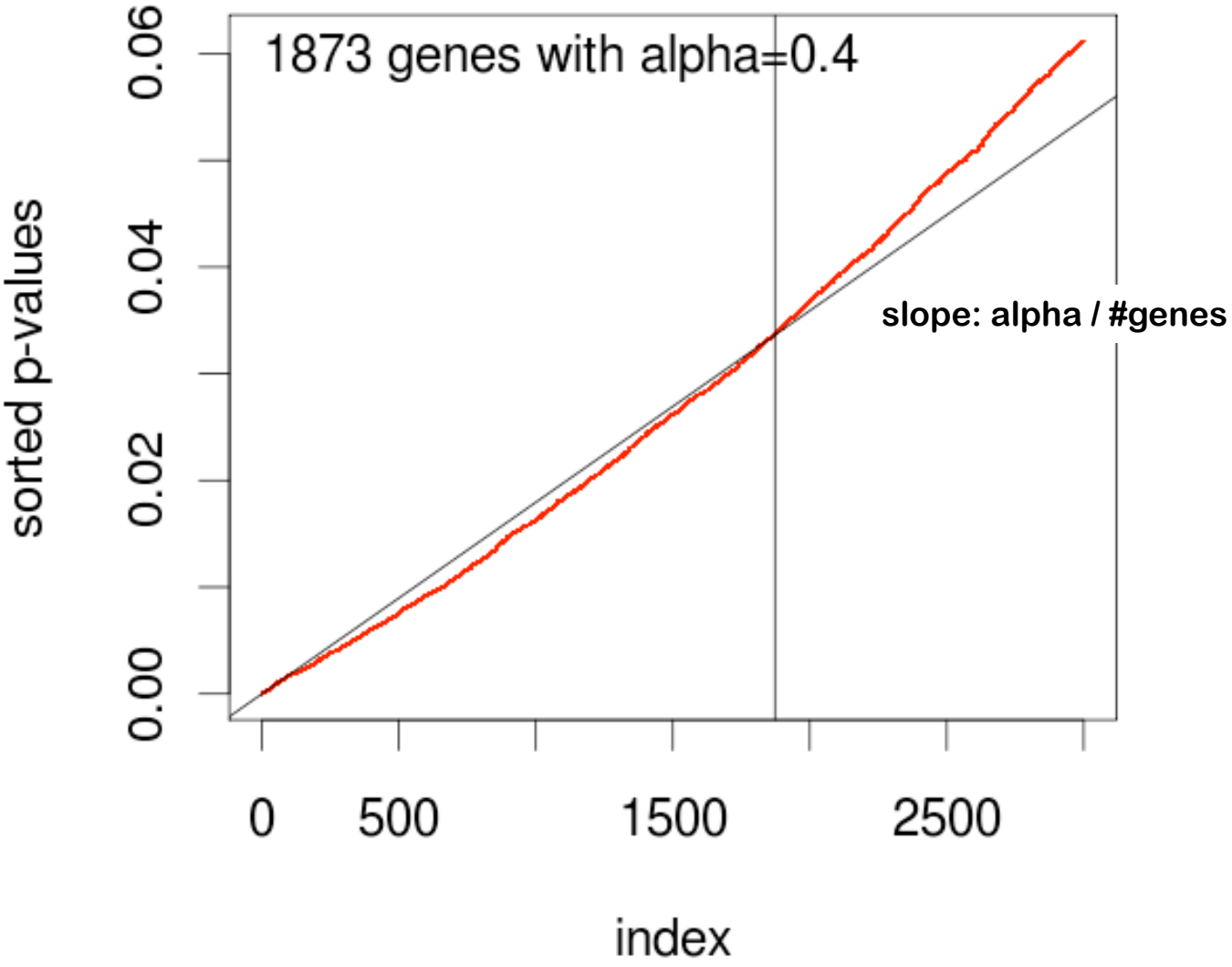
❍ To control $FDR = E(V/R)$ at level α, let

