Multicriteria Gene Screening for Analysis of Differential Expression with DNA Microarrays

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Abstract

This paper introduces a statistical methodology for identification of differentially expressed genes in DNA microarray experiments based on multiple criteria. These criteria are: false discovery rate (FDR); variance-normalized differential expression levels (paired t statistics); and minimum acceptable difference (MAD). The methodology also provides a set of simultaneous FDR confidence intervals on the true expression differences. The analysis can be implemented as a two stage algorithm in which there is an initial screen that controls only FDR, which is then followed by a second screen which controls both FDR and MAD. It can also be implemented by computing and thresholding the set of FDR p-values for each gene that satisfies the MAD criterion. We illustrate the procedure to identify differentially expressed genes from a wild-type vs. knockout comparison of microarray data.

Keywords: bioinformatics, gene filtering, gene profiling multiple comparisons, familywise error rates.

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1. INTRODUCTION

Since Watson and Crick discovered DNA more than fifty years ago, the field of genomics has progressed from a speculative science to one of the most thriving areas of current research and development.[31] After successful completion (99%) of the Human Genome project in 2002,[11] attention is turning to "functional genomics" and "proteomics," thanks principally to remarkable advances in computations and technology. These disciplines encompass the greater challenge of understanding the complex functional behavior and interaction of genes and their encoded proteins at the cellular level. This task has been significantly aided by the advent of DNA microarray technology and associated algorithms that enable researchers to filter through daunting amounts of data and genetic information. In this paper, we describe a new approach to extracting a subset of differentially expressed genes from DNA microarray data.

A DNA microarray consists of a large number of DNA probe sequences that are put at defined positions on a solid support such as a glass slide or a silicon wafer.[10,4] After hybridization of a fluorescently labelled sample (gene transcripts) to DNA microarrays, the abundance of each probe present (called probe response) in the sample can be estimated from the measured levels of hybridization (i.e., the intensity of fluorescent signal). Two main types of DNA microarrays are in wide-use for gene expression profiling: Affymetrix GeneChips,[1] which are generated by photo-lithography; and spotted cDNA (or oligonucleotide) arrays on glass slides.[25]

DNA microarrays enable biologists to study global gene expression profiles in tissues of interest over time periods and under specific conditions or treatments. For these cases a large set of samples, consisting of several biological replicates, are hybridized to a set of microarrays. The objective is to identify subsets of genes whose expression profile over time exhibit salient behavior(s), e.g., differ in response to different treatments. A crucial aspect of selecting the genes of interest is the specification of a preference ordering for ranking the probe responses. Many gene selection and ranking methods are based on testing fitness criteria such as: the eigenvalue spread in a principal components analysis (PCA) of all pairs of gene expression profiles; the ratio of between-population-variation to within-population-variation; or the cross correlation between profiles.[16,2,9]

These methods have deficiencies which have impeded their use for practical experiments. First, is the need for improved relevance of the fitness criterion to the scientific objectives of the experiment. It is

often difficult for an experimenter to choose quantitative criteria that characterize the aspects of a gene expression profile of interest. Second, is the need for simultaneous control of the biological significance (MAD) and the statistical significance (FDR) of differential responses discovered in the selected gene probes. A probe response difference which is too small is not of much use to the experimenter even if the difference is statistically significant. This is because the microarray experiment is usually only the first step in gene discovery; each microarray probe difference that is discovered must be validated by painstaking followup analysis that may have limited sensitivity to small differences. Third, is the need for tight confidence intervals on these differences. The size of a confidence interval provides useful information on the statistical precision of an estimate of differential response.

The method we present in this paper adopts a statistical multicriteria framework for gene microarray analysis with MAD constraints on differential expression. The framework allows the experimenter to adopt multiple fitness criteria, explicitly incorporate control on biological significance in addition to statistical significance, and generate confidence intervals on discovered gene expression differences. Our method is strongly influenced by the FDR-adjusted confidence interval (FDR-CI) approach recently introduced by Benjamini and Yekutieli.[7] We illustrate our methods for a differential expression experiment designed to probe the genetic basis of retinal development. This experiment involves two populations, called wild-type and knockout, and the objective is to find genes that exhibit biologically and statistically significant differences between these populations. The purpose of this article is to illustrate methodology and not to report scientific findings, which will be reported elsewhere.

It is worthwhile to compare the framework developed in this paper to related work. Liu and Iba have proposed an interesting multicriteria evolutionary approach to gene selection and classification in gene microarray experiments.[20] Similarly, Fleury and Hero have proposed Pareto-optimality for selecting subsets of genes using a combination of bootstrap resampling and Bayes decision theory.[14,17,13] Single stage[26] and multi stage[23,3,6] screening methods which control FWER or FDR have been proposed by several authors for similar problems to ours. However, none of the above approaches account for a MAD constraint or provide confidence intervals on the differential expression levels of the discovered genes. In contrast, our approach accounts for both FDR and MAD constraints and generates such confidence intervals using the FDR-CI framework.[7] Furthermore, we specify an algorithm for computing FDR p-

gene g	Pn2	Pn10	M2
W	4 samples	4 samples	4 samples
K	4 samples	4 samples	4 samples

Table 1. The knockout vs wild-type experiment is equivalent to a two way layout of treatment (W or K) and time (t=Pn2,Pn10,M2).

values for all genes at any prescribed MAD level.

The outline of the paper is as follows. In Sec. 2 we give a general description of the type of differential gene microarray experiment that will be illustrated in Sec. 4. In Sec. 3 we describe the proposed two-stage multicriteria approach. Finally, in Sec. 4 we illustrate these techniques for experimental data.

2. DIFFERENTIAL EXPRESSION PROFILE EXPERIMENTS

This type of experiment is very common in genetics research[32,27] and involves comparing gene expression profiles of a set of G genes expressed in two or more populations. The data from this experiment fall into the category of a two way layout[18] where each cell in the layout corresponds to a set of replicates of samples from one of the two populations (row) and one of T time points (column) (see Table. 1).

