Exploring the Information in P-values for the Analysis and Planning of Multiple-Test Experiments

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Abstract

A new methodology is proposed for estimating the proportion of true null hypotheses in a large collection of tests. Each test concerns a single parameter δ whose value is specified by the null hypothesis. We combines a parametric model for the conditional CDF of the p-value given δ with a nonparametric spline model for the density $g(\delta)$ of δ under the alternative hypothesis. The proportion of true null hypotheses and the coefficients in the spline model are estimated by penalized least-squares subject to constraints that guarantee that the spline is a density. The estimator is computed efficiently using quadratic programming. Our methodology produces an estimate $\widehat{g}(\delta)$ of the density of δ when the null is false and can address such questions as "when the null is false, is the parameter usually close to the null or far away?" This leads us to define a "falsely interesting discovery rate" (FIDR), a generalization of the false discovery rate. We contrast the FIDR approach to Efron's "empirical null hypothesis" technique. We discuss the use of $\widehat{g}$ in sample size calculations based on the expected discovery rate (EDR). Our recommended estimator of the proportion of true nulls has less bias compared to estimators based upon the marginal density of the p-values at 1. In a simulation study, we compare our estimators to the convex, decreasing estimator of Langaas, Ferkingstad, and Lindqvist. The most biased of our estimators is very similar in performance to the convex, decreasing estimator. As an illustration, we analyze differences in gene expression between resistant and susceptible strains of barley.

Key words: Expected discovery rate, False discovery rate, Inverse problem, Microarray, Penalty, Power and sample size, Quadratic programming, Simultaneous tests, Splines.

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1 Introduction

We consider testing $H_{0i} : \delta_i = 0$ for $i = 1, \ldots, n$. Let π_0 be the proportion of $\delta_1, \ldots, \delta_n$ equal to the null value 0. We assume that the remaining proportion $(1 - \pi_0)$ of the δ_i have an empirical distribution G_n well approximated by a continuous G with density g. We assume that, under H_{0i}, the p-value is uniformly distributed, as is true in many simple but important cases, for example, of t- and F-tests.

A major advantage of our approach that we estimate G as well as π_0. We use the estimate of G to address the problem that if the number of false null hypotheses is large, then one may not wish to discover all false nulls (Efron, 2004). We partition the parameter space into three subspaces: the region where the null hypothesis is true, the region where the null is false but the parameter value is close to the null, and the region where the null is false and the parameter value is sufficiently far from the null to be of interest. Specifically, we define a non-null value of δ to be "interesting" if it exceeds a user-defined bound δ'. The falsely interesting discovery rate (FIDR) is defined as the conditional probability, given that the null has been rejected, that either the null is true or that it is false but the value of δ is not interesting. Using our estimate of G, we are able to estimate the FIDR; see Section 10. Also, we illustrate how and estimate of G can be used to plan sample sizes for future experiments.

Another advantage of our methodology is that it reduces bias when estimating π_0. Estimates of π_0 are useful for several purposes such as selection of a sample size to control the FDR (Jung, 2005 and Liu and Hwang, 2005). Estimation of the false discovery rate (FDR) requires estimates of π_0 and of the probability of rejecting the null. An estimate of G can be used for sample size calculations based on the expected discovery rate (EDR) and to determine the proportion of null hypotheses that are "false but uninteresting" meaning that the null is false but δ is close to the null value.

Suppose that the parameters $\delta_1, \ldots, \delta_n$ have associated p-values, $p_1, \ldots, p_n$, with p_i coming from a test of $H_{0i} : \delta_i = 0$ versus either a one or two-sided alternative. The conditional

CDF of p_i given δ_i will be denoted by $F_{p|\delta}(p\,;\delta_i)$, e.g., for a t-test $F_{p|\delta}$ would be derived from a non-central t-distribution with non-centrality parameter δ_i. Since the p-value is assumed to be uniformly distributed under H_0, the marginal CDF of p_i is

$$F_p(p\,;\pi_0) = \pi_0 p + (1-\pi_0)\text{EDR}(p) \tag{1}$$

where

$$\text{EDR}(p) = \int_{-\infty}^{\infty} F_{p|\delta}(p\,;\delta)dG(\delta) \tag{2}$$

is the Expected Discovery Rate (Gadbury et al., 2004). If one fixes α and varies δ, then $F_{p|\delta}(\alpha)$ is the power curve of a level-α test and $\text{EDR}(\alpha)$ is the expected power.

Much of the recent interest in estimation of π_0 is due to applications to false discovery rates. However, there are other interesting applications, e.g., Meinshausen and Rice (2005) discuss estimating the number of objects in the Kuiper Belt. These objects are detected by a reduction in light when they pass between a star and an observer. The null hypothesis is that there is no reduction, and the number of false null hypotheses gives information about the number of objects.

Currently, the most popular estimators of π_0 are equal to some estimator of the p-value density evaluating at 1, i.e., of $f_p(1;\pi_0) = F_p'(1;\pi_0)$. The underlying assumption is that p-values near 1 come from the null. However, this need not be true if $f_{p|\delta}(1;\delta) = F_{p|\delta}'(1;\delta) > 0$ for all δ in a set with positive probability, which is a common occurrence in applications; see Section 3. The difference between $f_p(1;\pi_0)$ and π_0 can be especially large if G has considerable probability near 0, as occurs in the example of Section 12. In one of the cases of the simulation study of Section 8, π_0 is 0.7 but estimates of $f_p(1;\pi_0) = F_p'(1;\pi_0)$ are near 0.85; thus, the probability $(1-\pi_0)$ of a false null is twice what one of the currently available estimators would report. The semiparametric estimators proposed in this paper are designed to reduce this positive bias and in our simulations the bias just mentioned is reduced from 0.15 to 0.05.

Our methodology is related to methods for estimation of mixing distributions, deconvolution, and other inverse problems, e.g., O'Sullivan (1986), Carroll and Hall (1988), Fan (1991), and Lesperance and Kalbfleisch (1992). Estimation of (π_0, g) is an inverse problem, which we approach similarly to O'Sullivan (1986) by using B-splines with a roughness penalty.

Our estimation methodology is described in Section 2–6. The promising convex, decreasing estimator of Langaas, Ferkingstad, and Lindqvist (2005), which we consider state-of-the-art, and two additional estimators based on our semiparametric approach are introduced in Section 7. A simulation study in Section 8 compares our estimators to the convex, decreasing estimator. Section 9 discusses estimation of the false discovery rate. In Section 10 the "falsely interesting discovery rate" is defined. Power and sample size calculations are discussed in Section 11. An example using gene expression data is in Section 12, and a summary is provided in Section 13.

2 The Semiparametric Estimator

We will model g as $g(\delta\,;\boldsymbol{\beta})$ where $g(\cdot\,;\cdot)$ is a spline and $\boldsymbol{\beta}$ is vector of coefficients. Let $F_p(\cdot\,;\pi_0,\boldsymbol{\beta})$ be given by (1) with $g(\delta)$ replaced by $g(\delta\,;\boldsymbol{\beta})$. We use penalized least squares to estimate $\boldsymbol{\beta}$ with constraints that guarantee that the estimate is a density.

In many applications, n is very large and for computationally efficiency it is useful to bin the p-values into, say, 2000 bins. Binning reduces computation both by data compression and by changing the estimation problem into a quadratic programming problem. Let N_{bin} be the number of bins; let l_i, c_i, r_i, and $w_i = r_i - l_i$ be the left edge, center, right edge, and width of the ith bin, $i = 1, \ldots, N_{\text{bin}}$; and let $M_1, \ldots, M_{N_{\text{bin}}}$ be the bin counts. Then $y_i = M_i/(nw_i)$ is an unbiased estimate of

$$m_i(\pi_0,\boldsymbol{\beta}) = \frac{F_p(r_i\,;\pi_0,\boldsymbol{\beta}) - F_p(l_i\,;\pi_0,\boldsymbol{\beta})}{w_i} \approx f_p(c_i\,;\pi_0). \tag{3}$$

We will estimate $(\pi_0, \boldsymbol{\beta})$ by minimizing the penalized weighted sum of squares,

$$SS(\pi_0, \boldsymbol{\beta}\,; \lambda) = \sum_{i=1}^{N_{\text{bin}}} \omega_i^2 \left\{y_i - m_i(\pi_0, \boldsymbol{\beta})\right\}^2 + \lambda Q(\boldsymbol{\beta}) \tag{4}$$

where ω_i^2 is a weight, $Q(\boldsymbol{\beta})$ is a penalty to be discussed later, and $\lambda \geq 0$ is a penalty parameter. This estimator of π_0 will be called the "semiparametric" estimator since it combines $f_{p|\delta}(p\,;\delta)$ with a nonparametric spline model for g. The weights could be $\omega_i^2 \equiv 1$ or they could be the reciprocals of the estimated variances of the y_i. In the latter case, the weighted least-squares estimator is an approximate minimum chi-squared statistic.