$$j^\star = \max\{j : p_{r_j} \leq (j/m)\alpha\}.$$

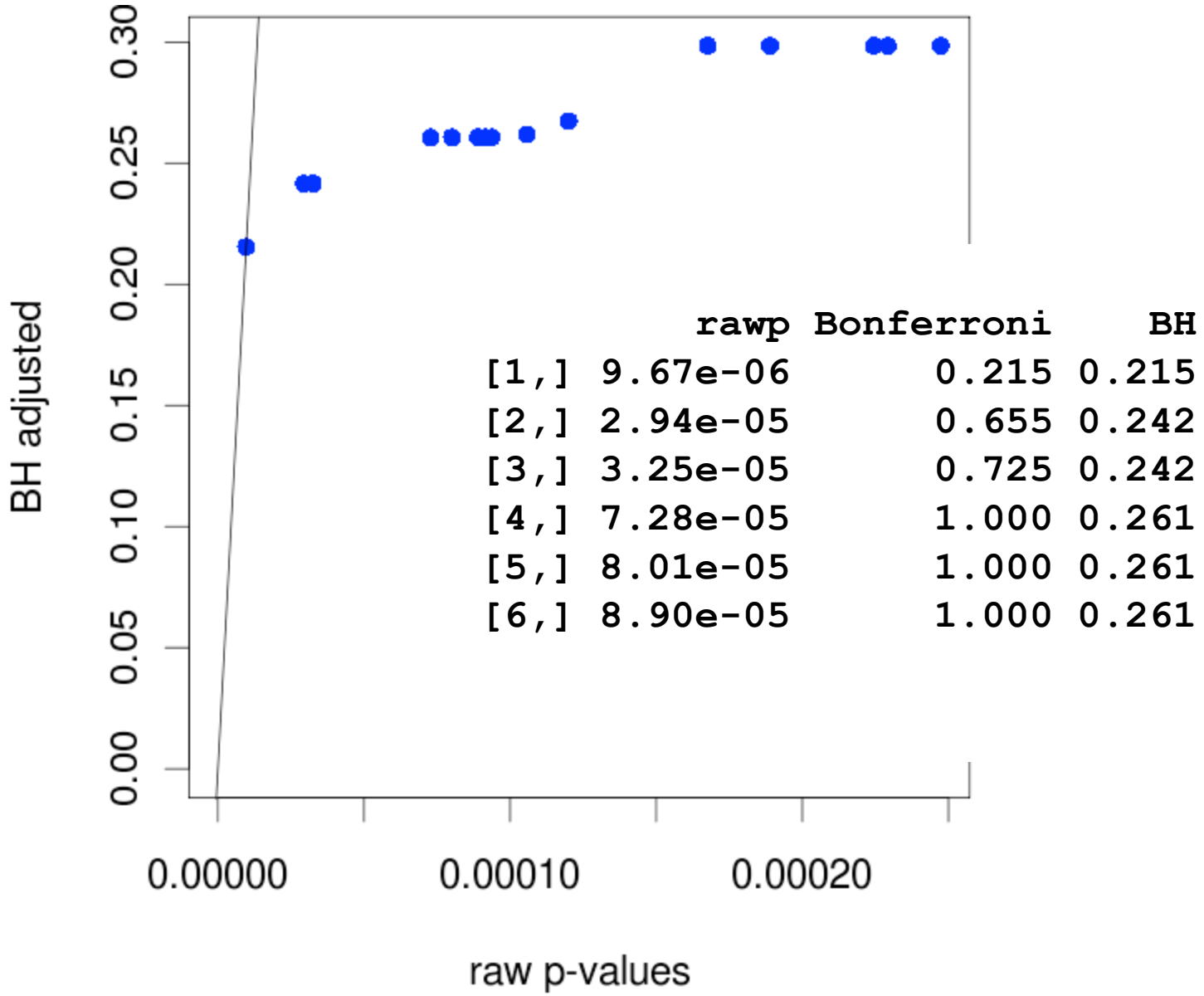
Reject the hypotheses H_{r_j} for $j = 1, \ldots, j^\star$.

❍ Is valid for independent test statistics and for some types of dependence. Tends to be conservative if many genes are differentially expressed. Implemented in multtest.

Benjamini Hochberg multiple testing adjustment

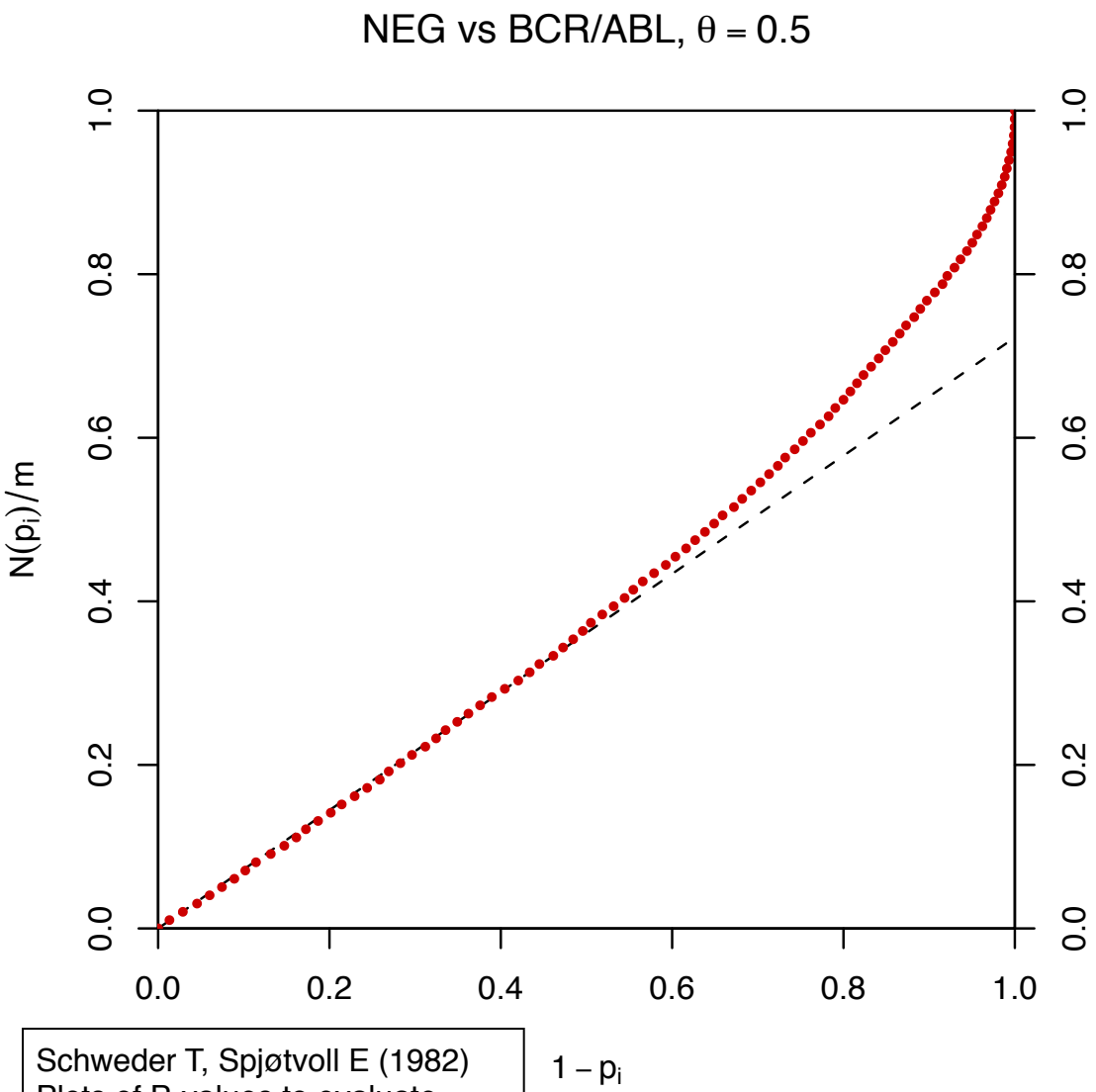


Benjamini Hochberg multiple testing adjustment



	rawp	Bonferroni	BH
[1,]	9.67e-06	0.215	0.215
[2,]	2.94e-05	0.655	0.242
[3,]	3.25e-05	0.725	0.242
[4,]	7.28e-05	1.000	0.261
[5,]	8.01e-05	1.000	0.261
[6,]	8.90e-05	1.000	0.261

Schweder and Spjøtvoll p-value plot



Schweder T, Spjøtvoll E (1982) Plots of P-values to evaluate many tests simultaneously. Biometrika 69:493–502.