Any gene whose temporal profile differs from wild-type to knockout populations is called "differentially expressed" in the experiment. One variant of this experiment is called the wild-type vs knockout experiment. In such an experiment one has a control population (wild-type) of subjects and a treated population (knockout) of subjects whose DNA has been altered in some way. Each population is comprised of T different age groups arranged in T subpopulations. M independent samples are taken from each subpopulation and are hybridized to a different microarray yielding G pairs of expression profiles (see Fig. 1 for profiles of gene having probeset number 101996_at). This generates a total of $2MT$ microarrays. It is common to express the differential response between wild-type and knockout responses in terms of *foldchange* expressed as the ratio of these responses. For example, a foldchange of 2.0, or 1.0 in log base 2, at a given time corresponds to a wild-type response which is twice as large as the knockout response. We denote by $\{\mu_t(g)\}_{t=1}^T$ and $\{\eta_t(g)\}_{t=1}^T$ the true log wild-type and log knockout expression profiles, respectively, expressed as log base 2 of the true hybridization abundances.

Fig. 2 illustrates the 3 dimensional multicriteria space of mean differential responses $\{\mu_t(g) - \eta_t(g)\}_{t=1}^3$ for the three time point experiment described in Sec. 4. Also indicated is a "MAD box" which defines

unacceptably small (inside box) vs acceptably large (outside box) differential responses, and a scatter of a small subset of all the sample mean differential responses (dots) from the experiment. Our objective is to discover which genes are likely to have a "positive differential response" falling outside of the box in Fig. 2. A very commonly used method is to simply apply a threshold to the sample means to detect those who fall outside of the box in Fig. 2 as positive responses. However, as will be shown, this method does not account for statistical sampling uncertainty and can lead to many false positives.

The objective can be stated mathematically as follows: find a set of gene probes which satisfy the MAD constraint: $|\mu_t(g) - \eta_t(g)| > \mathrm{fcmin}$ for at least one $t \in \{1, \ldots, T\}$. Here the MAD constraint is quantified by the user-specified minimum magnitude foldchange fcmin (expressed in log base 2). Thus we need to simultaneously test the G pairs of two-sided hypotheses

$$\begin{aligned} H_0(g) &: |\mu_1(g) - \eta_1(g)| \leq \mathrm{fcmin} \textbf{ and } \cdots \textbf{ and } |\mu_T(g) - \eta_T(g)| \leq \mathrm{fcmin}, \\ H_1(g) &: |\mu_1(g) - \eta_1(g)| > \mathrm{fcmin} \textbf{ or } \cdots \textbf{ or } |\mu_T(g) - \eta_T(g)| > \mathrm{fcmin}, \end{aligned} \tag{1}$$

$g = 1, \ldots, G$. Of course when we must decide between $H_0(g)$ and $H_1(g)$ based on a random sample there will generally be decision errors in the form of false positives (decide $H_1(g)$ when $H_0(g)$ is true) and false negatives (decide $H_0(g)$ when $H_1(g)$ is true). For any test the experimenter needs to be able to control both its statistical and biological level of significance. The *statistical level of significance* of the test is specified by the false positive rate. In contrast the *biological level of significance* of the test is specified by fcmin.

There are three aspects to the hypothesis testing problem (1) which make it non-standard: (i) standard tests on differences in means, such as the paired-t test, treat any non-zero difference as significant whereas (1) specifies that only differences exceeding the specified MAD level of fcmin are significant; (ii) a positive response ($H_1(g)$) is described by multiple criteria, here equal to the T magnitude log response ratios at each point in time; (iii) the G pairs of hypotheses must be tested simultaneously. For the case $G = T = 1$ the first aspect can be treated by applying methods for composite hypothesis testing such as generalized likelihood ratio tests, unbiased tests, and confidence interval test procedures.[8,30] When $\mathrm{fcmin} = 0$, (ii) and (iii) can be handled by applying a standard method, like paired t-test, to (1) for each gene probe g, implemented with a multiplicity error correction factor, e.g., Bonferroni, familywise error rate, or false

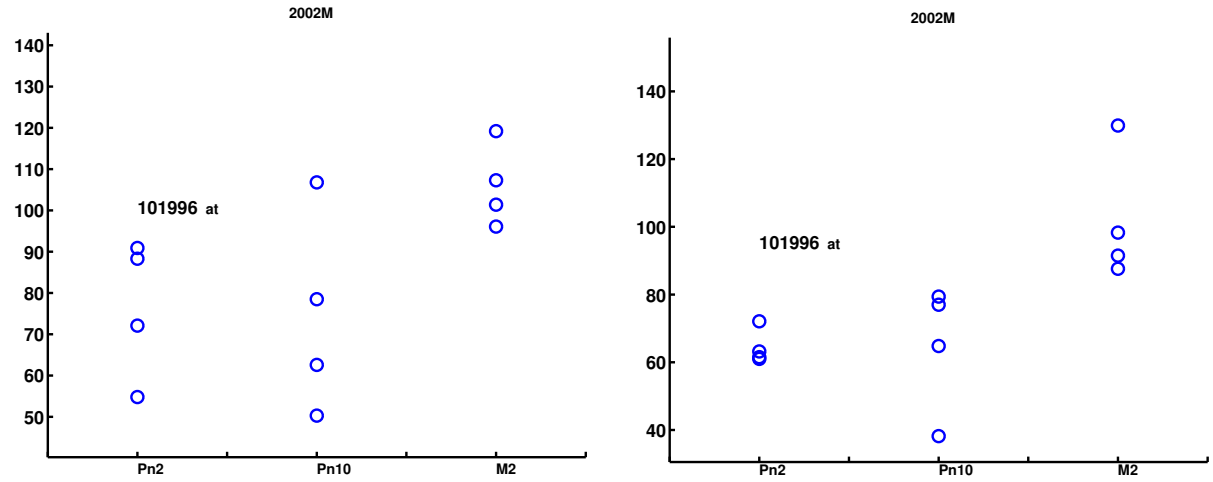

Figure 1. *Responses for a particular gene (probeset number* 101996_at*) in knockout mouse (left) vs wild-type mouse (right) for the differential expression study discussed in Sec. 2. There are 3 time points (labeled Pn2, Pn10 and M2) and at each time point there are 4 replicates.*