3 The Conditional CDF $F_{p|\delta}$

To evaluate $m_i(\pi_0, \boldsymbol{\beta})$ in (3), we need $F_{p|\delta}$. Suppose that we observe iid $X_1, \ldots, X_n$ with conditional CDF $F_x(x\,;\delta)$ and that the rejection regions are $X_i > \kappa$ for some κ. Then the ith p-value is $1 - F_x(X_i\,;0)$. The CDF of the p-value under δ is

$$F_{p|\delta}(p\,;\delta) = 1 - F_x\{F_x^{-1}(1-p\,;0)\,;\delta\},\ 0 < p < 1. \tag{5}$$

Ruppert, Nettleton, and Hwang (2005) apply (5) to t-tests as well as one- and two-sided location problem, including z-tests. Here we focus on t-tests.

Let T be a statistic whose CDF is $F_t(\cdot\,;\nu,\delta)$, the non-central-t CDF with ν degrees of freedom and non-centrality parameter δ. By (5), the CDF of the p-value for is $F_{p|\delta}(p\,;\delta_i) = 1 - F_t\left\{F_t^{-1}(1-p\,;\nu,0)\,;\nu,\delta\right\}$ for one-sided tests and, for two-sided tests, $F_{p|\delta}(p\,;\delta_i) = 1 - \{F_t(t\,;\nu,\delta) - F_t(-t\,;\nu,\,\delta)\}\big|_{t=F_t^{-1}(1-p/2\,;\nu,0)}$, which depend on δ_i only through $|\delta_i|$. Since we will focus on t-tests, there is no loss in generality by assuming that

$$\delta \geq 0, \tag{6}$$

or, alternatively, of viewing $|\delta|$ rather than δ as the parameter. Assumption (6) is especially convenient for modeling g and will be made throughout this paper.

In both one- and two-sided t-tests, $f_{p|\delta}(1\,;\delta) > 0$ for all δ.

4 The Spline Model for $g(\cdot)$

The density g will be modeled as a linear spline and estimated using the B-spline basis. We will be using assumption (6). Let δ^* be an upper bound for δ so that g is assumed to have support contained in $[0, \delta^*]$. The spline will have K knots, $0 = \kappa_1, \ldots, \kappa_K = \delta^*$, equally spaced between 0 and δ^*, so that the distances between adjacent knots are all equal to $d = \delta^*/(K-1)$. The choice of K is not critical as long as it is large enough. Because the spline is penalized, the "effective" number of parameters is controlled by the penalty parameter and K only provides an upper bound. We have experimented with $K = 8$ and 16 and found that both choices work well, because data-driven methods for choosing the effective number of parameters choose a value less than the upper bound of 8 when $K = 8$. For example, in an experiment with 5000 p-values, the approximate generalized cross validation method we introduce in Section 6 chose between 4 and 5 effective parameters when using either $K = 8$ or $K = 16$. In our numerical examples of Sections 8 and 12, we use $K = 12$.

Another issue is the choice of δ^*, the upper bound for δ. We have used $\delta^* = 6$ in our empirical studies and this choice proved satisfactory. The explanation for this is that the tests we studied were t-tests with δ the non-centrality parameter. Thus, δ is the deviation of a parameter from its null value expressed in standard deviation units, so that 6 is a reasonable upper bound for δ. If we bin the p-values into 2000 bins, say, then there is virtually no information about the exact value of δ once it exceeds 6, for any δ above 6 is almost certain to produce a p-value in the [0, 1/2000] bin.

The B-splines are plotted in Web Figure 1 for the case $\delta^* = 6$ and $K = 7$. The first B-spline, B_1, decreases linearly from $2/d$ to 0 on the interval $[0, \kappa_2] = [\kappa_1, \kappa_2]$ and is zero elsewhere. The remaining B-splines $B_2, \ldots, B_{K-1}$ are such that B_k increases linearly from 0 to $1/d$ on $[\kappa_{k-1}, \kappa_k]$ and then decreases linearly from $1/d$ to 0 on $[\kappa_k, \kappa_{k+1}]$ and is 0 elsewhere. The B-splines span the space of linear splines with knots $\kappa_1, \ldots, \kappa_K$ and constrained to be zero at the last knot. This constraint forces the splines to be continuous on $[0, \infty)$, which seems reasonable. The constraint could be removed by adding an additional B-spline that increases linearly from κ_{K-1} to κ_K and is zero elsewhere. This B-spline is shown as a dashed line in Web Figure 1. Each B-spline has been normalized so that it is a density, and therefore any convex combination of the B-splines is also a density. Thus, our model for g will be $g(\delta, \boldsymbol{\beta}) = \sum_{k=1}^{K-1} \beta_k B_k(\delta)$, where $\beta_k \geq 0$ for all k and $\sum_{k=1}^{K-1} \beta_k = 1$.

5 The Penalized Least-Squares Estimator

To find a more explicit expression for the middle expression in (3), we now write $F_p(\cdot\,; \pi_0, \boldsymbol{\beta})$ in terms of the B-splines. It is convenient to reparameterize to a parameter vector $\boldsymbol{\theta}$ as follows. Define $\theta_1 = \pi_0$ and $\theta_{k+1} = (1-\pi_0)\beta_k$ for $k = 1, \ldots, K-1$, and define $\boldsymbol{\theta} = (\theta_1, \ldots, \theta_K)^\mathsf{T}$. Let $Z_1(p) = p$ be the (uniform) CDF of the p-values under H_0, and for $k = 1, \ldots, K-1$ let

$$Z_{k+1}(p) = \int F_{p|\delta}(p; \delta) B_k(\delta) d\delta \tag{7}$$

be the marginal CDF of a p-value if the density of δ is B_k. Then the marginal CDF of a p-value is modeled as $F_p(p\,; \boldsymbol{\theta}) = \sum_{k=1}^{K} \theta_k Z_k(p)$ where $\theta_k \geq 0$ for all k and $\sum_{k=1}^{K} \theta_k = 1$. The roughness penalty we will use penalizes deviations of $\widehat{g}$ from a linear function using a finite difference approximation to the second derivative of g. It is convenient if the roughness penalty is expressed in terms of $\boldsymbol{\theta}$. The value of g at the knots is $g(\kappa_1) = g(0) = 2\beta_1/d = 2(1-\pi_0)^{-1}\theta_2/d$, $g(\kappa_k) = \beta_k/d = (1-\pi_0)^{-1}\theta_{k+1}/d$ for $k = 2, \ldots, K-1$, and $g(\kappa_K) = g(\delta^*) = 0$. The roughness penalty is

$$\begin{aligned} Q(\boldsymbol{\theta}) &= (2\theta_2 - 2\theta_3 + \theta_4)^2 + \sum_{k=3}^{K-2} (\theta_k - 2\theta_{k+1} + \theta_{k+2})^2 \\ &= \{d(1-\pi_0)\}^2 \sum_{k=1}^{K-3} \{g(\kappa_k) - 2g(\kappa_{k+1}) + g(\kappa_{k+2}))\}^2. \end{aligned} \tag{8}$$

Now define $\boldsymbol{y} = (y_1, \ldots, y_{N_{\text{bin}}})^\mathsf{T}$ and let $\boldsymbol{Z}$ be the $N_{\text{bin}} \times K$ matrix whose i,jth element is

$$Z_{i,j} = \{Z_j(r_i) - Z_j(l_i)\}/w_i. \tag{9}$$

Then the sum of squares is

$$\begin{aligned} SS(\boldsymbol{\theta};\lambda) &= \sum_{i=1}^{N_{\text{bin}}} \omega_i^2 \left\{ y_i - \sum_{k=1}^{K} \theta_k Z_{i,k} \right\}^2 \\ &+ \lambda \left\{ (2\theta_2 - 2\theta_3 + \theta_4)^2 + \sum_{k=3}^{K-2} (\theta_k - 2\theta_{k+1} + \theta_{k+2})^2 \right\} \\ &= (\boldsymbol{y} - \boldsymbol{Z\theta})^{\mathsf{T}} \Omega (\boldsymbol{y} - \boldsymbol{Z\theta}) + \lambda \boldsymbol{\theta}^{\mathsf{T}} \left\{ (\boldsymbol{DA})^{\mathsf{T}} \boldsymbol{DA} \right\} \boldsymbol{\theta}, \qquad (10) \end{aligned}$$

where $\boldsymbol{\Omega} = \text{diag}(\omega_1^2, \ldots, \omega_n^2)$, $\boldsymbol{A} = \text{diag}(0, 2, 1, \ldots, 1)$, and $\boldsymbol{D}$ is a $(K-3) \times K$ "differencing matrix" whose ith row has $+1$ in the columns $i+1$ and $i+3$, -2 in column $i+2$ and zeros elsewhere. Minimizing (10) is equivalent to minimizing $\boldsymbol{f}^{\mathsf{T}}\boldsymbol{\theta} + 0.5\, \boldsymbol{\theta}^{\mathsf{T}} \boldsymbol{H\theta}$ where $\boldsymbol{f}^{\mathsf{T}} = -\boldsymbol{y}^{\mathsf{T}}\boldsymbol{\Omega Z}$ and $\boldsymbol{H} = \boldsymbol{Z}^{\mathsf{T}}\boldsymbol{\Omega Z} + \lambda \boldsymbol{A}^{\mathsf{T}}\boldsymbol{D}^{\mathsf{T}}\boldsymbol{DA}$, with constraints $\boldsymbol{\theta} \geq 0$ and $\boldsymbol{1}^{\mathsf{T}}\boldsymbol{\theta} = 1$ where $\boldsymbol{1}$ is a K-dimensional vector of ones. The objective function and constraints are in the form used by the quadratic programming algorithm `quadprog` of MATLAB.