For a series of hypothesis tests $H_1 \ldots H_m$ with p-values p_i, plot

$$(1 - p_i, N(p_i)) \quad \text{for all } i$$

where N(p) is the number of p-values greater than p.

Example: differential expression testing

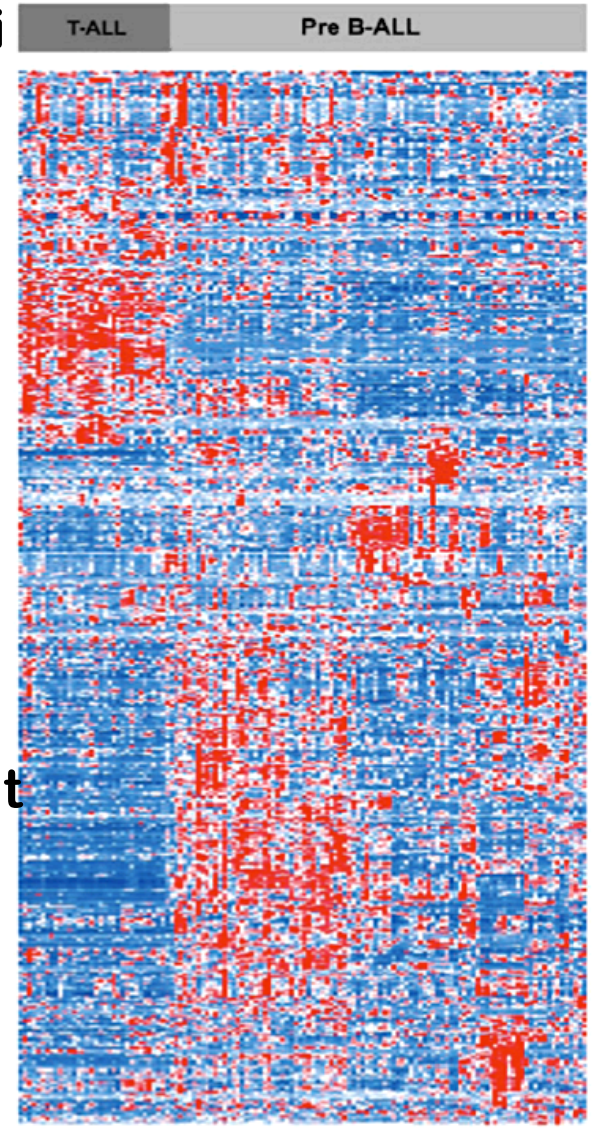
Acute lymphocytic leukemia (ALL) data, Chiaretti et al., Clinical Cancer Research 11:7209, 2005

Immunophenotypic analysis of cell surface markers identified

- T-cell derivation in 33,
- B-cell derivation in 95 samples

Affymetrix HG-U95Av2 3' transcript detection arrays with ~13,000 probe sets

Chiaretti et al. selected probesets with "sufficient levels of expression and variation across groups" and among these identified 792 differentially expressed genes.



Clustered expression data for all 128 subjects, and a subset of 475 genes showing evidence of differential expression between groups

Independent filtering

From the set of 13,000 probesets,

first filter out those that seem to report negligible signal (say, 40%),

then formally test for differential expression on the rest.

Conditions under which we expect negligible signal :

1. Target gene is absent in both samples. (Probes will still report noise and cross-hybridization.)
2. Probe set fails to detect the target.

Literature: von Heydebreck et al. (2004)