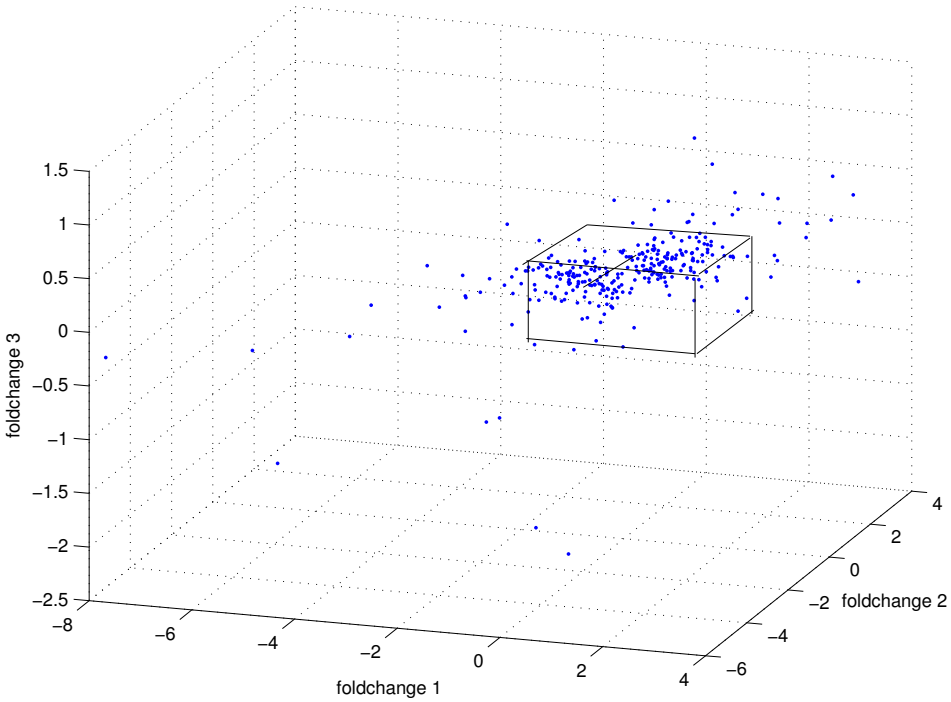

Figure 2. *Three dimensional multicriteria space for knockout and wild-type profiles over 3 time points shown in Fig. 1. The 3 criteria are the differential probe responses at each time point. Shown is a scatter plot of sample means of the differential responses along with a box of edge length* $2\mathrm{fcmin}$ *distinguishing biologically significant responses (outside box) from biologically insignificant responses (inside box).*

discovery rate,.[12] However, such a repeated test of significance will result in excessive false positives corresponding to small log response ratios that are biologically insignificant (do not satisfy the MAD constraint) but are statistically significant.

3. MULTICRITERIA GENE SCREENING METHOD

Define $\underline{\xi}(g) = [\xi_1(g), \ldots, \xi_T(g)]$ the true differential response vector associated with gene probe g, where $\xi_t(g) = \mu_t(g) - \eta_t(g)$. Given the DNA microarray data our objective is to test the G hypotheses (1) involving a total of $P = GT$ unknown parameters $\{\underline{\xi}(g)\}_{g=1}^G$.

Any test of (1) must test over multiple criteria $\{\underline{\xi}_t(g)\}_t$ and multiple genes at a given level of biological significance MAD=fcmin and a given level of statistical significance max FDR=α. Unless $\mathrm{fcmin} = 0$, this is a doubly composite hypothesis testing problem since the parameter values ξ_t are not specified under H_0 or H_1. Due to the presence of multiple criteria and multiple genes this problem falls into the area of multiple testing, simultaneous inference, and repeated tests of significance.[5,22] Two standard measures of statistical significance of a test of (1) are its *familywise error rate* (FWER) and its *false discovery rate* (FDR).[5] A mathematically convenient notation for a test of (1) is $\phi(g)$, which is called a *test function*, taking on values 0 or 1 depending on whether the test declares H_0 or H_1 for probe g, respectively. With $\mathcal{G}_0$ denoting the probes not having positive responses, the FWER and FDR of a test ϕ can be mathematically defined as:

$$\begin{aligned} \mathrm{FWER}(\mathcal{G}_0) &= 1 - E\left[\Pi_{g=1}^G (1 - \phi(g))\psi_{\mathcal{G}_0}(g)\right] \\ \mathrm{FDR}(\mathcal{G}_0) &= E\left[\frac{\sum_{g=1}^G \phi(g)\psi_{\mathcal{G}_0}(g)}{\sum_{g=1}^G \phi(g)}\right], \end{aligned}$$

where $E[Z]$ denotes statistical expectation of a random variable Z and $\psi_{\mathcal{G}_0}(g)$ is the indicator function of the set $\mathcal{G}_0$. In words, the FWER is the probability that the test of all G pairs of hypotheses (1) yields at least one false positive in the set of declared positive responses. In contrast, the FDR is the average proportion of false positives in the set of declared positive responses. The FDR is dominated by the FWER and is therefore a less stringent measure of significance. Both FWER and FDR have been widely used for gene microarray analysis.[12,28,23,3]