If λ is chosen by cross-validation, then the quadratic program must be solved for each value of λ on some grid. Much of the effort is devoted to computing $\boldsymbol{f}$ and $\boldsymbol{Z}^{\mathsf{T}}\boldsymbol{\Omega Z}$ since $\boldsymbol{y}$ is $N_{\text{bin}} \times 1$ and $\boldsymbol{Z}$ is $N_{\text{bin}} \times K$. However, these matrices can be computed once.

Computation of the $Z_{i,j}$'s defined by (9) requires that we compute $Z_j(p)$ given by (7) with p equal to each of the bin edges. We computed the integral in (7) numerically using 500 values of δ. Doing this required that $F_{p|\delta}(p\,;\delta)$ be valued at $N_{\text{bin}} \times 500$ combinations of p and δ. For the t-tests this takes several minutes of clock time. To speed up computations, we computed these values of $F_{p|\delta}(p\,;\delta)$ once, saved them, and then loaded them into memory as needed. With this device, our estimators can be computed in about 10 seconds of clock time using a MATLAB program run on a 2.2 GHz PC.

The fitted value

$$\widehat{f_p}(c_i) = \widehat{y}_i = \sum_{k=1}^{K} \widehat{\theta}_k Z_{i,k} \qquad (11)$$

estimates $m_i(\pi_0, \boldsymbol{\beta})$ given by (3), which is an approximation to $f_p(c_i)$, the marginal density of the p-values at the center of the ith bin. Also, $F_p(p)$ can be estimated by

$$\widehat{F}_p(r_i) = \sum_{i'=1}^{i} w_{i'} \left\{ \sum_{k=1}^{K} \widehat{\theta}_k Z_{i',k} \right\} \tag{12}$$

when p is some right bin edge r_i and then interpolated to other values of p. The estimator of π_0 is

$$\text{“Semi, } \theta_1\text{”} = \widehat{\theta}_1. \tag{13}$$

The notation “Semi, θ_1” is intended to remind the reader that this is a semiparametric estimator based only on $\widehat{\theta}_1$. Two other semiparametric estimators based on $\widehat{\boldsymbol{\theta}}$ will be introduced in Section 7. Also, let $\widehat{g}(\delta) = \sum_{k=1}^{K-1} \widehat{\beta}_k B_k(\delta)$ and $\widehat{G}(\delta) = \int_0^{\delta} \widehat{g}(u)du$.

6 Approximate Cross-Validation

An obvious method for choosing λ is cross-validation (CV). However, exact cross-validation would be slow to compute, so instead we used an approximation to the generalized cross-validation (GCV) statistic. The GCV statistic itself is not defined for our estimator because the constraints make the estimator nonlinear in $\boldsymbol{y}$. Thus, there is no hat matrix and the usual method of defining the degrees of freedom of the fit (DF) does not apply—see Chapter 3 and Section 5.3 of Ruppert, Wand, and Carroll (2003) for an introduction to GCV, linear estimators, the hat matrix, GCV, and DF for penalized least-squares estimators. Therefore, we use the DF parameter from estimating $\boldsymbol{\theta}$ by minimizing (10) without constraint—this is a poor estimator of $\boldsymbol{\theta}$ but gives a DF value that worked well in our simulations when put into the GCV formula.

The unconstrained minimizer of (10) is $\left\{\boldsymbol{Z}^{\mathsf{T}}\boldsymbol{\Omega}\boldsymbol{Z} + \lambda(\boldsymbol{D}\boldsymbol{A})^{\mathsf{T}}(\boldsymbol{D}\boldsymbol{A})\right\}^{-1}\boldsymbol{Z}^{\mathsf{T}}\boldsymbol{\Omega}\boldsymbol{y}$, and has hat matrix $\boldsymbol{H}(\lambda) = \boldsymbol{Z}\left\{\boldsymbol{Z}^{\mathsf{T}}\boldsymbol{\Omega}\boldsymbol{Z} + \lambda(\boldsymbol{D}\boldsymbol{A})^{\mathsf{T}}(\boldsymbol{D}\boldsymbol{A})\right\}^{-1}\boldsymbol{Z}^{\mathsf{T}}\boldsymbol{\Omega}$. Then $\text{DF}(\lambda) = \text{trace}\{\boldsymbol{H}(\lambda)\} = \text{trace}\left[\left\{\boldsymbol{Z}^{\mathsf{T}}\boldsymbol{\Omega}\boldsymbol{Z} + \lambda(\boldsymbol{D}\boldsymbol{A})^{\mathsf{T}}(\boldsymbol{D}\boldsymbol{A})\right\}^{-1}\boldsymbol{Z}^{\mathsf{T}}\boldsymbol{\Omega}\boldsymbol{Z}\right]$, and the approximate GCV statistic we use is $\text{GCV}(\lambda) = \|\boldsymbol{y} - \boldsymbol{Z}\widehat{\boldsymbol{\theta}}(\lambda)\|^2/\{N_{\text{bin}} - \text{DF}(\lambda)\}^2$, where $\boldsymbol{\theta}(\lambda)$ is the estimator of Section 5 that minimizes (10) *with* constraints. The smoothing parameter λ is chosen by computing $\text{GCV}(\lambda)$ on a grid on λ values and choosing the value that minimizes $\text{GCV}(\lambda)$.

7 Alternative Estimators of π_0

Alternative estimators of π_0 can be obtained by minimizing estimators of f_p, the marginal density of the p-values. In this section, we describe three estimators in this class. The first two are based on our penalized least-squares fit. The third is the convex, decreasing estimator proposed by Langaas et al. (2005) and found by them to be best among estimators of π_0 that minimize an estimate of f_p.

7.1 Estimator Based On The Penalized Least-Squared Fit

An alternative semiparametric estimator, denoted by "Semi, $\min\{\widehat{f}\}$" is the minimum over i of (11), i.e.,

$$\text{“Semi, } \min\{\widehat{f}\}\text{”} = \min_i \widehat{f}_p(c_i) = \widehat{f}_p(c_{N_{\text{bin}}}) = \sum_{k=1}^{K} \widehat{\theta}_k Z_{N_{\text{bin}},k}. \tag{14}$$

The minimum occurs at $i = N_{\text{bin}}$ because the estimated density is decreasing.

We found that "Semi, θ_1" can biased downward and is somewhat more variable than "Semi, $\min\{\widehat{f}\}$". However, when a substantial proportion of the p-values near 1 come from the alternative hypothesis, then, because it is based on the incorrect assumption that $f_{p|\delta}(1\,;\delta) > 0$, "Semi, $\min\{\widehat{f}\}$" can be biased upwards to such an extend that nearly 100% of the MSE (mean squared error) is attributable to squared bias; see Section 8. These results motivated us to find an estimator that is a compromise between "Semi, θ_1" and "Semi, $\min\{\widehat{f}\}$". The former attempts to separate $f_p(1)$ into a component from the null and another component from the alternative and uses only the component from the null to estimate π_0. The latter uses both components. The problem with "Semi, θ_1" is that it is difficult to separate p-values coming from the null from those coming from alternative values of δ near the null. To circumvent this problem, we defined a new estimator, "Semi, compromise", which decomposes $f_p(1)$ in three components, one from the null, one from the alternative near the null, and the third from the alternative away from the null. Then "Semi, compromise" uses the first two components. This induces a slight upward bias which provides a margin of safety. It also decreases variability. More precisely, we define

$$\text{“Semi, compromise”} = \sum_{k=1}^{2} \widehat{\theta}_k Z_{N_{\text{bin}},k} \approx \text{“null and near null part” of } \widehat{f}_p(1) \tag{15}$$

One can see from (13), (14), and (15) and the fact that $Z_{i,k} \geq 0$ for all i, k, that “Semi, θ_1” $\leq$ “Semi, compromise”$\leq$ “Semi, $\min\{\widehat{f}\}$”.

We studied two versions each of “Semi, θ_1”, “Semi, $\min\{\widehat{f}\}$”, and “Semi, compromise”, an unweighted version where $\omega_i \equiv 1$ and a weighted version where $\omega_i = 1/\sqrt{\widehat{f}_p(c_i)}$, where $\widehat{f}_p$ is the unweighted estimator. The latter weights are based on the fact that the bin counts are approximately Poisson distributed. We found that weighting did not have a consistent effect on “Semi, θ_1”, “Semi, $\min\{\widehat{f}\}$”, and “Semi, compromise”, but that the weighted versions of these estimators often had a somewhat smaller mean squared error.

7.2 The Convex, Decreasing Estimator

Langaas et al. (2005) considered a number of different estimators of π_0. The best performing of these is the convex, decreasing density estimator applied to the p-values and evaluated at 1. These authors show that any twice differentiable, convex, and decreasing density f on $[0, 1]$ has a representation as $f(x) = \int_0^1 f_\theta(x)\gamma(\theta)\, d\theta + f_0(x)a_0 + f_1(x)a_1$, where f_0 is the uniform$(0, 1)$ density, $f_\theta(x) = 2\theta^{-2}(\theta - x)_+$, $0 \leq x \leq 1$, $0 < \theta \leq 1$, $a_0 = f(1)$, $a_1 = -1/2f'(1)$, and $\gamma = (1/2)\,\theta^2/f''(\theta)$. The nonparametric MLE (NPMLE) of a convex, decreasing density maximizes the likelihood over this class of densities. Langaas et al. (2005) suggest an iterative algorithm for approximating the nonparametric MLE by a discrete mixture, using only values of θ contained in some fine grid, e.g., $\{0, 0.01, 0.02, \ldots, 1\}$.