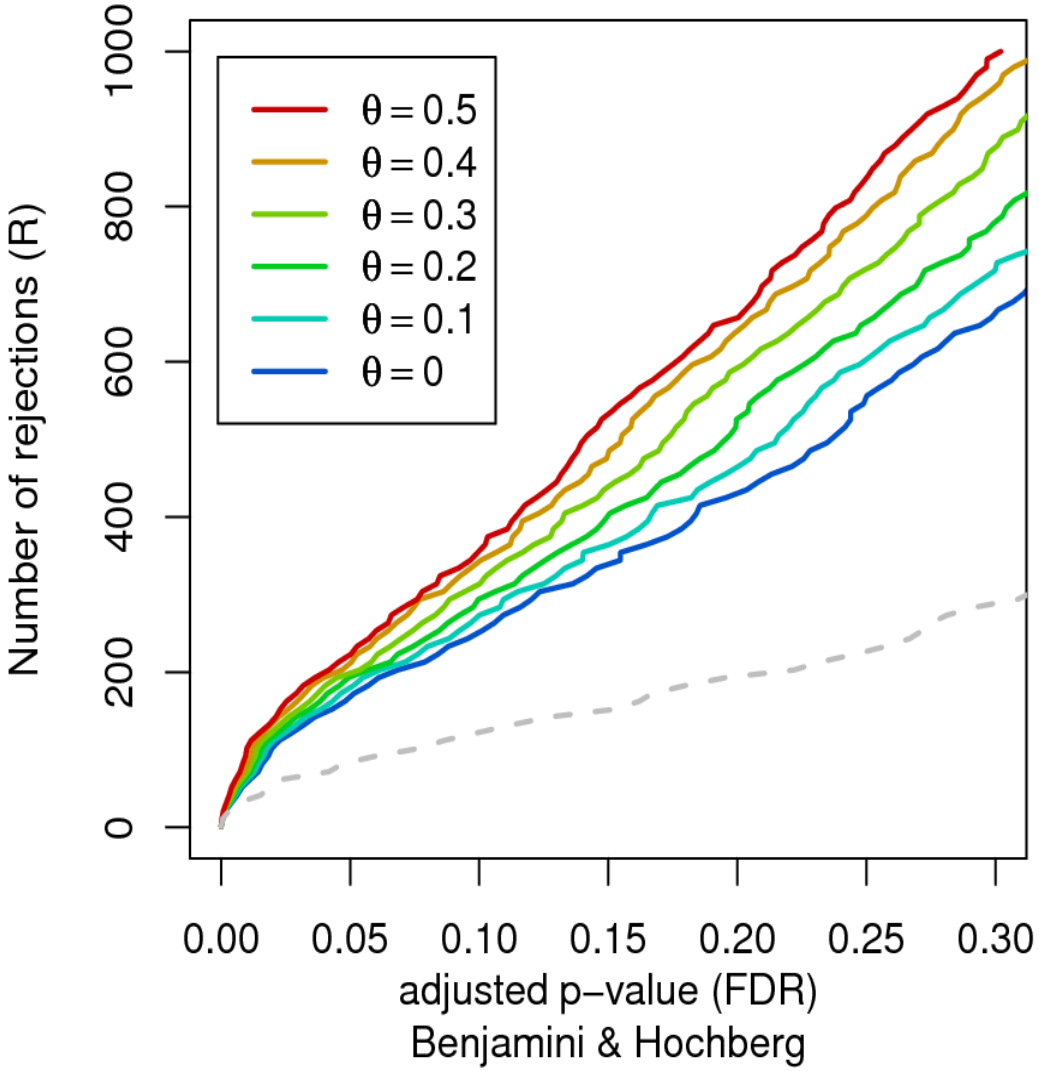
McClintick and Edenberg (BMC Bioinf. 2006) and references therein

Hackstadt and Hess (BMC Bioinf. 2009)

Many others.

Increased detection rates

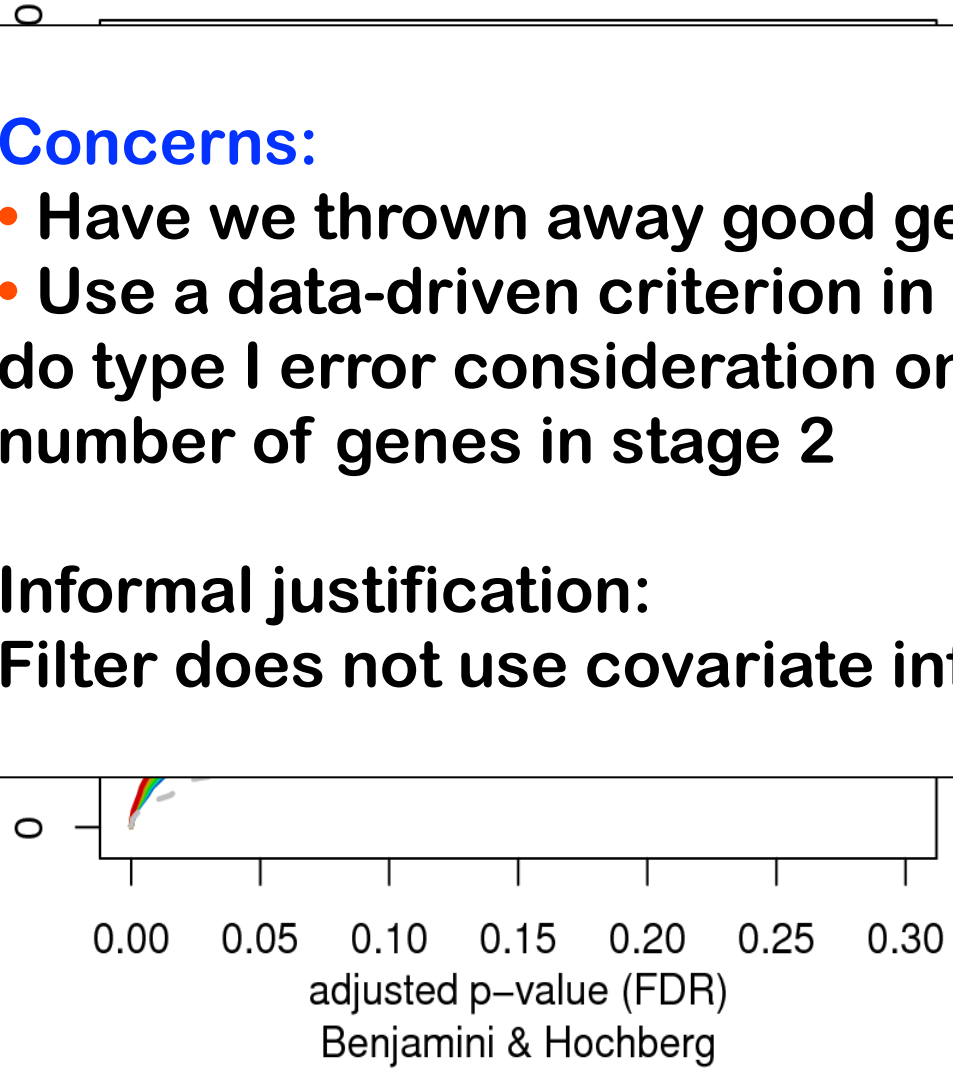
Stage 1 filter: compute variance, across samples, for each probeset, and remove the fraction θ that are smallest
Stage 2: standard two-sample t-test



Increased power?