It is useful to contrast the FWER and FDR to the per-comparison error rate (PCER). The PCER refers to the false positive error rate incurred in testing a single pair of hypothesis $H_0(g)$ vs. $H_1(g)$ for a single gene,

say gene $g = g_o$ and does not account for multiplicity of the hypotheses (1). The PCER is the probability that purely random effects would have caused g_o to be erroneously selected, generating a false positive, based on observing microarray responses for gene g_o only. If an experimenter were only interested in deciding on the biological significance of a single gene g_o based only on observing probes for that gene, then reporting PCER(g_o) would be sufficient for another biologist to assess the statistical significance of the experimenter's statement that g_o exhibits a positive response. In contrast to the PCER, FWER and FDR communicate statistical significance of an experimenter's finding of biological significance after observing all gene responses. The FWER is the probability that there are any false positives among the set of genes selected. On the other hand, the FDR refers to the expected proportion of false positives among the selected genes. The FDR is a less stringent criterion than the FWER.[15,5,28]

The FWER can be upper bounded as a function of $\{\text{PCER}(g)\}_{g=1}^{G}$ using Bonferroni-type methods[22] or it can be computed empirically from the sample by resampling methods.[33] The FDR can be computed by applying the step-down procedure of Benjamini and Hochberg[5] to the list of PCER p-values over all genes. For a given g the PCER p-value, denoted $p(g)$, of a test ϕ is a function of the microarray measurements and is defined as the minimum value of PCER for which $H_0(g)$ would be falsely rejected by the test. The set of gene responses which pass the test ϕ at a specified FDR can be simply determined after ordering the genes indices according to increasing PCER p-value $p(g_{(1)}) \leq \ldots \leq p(g_{(G)})$. Specifically, for a fixed value $\alpha \in [0, 1]$ of maximum acceptable FDR, the FDR constrained test will declare the following set $\mathcal{G}_1$ of genes as positive responses[15]:

$$\begin{aligned} \mathcal{G}_1 &= \{g_{(1)}, \ldots, g_{(K)}\} \\ K &= \max\{k : p(g_{(k)}) \leq k\alpha/G\ \nu\}, \end{aligned} \tag{2}$$

In this expression $\nu = 1$ if the decisions $\phi(g)$ can be assumed statistically independent over $g = 1, \ldots, G$, while $\nu = 1/\sum_{k=1}^{G} k^{-1}$ without the independence assumption.

A test which controls a maximum level α of acceptable FDR is said to be a FDR test of level-α. We propose a test ϕ of (1) at FDR level α and MAD level fcmin based on intersecting simultaneous confidence intervals on the T differences $\underline{\xi}(g)$ with the unacceptable difference region $[-\mathrm{fcmin}, \mathrm{fcmin}]$. We will specify a two stage direct implementation and a single stage inverse implementation in the following subsections. First, however, we recall some facts about simultaneous CIs.

Let θ be an unknown parameter, e.g., a gene's foldchange $\xi_1(g)$ at time $t = 1$. A PCER $(1-\alpha)\times 100\%$ CI on θ, is an interval $I(\alpha) = [a, b]$ with random data-dependent endpoints that covers the true θ value, say θ_o, with probability at least $1-\alpha$:

$$P(a \le \theta_o \le b | \theta = \theta_o) \ge 1 - \alpha.$$

There is always a tradeoff between confidence level $1-\alpha$ and precision (CI length) since the length $b-a$ of $I(\alpha)$ generally increases as α decreases. Let $\mathcal{A}$ be any subset of $\mathbb{R}$. A PCER CI on θ can be converted to a PCER level-α test of the hypotheses $H_0(g) : \theta \in \mathcal{A}$ vs. $H_1(g) : \theta \notin \mathcal{A}$ by the simple procedure: "reject H_0 if the $(1-\alpha)\times$ 100% CI on θ does not intersect $\mathcal{A}$".[8]

Multiple parameters, $\theta_1, \ldots, \theta_P$, can be simultaneously covered by FWER $(1-\alpha)\times 100\%$ CIs $\{I^p(1-(1-\alpha)^{1/P})\}_{p=1}^P$, where $I^p(\alpha)$ is a PCER $(1-\alpha)\times$100% CI on θ_p. Under the assumption that each of the P PCER CIs are statistically independent, the FWER intervals cover all the parameters with probability at least $1-\alpha$.[22] A less stringent set of CIs $\{I^p(\alpha/P)\}_{p=1}^P$, which can be applied to dependent sets of PCER CIs, is guaranteed to cover at least $(1-\alpha)P$ of the unknown parameters.[22,34] When the number of P of parameters is random, as occurs when the number of parameters results from some initial screening, the above methods cannot be applied. It was for this situation that the FDR-CI approach was developed.[7] If P is the result of initial screening at a FDR level α of Q parameters having PCER CIs $\{I^p(\alpha)\}_{p=1}^Q$ then the FDR-CIs on the P parameters are defined as $\{I^p(P\alpha/Q)\}_{p=1}^P$. The FDR-CIs are guaranteed to cover at least $(1-\alpha)\times 100\%$ of the P unknown parameters.

Below we give two equivalent FDR-CI procedures for screening differentially expressed genes with FDR and MAD constraints.

3.1. Direct Two Stage Screening Procedure

Stage 1: Gene screening at MAD level 0 extracts a set of G_1 genes $\mathcal{G}_1$ by testing (1) under the relaxed MAD constraint $\mathrm{fcmin} = 0$ using a FDR level-α test via the step-down procedure (2).

Stage 2: Gene screening at MAD level $\mathrm{fcmin} > 0$ extracts a set $\mathcal{G}_2$ of positive genes from those in $\mathcal{G}_1$ as follows. For each gene $g \in \mathcal{G}_1$ construct T simultaneous CI's, denoted $\{I_t^g(\alpha)\}_{t=1}^T$, of FWER level $(1-\alpha)\times$100% on the true foldchanges $\{\mu_t(g) - \eta_t(g)\}_{t=1}$. Convert these into $(1-\alpha)\times$100% FDR-CI's

by the method of Benjamini and Yekutieli[7]: $I_t^g(\alpha) \rightarrow I_t^g(G_1\alpha/G)$, $t = 1, \ldots, T$, $g = 1, \ldots, G$. Finally, define the set of indices $\mathcal{G}_2$ of gene profiles having at least one time point where the FDR-CI does not intersect $[-\mathrm{fcmin}, \mathrm{fcmin}]$:

$$\mathcal{G}_2 = \{\ g \in \mathcal{G}_1 : (\cup_{t=1,2,3} I_t^g(G_1\alpha/G)\ \cap\ [-\mathrm{fcmin}, \mathrm{fcmin}]) = \emptyset\ \}\,, \tag{3}$$

where $\emptyset$ denotes the empty set. It follows from Sec 3.1 of Benjamini and Yekutieli[7] that the set $\mathcal{G}_2$ has FDR less than or equal to α at MAD level fcmin.