We developed a algorithm for approximating the NPMLE that differed in a few ways from the Langaas et al. algorithm. First, we minimized a chi-squared statistic rather than maximizing the likelihood. Second, we used all θ on the grid $\{0, 0.01, 0.02, \ldots, 1\}$. Finally, we used quadratic programming to optimize. Our estimators were of the form

$$\sum_{i=0}^{100} b_i f_{i/100}(x), b_i \geq 0\ \forall\ i,\ \sum_{i=0}^{100} b_i = 1. \tag{16}$$

The minimum chi-squared statistics is a quadratic function of $(b_0, \ldots, b_{100})$ and the constraints in (16) are linear. Thus, our estimator can be calculated by quadratic programming in the same way that (10) was minimized. Computation is very fast using this algorithm taking about 4 seconds of clock time. Following Langaas et al. (2005), we denote this estimator evaluated at 1 by "Convex". It is well known that minimum chi-square estimators are asymptotically equivalent to MLE based on grouped data, e.g., see Holland (1967) or Rao (1973), and there should be little loss of information in grouping data into a large number, e.g., 2000, bins. Therefore, the estimate computed by our algorithm is expected to be nearly equal to the MLE.

8 Simulation Studies

We performed simulations of the two-sided t-test to compare "Convex", "Semi, θ_1", "Semi, $\min\{\widehat{f}\}$", and "Semi, compromise". We generated $t_1, \ldots, t_n$ that were independent non-central-t variates with non-centrality parameters $\delta_1, \ldots, \delta_n$ and each with 4 degrees of freedom. The null hypotheses was $H_0 : \delta_i = 0$ and under the alternative the δ_i were generated from a Beta(b_1, b_2) density on $[\delta_{\min}, \delta_{\max}]$ where $(\delta_{\min}, \delta_{\max}, b_1, b_2, \pi_0)$ varied across several cases. In each simulation, we generated 10,000 p-values and exactly 10,000π_0 came from the null.

The simulations used six cases of (π_0, g). In Cases 1–3 $\pi_0 = 0.95$ and in Cases 4–6 $\pi_0 = 0.7$. Three densities were used for g and their parameters were, respectively, $(\delta_{\min}, \delta_{\max}, b_1, b_2)$ = (0, 4, 1, 2), (0, 4, 2, 2), and (0.5, 4.5, 3, 2). The first density, used in Cases 1 and 4, has support [0, 4] and is concentrated around 0, making it difficult to distinguish p-values from the null and from the alternative. This density is similar to the estimates of g in the gene expression study in Section 12, which suggests that difficulty distinguishing p-values from the null and alternative might be common, at least in gene expression studies. The second density, used in Cases 2 and 5, also has support [0, 4], but has a mode away from 0. The third density, used in Cases 3 and 6, has support [0.5, 4.5] which is separated from the null hypothesis. The three densities are labelled, respectively, “near,” “moderately near,” and “far” from the null in the Table 1.

For a two-sided t-test, the distribution of the p-value depends only on $|\delta|$, so there is no loss in generality in having g supported on a positive interval. For the semiparametric estimator, N_{bin} was fixed at 2000 and K was 12.

We also found that the weighted versions of “Semi, θ_1” and “Semi, $\min\{\widehat{f}\}$” did not dominate unweighted versions, but generally the weighted estimators were somewhat better. To save space, only results for the weighted estimators will be presented.

The results are in Table 1. From these results, we conclude that:

- In Cases 1, 2, 4, and 5 where g is near or moderately near the null, “Semi, compromise” has the smallest RMSE of the four estimators.
- In Case 4, “Semi, $\min\{\widehat{f}\}$” and “Convex” have severe positive bias because many of the p-values near 1 are from the alternative. In this case, “Semi, compromise” is far superior to “Semi, $\min\{\widehat{f}\}$” and “Convex”.
- In Cases 3 and 6 where g is far from the null, “Semi, $\min\{\widehat{f}\}$” has the smallest RMSE of the four estimators.
- “Semi, θ_1” has large RMSE values.

In many applications, the test statistics will not be independent. In microarray experiments, for example, between-gene correlations of varying magnitude are expected among genes functioning together in biological pathways. To investigate the effects of varying between-gene correlation levels, we simulated two-sided t-tests with an autoregressive type correlation and DF=4 and n = 10,000. More specifically, the ith p-value was based on $t_i = (\delta_i + e_i)/\sqrt{s_i^2/DF}$ where e_i is a Gaussian AR(1) process and s_i^2 is independent of e_i and χ^2_{DF} distributed with $DF = 4$. Specifically, $e_i = \rho e_{i-1} + u_i$ where the u_i are independent

$N(0, 1-\rho^2)$, so that the e_i are $N(0,1)$ and e_i and e_j have correlation $\rho^{|i-j|}$. The s_i^2 were mutually independent. When $\rho \neq 0$, the joint distribution of the p-values will depend on how the δ_i are ordered. We considered two orderings of the δ_i, "permute" where the δ_i were randomly permuted and "sort" where the δ_i were sorted from smallest to largest, so, in particular, all the p-values from true nulls came first. Under "sort" p-values with similar values of δ will be more highly correlated. The proportion of true nulls, π_0, was fixed at 0.9 and $(\delta_{\min}, \delta_{\max}, b_1, b_2)$ was fixed at (0, 4, 2, 2). The results are in Web Table 1 and will be summarized here. We were at first surprised to see that the RMSE's of "Semi, $\min\{\widehat{f}\}$", "Semi, compromise", and "Convex" were nearly independent of ρ and also of whether the δ_i were permuted or sorted. However, there is a simple explanation. For these estimators, the largest component of RMSE is squared bias, not variance, and bias should depend little, if at all, on the amount of autocorrelation. In contrast, "Semi, θ_1" has a larger component due to variance and its RMSE is larger when ρ is larger. However, the RMSE of "Semi, θ_1" also depends very little upon whether the δ_i were permuted or sorted. It was interesting that "Semi, compromise" had a smaller RMSE than "Convex" and "Semi, $\min\{\widehat{f}\}$" in all five cases.

How much one is willing to tolerate bias will influence the choice of estimator. When using an estimate of π_0 for determining the false discovery rate, a positive bias is often considered to be less serious than a negative bias, because it leads to conservative false discovery rates. However, a bias of 0.15 seen in "Semi, $\min\{\widehat{f}\}$" and "Convex" may be too conservative, and it is unnecessary now that "Semi, compromise" is available. In other applications, such as determining the number of Kuiper Belt objects (Meinshausen and Rice, 2005), bias in either direction is undesirable.

In general, bias is a major component of the RMSE of the estimators. The amount of bias depends on both π_0 and g. Obviously, there can be little positive bias if π_0 is close to 1, but if π_0 is 0.7 then bias can be severe. Fortunately, our semiparametric methodology provides estimates of both of π_0 and g, so one can be alerted to situations where bias may be severe. Web Figures 2 and 3 show estimates of f_p and g from 10 independent simulated data sets for Cases 4 and 6, respectively. The estimates of g are rather close to g itself, showing that it is possible to determine whether values of δ under the alternative are mostly close to or far from the null; it is only when they are mostly close to the null that severe bias should be expected. In Cases 4–6, $\pi_0 = 0.7$ so there is more information about g than in Cases 1–3, where g is not estimated quite so well. However, when π_0 is close to 1 as in Cases 3–6, the estimate of π_0 will indicate both that g may not be estimated accurately and that, fortunately, positive bias will not be severe.

A referee mentioned that in SNP association studies, one expects that π_0 will increase with n and, in general, to be close to 1. To investigate such cases, we simulated with $n = 25,000$ and $\pi_0 = 0.99$. The results are in Table 2. The performances of "Semi, $\min\{\widehat{f}\}$", "Semi, compromise", and "Convex" are very good. As might be expected, upward bias is not a serious problem when estimating a probability that is close to 1.

Another referee was interested in cases where most of the nulls are false. To investigate this situation, we added three cases to Table 2 where $n = 5,000$ and $\pi_0 = 0.3$. We see from that table that when π_0 is this small, then "Convex" is very biased but "Semi, compromise" works reasonably well.

9 Estimating the False Discovery Rate

Benjamini and Hochberg (1995) introduced the False Discovery Rate (FDR) for multiple testing problems. A variety of methods have been proposed for estimating the FDR when rejecting all null hypotheses with a p-value below some fixed α. Benjamini and Hochberg (2000), Storey (2002), and Storey and Tibshirani (2003) among others have proposed FDR estimators of the form

$$\widehat{\mathrm{FDR}} = \frac{\alpha \widehat{\pi}_0}{\widehat{F}_p(\alpha)}, \tag{17}$$

where $\widehat{F}_p(\alpha)$ is estimated simply by the proportion of observed p-values that fall below α, and $\widehat{\pi}_0$ is an estimator of π_0 that differs among methods. The method of Storey and Tibshirani (2003), which is perhaps the most widely used in practice, estimates π_0 by approximating the p-value density at $p = 1$. As discussed in the Introduction and in Section 8, π_0 can be substantially less than estimates of the p-value density at $p = 1$. From (17) we see that upward bias in $\widehat{\pi}_0$ will cause upward bias in $\widehat{\text{FDR}}$. To improve the situation, we propose to estimate FDR by (17) where $\widehat{F}_p(\alpha)$ is estimated by (12) and "Semi, compromise" is used to estimate π_0. Our least-squares fitting method will minimize the difference between our denominator and that of Storey and Tibshirani (2003), so the main difference will be in the numerators, where the results of Section 8 suggest that our method will exhibit less positive bias.