Increased detection rate implies increased power

only if we are still controlling type I errors at the same level as before.



Concerns:

- **Have we thrown away good genes?**
- **Use a data-driven criterion in stage 1, but do type I error consideration only on number of genes in stage 2**

Informal justification:
Filter does not use covariate information

What do we need for type I error control?

I. For each individual (per gene) test statistic, we need to know its correct null distribution

II. If and as much as the multiple testing procedure relies on certain (in)dependence structure between the different test statistics, our test statistics need to comply.

I.: one (though not the only) solution is to make sure that by filtering, the null distribution is not affected - that it is the same before and after filtering

II.: See later

Result: independence of stage 1 and stage 2 statistics under the null hypothesis

For genes for which the null hypothesis is true (X_1 ,…, X_n exchangeable), f and g are statistically independent in both of the following cases:

- **Normally distributed data:**

  f (stage 1): overall variance (or mean)

  g (stage 2): the standard two-sample t-statistic, or any test statistic which is scale and location invariant.

- **Non-parametrically:**

  f: any function that does not depend on the order of the arguments. E.g. overall variance, IQR.

  g: the Wilcoxon rank sum test statistic.

Both can be extended to the multi-class context: ANOVA and Kruskal-Wallis.

Derivation

Non-parametric case:

Straightforward decomposition of the joint probability into product of probabilities using the assumptions.

Normal case:

Use the spherical symmetry of the joint distribution, p-dimensional $N(0, 1\sigma^2)$, and of the overall variance; and the scale and location invariance of t.

This case is also implied by Basu's theorem

(V complete sufficient for family of probability measures P, T ancillary $\Rightarrow$ T, V independent)

What do we need for type I error control?

The distribution of the test statistic under the null.

I. Marginal: for each individual (per gene) test statistic

II. Joint: some (though not all) multiple testing procedures relies on certain independence properties of the joint distribution

I.: one (though not the only) solution is to make sure that by filtering, the marginal null distribution is not affected - that it is the same before and after filtering

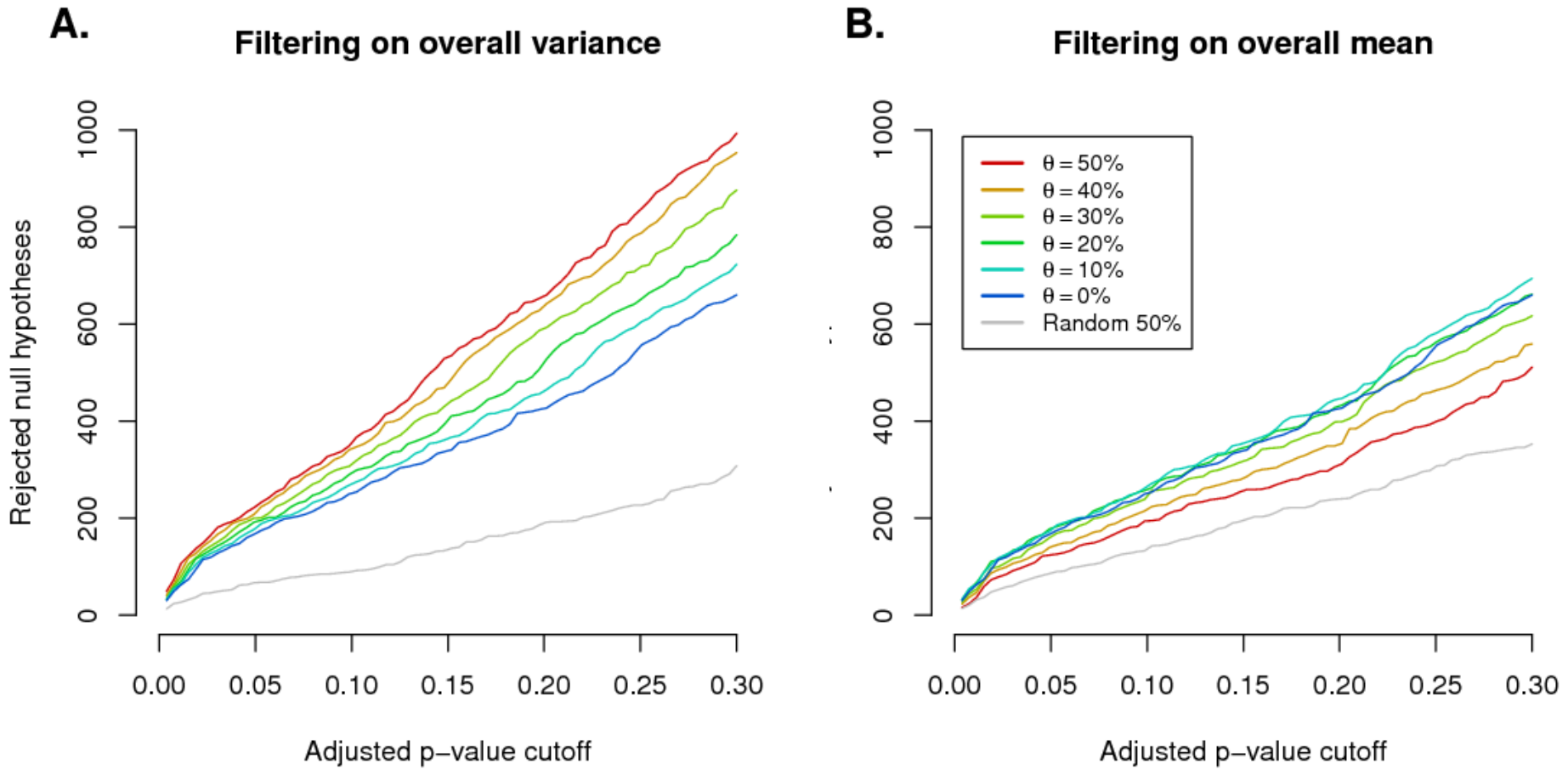
Multiple testing procedures and dependence