3.2. Inverse Screening Procedure: FDR p-values

In many practical situations the experimenter may not be comfortable specifying a MAD or FDR criterion in advance. In these situations it is more useful to solve the following "inverse problem:" what is the most stringent pair of criteria (α, fcmin) that would cause a particular subset of genes among the positives $\mathcal{G}_2$? For fixed fcmin the most stringent (minimum) value α for which a gene would fall into $\mathcal{G}_2$ is called the FDR p-value. The FDR p-value for a gene g_o can be computed by: (1) computing the PCER p-value sequence $\{p(g)\}_{g=1}^G$; (2) arranging the PCER p-value sequence in increasing order $p(g_{(1)}) \leq \ldots \leq p(g_{(G)})$; (3) finding the minimum value $\alpha = \alpha(g_o)$ for which at least one of the PCER CIs $\{I_t^{g_o}(\alpha)\}_{t=1}^T$ does not intersect $[-\mathrm{fcmin}, \mathrm{fcmin}]$; (4) computing the integer index

$$N(\alpha(g_o)) = \sum_{k=1}^{G} I\left(p(g_{(k)})k/G \leq 1 - (1-\alpha(g_o))^T\right),$$

where $I(A) = 1$ if statement A is true and $I(A) = 0$ otherwise; (5) the FDR p-value of g_o is then simply $p(g_i)$, where $i = N(\alpha(g_o))$. Repeating this as g_o ranges over $1, \ldots, G$ gives a sequence of FDR p-values at MAD level fcmin that can be thresholded to determine the set of positive genes $\mathcal{G}_2$ at any desired FDR level of significance.

4. APPLICATION TO A WILDTYPE VS KNOCKOUT EXPERIMENT

These experiments were performed to investigate the role of a specific retinal transcription factor, Nrl,[29] in the development of mouse retina. The retinal samples were taken from four pairs ("biological replicates") of wild-type and knockout (Nrl deficient) mice[21] at three different time points: postnatal day 2 (Pn2), postnatal day 10 (Pn10) and 2 months of age (M2). The samples were then hybridized to a total of

twenty-four MGU74Av2 Affymetrix Gene Chips. The log base 2 probe responses were extracted from Affymetrix GeneChips using the Robust Microarray Analysis (RMA) package.[19] We denote the measured wild-type and knockout responses by $W_{t,m}(g)$ and $K_{t,m}(g)$, where $m = 1, \ldots, M$, $t = 1, \ldots, T$, and $g = 1, \ldots, G$ index microarray replicate, time, and gene probe location on the microarray, respectively. For this experiment $G = 12421$, $M = 4$, and $T = 3$. To construct confidence intervals on foldchanges we define the vector of paired t-test statistics:

$$\hat{\underline{\xi}}(g) = \left[\frac{|\overline{W_1}(g) - \overline{K_1}(g)|}{\mathrm{s}_1(g)/\sqrt{M/2}}, \frac{|\overline{W_2}(g) - \overline{K_2}(g)|}{\mathrm{s}_2(g)/\sqrt{M/2}}, \frac{|\overline{W_3}(g) - \overline{K_3}(g)|}{\mathrm{s}_3(g)/\sqrt{M/2}} \right], \quad g = 1, \ldots, G.$$

Here $\overline{W_t}(g) = M^{-1} \sum_{m=1}^{M} W_{t,m}(g)$ and $\overline{K_t}(g) = M^{-1} \sum_{m=1}^{M} K_{t,m}(g)$ denote the sample mean of the M replicates at time t for wild-type and knockout treatments, respectively, and

$$\mathrm{s}_t^2(g) = (2(M-1))^{-1} \left(\sum_{m=1}^{M} (W_{t,m}(g) - \overline{W_t}(g))^2 + \sum_{m=1}^{M} (K_{t,m}(g) - \overline{K_t}(g))^2 \right)$$

denotes the pooled sample variance at time t.

For stage 1 of the screeing procedure we consider the simple and standard[22] simultaneous test of (1) at MAD level $\mathrm{fcmin} = 0$: "decide $H_1(g)$ if $\max_{t=1,2,3} \frac{|\overline{W_t}(g) - \overline{K_t}(g)|}{\mathrm{s}_t(g)/\sqrt{M/2}} > \mathrm{fcmin}$". Under the large M approximation that the paired t-test statistic has a Student t distribution,[24] and assuming time independence of cells in the two way layout of Table 1, we can easily compute both the PCER p-value for this test

$$p(g) \;\; = \;\; 1 - \left[2\mathcal{T}_{2(M-1)}(\hat{\xi}(g)) - 1 \right]^3, \tag{4}$$

and simultaneous $(1-\alpha)\times 100\%$ confidence intervals, $I_1^g(\alpha), I_2^g(\alpha), I_3^g(\alpha)$, for the temporal foldchanges $\{\mu_t(g) - \eta_t(g)\}_{t=1,2,3}$ of gene g

$$\begin{aligned} \overline{W_t}(g) - \overline{K_t}(g) - \mathrm{s}_t(g)/\sqrt{M/2}\; \mathcal{T}_{2(M-1)}^{-1}(1-\alpha/2) \leq & \\ \mu_t(g) - \eta_t(g) & \\ \leq \overline{W_t}(g) - \overline{K_t}(g) + \mathrm{s}_t(g)/\sqrt{M/2}\; \mathcal{T}_{2(M-1)}^{-1}(1-\alpha/2), & \end{aligned} \tag{5}$$

$t = 1, 2, 3$. In the above $\mathcal{T}_\nu : \mathbb{R} \mapsto [0,1]$ denotes the Student t cumulative distribution function with ν degrees of freedom and $\mathcal{T}_\nu^{-1}$ denotes its functional inverse, i.e. the Student-t quantile function.