10 When is a p-value Interesting?

Efron (2004) discusses a potential problem when one has a large number of tests—the number of false nulls is often very large and we do not necessarily want to "discover" every one of them. This problem does not always occur. In the astronomy example of Meinshausen and Rice (2005) mentioned in Section 1, we are not really interested in which nulls are false, only in how many nulls are false, so there is no danger of discovering too many false nulls. However, in gene expression studies one is primarily interested in finding nulls that are both false and "important" or "interesting" biologically. For example, in the microarray experiment described in Section 12, the biologists were looking for barley genes that are involved in resistance to a fungal pathogen. Many genes are likely to change expression during attack by a pathogen, but some changes may be quite small and play only a minor role in a plant's defense response. While all changes, regardless of size, are potentially of interest, researchers may wish to focus attention initially on the genes that exhibit the largest and most consistent changes in expression. In some cases this may provide a clearer picture of the biology than attempting to simultaneously interpret the meaning of small changes in thousands of genes.

10.1 The Falsely Interesting Discovery Rate

If "interesting" is interpreted as an especially unusual p-value as in Efron (2004), then we are assuming that the δ values farthest from the null are of greatest interest. This assumption is debatable, of course, and we do not think that making this assumption is always a good idea. However, if we are willing to make it, then our estimates of π_0 and g can be useful for determining the number of interesting δ values. Suppose we want to know what proportion of the p-values come either from a true null or from a null that is false but with a δ value that is "uninteresting," where "uninteresting" is defined by subject-matter considerations to mean that $\delta < \delta'$ for some fixed $\delta' > 0$. This is proportion can be estimated by

$$\widehat{\pi}_0 + (1 - \widehat{\pi}_0) \int_0^{\delta'} \widehat{g}(\delta)\, d\delta. \tag{18}$$

We define the "falsely interesting discovery rate" (FIDR) as the conditional probability that a null hypothesis is either true or false but with an uninteresting value of δ, given that it has been rejected, i.e., again assuming that a null hypothesis is rejected if the p-value is its less than α,

$$\mathrm{FIDR}(\alpha, \delta') = \frac{P(\delta < \delta' \text{ and } p\text{-value} < \alpha)}{P(p\text{-value} < \alpha)}. \tag{19}$$

The denominator of (19) can be estimated by $\widehat{F}_p(\alpha)$. If δ' is one of the knots, say the k'th, then the numerator of (19) can be estimated when α is a right bin edge, say r_i, by $\sum_{i'=1}^{i} w_{i'} \left\{ \sum_{k=1}^{k'} \widehat{\theta}_k Z_{i',k} + (1/2)\widehat{\theta}_{k'+1} Z_{i',k'+1} \right\}$ and then interpolated for other values of α. Here we use the facts that θ_1 is the probability that the null is true, that θ_{k+1} is the coefficient of the kth B-spline, and that the kth B-spline peaks at the kth knot and has half of its probability to the left of that knot.

Efron (2004) has a rather different approach to the problem of rejecting too many nulls. He replaces the "theoretical null hypothesis" by an "empirical null hypothesis." Efron applies the inverse probit transformation to the p-values so that those "z-values" coming from the null will have an exact $N(0,1)$ distribution. He then finds an estimate, $\widehat{f}_z$, of the density of the z-values. The "empirical null" is that the z-value is $N(\delta_z, \sigma_z^2)$ where δ_z is the mode of the estimated density and σ_z^2 is the $-1/\{\log(\widehat{f}_z)\}''(\delta_z)$.

Subject-matter specialists might find confusing a null hypothesis estimated from the data and without the clear scientific meaning typical of a theoretical null. In contrast, the idea that "the null is false but not by much" seems natural.

11 Power and Sample Sizes

Gadbury et al. (2004) define the Expected Discovery Rate (EDR) to be the probability of a "discovery," given that the effect is real, i.e., the probability that an effect is declared significant, given that the null hypothesis is false. The EDR in our notation was given by (2). We assume that g has been estimated and we are now contemplating a repetition of the same experiment, or perhaps a similar experiment, with new sample sizes that differ from the old by a factor η. We assume that δ represents the non-centrality parameter of a test that changes from δ to $\delta^* = \sqrt{\eta}\delta$ with the new sample size. This would be the case, for example, if we were considering two-sample t-tests with n observations per sample.

It is of interest to see how EDR changes with η. Since G is the conditional distribution of δ given that the null is false, the EDR for any η is defined by

$$\mathrm{EDR}(\alpha, \eta) = \int_0^\infty F_{p|\delta}(\alpha\,;\sqrt{\eta}\delta)\,dG(\delta). \tag{20}$$

Let $\widehat{\mathrm{EDR}}(\alpha,\eta)$ be (20) with G replaced by $\widehat{G}$. Gadbury et al. also define TN (True Negative) as the probability an effect is not real given that it is declared not significant and TP (True Positive) as the probability an effect is real given that it is declared significant. In our notation

$$\mathrm{TN}(\alpha,\eta) = \frac{(1-\alpha)\pi_0}{(1-\alpha)\pi_0 + (1-\pi_0)\{1-\mathrm{EDR}(\alpha,\eta)\}} \tag{21}$$

and

$$\mathrm{TP}(\alpha,\eta) = \frac{(1-\pi_0)\mathrm{EDR}(\alpha,\eta)}{\alpha\pi_0 + (1-\pi_0)\mathrm{EDR}(\alpha,\eta)}. \tag{22}$$

$\mathrm{TN}(\alpha,\eta)$ and $\mathrm{TP}(\alpha,\eta)$ can be estimated by plugging $\widehat{\mathrm{EDR}}(\alpha,\eta)$ and "Semi, $\min\{\widehat{f}\}$" or "Semi, compromise" into (21) and (22).

We also define an Expected Interesting Discovery Rate (EIDR) as the probability of a "discovery" given that the null is false and interesting meaning that $\delta > \delta'$. Thus, $\mathrm{EIDR}(\alpha,\eta,\delta') = \int_{\delta'}^{\infty} F_{p|\delta}(\alpha\,;\sqrt{\eta}\delta)g(\delta)d\delta/(\int_{\delta'}^{\infty} g(\delta)d\delta)$. Note that EIDR can be viewed as the sensitivity of the test for detecting departures from the null that are interesting ($\delta > \delta'$). It is straightforward to extend the usual definition of specificity in a similar manner to the probability that a gene will be declared not significant, given that the gene is null or near null ($\delta \leq \delta'$).

Examining estimates of EDR, EIDR, TP, and TN as a function of α for varying choices or η will help researchers determine appropriate sample sizes for future microarray experiments. For example, a researcher may have the goal of identifying 90% of all "interesting" gene expression differences, where "interesting" is defined by specifying a value for δ'. Furthermore, suppose this level of discovery is to be achieved while maintaining a true positive rate (TP) in excess of 0.95. By estimating EIDR and TP from pilot data, we can estimate the sample size relative to that in the pilot experiment (η) that will be required to meet the desired performance criteria. Such information will prevent researchers from wasting effort and resources on experiments that are likely to fall far short of their performance goals, or from using more resources than necessary to achieve their performance goals. These calculations are particularly valuable for microarray experiments where labor and supply costs are quite high.

12 Example: Gene Expression in Barley

Caldo, Nettleton, and Wise (2004) conducted a microarray experiment to identify barley genes that play a role in resistance to a fungal pathogen. To illustrate our methods, we describe the analysis of a subset of the data they considered.

Two genotypes of barley seedlings, one resistant and one susceptible to a fungal pathogen, were grown in separate trays randomly positioned in a growth chamber. Each tray contained six rows of 15 seedlings each. The six rows in each tray were randomly assigned to six tissue collection times: 0, 8, 16, 20, 24, and 32 hours after fungal inoculation. After simultaneously inoculating plants with the pathogen, each row of plants was harvested at its randomly assigned time. One Affymetrix GeneChip was used to measure gene expression in the plant material from each row of seedlings. The entire process was independently repeated a total of three times, yielding data on 22,840 probe sets (corresponding to barley genes) for each of 36 GeneChips (2 genotypes × 6 time points × 3 replications). This can be viewed as a split-plot experimental design with replications as blocks, trays as whole plots, and rows of seedlings as split plots.

A mixed linear model corresponding to the split-plot design was separately fit to the 36 log-scale measures of expression for each gene. Specifically, each mixed linear model included fixed effects for genotypes, times, and genotype-by-time interaction along with random effects for replications, replication-by-genotype terms (i.e., trays), and residuals corresponding to rows of seedlings. The usual assumptions regarding independence, normality, and constant variance were assumed for the random effects within a gene.