1. Methods that work on the p-values only and allow general dependence structure: Bonferroni, Bonferroni-Holm (FWER), Benjamini-Yekutieli (FDR)

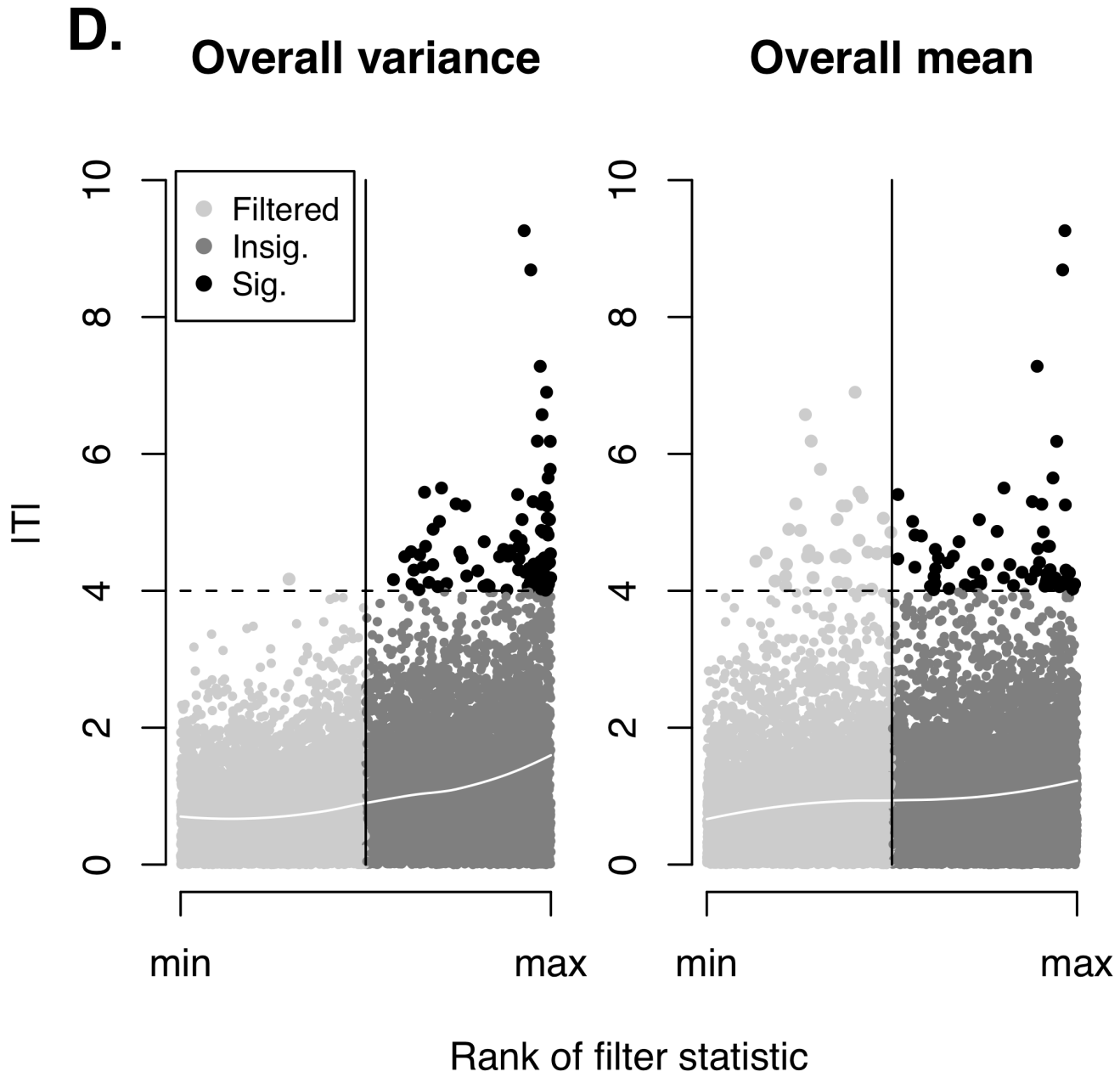
2. Those that work on the data matrix itself, and use permutations to estimate null distributions of relevant quantities (using the empirical correlation structure): Westfall-Young (FWER)

3. Those that work on the p-values only, and make dependence-related assumptions: Benjamini-Hochberg (FDR), q-value (FDR)