Stage 1	Compute and sort PCER p-values according to (4) Select gene indices $\mathcal{G}_1$ according to (2)
Stage 2	Construct simultaneous PCER CIs using (5) Select gene indices $\mathcal{G}_2$ according to (3)

Table 2. Two stage FDR-CI algorithm for screening genes from the knockout vs wild-type experiment.

With the above expressions we can find the set $\mathcal{G}_1$ of gene indices which pass stage 1 FDR screening by substituting the sorted PCER p-values (4) into the step-down algorithm (2). Stage 2 of screening selects gene indices according to the FDR-CI's from (3). This direct two stage screening stage procedure is summarized in Table 2. Alternatively, the inverse procedure of Sec. 3.2 can implemented using (4) and the explicit expression for the $\alpha(g)$ sequence

$$\alpha(g) = 2\left[1 - \mathcal{T}_{2(M-1)}\left(\frac{\max|\overline{W_t}(g) - \overline{K_t}(g)| - \mathrm{fcmin})}{\mathrm{s}_t(g)/\sqrt{M/2}}\right)\right],$$

$g = 1, \ldots, G$.

4.1. Experimental Results

Figures 3 and 4 illustrate the direct and inverse implementations of the FDR-CI screening procedure. In Fig. 3 the direct screening procedure is constrained by MAD and FDR criteria $\mathrm{fcmin} = 2.0$ and $\alpha = 0.2$, respectively. As there are $T = 3$ time points and $G = 12,421$ genes there are $GT = 37,263$ parameters for which FDR-CI's are constructed. A gene passes the screening if at least one of the 3 time instants has a FDR-CI that does not intersect the interval $[-\mathrm{fcmin}, \mathrm{fcmin}]$. The test is implemented by defining two rank orderings of the FDR-CIs of the genes according to: (1) the FDR-CI with minimum upper boundary over the 3 time points; and (2) the FDR-CI with maximum lower boundary over the time points. Figure 3.a and b show relevant segments of these two ordered sequences of CI's. Screening all genes with maximum lower endpoints $> \mathrm{fcmin}$ and minimum upper endpoints $< -\mathrm{fcmin}$ generates the set of declared positive genes $\mathcal{G}_2$.

Figure 4 illustrates the inverse procedure specified in Sec. 3.2 for screening differentially expressed genes. First the FDR p-values are computed for each gene at several MAD levels of interest. For each MAD level fcmin we plot the ordered FDR p-values. These can be plotted on the same gene index axis since the induced gene ordering is independent of MAD level. FDR p-value curves for four different levels