Genes that exhibit different patterns of expression over the time course following inoculation are of primary interest because this type of differential gene activity may help to explain why the one genotype is resistant to the fungus while the other is susceptible. Thus interaction between genotype and time is of primary interest in this experiment. We focus here on t-tests intended to detect specific sub-interactions within the overall genotype-by-time interaction. In particular, for each gene indexed by i and for each time $t = 8$, 16, 20, 24, and 32 hours after inoculation, we test $H_{0i}^{(t)} : \mu_{irt} - \mu_{ist} = \mu_{ir0} - \mu_{is0}$ where μ_{irt} and μ_{ist} denote the mean expression of gene i in resistant and susceptible barley genotypes, respectively, at t hours after inoculation. Note that rejection of $H_{0i}^{(t)}$ suggests that the expression difference between genotypes at time t during fungal attack has changed from the baseline difference between the genotypes at the initial time point.

According to our mixed-linear model, the test statistic for $H_{0i}^{(t)}$ will have a non-central t distribution with 20 degrees of freedom and non-centrality parameter $\delta_i^{(t)} = \sqrt{n}(\mu_{irt} - \mu_{ist} - \mu_{ir0} + \mu_{is0})/(\sqrt{4\sigma_e^2})$, where n denotes the number of replications ($n = 3$ in this case) and σ_e^2 denotes the residual variance component. Clearly $H_{0i}^{(t)}$ is equivalent to $\delta_i^{(t)} = 0$. We now present results for the five sets of p-values obtained by testing $H_{0i}^{(t)} : \delta_i^{(t)} = 0$ for all $i = 1, \ldots, 22,840$ at each time $t = 8$, 16, 20, 24, and 32 hours after inoculation.

12.1 Estimating π_0 and g

Table 3 contains the "Semi, θ_1", "Semi, compromise", "Semi, $\min\{\widehat{f}\}$", and "Convex" estimates of π_0 for tests of the 0-8, 0-16, 0-20, 0-24, and 0-32 interactions. The "Semi, compromise" estimates for the 0-t interaction decreases as t increases, indicating that more genes are being differentially expressed as the time since exposure increases. The "Convex" and "Semi, $\min\{\widehat{f}\}$" estimates are similar to each other and both are larger than the "Semi, compromise" estimates. Moreover, the differences between the "Convex" or "Semi, $\min\{\widehat{f}\}$" estimate and the "Semi, compromise" estimate increases as t gets larger. Table 3 also has results from bootstrapping "Semi, compromise" by resampling p-values. There is little variability in the estimator and the bootstrap mean is near the estimate from the original sample.

Figure 1 shows the estimates of f_p and g for each set of tests. Note that the estimates of g peak at 0, indicating that δ is typically near the null. This is another reason why the "Convex" and "Semi, $\min\{\widehat{f}\}$" have a large positive bias. In the plots on the left side of this figure, we also show the component of f_p coming from the null hypothesis, which is

$$\widehat{\theta}_1 I\{0 \leq p \leq 1\} + \sum_{k=2}^{L} \widehat{\theta}_k \int f_{p|\delta}(p\,;\delta) B_k(\delta) d\delta, \tag{23}$$

with $L = 1$ (the sum from 2 to L is defined to be 0 if $L = 1$) and the component of f_p coming from or near the null which is (23) with $L = 2$. The semiparametric estimate of f_p is (23) with $L = K$.

Figure 2 contain 30 bootstrap estimates of g and histograms of 250 bootstrap "Semi, compromise" estimates. The bootstrap results suggest that g and π_0 can be estimated with reasonably good accuracy. However, the bootstrap may overestimate accuracy if the p-values are not conditionally independent, so these results should be interpreted cautiously.

Web Figure 4 is a plot of (18), the estimated proportion of δ_i less than δ', versus δ' for the 0-8, 0-20, and 0-32 hour interactions. The estimate of π_0 is "Semi, compromise", which is 0.57 in this example. If $\delta' = 1$, then one can see in the figure that for the 0-32 hour interaction about 82% of the null hypotheses are either true or "false but with δ uninteresting." Since, from Table 3, "Semi, compromise" = 0.57, it appears that about 25% of the null hypotheses are "false but with δ uninteresting" and about 18% are "false and δ is interesting."

12.2 Estimating the FDR and FIDR

Web Figure 5 shows estimates of FDR as functions of the critical value α for the p-value (α has been multiplied by 100 to make the figure more legible). That figure has estimates using both "Semi, compromise" and "Convex" for the 0-8 and 0-32 hour interactions. For the 0-8 hour interaction, "Semi, θ_1" and "Convex" are very close to each other and therefore give similar FDR estimates. For the 0-32 hour interaction, the upward bias of "Convex" causes a some overestimation of the FDR; if $100 \times \alpha = 0.2$, then the estimated FDR is about 0.038 using "Semi, compromise" but 0.046, about 21% higher, using "Convex".

Web Figure 6 shows the estimate of the FIDR(α, δ') for the 0-32 hour interaction data.

Here $\delta' = 0.55$, 1.09, and 2.18 which are the second, third, and fifth of 12 knots. Suppose we use $100 \times \alpha = 0.2$, that is, we reject the null if p-value < 0.002. Then one can see from Web Figure 4 that the FIDR(0.002,1.09) is about 0.13, more than three times the FDR of 0.038. However, FIDR(0.002,0.55) is only 0.05, much closer to the FDR.

If we wanted to have the FIDR$(\alpha, 1.09)$ close to 0.1, for example, then Web Figure 4 suggests $\alpha = 0.001$. For the 0-32 hour interaction, about 2.3% (534 of 22,840) of the p-values are below 0.001.

12.3 Estimating EDR, TN, and TP

Estimates of the EDR(α, η), EIDR$(\alpha, \eta, 1)$, EIDR$(\alpha, \eta, 2)$, TP(α, η), and TN(α, η) for the 0-32 hour interaction are shown in Figure 3 for $0.001 \le \alpha \le .02$ and $\eta = 1$, 2, and 4. There are vertical lines in these plots through $\alpha = 0.01$. Suppose we use this value of α. Then from the top plot in Figure 3 we see that the EDR is 0.1 if the current number of replicates, three, is maintained. If six replicates are used, then the EDR rises to about 0.18, and if twelve replicates are used then the EDR is about 0.3. These numbers somewhat discouraging—even with twelve replicates only about 30% of the genes with a 0-32 interaction will be discovered. The problem here is that most of these expressed genes are difficult to discover because δ is near 0. If we only consider "interesting" genes with $\delta > 1$ then the value of EIDR$(0.01, \eta, 1)$ is nearly double the value of EDR$(0.02, \eta)$, i.e., about 0.2, 0.38, and 0.6 for three, six, and twelve replicates, respectively. Moreover, EIDR$(0.01, \eta, 2)$ is even larger, approximately 0.4, 0.7, and 0.95 for three, six, and twelve replicates, respectively. Thus, with twelve replicates, we can expect to discover 95% of the genes with a 0-32 hour interaction so large that the non-centrality parameter is 2 or larger.

13 Summary

The barley gene expression data suggests that g is close to the null for these data. In such situations, the simulation results show that "Convex" and "Semi, $\min\{\widehat{f}\}$" are positively biased and “Semi, compromise” is the best of these three estimators, especially when π_0 is not close to 1.

In other studies, g may be far from the null and then the simulation results suggest that “Convex” and “Semi, $\min\{\widehat{f}\}$” will outperform “Semi, compromise”. The simulation studies also suggest that “Semi, $\min\{\widehat{f}\}$” will be somewhat superior to “Convex” is such cases. Since our semiparametric methodology produces an estimator of g, in any application we can assess whether g is near the null or not. This assessment will provide guidance as to whether “Semi, compromise” or “Semi, $\min\{\widehat{f}\}$” should be used.

In our example, we found that in all five cases the alternative was poorly separated from the null in that g peaked at 0 with high probability that δ was less than 1. This is different from the alternatives used in simulation studies by other investigators, e.g., Broberg (2005) and Langaas et al. (2005). In our Monte Carlo study, we used three different g which range from being poorly to well separated from H_0 and that bias depends strongly on g. We suggest that other researchers estimate g and that future studies investigate g poorly separated from the null. Langaas et al. (2005) state that they use a g separated from the null “to make the estimable upper bound $\overline{\pi}_0$ close to the true π_0,” that is, to ameliorate the positive bias of “Convex” and the other estimators they consider, and they also state that this “does not mean that we imply that smaller changes are biologically uninteresting.” Our results suggest that one can target π_0 itself as the quantity to estimate rather than the upper bound of $\overline{\pi}_0 = f_p(1)$, and then there is no need to restrict g as they have done.

If one must choose a single estimator among those studied, we recommend “Semi, compromise” since it had generally good performances in all cases in our simulations study, for both one- and two-sided tests and for z-tests as well as t-tests. No other estimator in our study performed well across all cases.

There are many other estimators of π_0 beside those we have studied. Broberg (2005) describes and compares eight of them in a simulation study. However, the estimators in

Langaas et al. (2005) are not included in Broberg's study. A full comparison of all available estimators is beyond the scope of this paper. As can be seen in the Table 1 as well as Table 3 in Broberg, bias is often the major component of RMSE and the size and direction of bias depends heavily upon π_0 and g. Finding an estimator with a consistently small bias would be a desirable, but perhaps unattainable, goal.