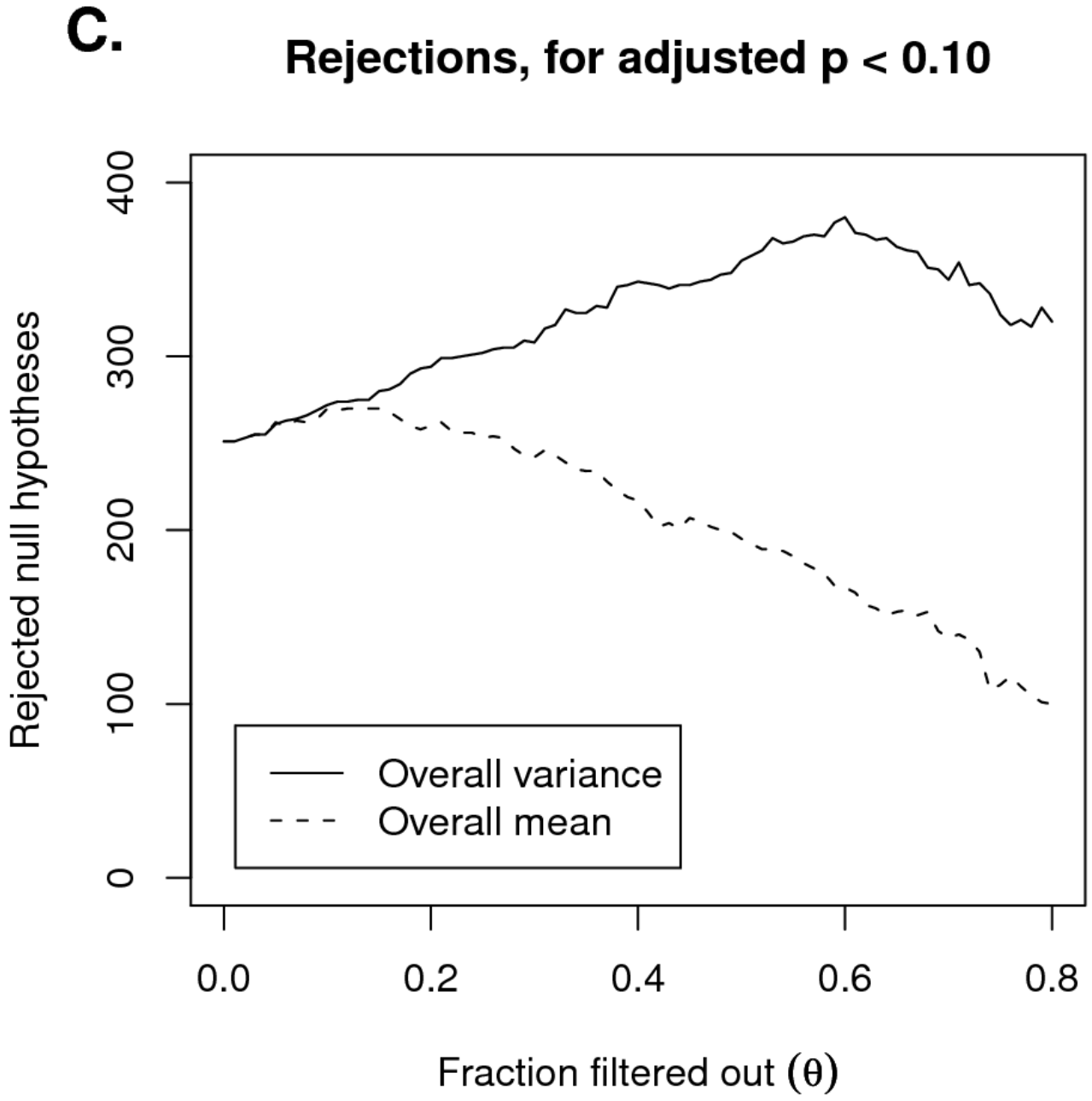
Now we are confident about type I error, but does it do any good? (power)



Diagnostics



θ


C.
Rejections, for adjusted p < 0.10
Rejected null hypotheses
0
100
200
300
400
0.0
0.2
0.4
0.6
0.8
Fraction filtered out (θ)
Overall variance
Overall mean

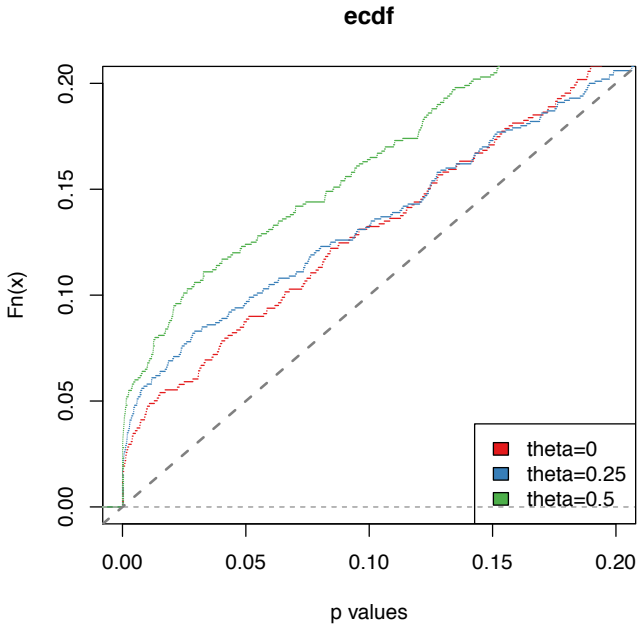
High-Throughput sequencing data

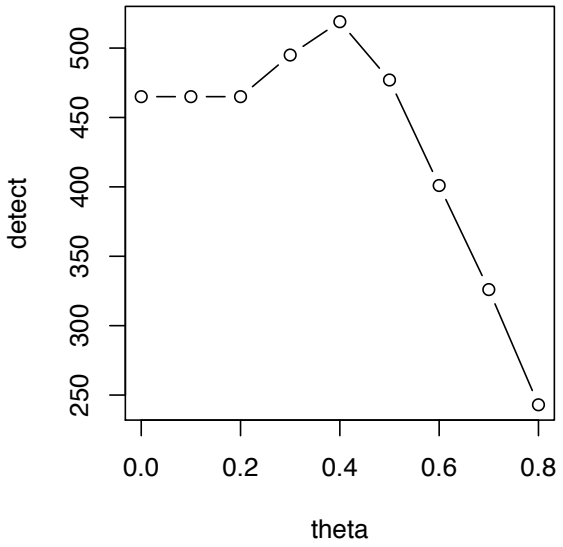
Filtering by overall sum of counts S is compatible with differential expression methods such as edgeR, DESeq.

Power gain?

Filtering of genes with $S = 0$: trivial.

Filtering with $S > S_{min}$: 15% gain for pasilla data - larger datasets, potentially more





Results summary

If done improperly, "filtering" invalidates type-I error control.

One way to do it properly is to make sure that stage-one (filter) and stage-two (differential expression) statistics are marginally independent:

1. (Normal distributed data): overall variance or mean, followed by t-test
2. Any permutation invariant statistic, followed by Wilcoxon rank sum test

Marginal independence is sufficient to maintain control of FWER at nominal level.

Control of FDR is usually also maintained.

(It could in principle be affected by filter-induced changes to correlation structure of the data. Check your data for indications of that. We have never seen it to be a problem in practice.)