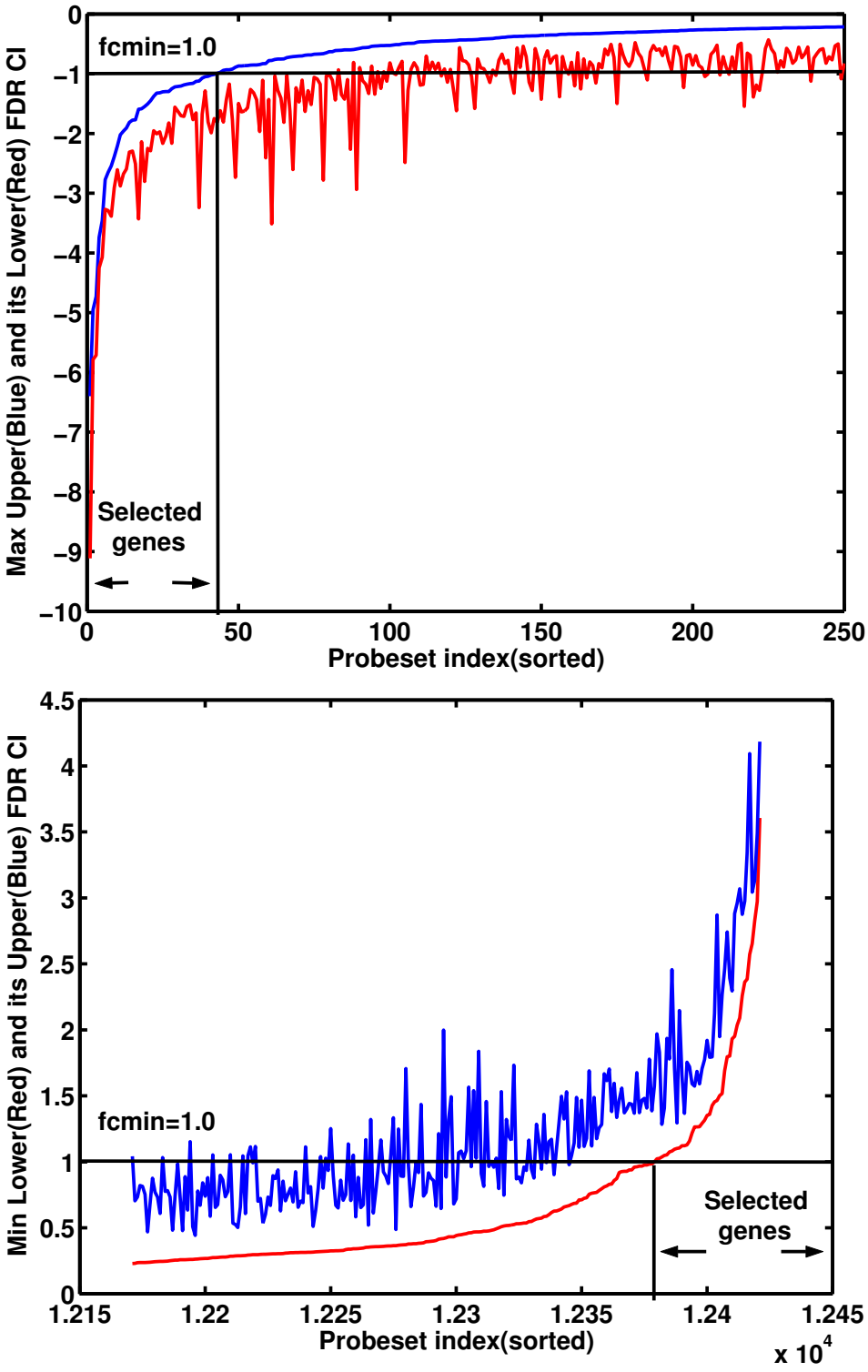

Figure 3. *Segments of upper and lower curves specifying the 80% FDR confidence intervals (FDR-CI) on the foldchanges* $\{\mu_t(g) - \eta_t(g)\}_{t=1,2,3}$ *for the knockout vs. wild-type study. Upper and lower curves in each figure sweep out FDR-CI upper and lower boundaries on foldchange for all genes (indexed by probeset number). In the top graph the curves sweep out the sequence of FDR-CIs indexed in increasing order of the (maximum) lower CI boundary. In the lower graph the ordering is in increasing order of the (minimum) upper CI boundary. Only those genes whose 3 FDR-CIs do not intersect* $[-\mathrm{fcmin}, \mathrm{fcmin}]$ *are selected by the second stage of screening. When the MAD foldchange criterion is* $\mathrm{fcmin} = 2.0$ $(1.0$ *in log base 2) these genes are obtained by thresholding the curves as indicated.*

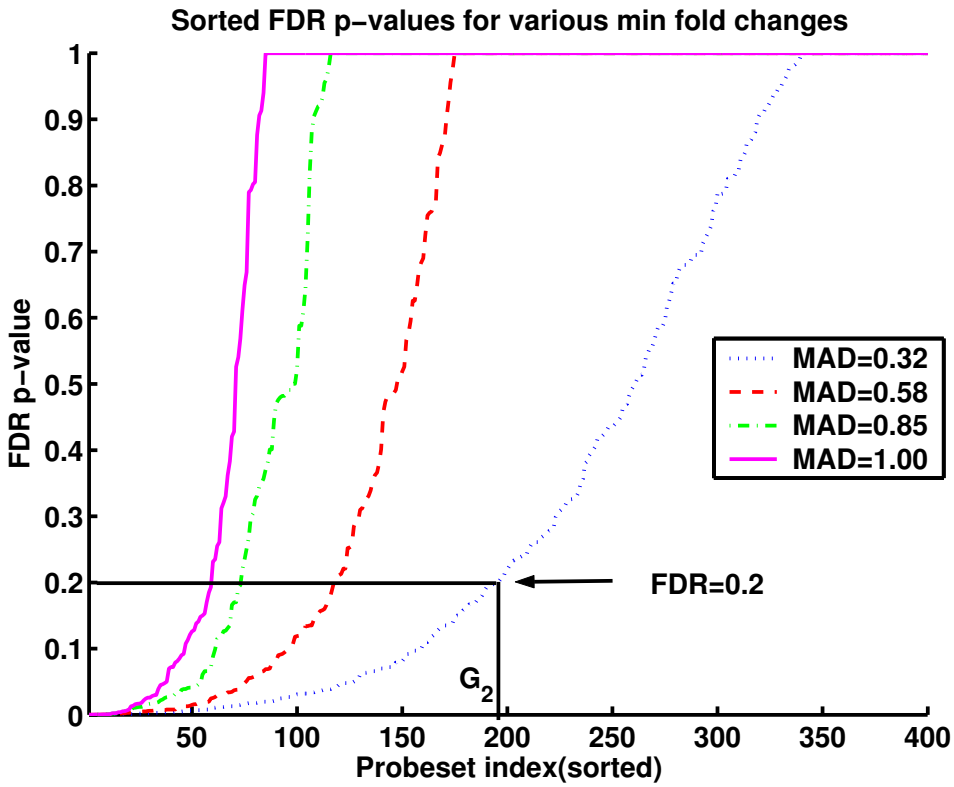

Figure 4. *Plots of FDR p-value curves over sorted list of gene indices for 4 values of the minimum acceptable difference (MAD) criterion:* $\mathrm{fcmin} = 0.32, 0.58, 0.85, 1.0$ *(log base 2) corresponding to wild-type/knockout MAD ratios of* $1.25, 1.5, 1.8$*, and* 2.0*, respectively. Constraints FDR*≤ 0.2 *and foldchange*> 0.32 *determine a set* $\mathcal{G}_2$ *of* G_2 *differentially expressed genes by thresholding the corresponding curve as indicated.*

of fcmin are illustrated in Figure 4. The figure also illustrates how for FDR and MAD constraints $\alpha = 0.2$ and $\mathrm{fcmin} = 0.32$, respectively, the G_2 positive responses $\mathcal{G}_2$ can be extracted from the FDR p-value curve by thresholding. Notice that for fixed α, the size G_2 decreases rapidly as the MAD criterion becomes more stringent, i.e., as fcmin increases.

Figure 5 shows nine of the top ranked (in FDR p-value) differentially expressed gene profiles in (log base 2 scale) among the 59 genes selected by either the direct or inverse implementations of the FDR-CI screening procedure. In the figure the level of significance constraint is FDR$\leq \alpha = 0.2$ and the minimum foldchange constraint is MAD$> \mathrm{fcmin} = 1.0$.

In Table 3 we compare the performance of the proposed screening algorithm, labeled "Two-stage FDR-CI," to two other algorithms, called "Thresholded FDR" and "Thresholded RMA." All three algorithms

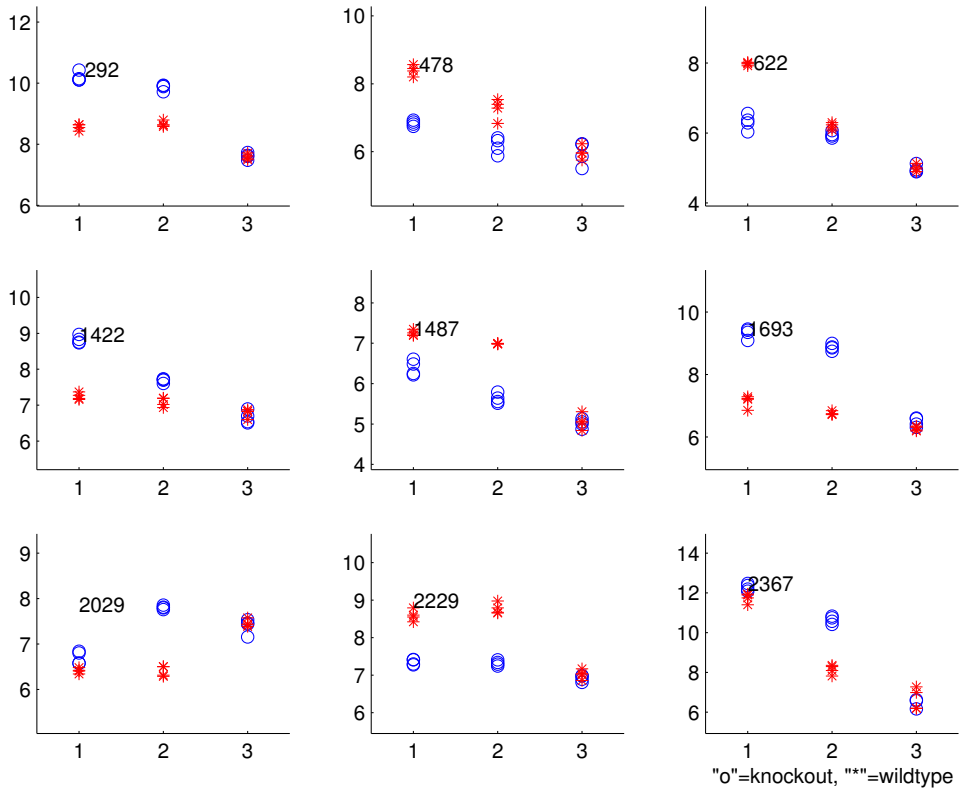

Figure 5. *Gene profiles of 9 of the differentially expressed genes discovered using proposed two stage FDR-CI procedure with constraints on level of significance* $\alpha = 0.2$ *and minimum foldchange* $\mathrm{fcmin} = 1.0$. *Knockout "o" and Wildtype "*" are as indicated.*

aim to control MAD at a level of $\mathrm{fcmin} = 1.0$(log base 2). The "Two-stage FDR-CI" and "Thresholded FDR" algorithms aim to control FDR at a level of $\alpha = 0.2$ in addition to MAD. Both of these latter algorithms were implemented as two stage algorithms with common stage 1, which is to select the gene responses $g \in \mathcal{G}_1$ that pass the paired-t test of hypotheses (1) with $\mathrm{fcmin} = 0$ at a FDR level of 20%. The second stage of the "Two-stage FDR-CI" algorithm selects $\mathcal{G}_2$ as a subset of $\mathcal{G}_1$ at the prescribed CI FDR level of 20%. Stage 2 of the "Thresholded FDR" algorithm simply selects the subset of genes $g \in \mathcal{G}_1$ having at least one sample mean foldchange exceeding $\mathrm{fcmin} = 1.0$, i.e., it implements the following filter

$$\max_{t=1,2,3} |\overline{W_t}(g) - \overline{K_t}(g)| > 1.0, \tag{6}$$

on probes $g \in \mathcal{G}_1$. The single stage "Thresholded RMA" algorithm, a non-statistical method commonly used in many microarray studies, implements the filter (6) on the responses of each g in the original set of 12,421 genes as indicated in Fig. 2.

	# Screened	# Discovered	max(pv)	median(pv)	avg(FDR-CI length)
Thresholded RMA	12,421	159	1.0	0.80	1.52
Thresholded FDR	303	127	1.0	0.31	1.17
Two-stage FDR-CI	303	59	0.19	0.02	1.09

Table 3. Performance comparison for three algorithms for selecting genes with magnitude (log base 2) foldchange > 1.0. Thresholded RMA and Thresholded FDR are significantly worse in terms of statistical significance (p-value) than the proposed Two-stage FDR-CI algorithm (columns 4 and 5). Furthermore, the average length of the CIs on foldchanges of the discovered genes are shorter for the Two Stage FDR-CI algorithm than for the other algorithms (column 6).

The number of screened and discovered genes for the three algorithms is indicated in the first two columns of Table 3. The maximum and median of the FDR p-values of the discovered genes is indicated in the third and fourth columns for each algorithm. The last column indicates the maximum length of the FDR-CI's on foldchanges of the discovered genes. We conclude from Table 3 that the proposed "Two stage FDR-CI" algorithm outperforms the other algorithms in terms of: 1) maintaining the FDR requirement that false positives not exceed 20% (column 4); 2) ensuring a substantially lower median FDR p-value than the others (column 5); 3) discovering genes that have tighter (on the average) confidence intervals on biologically significant (> 1.0) foldchange (column 6).

5. CONCLUSION

Signal processing for analysis of DNA microarrays for gene expression profiling is a rapidly growing area and there are enough challenges to keep the community busy for years. It is essential that signal processing methods be relevant and capture the biological aims of the experimenter. In this paper we developed a flexible multicriteria approach to gene selection and ranking for screening differentially expressed gene profiles. The criteria, selected here for analysis, capture the expression differences at multiple time points, account for minimum acceptable difference constraints, and control false discovery rate.

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