No other estimator that we are aware of also provides an estimate of g. We believe that this is an important advantage of our methodology, since $\widehat{g}$ can be used to assess the possible size and direction of bias, to estimate how many false nulls are close to the null, and to determine sample sizes appropriate for future studies.

Genovese and Wasserman (2004) discuss identifiability of π_0 and mention that π_0 is identified under parametric assumptions. We make a parametric assumption only about f_p and this seems enough to identify π_0, though we know of no proof. We intend future study of robustness to this parametric assumption. Robustness is an issue even for nonparametric estimators, e.g., "Convex", that assume that the null distribution of the p-value is uniform.

14 Supplementary Materials

Web Tables and Figure referenced in Sections 4, 8, and 12 are available under the Paper Information link at the Biometrics website http://www.tibs.org/biometrics.

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Table 1: Two-sided t-tests with $DF = 4$ and $n = 10{,}000$ p-values per data set. RMSE (root mean squared error) and bias. 600 Monte Carlo simulated data sets per case. $(\delta_{\min}, \delta_{\max}, b_1, b_2)$ is (0, 4, 1, 2) in Cases 1 and 4, (0, 4, 2, 2) in Cases 2 and 5, and (0.5, 4.5, 3, 2) in Cases 3 and 6.

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
π_0	0.95	0.95	0.95	0.7	0.7	0.7
nearest of g from null	near	moderate	far	near	moderate	far
			RMSE			
"Semi, θ_1"	0.0251	0.0348	0.0346	0.0503	0.1035	0.0732
"Semi, $\min\{\widehat{f}\}$"	0.0276	0.0145	0.0066	0.1519	0.0748	0.0187
"Semi, compromise"	0.0206	0.0124	0.0269	0.0492	0.0268	0.0458
"Convex"	0.0238	0.0148	0.0121	0.1485	0.0767	0.0214
			Bias			
"Semi, θ_1"	0.0001	−0.0228	−0.0286	−0.0063	−0.0703	−0.0233
"Semi, $\min\{\widehat{f}\}$"	0.0266	0.0123	−0.0014	0.1517	0.0743	0.0166
"Semi, compromise"	0.0076	0.0007	−0.0221	0.0367	0.0158	−0.0169
"Convex"	0.0208	0.0089	−0.0020	0.1476	0.0749	0.0167

Table 2: Two-sided t-tests with $DF = 4$ and $n = 25{,}000$ (Cases 7–9) or 5,000 (Cases 10–12) p-values per data set. RMSE and bias. 600 Monte Carlo simulated data sets per case. $(\delta_{\min}, \delta_{\max}, b_1, b_2)$ is (0, 4, 1, 2) in Cases 7 and 10, (0, 4, 2, 2) in Cases 8 and 11, and (0.5, 4.5, 3, 2) in Cases 9 and 12.

	Case 7	Case 8	Case 9	Case 10	Case 11	Case 12
π_0	0.99	0.99	0.99	0.3	0.3	0.3
nearest of g from null	near	moderate	far	near	moderate	far
			RMSE			
"Semi, θ_1"	0.0123	0.0156	0.0175	0.2100	0.0750	0.0112
"Semi, $\min\{\widehat{f}\}$"	0.0069	0.0044	0.0039	0.3468	0.1738	0.0357
"Semi, compromise"	0.0065	0.0057	0.0081	0.2075	0.0740	0.0112
"Convex"	0.0060	0.0067	0.0066	0.3496	0.1794	0.0453
			Bias			
"Semi, θ_1"	0.0000	−0.0087	−0.0144	0.1266	0.0705	−0.0043
"Semi, $\min\{\widehat{f}\}$"	0.0063	0.0027	−0.0021	0.3466	0.1734	0.0348
"Semi, compromise"	0.0046	−0.0005	−0.0063	0.2045	0.0709	−0.0043
"Convex"	0.0017	−0.0007	−0.0029	0.3489	0.1783	0.0422

Table 3: Estimates of π_0 for barley gene expression interaction tests. "0-t" is the interaction between resistant/susceptible and time at 0 and t hours after exposure. The bootstrap results are for "Semi, compromise".

Interaction	0-8	0-16	0-20	0-24	0-32
"Semi, θ_1"	0.8639	0.6497	0.2734	0.1644	0.2307
"Semi, $\min\{\widehat{f}\}$"	0.9435	0.9074	0.8743	0.8605	0.7097
"Convex"	0.9324	0.9087	0.8668	0.8468	0.7032
"Semi, compromise"	0.9195	0.8519	0.8075	0.7884	0.5728
bootstrap mean	0.9197	0.8522	0.8133	0.7885	0.5721
bootstrap std dev	0.0067	0.0101	0.0100	0.0114	0.0116
bootstrap 2.5 %	0.8885	0.8277	0.7907	0.7592	0.5395
bootstrap 97.5 %	0.9412	0.9003	0.8592	0.8185	0.6042

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Figure 1: Barley gene expression data. Top to bottom rows: 0-8, 0-16, 0-20, 0-24, and 0-32 hour interactions. Left plots show the semiparametric (semipar) and convex, decreasing (conv-decr) estimates of f_p and a histogram of the p-values—the "o" are at the tops of the 50 bins. "From null" shows the estimated component of f_p coming from the null hypotheses—it is the uniform (0,1) density multiplied by "Semi, θ_1". "Compromise" shows the estimated component of f_p coming from the null hypotheses or δ close to the null value—see text. The height of the "compromise" estimate of f_p at 1 is the "Semi, compromise" estimate of π_0. The right plots are the estimates of g.

Figure 2: Plot of 30 bootstrap estimates of g (left) and histogram of 250 bootstrap estimates of π_0 (right). Top to bottom: 0-8, 0-16, 0-20, 0-24, and 0-32 hour interactions.

Figure 3: Barley data. 0-32 hour interaction. Estimates of EDR(α, η) (Expected Discovery Rate), EIDR$(\alpha, \eta, 1)$ (Expected Interesting Discovery Rate with $\delta' = 1$), TP(α, η) (True Positive), and TN(α, η) (True Negative) curves for $\eta = 1$, 2, 4.