Conclusion

Correct use of this two-stage approach can substantially increase power at same type I error.

References

Bourgon R., Gentleman R. and Huber W. Independent filtering increases detection power for high-throughput experiments, PNAS (2010)

On pseudo-ROC:Richard Bourgon's PhD thesis

Bioconductor packages genefilter, multtest

Simon Anders
Richard Bourgon
Bernd Fischer
Gregoire Pau

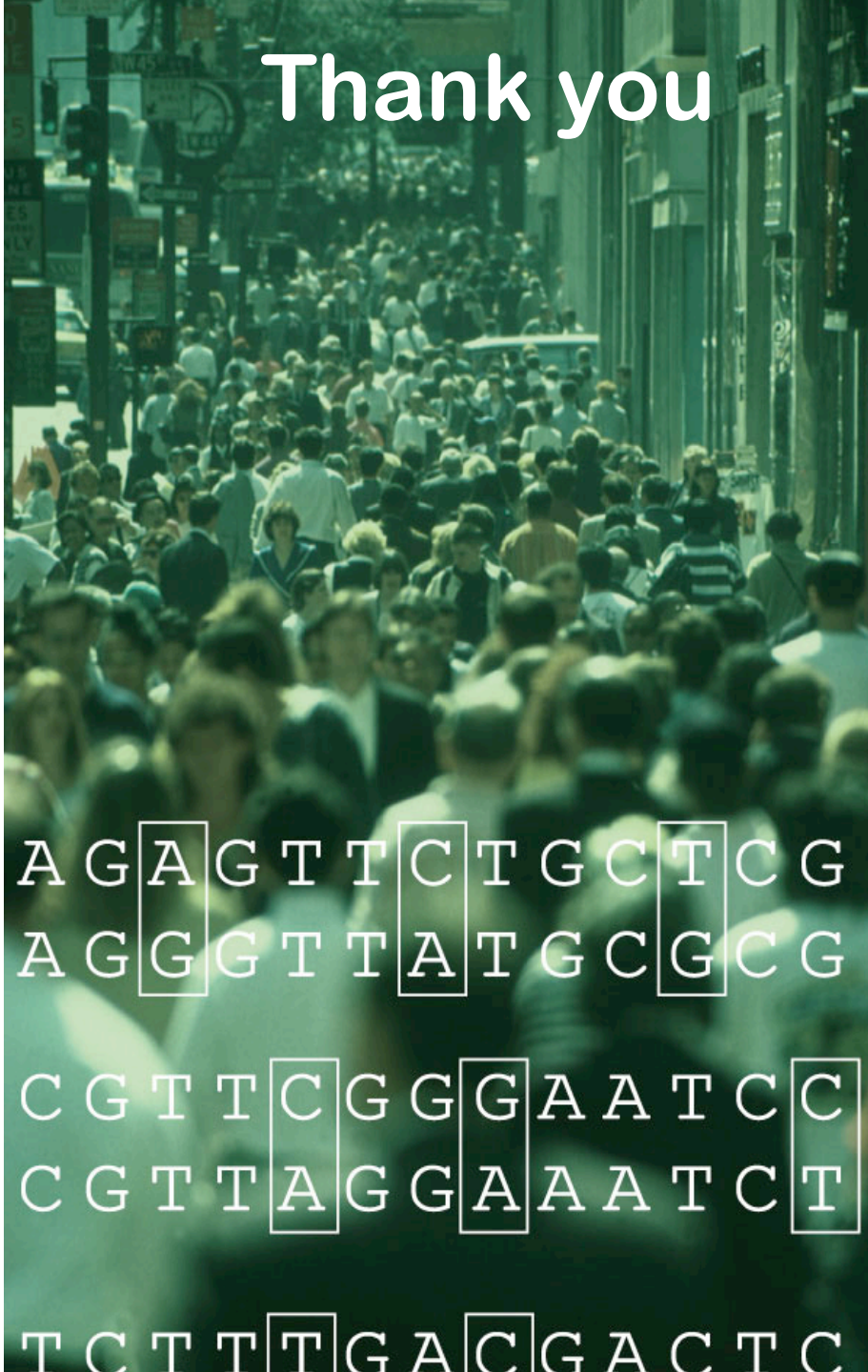
Robert Gentleman, F. Hahne, M. Morgan (FHCRC)

Lars Steinmetz, J. Gagneur, Z. Xu, W. Wei (EMBL)

Michael Boutros, F. Fuchs, D. Ingelfinger, T. Horn, T. Sandmann (DKFZ)

Steffen Durinck (Illumina)

All contributors to the R and Bioconductor projects



Derivation (non-parametric case)

$$P(f \in A, g \in B)$$

A, B: measureable sets
f: stage 1, g: stage 2

$$= \int_{\mathbb{i}^n} \delta_A(f(X))\ \delta_B(g(X))\ dP_X$$

exchangeability

$$= \frac{1}{n!} \sum_{\pi \in \Pi_n} \int_{\mathbb{i}^n} \delta_A(f \circ \pi(X))\ \delta_B(g \circ (X))\ dP_X$$

f's permutation invariance

$$= \int_{\mathbb{i}^n} \delta_A(f(X)) \left(\frac{1}{n!} \sum_{\pi \in \Pi_n} \delta_B(g \circ (X)) \right) dP_X$$

distribution of g generated by permutations

$$= \int_{\mathbb{i}^n} \delta_A(f(X))\ P(g \in B)\, dP_X$$

$$= P(f \in A) \cdot P(g \in B) \qquad \#$$

Positive Regression Dependency

On the subset of true null hypotheses:

If the test statistics are $X = (X_1, X_2, \ldots, X_m)$:

For any increasing set D (the product of rays, each infinite on the right), and H_{0i} true, require that

Prob(X in D | X_i = s) is increasing in s, for all i.

Important Examples

Multivariate Normal with positive correlation

Absolute Studentized independent normal