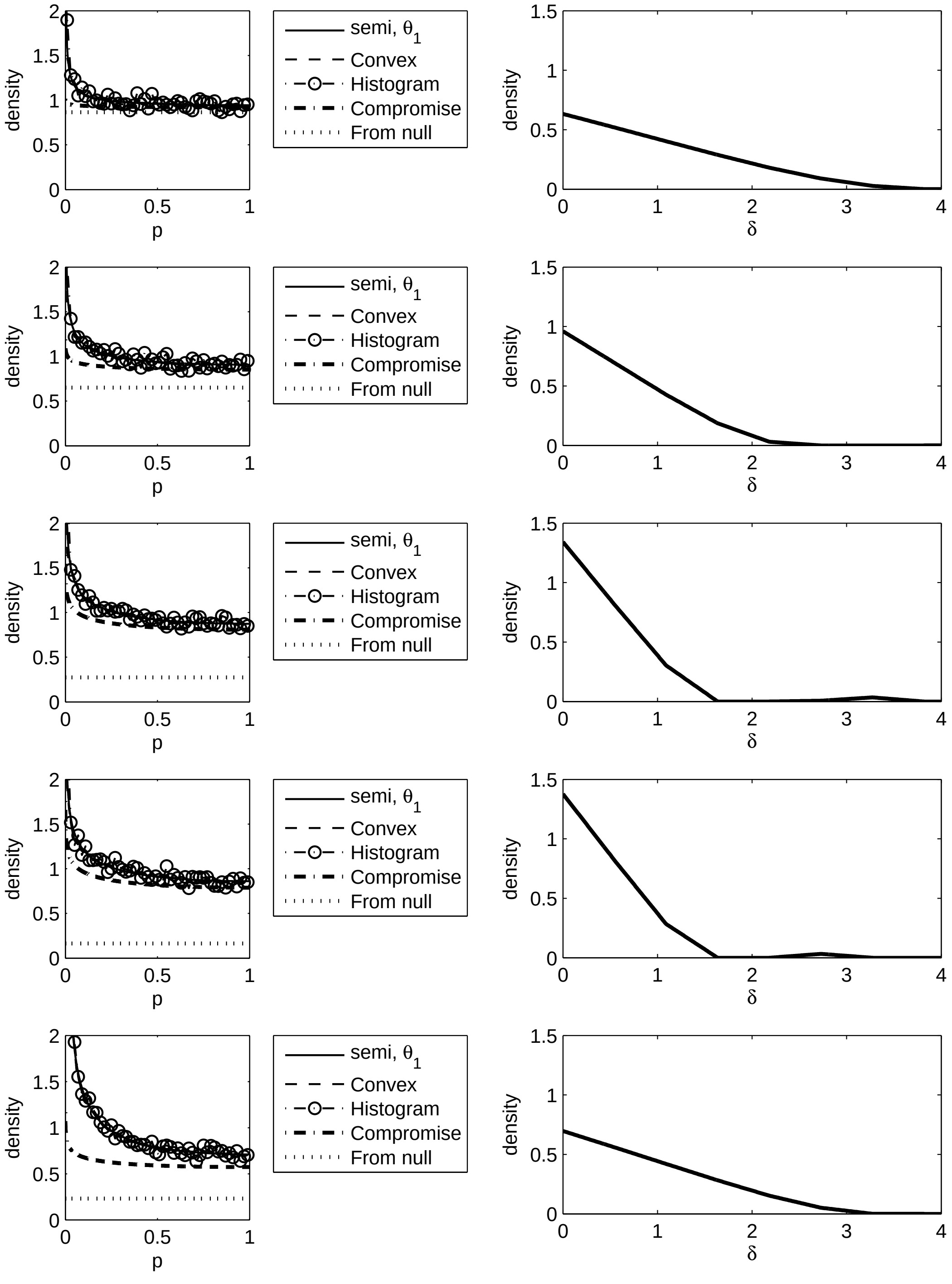
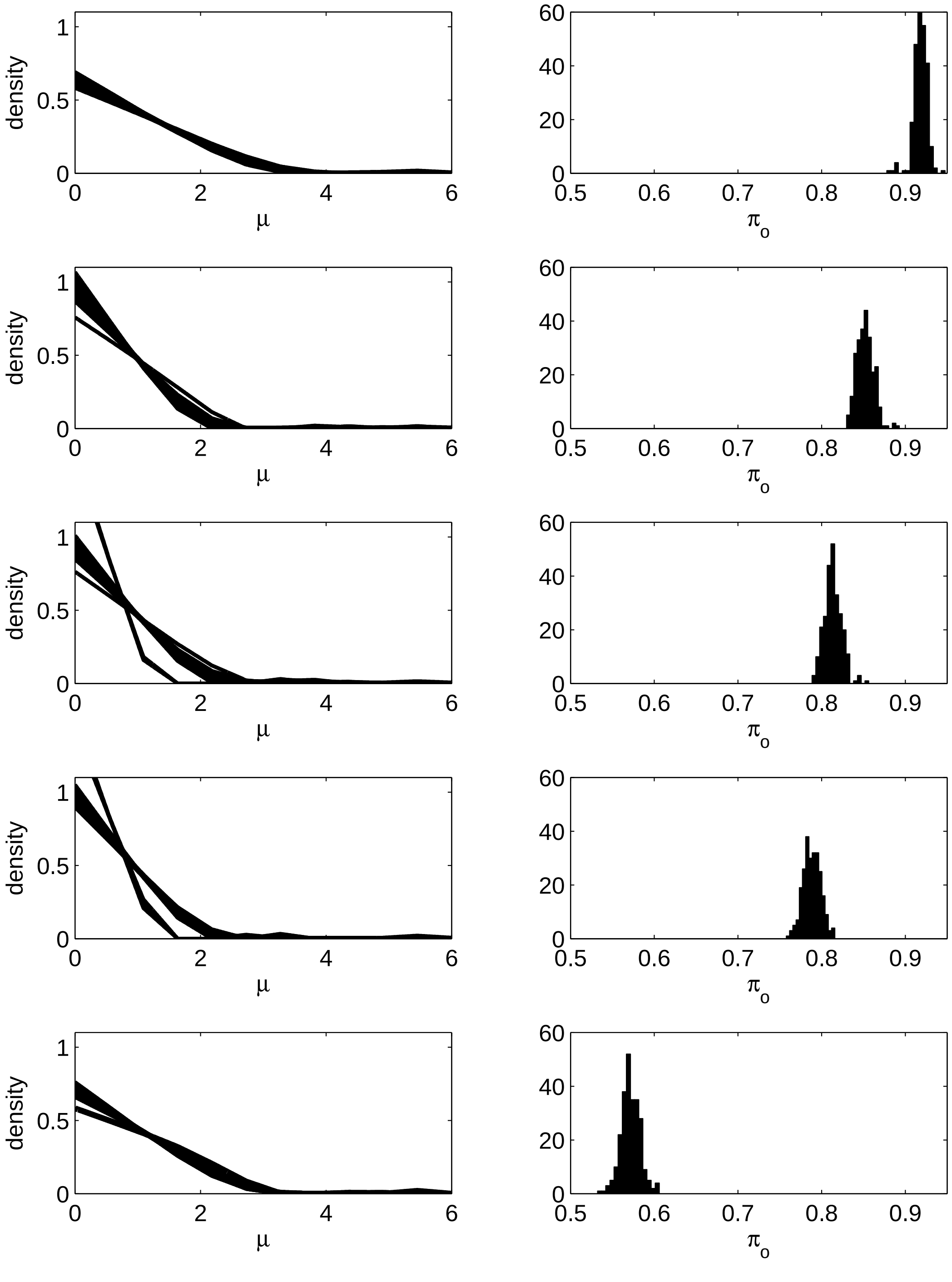

density
p
semi, θ_1
Convex
Histogram
Compromise
From null
density
δ
0
0.5
1
1.5
2
0
1
2
3
4

Figure 1:


density
1
0.5
0
0
2
4
6
μ
60
40
20
0
0.5
0.6
0.7
0.8
0.9
π_o
density
1
0.5
0
0
2
4
6
μ
60
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20
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0.5
0.6
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0.9
π_o
density
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π_o
density
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π_o
density
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4
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μ
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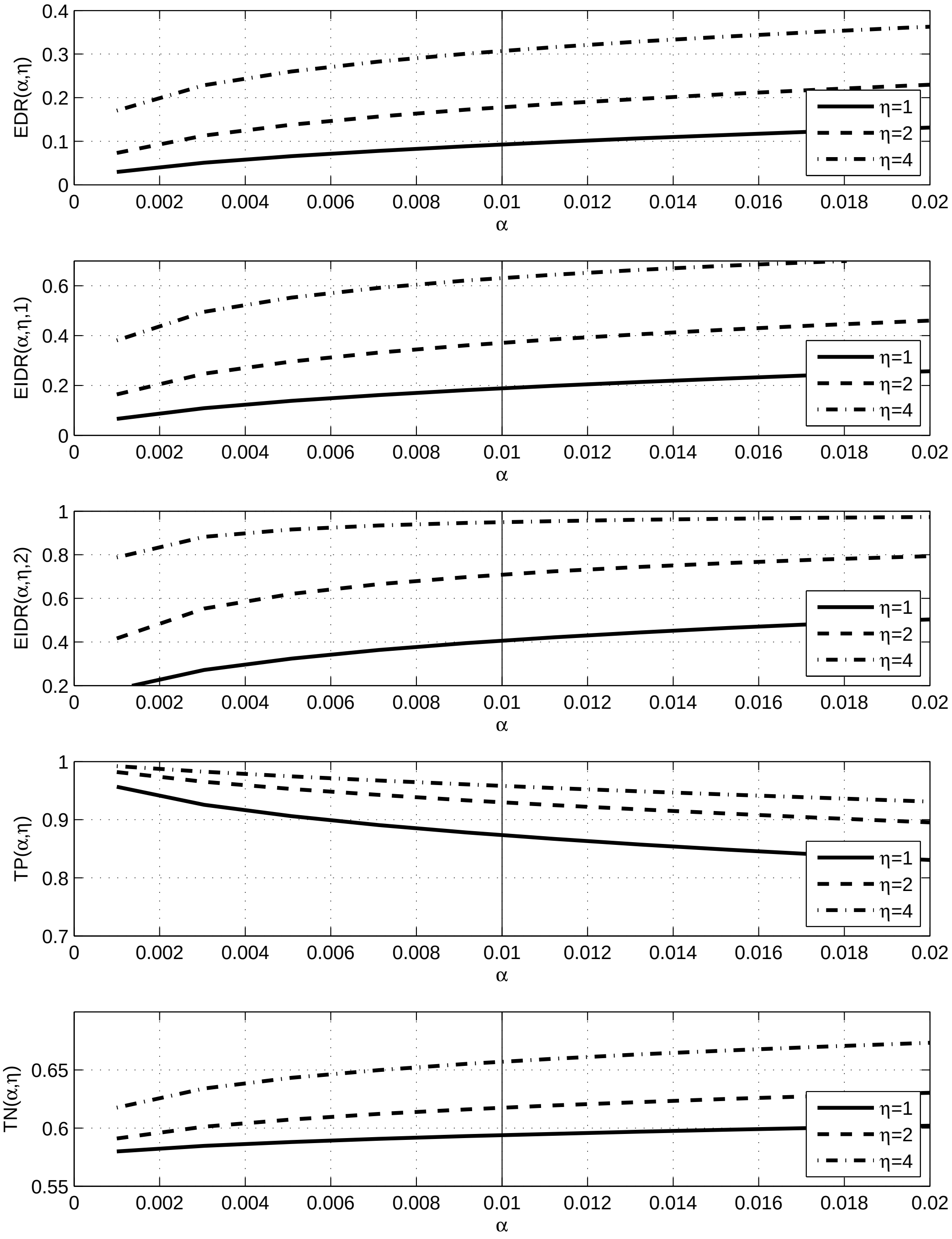
40
20
0
0.5
0.6
0.7
0.8
0.9
π_o

Figure 2:



EDR(α,η)
0.4
0.3
0.2
0.1
0
EIDR(α,η,1)
0.6
0.4
0.2
0
EIDR(α,η,2)
1
0.8
0.6
0.4
0.2
TP(α,η)
1
0.9
0.8
0.7
TN(α,η)
0.65
0.6
0.55
0
0.002
0.004
0.006
0.008
0.01
0.012
0.014
0.016
0.018
0.02
α
η=1
η=2
η=4

Figure 3: