Optimal Sample Size for Multiple Testing: the Case of Gene Expression Microarrays

Peter Müller,[1] **Giovanni Parmigiani,**[2] **Christian Robert,**[3] and **Judith Rousseau**[4]

Abstract

We consider the choice of an optimal sample size for multiple comparison problems. The motivating application is the choice of the number of microarray experiments to be carried out when learning about differential gene expression. However, the approach is valid in any application that involves multiple comparisons in a large number of hypothesis tests. We discuss two decision problems in the context of this setup: the sample size selection and the decision about the multiple comparisons. We adopt a decision theoretic approach, using loss functions that combine the competing goals of discovering as many differentially expressed genes as possible, while keeping the number of false discoveries manageable. For consistency, we use the same loss function for both decisions. The decision rule that emerges for the multiple comparison problem takes the exact form of the rules proposed in the recent literature to control the posterior expected false discovery rate (FDR). For the sample size selection, we combine the expected utility argument with an additional sensitivity analysis, reporting the conditional expected utilities, and conditioning on assumed levels of the true differential expression. We recognize the resulting diagnostic as a form of statistical power, facilitating interpretation and communication.

As a sampling model for observed gene expression densities across genes and arrays, we use a variation of a hierarchical Gamma/Gamma model. But the discussion of the decision problem is independent of the chosen probability model. The approach is valid for any model that includes positive prior probabilities for the null hypotheses in the multiple comparisons, and that allows for efficient marginal and posterior simulation, possibly by dependent Markov chain Monte Carlo simulation.

Key words: Genomic data analysis; False discovery rate; Multiple comparison.

[1]Department of Biostatistics, The University of Texas M. D. Anderson Cancer Center, Houston, TX.
[2]Department of Oncology, Biostatistics and Pathology, Johns Hopkins University, Baltimore, MD
[3]CEREMADE, Université Paris Dauphine, and CREST, INSEE, France
[4]Université Rene Descartes, Paris, and CREST, INSEE, France
The order of the authors is strictly alphabetical.

1 Introduction

We consider the problem of sample size selection for experiments involving massive multiple comparisons. Approaching the sample size question as a decision problem, we argue that a solution needs to address the choice of sample size as part of a larger decision problem, involving both the sample size decision before carrying out the experiment and the later decision about the multiple comparisons once the data has been collected. We consider loss functions that combine the competing goals of controlling false-positive and false-negative decisions. For a variety of reasonable loss functions, we show that the form of the terminal decision is the same: reject all comparisons with marginal posterior probability beyond a certain threshold. We prove a formal result about the slow rate of change of the expected utility over a range of practically relevant sample sizes; this suggests that the preposterior expected utility alone may not suffice for a decisive sample size recommendation. Motivated by this, we conclude by recommending appropriate summaries of sensitivity of the expected utility with respect to the parameters of interest. The discussion includes specific algorithms to evaluate the proposed diagnostics. With a view towards the motivating application, we propose a nonparametric probability model that allows the use of pilot data to learn about the relevant sampling distribution for the sample size decision. Formally, this amounts to using the posterior predictive distribution from the pilot data as the prior model that is used in the sample size calculation.

Our discussion is motivated by the specific problem of choosing the number of replications in microarray experiments. Gene expression microarrays are technologies for simultaneously quantifying the level of transcription of a large portion of the genes in an organism (Schena et al., 1995; Duggan et al., 1999). (For a recent review of microarray technology and related statistical methods see, for example, Kohane et al., 2002.) The range of applications is broad. Here we focus on controlled experiments that aim to search or screen for genes whose expressions are regulated by modifying the conditions of interest, either environmentally or genetically. There are a number of pressing biological questions that can be addressed using this type of genomic screening. Because microarrays are costly, the design of the experiment and choice of sample size result in a difficult trade-off between the allocation of limited research resources and statistical learning. Our approach is applicable to the process of selecting the number of biological replicates (such as the number of mice to be assigned to each group), as well as the selection of the number of technological replicates (such as the

number of separate aliquots of RNA to be extracted from two biological samples that are being compared). In our theoretical discussion, we use the term "replicate" to refer to either type. Each situation requires a different interpretation of the array-to-array variability, as well as different priors, or different pilot samples. Our illustration specifically concerns biological replicates. General issues of experimental design in microarray experiments, beyond the sample size selection considered in this article, are discussed by Kerr and Churchill (2001), Yang and Speed (2002) and Simon et al. (2002).

The general goal of the genomic screening is to discover as many as possible of the genes that are differentially expressed across the experimental conditions, while keeping the number of false discoveries at a manageable level. The consequences of a false discovery are often similar across genes. The decision-making process when selecting a sample size can benefit from explicitly acknowledging these experimental goals by following a formal decision theoretic approach. Sample size selection using decision theoretics, including the multistage nature central to our discussion, was formalized within a Bayesian framework as early as 1961 through the work of Raiffa and Schlaifer (1961). (See also Lindley, 1997 or Adcock, 1997 and references therein for discussions of sample size determination.)

Following this paradigm, we present a general decision theoretic framework for the choice of sample size for genomic screening or for use in a similar selection problem. Central to our analysis is the concept of the false-discovery rate (FDR), introduced by Benjamini and Hochberg (1995). In controlled experiments, it is plausible to assume that genes can be divided into two groups: truly altered and truly unaltered genes. For a given approach to selecting a set of putatively altered genes, the FDR is the fraction of truly unaltered genes among the genes classified as differentially expressed. Commonly used microarray software uses the FDR to guide gene selection (see, for example, Tusher et al., 2001). Applications of FDRs to microarray analysis are discussed by Storey and Tibshirani (2003). Extensions are discussed by Genovese and Wasserman (2002), who also introduce the definition of the posterior expected FDR as we use it here. We show that the decision theoretic approach leads to a multiple comparison decision of the form described in Genovese and Wasserman (2002). They focus on decision rules of the following kind. Assume that for each comparison some univariate summary statistic v_i is available. This could be, for example, a p-value or any other univariate statistic related to the comparison of interest. All comparisons with v_i beyond a certain cutoff t are considered discoveries. Central to their approach is the use of an upper bound on the FDR to calibrate that cutoff t.

In the context of microarray experiments, important initial progress towards the evaluation of sample sizes has been made by Pan et al. (2002), who developed traditional power analyses for use in the context of microarray experiments. Their modeling is realistic in that it acknowledges heterogeneity in gene-specific noise, and specifies a mixture model for regulated and unregulated genes. Further progress, however, is necessary. Pan et al. (2002) do not exploit heterogeneity in developing screening statistics, as done by hierarchical models. This can potentially underestimate the power, especially in the critical range of experiments with very few replicates. Also, their power analysis considers a single effect size for all regulated genes. Finally, explicit consideration of properties of the entire selection, such as FDR, is preferable in the context of multiple testing. Zien et al. (2002) propose an alternative approach to an informed sample size choice. They consider ROC-type curves, showing achievable combinations of false-negative and false-positive rates. Mukherjee et al. (2003) discuss sample size considerations for classification of microarray data. They assume a parametric learning curve for empirical error as a function of the sample size. Their approach is based on estimating the parameters in that learning curve. Lee and Whitmore (2002) consider an ANOVA setup, including, among other parameters, interaction effects for the gene and biologic conditions. Hypothesis testing for these interactions formalizes the desired inference about differential expression. They assume approximate normality for an estimator of these interaction effects and proceed with a conventional power analysis. Bickel (2003) proposes a framework for inference on differential gene expression that includes a loss function consisting of a payoff for correct discoveries and a cost for false discoveries. (See Section 2.1 for a definition of these events.) The net desirability function defined in Bickel (2003) is equivalent to one of the loss functions introduced in Section 2.1.

As in many traditional sample size problems, the practical use of the proposed approach will be as a decision support tool. We do not expect investigators to blindly trust the proposed solution. Rather, we envision that an investigator may be operating under budget and resource constraints that allow for a narrow range of sample size choices. The proposed methods can guide the choice within that range by informing the investigator about the likely payoffs and decision summaries.

In Section 2 we outline the decision problem and our approach to the solution in a general form, without referring to a specific probability model. In Section 3 we develop an efficient simulation approach for evaluating the required sample size selection criteria. We define a Monte Carlo simulation method that allows us to evaluate the expected false-

negative rate (FNR) and power across the sample sizes. We demonstrate that, because of their preposterior nature, the required simulations are easier and less computation-intensive compared to posterior simulation in the underlying probability model. In Section 4 we introduce a specific probability model that is used in Section 5 to show results in an example. Section 6 concludes with a final discussion.

2 The Decision Problems

To highlight the general nature of the proposed approach, we first proceed without reference to a specific probability model or comparison of interest. We let ω and y denote the model parameters and expression measurements, and let $z_i \in \{0, 1\}$ denote an indicator for the regulation of gene i. Regulation is broadly defined to include any of the typical questions of interest, such as differential expression across two conditions; time trends; sensitivity to at least one out of a panel of compounds; and so forth. We assume that the probability model includes indicators z_i as parameters, or as easily imputed latent variables. We will write y_J when we want to highlight that the data y is a function of the sample size J. We assume that the underlying probability model allows for efficient posterior simulation. Let $v_i = P(z_i = 1|y)$ denote the marginal posterior probability for the i-th comparison. Computation of v_i could involve some analytical approximations, like empirical Bayes estimates for hyperparameters, etc. In Section 4, we will introduce the probability model used in our implementation and discuss posterior inference in that model.

An important aspect of the problem is that the earlier decision about the sample size needs to take into account the later decision about gene selection. This will be either a selection (also referred to as discovery, or rejection, and denoted as $d_i = 1$ for comparison i), or not (also referred to as a negative and denoted as $d_i = 0$). Decision theoretic approaches to sample size selection assume that the investigator is a rational decision maker choosing an action that minimizes the loss of the possible consequences – averaging with respect to all the relevant unknowns (Raiffa and Schlaifer, 1961; DeGroot, 1970). At the time of the sample size decision the relevant unknowns are the data y, the indicators $z = (z_1, \ldots, z_n)$ and the model parameters ω. The relevant probability model with respect to which we average is the prior probability on (z, ω) and the conditional sampling distribution on y given (z, ω). At the time of the decision about multiple comparisons the data y is known and the relevant probability model is the posterior distribution conditional on y.

In the traditional backward induction fashion the solution proceeds by first considering the terminal multiple comparison decision of the gene selection. Knowing the optimal policy for the eventual gene selection we can then approach the initial sample size problem. It is thus natural to first discuss inference about the multiple comparison decisions d_i, $i = 1, \ldots, n$.

2.1 Terminal Decision

The choice of a decision rule for multiple comparisons is driven by the following considerations. First, the rule should have a coherent justification as the solution that minimizes the expected loss under a sensible loss function. Second, inference about the multiple comparison decision is nested within the sample size selection, making computational efficiency an important issue. In the type of experiments considered here, a relatively small number of genes are regulated, and the noise levels are relatively high. Finally, although our approach is based on joint probability models on data and parameters, i.e., in essence Bayesian, we are concerned about frequentist operating characteristics for the proposed rule. The use of frequentist properties to validate Bayesian inference is common practice in the context of medical decision making.

With these considerations in mind, we propose loss functions that have the following characteristics: they capture the typical goals of genomic screening; they are easy to evaluate; lead to simple decision rules; and can be interpreted as generalizations of frequentist error rates. We consider four alternative loss functions that all lead to terminal decision rules of the same form. We start with a notation for various summaries that formalizes the two competing goals of controlling the false-negative and false-positive decisions. Writing $D = \sum d_i$ for the number of discoveries, we let

$$\mathrm{FDR}(d, z) = \frac{\sum d_i(1 - z_i)}{D + \epsilon} \text{ and } \mathrm{FNR}(d, z) = \frac{\sum (1 - d_i) z_i}{n - D + \epsilon} \tag{1}$$

denote the realized false-discovery rate and false-negative rate, respectively. $\mathrm{FDR}(\cdot)$ and $\mathrm{FNR}(\cdot)$ are the percentage of wrong decisions, relative to the number of discoveries and negatives, respectively (the additional term ϵ avoids a zero denominator). (See, for example, Genovese and Wasserman, 2002, for a discussion of FNR and FDR.) Conditioning on y and marginalizing with respect to z, we obtain the posterior expected FDR and FNR

$$\overline{\mathrm{FDR}}(d, y) = \int \mathrm{FDR}(d, z)\, \mathrm{d}p(z \mid y) = \sum d_i(1 - v_i)/(D + \epsilon)$$

and

$$\overline{\mathrm{FNR}}(d, y) = \int \mathrm{FNR}(d, z)\, \mathrm{d}p(z \mid y) = \sum (1 - d_i) v_i / (n - D + \epsilon).$$

Let $\overline{\mathrm{FD}} = \sum d_i(1 - v_i)$ and $\overline{\mathrm{FN}} = \sum (1 - d_i) v_i$ denote the posterior expected count of false discoveries and false negatives. We consider four ways of combining the goals of minimizing false discoveries and false negatives. The first two specifications combine false-negative and false-discovery rates and numbers, leading to the following posterior expected losses:

$$L_N(d, y) = c\,\overline{\mathrm{FD}} + \overline{\mathrm{FN}},$$

and $L_R(d, y) = c\,\overline{\mathrm{FDR}} + \overline{\mathrm{FNR}}$. The loss function L_N is a natural extension of $(0, 1, c)$ loss functions for traditional hypothesis testing problems (Lindley, 1971). From this perspective the combination of error rates in L_R seems less attractive. The loss for a false discovery and false negative depends on the total number of discoveries or negatives, respectively. Alternatively, we consider bivariate loss functions that explicitly acknowledge the two competing goals, leading to the following posterior expected losses:

$$L_{2R}(d, y) = (\overline{\mathrm{FDR}}, \overline{\mathrm{FNR}}), \quad L_{2N}(d, y) = (\overline{\mathrm{FD}}, \overline{\mathrm{FN}}).$$

Using posterior expectations we marginalize with respect to the unknown parameters, leaving only d and y as the arguments of the loss function. The sample size is indirectly included in the dimension of the data vector y. For the bivariate loss functions we need an additional specification to define the minimization of the bivariate functions. A traditional approach to select an action in multicriteria decision problems is to minimize one dimension of the loss function while enforcing a constraint on the other dimensions (Keeney et al., 1976). We thus define the optimal decisions under L_{2N} as the minimization of $\overline{\mathrm{FN}}$ subject to $\overline{\mathrm{FD}} \leq \alpha_N$. Similarly, under L_{2R} we minimize $\overline{\mathrm{FNR}}$ subject to $\overline{\mathrm{FDR}} \leq \alpha_R$.

Under all four loss functions the optimal decision for the multiple comparisons takes the same form.

Theorem 1 *Under all four loss functions the optimal decision takes the form*

$$d_i = I(v_i \geq t^*).$$

The optimal cutoff t^* is $t^*_N = c/(c+1)$, $t^*_R(y) = v_{(n-D^*)}$, $t^*_{2N}(y) = \min\{s : \overline{FD}(s, y) \leq \alpha_N\}$, and $t^*_{2R}(y) = \min\{s : \overline{FDR}(s, y) \leq \alpha_R\}$, under L_N, L_R, L_{2N} and L_{2R}, respectively. In the expression for t^*_R, $v_{(i)}$ is the i-th order statistic of $\{v_1, \ldots, v_n\}$, and D^* is the optimal number of discoveries. See the proof in the appendix for a constructive definition of D^*.

The proof proceeds by straightforward algebra. (See the appendix for details.) Under L_R, L_{2N} and L_{2R}, the optimal threshold t^* depends on the observed data. The nature of the terminal decision rule d_i is the same as that in the work of Genovese and Wasserman (2002), which is a more general rule, allowing the decision to be determined by cutoffs on any univariate summary statistic v_i. Using $v_i = P(z_i = 1|y)$ is a special case.

For simplicity we will focus on $L = L_{2N}$ only in the upcoming discussion (omitting the subscript $2N$ to simplify the notation). In Section 5.3 we will revisit the other three loss functions. Also, by a slight abuse of the notation, we write $d = t$ for the decision rule $d_i = I(v_i \geq t)$. Finally, we note that not all loss functions lead to decisions $d_i = I(v_i \geq t)$. For example, assuming a loss of a false negative that depends on the true level of differential expression would lead to different rules. One could argue that discovering a gene that shows a very small differential expression in a given experiment may not be as interesting as discovering one that shows a major change in its expression.

2.2 Sample Size

2.2.1 Marginal FN and FNR

In contrast to the terminal decision of the gene selection, which is made conditional on the observed data, the sample size is decided prior to conducting the experiment. Thus we now consider the marginal prior mean of the proposed loss functions, also known as the preposterior expected loss (Raiffa and Schlaifer, 1961), after substituting the optimal terminal decision for the multiple comparison decision. The relevant loss function $L^m(J)$ for the sample size selection is

$$L^m(J) \;=\; E[\min_d\{L(d,y)\}] \;=\; E[\min_d\{\overline{\mathrm{FN}}(d,y_J) \;|\; \overline{\mathrm{FD}} \;\leq\; \alpha_N\}] \;=\; E[\overline{\mathrm{FN}}(t^*(y_J),y_J)\}] \quad (2)$$

The conditioning bar in the nested optimization indicates that the minimization is subject to the bound on $\overline{\mathrm{FD}}$. The sequence of alternating between the expectation and the optimization is characteristic for sequential decision problems. (See, for example DeGroot, 1970, and Berger, 1985, for a discussion of sequential decision problems in general.) The expectation is determined with respect to the prior probability model on the data y_J under a given sample size J. The only argument left after determining the expectation and the minimization is the sample size J. The nested minimization with respect to d is the solution of the multiple comparison problem. It reduces to $d_i = I\{v_i > t^*(y_J)\}$. We will denote the preposterior

expected FN by $\overline{\text{FN}}_m(J) = E[\overline{\text{FN}}(t^*(y_J), y_J)]$, with the bound on $\overline{\text{FD}}$ being implicit in the definition of $t^*(y_J)$. Thus we could alternatively write (2) as $L^m(J) = \overline{\text{FN}}_m(J)$. We use analogous definitions for $\overline{\text{FNR}}_m$, $\overline{\text{FDR}}_m$ and $\overline{\text{FD}}_m$. The latter is equal to α_N by definition of $t^*(y_J)$.

In Section 3, we will introduce a simulation-based algorithm for a practical evaluation of the expectation and nested optimization in (2). Using the algorithm one could evaluate and then plot the marginal expected utility, i.e., $\overline{\text{FN}}_m$, against J to select a sample size. At this time one could add a (deterministic) sampling cost, if desired. But in practical application this would require the difficult choice of a relative weight for the sampling cost versus an inference loss. Alternatively, we take a goal-oriented perspective and use the plot of $L^m(J)$ versus the sample size J to find a sample size for any set goal of $L^m(J)$.

However, in doing so a practical complication arises. For relevant sample sizes of $J \leq 20$ the decrease in $L^m(J)$ is too flat to allow a conclusive choice of sample size. (See Figure 1a for an example.) The slow rate of decrease is a general feature of $\overline{\text{FNR}}$ and $\overline{\text{FN}}$.

Theorem 2 *Consider the three loss functions $L = L_{2N}$, L_N, and L_{2R}. The false-negative rate and counts of $\overline{FNR}$ and $\overline{FN}$ decrease asymptotically as*

$$\overline{FNR}(t^*, y_J) = O_P(\sqrt{\log J/J}),$$

where t^* generically indicates the optimal cutoff under each of the three loss functions, and

$$\overline{FN}(t^*, y_J) = O_P(n\sqrt{\log J/J}).$$

For both results we have to assume that the genes are "randomly chosen," i.e., that a fraction p, $0 < p < 1$, of the genes are truly differentially expressed. In other words, we assume that the level of differential expression is neither always equal to zero (or very small), nor always different from zero. A formal argument is given in the appendix. The argument starts with a Laplace approximation for $v_i = P(z_i = 1 \mid y_J)$. Based on this approximation we show that only genes with a low or zero differential expression are included in the negative set, i.e., the set of genes with $d_i = 0$. We then approximate the average in $\overline{\text{FNR}}$ (or $\overline{\text{FN}}$) by an integral, exploiting the fact that these are genes with small differential expressions. Finally, we recognize that the integral expression is on the order of $\sqrt{\log J/J}$.

2.2.2 Conditional Preposterior Expected Utility

The relatively flat nature of the expected utility $L^m(J)$ does not allow for a conclusive sample size recommendation. A natural next step is to investigate the expected utility as a function of an assumed true value for some key parameters of the probability model. Specifically, we assume that the probability model includes a parameter ρ_i that represents the level of differential expression for gene i, with $\rho_i = 0$ if $z_i = 0$ and $\rho_i \neq 0$ when $z_i = 1$. For example, in the probability model discussed in Section 4 we would use $\rho = \log \theta_1/\theta_0$. We thus proceed by considering the expected utility, conditional on an assumed true level of ρ_i. Recall the definition of FN as the false-negative count. Conditioning on ρ_i only changes the term that is related to gene i. For a large n, conditioning ρ_i for one gene leads to only negligible changes in inference for other genes (*a posteriori*, as well as *a priori*). Finally, note that for $\rho_i \neq 0$ gene i only contributes to FN, not to FD. Thus, we can characterize the conditional expected utility as a function of ρ_i by considering the relevant term in $\overline{\text{FN}}_m$:

$$\beta(J, \rho_i) = P\{v_i(y_J) > t^* \mid \rho_i\} = \int I(v_i(y_J) > t^*) \, \mathrm{d}p(y_J \mid \rho_i), \tag{3}$$

writing $v_i(y_J)$ to highlight the nature of the marginal posterior probability v_i as a function of the data. The expectation is determined with respect to the joint probability model on data y. In particular, the expected utility appropriately adjusts for dependencies, uncertainties on other model parameters, and the entire process of finding and applying $t^*(y_J)$. Assuming that the genes are *a priori* exchangeable, the marginal expectation is the same for all i, allowing us to drop the i subindex.

The diagnostic $\beta(J, \rho)$ has interesting interpretations. We define it as the term in the conditional expected utility that varies as a function of ρ_i. Our main reason to propose it is due to its link with the traditional notion of power. The definition of β is essentially the power to test one hypothesis in the multiple comparison decision, although with an added twist of marginalizing it with respect to all other parameters. To simplify the terminology, we will refer to $\beta(J, \rho)$ as "power," with the understanding that the definition includes the mentioned marginalizations. Figure 2 shows a typical example.

Thus the following modification to the approach outlined in Section 2.2.1 emerges. The investigator fixes a minimum level of differential expression that is of interest in the given experiment, and the desired probability of discovering a gene that is differentially expressed at that level. Inspection of a power plot like Figure 2, together with $\overline{\text{FN}}_m$ and $\overline{\text{FD}}_m$ in the marginal loss function allows the investigator to obtain an informed sample size choice. The

$\overline{\text{FD}}_m$ and $\overline{\text{FN}}_m$ plots, and plots of related summaries $\overline{\text{FDR}}_m$ and $\overline{\text{FNR}}_m$, add the experiment-wise dimension to the marginal, comparison-wise summary that provided by the power plot. It tells the investigator how many false negatives might be missed, averaging over the range of likely differential expression levels and summing over all genes. Computation of $\beta(J, \rho)$ is achieved within the same preposterior simulation that is used to evaluate $\overline{\text{FN}}_m$ and $\overline{\text{FD}}_m$.

3 Simulation

The described approach to sample size selection involves several calculations that are typically analytically intractable. Details depend on the specific probability model. Often the posterior mean probabilities v_i, the threshold $t^*(y_J)$, and the expected FNR are not available in closed form. However, all can be computed by Monte Carlo simulation. In this section we describe how such Monte Carlo simulation is implemented. Before we give a step-by-step algorithm, we introduce the notations and review the important steps in the algorithm in words. The discussion is still possible without reference to a particular probability model.

For a given sample size J we simulate data $y_J \sim p(y_J)$. Simulating from the marginal $p(y_J) = \int p(y_J \mid \omega, z)\, \mathrm{d}p(\omega, z)$ is conveniently implemented by first generating "true" parameters (ω, z) from the prior, and then generating from the assumed sampling model $p(y_J \mid \omega, z)$ given the simulated parameter. To distinguish this prior simulation from a posterior MCMC simulation that will be required later in the algorithm, we mark the realizations of this prior simulation by a superindex as in ω^o, etc.

For each simulated data set y_J we compute the marginal posterior probabilities $v_i = p(z_i = 1 \mid y_J)$ and evaluate $\overline{\text{FD}}(t, y_J)$ and $\overline{\text{FDR}}(t, y_J)$ on a grid over t to find the optimal cutoff $t^*(y_J)$. Plugging in the optimal cutoff t^* in $d_i = I(v_i > t)$, we evaluate the posterior means $\overline{\text{FN}}(t^*, y_J)$ and $\overline{\text{FNR}}(t^*, y_J)$. Averaging over y_J by (independent) Monte Carlo simulation, with repeated simulation of $y_J \sim p(y_J)$, we compute

$$L^m(J) = E_{y_J}\left\{\overline{\text{FN}}(t^*, y_J)\right\}. \tag{4}$$

The nonlinear thresholding $d_i = I(v_i > t^*)$ implicit in the definition of $\overline{\text{FN}}$ hinders the interpretation of (4) as one joint integral with respect to the joint distribution $p(\omega, y_J)$ on parameters and data. Instead we need to proceed with two nested steps, as described above. Finally, evaluating (4) across J we find the sample size J^*, which allows us to achieve a desired marginal expected loss.

The information in the marginal loss $L^m(J)$ is supplemented by power curves $\beta(J, \rho)$. Power $\beta(J, \rho)$ as defined in (3) is a summary of the preposterior expected utility. It is evaluated as part of the same simulation described above to find L^m. For each simulated experiment we record (J, ρ_i^o, v_i, d_i), $i = 1, \ldots, n$. Here ρ_i^o is the true simulated level of the differential expression. The recorded simulations are then arranged by J to compute $\overline{\text{FN}}_m$ as described above. Arranging the same simulations by J and ρ (possibly on a grid) we estimate $\beta(J, \rho)$, which can be summarized in plots like those in Figure 2.

Implementation is facilitated by several simplifications that increase the computational efficiency. First, we will use common random numbers across J, in the following sense. We consider sample sizes on the interval $J_0 \leq J \leq J_1$. We start by generating one large sample y_{J_1}, and use appropriate subsamples $y_J \subset y_{J_1}$ to compute $\overline{\text{FN}}_m(J)$, $\overline{\text{FD}}_m(J)$, $\overline{\text{FNR}}_m(J)$ and $\overline{\text{FDR}}_m(J)$, for J over a grid $J_0 \leq J \leq J_1$. Using the common underlying data reduces sampling variation across J.

Another simplification arises in the setup of the posterior simulations required to evaluate the posterior expected $\overline{\text{FN}}(t, y_J)$ and $\overline{\text{FD}}(t, y_J)$. Both require posterior simulation $\omega \sim p(\omega|y_J)$ by MCMC. In the context of the preposterior simulation we can start the MCMC at the true parameter values ω^o used to simulate the data y_J. Details are explained in the step-by-step algorithm below.

Finally, when computing $L^m(J)$, we borrow strength across different sample sizes. Instead of averaging separately for each J the computed values $L(t^*, y_J)$ for that J, we proceed as follows. Consider a scatterplot of all pairs $(J, L(t^*, y_J))$. We fit a smooth curve $\widehat{L}^m(J)$ through all points of the scatterplot. This formalizes the borrowing strength across different sample sizes J, exploiting the fact that $L^m(J)$ is smooth across J. In fact, we recommend enforcing the smooth fit $\widehat{L}^m$ to be monotone, decreasing, and to follow the $(\log J/J)$ asymptotics. We used a least squares fit of a linear regression of the observed $\overline{\text{FN}}(t^*, y_J)$ values on $\sqrt{\log J/J}$. For comparison, we fit a smoothing spline without any such constraints. The spline fit is practically indistinguishable from the simple regression, validating the use of the asymptotic law for the curve fitting. (See Section 5.)

Algorithm 1: Sample Size Determination

1. *Simulation:* Loop over repeated simulations $y_{J_1} \sim p(y_{J_1})$.

    1.1. *Prior simulation* $(\omega^o, z^o) \sim p(\omega, z)$.

1.2. *Data simulation:* $y_{J_1} \sim p(y_{J_1} \mid \omega^o, z^o)$.

We simulate data for the largest sample size J_1 considered in the design.

1.3. *Loop over J:* loop over a grid of sample sizes $J = J_1, \ldots, J_0$.

Let $y_J \subset y_{J_1}$ denote the size J subset of the maximal data set.

1.3.1. *Posterior simulation* $\omega \sim p(\omega|y_J)$.

a. Initialize MCMC posterior simulation with $(\omega, z) = (\omega^o, z^o)$.

b. Simulate S transitions of the posterior MCMC.

1.3.2. *Posterior probabilities:*

Compute $v_i = P(z_i = 1|y_J)$ as the appropriate ergodic average and evaluate

$$\overline{\text{FD}}(t, y_J) = \sum (v_i > t)\,(1 - v_i) \text{ and } \overline{\text{FDR}}(t, y_J) = \overline{\text{FD}}(t, y_J)/(D + \epsilon)$$

for $t \in \{v_1, \ldots, v_J\}$ and find the optimal cutoff $t^*(y_J)$.

Record $\big(J, \overline{\text{FD}}(t^*, y_J), \overline{\text{FN}}(t^*, y_J), \overline{\text{FDR}}(t^*, y_J), \overline{\text{FNR}}(t^*, y_J)\big)$.

1.3.3. *Power:* Let $d_i = I(v_i > t^*)$ and record the triples (J, ρ_i^o, d_i).

2. *Curve Fitting of Monte Carlo Experiments:*

2.1. Preposterior expectations $L^m(J)$, $\overline{FD}_m$, $\overline{FN}_m$, $\overline{FDR}_m$ *and* $\overline{FNR}_m$*:* For each of the last four quantities fit a curve through the observed pairs $(J, \overline{\text{FN}})$, etc. Use the asymptotic expressions reported in Theorem 1 to guide the curve fitting.

2.2. Power v_i*:* Use the triples (J, ρ_i^o, d_i) computed in step 1 to estimate $\beta(J, \rho)$.

3. *Optimal sample size:*

Use $\widehat{L}^m(J)$ and power curves as in Figure 2 to make an informed sample size choice.

4 The Probability Model

Our approach to sample size selection assumes an encompassing probability model that specifies a joint distribution across comparisons and across repeated experiments. In general, the model should be sufficiently structured and detailed to reflect the prior expected levels of noise, a reasonable subjective judgment about the likely numbers of differentially expressed genes, and some assumption about dependencies, if relevant. It should also be easy to include prior data when available.

The design argument can be developed with a simplified model, ignoring all details of the data cleaning process, including the spatial dependence of measurement errors across the microarray, correction for misalignments, etc. While such detail is critical for the analysis of the observed microarray data, it is an unnecessary burden for the design stage. The variability resulting from preprocessing and normalization can be subsumed as an aggregate in the prior description of the expected noise. In the following discussion we thus assume that the data are appropriately standardized and normalized and that the noise distribution implicitly includes the consideration of those processes. (See, for example, Tseng et al., 2001; Baggerly et al., 2001; or Yang et al., 2002, for a discussion of the process of normalization.)

For the implementation in the example we choose a variation of the model introduced in Newton et al. (2001) and Newton and Kendziorski (2003). We focus on the comparison of two conditions and assume that data will be available as arrays of appropriately normalized intensity measurements X_{ij} and Y_{ij} for gene i, $i = 1, \ldots, n$, and experiment j, $j = 1, \ldots, J$, with X and Y denoting the intensities in the two conditions.

Newton et al. (2001) propose a hierarchical Gamma/Gamma model. The model starts by assuming that the observed intensities are sampled from Gamma distributions, with a conjugate Gamma prior on the scale parameters. The model includes a positive prior probability mass for matching the means of the Gamma distribution for the same gene under the two conditions of interest. For the purpose of the sample size design we extend the model to multiple experiments, $j = 1, \ldots, J$. We assume a Gamma sampling distribution for the observed intensities X_{ij}, Y_{ij} for gene i in sample j,

$$X_{ij} \sim \mathrm{G}amma(a, \theta_{0i}) \text{ and } Y_{ij} \sim \mathrm{G}amma(a, \theta_{1i}). \tag{5}$$

The scale parameters are gene-specific random effects $(\theta_{0i}, \theta_{1i})$, with positive prior probability for a tie,

$$Pr(\theta_{0i} = \theta_{1i}) = Pr(z_i = 0) = p.$$

Conditional on latent indicators z_i for differential gene expression, $z_i = I(\theta_{0i} \neq \theta_{1i})$, we assume conjugate Gamma random effects distributions

$$\begin{aligned} \theta_{0i} &\sim \mathrm{G}amma(a_0, \nu) \\ (\theta_{1i}|z_i = 1) &\sim \mathrm{G}amma(a_0, \nu) \text{ and } (\theta_{1i}|z_i = 0) \sim \mathbb{I}_{\theta_{0i}}(\theta_{1i}). \end{aligned} \tag{6}$$

The model is completed with a prior $p(\eta)$ for the parameters $\eta = (a, a_0, \nu, p)$. In the implementation for the example in Section 5 we fix ν. We assume *a priori* independence and use

marginal Gamma priors for a_0 and a, and a conjugate Beta prior for p. As in Newton et al. (2001), the above model leads to closed-form marginal likelihoods $p(X_i, Y_i \mid z_i = 0, \eta)$ $p(X_i, Y_i \mid z_i = 1, \eta)$ and $p(X_i, Y_i \mid \eta)$ after integrating out θ_{1i}, θ_{0i}; but which are still conditional on $\eta = (p, a, a_0)$. This greatly simplifies the posterior simulation.

We add two more generalizations to the model. First, we want to modify the model to allow the use of a pilot data set to learn about the sampling distribution of the observed gene expressions across genes and repeated samples. We envision a system where the investigator collects some pilot data (on control tissue) before going through the sample size argument. These pilot data could then be used to learn about the important features of the sampling distribution. If the observed pilot data can be adequately fit by the marginal model $p(X_i|z_i = 0)$ under the Gamma/Gamma hierarchical model, then the sample size design should proceed as before. If, however, the pilot data show evidence against the Gamma/Gamma model, then the system should estimate a model extension and proceed with the extended model. A convenient way to achieve the desired extension is a scale mixture extension of the basic model (5). In particular, we assume

$$X_{ij} \sim \int Ga(a, \theta_{0i}\, r_{ij})\ \mathrm{d}p(r_{ij} \mid w, m) \text{ and } Y_{ij} \sim \int Ga(a, \theta_{1i}\, s_{ij})\ \mathrm{d}p(s_{ij} \mid w, m) \qquad (7)$$

where $p(r \mid w, m)$ is a discrete mixing measure with $P(r = m_k) = w_k$ $(k = 1, \ldots, K)$. Locations $m = (m_1, \ldots, m_K)$ and weights $w = (w_1, \ldots, w_K)$ parameterize the mixture. To center the mixture model at the basic model, we fix $m_1 = 1.0$ and assume a high prior probability for a large weight w_1. We use the same mixture for s_{ij}, $P(s_{ij} = m_k) = w_k$. The model is completed with $m_k \sim Ga(b, b)$, $k > 1$ and a Dirichlet prior $w \sim Dir_K(M \cdot W, W, \ldots, W)$. Selecting a large factor M in the Dirichlet priors assigns a high prior probability for a large w_1. By assuming a dominating term with $m_1 = 1.0$ and $E(m_2) = \ldots = E(m_K) = 1$, we allocate a large prior probability for the basic model and maintain the interpretation of $\rho_i = \theta_{0i}/\theta_{1i}$ as the level of differential expression.

A concern related to microarray data experiments prompts us to introduce a further generalization to allow for the occasional presence of slides that are outliers compared to the other arrays in the experiment. This happens for reasons unrelated to the biologic effect of interest, but needs to be accounted for in the modeling, nevertheless. We achieve this by adding a second mixture to (7)

$$(X_{ij}|r_{ij}, g_j) \sim Ga(a, \theta_{0i}\, g_j\, r_{ij}) \text{ and } (Y_{ij}|s_{ij}, g_j) \sim Ga(a, \theta_{1i}\, g_j\, s_{ij}), \qquad (8)$$

with an additional slide-specific scale factor g_j. Paralleling the definition of $p(r_{ij} \mid w, m)$, we use a finite discrete mixture $P(g_j = m_{gk}) = w_{gk},\ k = 1, \ldots, L$ with a Dirichlet prior $(w_{g1}, \ldots m_{gL}) \sim Dir_L(M_g \cdot W_g, W_g, \ldots, W_g)$, $m_{g1} = 1$ and $m_{gk} \sim Ga(b_g, b_g)$ for $k = 2, \ldots, L$.

An important feature of the proposed mixture model is its computational simplicity. We will proceed in two stages. In a first stage we use the pilot data to fit the mixture model. Let $X^o_{ij},\ j = 1, \ldots, J^o$, denote the pilot data. We will use posterior MCMC simulation to estimate the posterior mean model. This is done once, before starting the optimal design. Posterior simulation in mixture models like (8) is a standard problem. We include reversible jump moves to allow for random size mixtures (Green, 1995).

We then fix the mixture model at the posterior modes $\widehat{K}$ and $\widehat{L}$, and the posterior means $(\bar{w}, \bar{m}, \bar{w}_g, \bar{m}_g) = E(w, m, w_g, m_g \mid X^o, \widehat{K}, \widehat{L})$. We proceed with the optimal sample size approach, using model (8) with the fixed mixtures. The procedure, including all posterior and marginal simulation, is done exactly as before, with only one modification. We add a step to impute r_{ij}, s_{ij} and g_j. Conditional on (r_{ij}, s_{ij}, g_j), we replace X_{ij} by $X_{ij}/(r_{ij}\, g_j)$ and Y_{ij} by $Y_{ij}/(s_{ij}\, g_j)$. Everything else remains unchanged. Updating the mixture variables r_{ij}, s_{ij} and g_j is straightforward. The following algorithm summarizes the proposed approach with the pilot data.

Algorithm 2: Sample Size Determination with Pilot Data

1. *Pilot data:* Assume pilot data $X^o = \{X^o_{ij}, i = 1, \ldots, n, j = 1, \ldots, J^o\}$, from control tissue is available.

2. *Mixture model:* Estimate the mixture model and report the posterior modes $(\widehat{K}, \widehat{L})$, and the conditional posterior means $(\bar{w}, \bar{m}, \bar{w}_g, \bar{m}_g) = E(w, m, w_g, m_g \mid X^o, \widehat{K}, \widehat{L})$. Both are computed by posterior MCMC simulation for the mixture model (8).

3. *Optimal Sample Size:* Proceed as in Algorithm 1, replacing X_{ij} with $X_{ij}/(r_{ij}g_j)$ and Y_{ij} by $Y_{ij}/(s_{ij}g_j)$, and adding an additional step in the posterior MCMC to update the mixture indicators r_{ij} and s_{ij} (Step 1.2. in Algorithm 1).

   The indicators are initialized with the (true) values from the data simulation. The mixture model parameters remain fixed at $(\bar{w}, \bar{m}, \bar{w}_g, \bar{m}_g, \widehat{K}, \widehat{L})$.

Rescaling with the iteratively updated latent scale factors $r_{ij}g_j$ and $s_{ij}g_j$ formalizes the use of the pilot data to inform the sample size selection by changing the prior simulation (as in

Algorithm 1) to the preposterior simulation, conditional on the pilot data.

5 Example

We analyze the data reported in Richmond et al. (1999). The data are also used as an illustration in Newton et al. (2001). We use the control data to plan for a hypothetical future experiment.

5.1 Implementation

We proceed as proposed in Algorithm 2. First, we estimate the mixture model (8), using the available control data as a pilot data set X^o. Estimation of (8) is implemented as a Markov chain Monte Carlo posterior simulation with reversible jump (RJ) moves. We use split-merge moves (Richardson and Green, 1997) for both mixtures defined in (8). Recall that the mixtures are defined with respect to the discrete mixing measures $p(r_{ij} \mid w, m)$ and $p(g_j \mid w_g, m_g)$. The third mixture, with respect to s_{ij}, does not appear in the model since the pilot data includes only the control data. We find the posterior mode for the size of the mixture models at $\widehat{K} = 3$ and $\widehat{L} = 2$.

To define the probability model for the design calculations, we fix $K = 3$ and $L = 2$ and set the mixture model parameters (m, w, m_g, w_g) at their posterior means (conditional on the fixed size of the mixture). Maintaining the randomness of the mixture parameters in the design model would not significantly complicate the procedure, but it would also not contribute much to the final inference. Implementing Algorithm 1, we compute the expected losses, and power $\beta(J, \rho)$ across a grid of sample sizes J.

Algorithm 1 is implemented as three nested loops. The outer loop is simply a repeated simulation from model (8), with fixed mixture of gamma priors for the scale factors r_{ij}, s_{ij} and g_j. We start by generating the hyperparameters $\eta = (a, a_0, p)$ for the prior model, given in (5) through (8) (Step 1.1 of Algorithm 1). Let $Ga(\alpha, \beta)$ denote a Gamma distribution with a shape parameter α and a mean of α/β. We use a $Ga(2, 2)$ prior for a, a $Ga(12, 1)$ prior for a_0, and a $Be(1, 10)$ Beta prior for p. We include the additional prior constraints $a < 1$, $a_0 < 1$ and $0.01 < p < 0.15$. Next, we generate indicators z_i and random effects $(\theta_{0i}, \theta_{1i})$, $i = 1, \ldots, n$. Simulation for the outer loop is concluded by simulating hypothetical data (X_{ij}, Y_{ij}), $i = 1, \ldots, n$, $j = 1, \ldots, J_1$ (Step 1.2).

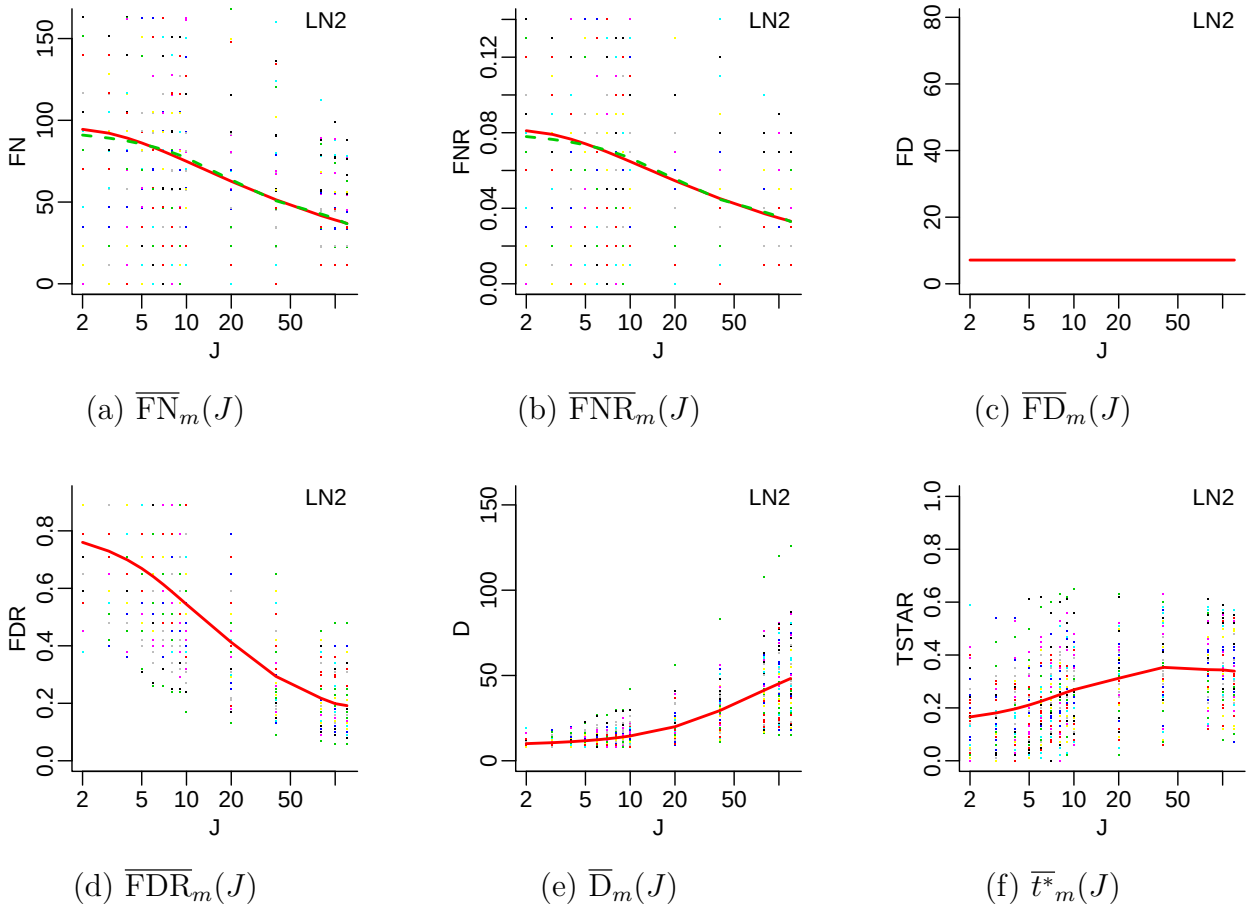
We then proceed with the second loop, nested within the first, by iterating over $J = J_1, \ldots, J_0$ (Step 1.3). For each J we generate a posterior Monte Carlo sample from $p(\omega \mid y_J)$. This is achieved by the third nested loop (Step 1.3.1), which implements a posterior MCMC simulation. Posterior simulation is initialized with the known true parameter values (saved from Step 1.1). In each iteration of the MCMC simulation we update r_{ij}, s_{ij}, g_j and the hyperparameters (a, a_0, p). The first three steps are draws from the multinomial complete conditional posterior for the respective indicators. The last three steps are implemented as random walk Metropolis-Hastings steps to update the hyperparameters. The random walk proposals are generated from a truncated univariate normal centered at the current values of the respective parameter, with normal standard deviations of 0.05, 0.1 and 0.05 for a, a_0 and p, respectively. Implementation of the MCMC is greatly simplified by noting that $p(\omega \mid y_J)$ can be analytically marginalized with respect to the random effects θ_{0i}, θ_{1i} and z_i. (See Newton et al., 2001, for a statement of the marginal likelihood.) At the end of each sweep of the posterior MCMC, we compute the posterior probabilities of the differential expression $p(z_i = 1 \mid \eta, r, g, s, y_J)$.

Upon completion of the innermost loop, we use ergodic averages of the conditional probabilities $p(z_i = 1 \mid \eta, r, g, s, y_J)$ to approximate $v_i = P(z_i = 1 \mid y_J)$ (Step 1.3.2). Using the marginal posterior probabilities v_i, we then evaluate the posterior false-discovery and false-negative counts and rates and corresponding decisions d_i and record them for later use. We also record the triples (J, ρ_i^o, d_i) (Step 1.3.3).

Upon completion of the outer loop, we summarize the observed $\overline{\text{FDR}}, \overline{\text{FD}}, \overline{\text{FNR}}, \overline{\text{FN}}$ and the triples (J, ρ_i^o, d_i). For example, the sample average over the simulated values of $\overline{\text{FDR}}$ under a given sample size J provides a Monte Carlo estimate of the preposterior expected $\overline{\text{FDR}}_m(J)$. The fraction of $d_i = 1$ under a given sample size and the true effect $\rho_i^o = \rho$ provides a Monte Carlo estimate for $\beta(J, \rho)$. To ensure sufficient Monte Carlo sample size, the latter is done for a grid on ρ.

5.2 Results

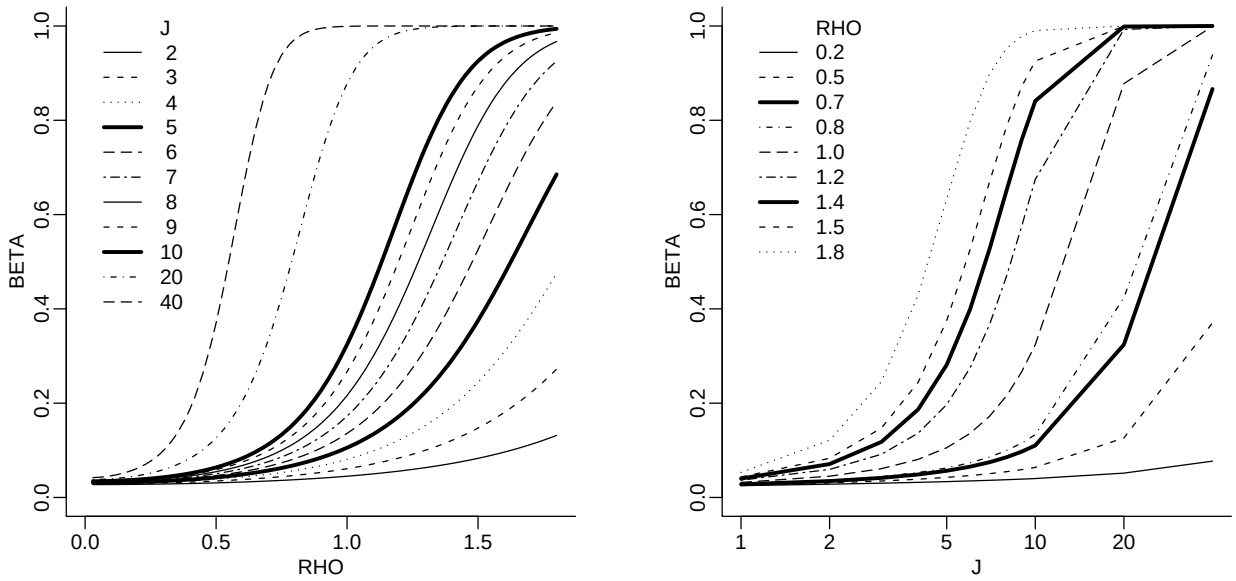
Recall that $\overline{\text{FN}}_m(J)$ and $\overline{\text{FNR}}_m(J)$ denote the preposterior expectations of $\overline{\text{FN}}$ and $\overline{\text{FNR}}$. We will use analogous notations $\overline{\text{D}}_m(J)$ and $\overline{t^*}_m(J)$ to denote the preposterior expectations of the number of discoveries D and the threshold t^*, computed under the loss L and sample size J, defined analogously to $\overline{\text{FNR}}_m$ and $\overline{\text{FN}}_m$. All inference was computed in one run of



(a) $\overline{\mathrm{FN}}_m(J)$ (b) $\overline{\mathrm{FNR}}_m(J)$ (c) $\overline{\mathrm{FD}}_m(J)$

(d) $\overline{\mathrm{FDR}}_m(J)$ (e) $\overline{\mathrm{D}}_m(J)$ (f) $\overline{t^*}_m(J)$

Figure 1: L_{2N}: Expected loss and other relevant summaries. Panel (a) shows the expected loss function $L^m = \overline{\mathrm{FN}}_m$ (FN in the plot). Panels (b) through (e) plot the expected false-negative rate $\overline{\mathrm{FNR}}_m$ (FNR in the plot), false-discovery count $\overline{\mathrm{FD}}_m$ (labeled FD) and rate $\overline{\mathrm{FDR}}_m$ (labeled FDR), the number of discoveries D and the threshold t^* (TSTAR). In each panel, the dots show the values recorded in each of the simulations (in panel (d) some dots fall outside the range of the figure). The false-discovery count $\overline{\mathrm{FD}}_m$ is fixed by design, leading to an increasing number of discoveries $\overline{\mathrm{D}}_m$. The dashed curves for $\overline{\mathrm{FNR}}_m$ and $\overline{\mathrm{FN}}_m$ (almost indistinguishable from the solid line) show an alternative curve fit. See the text for an explanation of the curve fit.



(a) $\beta = E_{y_J}\{P(d = 1|\rho, y_J)\}$ against ρ (by J)

(b) β against J (by ρ)

Figure 2: Power β (labeled BETA in the plot) against true effect ρ (labeled RHO) and against sample size J. In panel (b) power curves for two-fold ($\rho = \log 2$) and four-fold over expression ($\rho = \log 4$) are highlighted in bold. Power $\beta(J, \rho)$ is defined in (3) as the average posterior probability of discovery, conditional on the true level of differential expression $\rho_i = \log(\theta_{0i}/\theta_{1i})$.

Algorithm 1, collecting the necessary Monte Carlo averages for all summaries.

Figure 1 shows the expected loss $L^m(J) = \overline{\text{FN}}_m(J)$, and other summaries under the loss function $L = L_{2N}$. We set the threshold for $\overline{\text{FD}}$ as $\alpha_N = 7.1$. It is chosen to match a bound $\overline{\text{FDR}} \leq \alpha_R$ for $\alpha_R = 40\%$. The value is computed as $\alpha_N = 0.1\ n\bar{p}\alpha_R/(1 - \alpha_R)$, under the assumption that 10% of the true differential expressions are discovered. Under L_{2N}, the false-discovery count $\overline{\text{FD}}$, and thus also the preposterior expectation $\overline{\text{FD}}_m$, is fixed by definition at $\overline{\text{FD}} = \alpha_N$. To maintain the fixed $\overline{\text{FD}}$ the procedure has to eventually start lowering the threshold t^* to reject comparisons with increasingly lower posterior probabilities of differential expression. The estimated curves $\overline{\text{FNR}}_m(J)$ and $\overline{\text{FN}}_m(J)$ are derived by fitting a linear model with predictor $\sqrt{\log(J+1)/(J+1)}$ to the observed pairs $(J, \overline{\text{FNR}}(t^*_N, y_J))$. This is motivated by the asymptotic results of Theorem 1 (with the offset +1 to avoid a singularity at $J = 0$). For comparison we estimate the same curve using a cubic smoothing spline, using the `smoothing.spline` function in R with default parameters. The corresponding curves

for $\overline{\text{FNR}}_m$ and $\overline{\text{FN}}_m$ are shown as dashed lines. For $\overline{\text{FDR}}_m$, $\overline{\text{FD}}_m$, $\overline{\text{D}}_m$ and $\overline{t^*}_m$ we use cubic smoothing splines to estimate the mean value as a function of J.

The relatively flat nature of $\overline{\text{FN}}_m$ and $\overline{\text{FD}}_m$ does not allow a conclusive sample size choice. We propose to consider additional power curves, as defined in (3). Figure 2 shows $\beta(J, \rho)$ as a function of ρ and J. Panel (a) plots the power against the assumed true level of differential expression ρ, with a separate curve for each sample size J. Figure 2b plots the same summary against the sample size J, arranged by the level of differential expression ρ. In practice a sample size argument would then proceed as follows. First, the investigator determines a minimum level of differential expression that would be considered biologically meaningful, say two-fold expression at $\rho = \log 2$. Using a pilot data set, we proceed with Algorithm 2 to compute the expected FNR, FN, and power across the sample sizes. Inspection of the power plot for the level ρ of interest, together with the FNR and FN plots informs the investigator about the minimum sample size needed to achieve the desired power and/or error rates.

5.3 Alternative Loss Functions

While the general nature of the loss function as trading off false positives and false negatives is natural, the specific form of combining them is less clear. A strength of the proposed approach is that it allows us to evaluate the alternative loss functions that combine the basic summaries FN, FD, FDR, and FNR, in different ways with minimal computational effort. We discuss the results for three alternative loss functions, L_{2R}, L_N and L_R (introduced in Section 2.1).

We already established (in Theorem 1) the fact that there is a common optimal terminal decision rule under all four loss functions. This allows us to easily adapt Algorithm 1 for all four loss functions. The only required change is in step 1.3.2. For L_{2R}, L_N and L_R different definitions of t^* are required. The rest of the algorithm proceeds unchanged. It is possible to use a single implementation of Algorithm 1, recording $J, t^*, \overline{\text{FN}}, \overline{\text{FD}}, \overline{\text{FNR}}$ and $\overline{\text{FDR}}$ for all four loss functions. Appropriate summaries of the saved Monte Carlo samples allow us to produce summaries such as those shown in Figure 1 for all four loss functions, based on a single run of Algorithm 1.

An important implication of the different strategies for choosing the cutoff is the nature of $\overline{\text{FDR}}$ as a function of the sample size J. Under L_{2R} it remains, by design, constant over J. This has awkward implications. Imagine the asymptotic case with a large sample size

when the true z_i are practically known. To achieve the desired $\overline{\text{FDR}}$ we have to knowingly flag some genes as differentially expressed even when $v_i \approx 0$. By the same argument the loss L_{2N} leads to similar asymptotics. However, fixing the count $\overline{\text{FD}}$ instead of the rate $\overline{\text{FDR}}$ slows the awkward decrease in the threshold that is required to maintain the fixed false-discovery rate under L_{2R}. The number of discoveries is still increasing, but starts at a higher level and avoids the steep increase seen under L_{2R}. In contrast, under L_N the cutoff t is fixed across the sample size, leading to a vanishing $\overline{\text{FDR}}$ in the limit as $J \to \infty$, due to posterior consistency. However, these problems might only be of asymptotic relevance and not of concern for moderate sample sizes. Apart from these concerns, all three loss functions, L_{2R}, L_N and L_{2N}, are very similar with regard to their properties, nature of the inference, and implementation details.

Inference under L_R leads to different behaviors among the various summaries. In contrast to the other three loss functions the optimal decision under L_R does not constrain an error, the error rate or the cutoff. Considering plots similar to those in Figure 1, we find that at an intermediate sample size the threshold t^* swiftly moves from an initial value of $t^* \approx 0$ to the other extreme of $t^* \approx 1$. This unintuitive behavior confirms our initial reservations against L_R for including penalties for a false discovery and false negative that depend on the total number of discoveries or negatives, respectively.

In summary, inference under the four loss functions differs in how the competing goals of reducing false positives and false negatives are balanced. The loss functions L_{2R}, L_{2N} and L_N define the trade-off implicitly by fixing $\overline{\text{FDR}}$, $\overline{\text{FD}}$, and t^*, respectively. Under L_R the trade-off is explicitly included as a coefficient in the loss function. The constraint on $\overline{\text{FDR}}$ under L_{2R} has the awkward implication that with an increasing sample size we have to knowingly include an increasing number of false positives in the rejection region to maintain the set false-positive rate. The loss function L_R induces counterintuitive jumps in $\overline{\text{FDR}}$ and t^*. This leads us to favor L_{2N} and L_N. Both lead to very similar inference, with L_{2N} having the advantage that the constraint is on the practically more important $\overline{\text{FD}}$, rather than t^*, as in L_N.

6 Conclusion

The design of microarray experiments for measuring gene expression is a critical aspect of genomic analyses in biology and medicine. Microarrays are costly and difficult trade-offs

need to be evaluated in the allocation of resources to alternative investigations. Even in the simplest two-sample comparison setting, microrarray analyses pose difficult challenges to traditional sample size approaches: first, in terms of hypothesis testing, they present with a multitude of heterogeneous alternatives; second, they are generally performed with goals that are best captured by properties of the ensemble of the choices made; third, they mandate the incorporation of existing knowledge, as signal-to-noise ratios vary significantly with the specific technology, the source of RNA, and the overall experience of the laboratory's personnel.

Our goal in this article has been to develop a formal decision theoretic framework to address these challenges. This provides investigators the opportunity to quantify both the *a priori* uncertainty about the likely expression levels and the implications of sample size choices on the performance of inference about differential expression. The consequences of decisions are captured by loss functions related to genome-wise error rates. We argue for using posterior expected error rates for the terminal decision about the multiple comparisons, and marginal expected error rates for the design decision about the sample size, consistently with a Bayesian sequential approach. Similar issues recur in other high-dimensional multiple comparison problems and in the detection of faint signal levels in noisy data: the methods we propose are applicable more generally to those problems, as well.

In situations requiring complex decision making, decision models such as ours are best thought of as decision support tools. As is common in simpler settings, we envision investigators exploring various scenarios rather than simply eliciting input and blindly trusting the emerging sample size recommendation. A reasonable situation is also one in which an investigator has in mind a certain sample size that is feasible within given resource constraints. The proposed method informs the investigator about the effect sizes that she or he can realistically expect to discover with the proposed sample size, and about the ensuing error rates.

An interesting application of the proposed method is in a sequential framework. An investigator could proceed in steps, starting with an initial batch of experiments and stopping when a satisfactory balance of classification error rates is achieved. This could be implemented without preposterior calculations. Because genome-wise error rates refer to the ensemble of genes, an investigator could not sample to a foregone conclusion about any individual genes by using this stopping rule.

In our model, we assume that genes are from a discrete mixture in which some genes are

altered across the two samples, while others are completely unaltered. This assumption is realistic in tightly controlled experiments, but is less so in the comparison of RNA samples across organs, or across organisms. These broader comparisons are often made to produce exploratory analyses, such as clusters. The choice of sample sizes in these circumstances is different from that used in controlled experiments. Some insight into this issue is offered by Simon et al. (2002) and Bryan and van der Laan (2001).

An important practical indication for microarray design arises from the illustration described in Section 5. In particular, for a realistic set of parameters and pilot data, we show that the improvement in the genome-wide error rate appears to be non-concave, with a small initial plateau at very small sample size. In some cases the payoff of increasing the sample size from, say, two, to three appears to be negligible. This has implications for the common practice of planning experiments with only two or three replicates. We suggest that an analysis of the kind presented in Figure 1 would provide valuable information to investigators entertaining experiments with a very small number of replicates.

Appendix 1: Optimal Terminal Decision

We prove Theorem 1. We start by considering L_N, subject to a fixed total number of discoveries D. We find $L_N(d, y \mid D) = cD - (c+1)\sum d_i v_i + \sum v_i$. The last term does not involve the decision. For fixed D the rest is minimized by setting $d_i = 1$ for the D largest v_i. In other words, for any D the optimal rule is of the type $d_i = I(v_i > t)$, where t is simply the $(n-D)$-th order statistic of $\{v_1, \ldots, v_n\}$. Thus we conclude that the global minimum must be of the same form, and it only remains to find the globally optimal t. Straightforward algebra shows that the global minimum is achieved for $t^* = c/(c+1)$.

A similar argument holds under $L_R(d, y)$. We find

$$L_R(d, y \mid D) = C_1(D) - C_2(D) \sum_{i=n-D+1}^{n} v_{(i)} + C_3(D) \sum v_i. \tag{9}$$

with $C_1(D) = cD/(D+\epsilon)$, $C_2(D) = c/(D+\epsilon) + 1/(n-D+\epsilon)$, $C_3(D) = 1/(n-D+\epsilon)$, and $v_{(i)}$ the i-th order statistic of v_i. The global optimum is found by minimizing $L_R(d, y \mid D)$ with respect to D to find the optimal $D = D^*$. Thus the optimal decision is $d_i = (v_i > t)$ and $t_R^*(y) \equiv v_{(n-D^*)}$.

Under L_{2N} and L_{2R} we need an additional argument. To minimize $\overline{\mathrm{FNR}}$ subject to

$\overline{\text{FDR}} \leq \alpha$ we write the Lagrangian function

$$f_\lambda(d) = \overline{\text{FNR}} - \lambda(\alpha - \overline{\text{FDR}}).$$

Using Lagrangian relaxation (Fisher, 1985) we find a weight $\lambda^* \geq 0$ such that the minimization of $f_{\lambda^*}(d)$ provides an approximate solution to the original constrained optimization problem. (The solution is only approximate because of the discrete nature of the decision space.) But $f_{\lambda^*} = L_R$ with $c = \lambda^*$. Thus the solution must have the same form as described above. The only difference is that the implied coefficient c, itself, is a complicated function of the data. Knowing the structure of the solution we can solve the decision problem by finding the cutoff $t_{2R}(y) = \min\{s : \ \overline{\text{FDR}}(s, y) \leq \alpha\}$. A similar argument holds for L_{2N}, with $t_{2N}(y) = \min\{s : \ \overline{\text{FD}}(s, y) \leq \alpha\}$. Note that the optimal cutoff t^* in all three new loss functions is now a function of the data. We will write $t^*_L(y_J)$ for the optimal cutoff under loss L given data y_J.

Appendix 2: Asymptotic FNR

We now prove Theorem 2, assuming a model with the same structure as in Section 4. The specific distributional assumptions, including the Gamma sampling distribution for (X_{ij}, Y_{ij}) and the Gamma prior for $(\theta_{0i}, \theta_{1i})$, are not critical. We start the argument by establishing an asymptotic approximation for $P(z_j = 1|y_J)$. We will then use this result to argue that for a large J the rejection region has to necessarily include some genes with zero or a small level of true differential expression. This is true under all three loss functions, L_{2R}, L_{2N} and L_N. Thus the non-rejection region includes only small levels of true differential expression. We exploit this fact to approximate $\overline{\text{FNR}}$ by an integral that can be recognized as an expression of the order of $\sqrt{\log J/J}$. The integral approximation is valid only if a large number of genes are in the non-rejection region, allowing us to approximate the sum in the definition of $\overline{\text{FNR}}$ by an integral. We conclude the argument by showing that this is the case under all three loss functions, for a sufficiently large J.

We start with an asymptotic result for the posterior probability of differential expression. Let $\eta = (a, a_0, p)$ denote the hyperparameters, and let $y_i = \{X_{ij}, Y_{ij}, \ j = 1, \ldots, J\}$ denote

the data for gene i. As the number n of genes is very large, we have, for each gene:

$$P(z_i = 1|y) = \int P(z_i = 1|y_i, \eta) \mathrm{d}p(\eta|y_i) = P(z_i = 1|y_i, \hat{\eta})(1 + O_P(n^{-1/2}))$$
$$= P(z_i = 1|y_i, \eta)(1 + O_P(n^{-1/2})), \quad (10)$$

where $\hat{\eta}$ is the maximum likelihood estimator, and η are the true hyperparameters. Here $X_n = O_P(n^k)$ for a sequence of random variables X_n is defined as

$$\lim_{M \to \infty} \left\{ \limsup_n P[X_n/n^k > M] \right\} = 0$$

Moreover, for each gene i, the posterior probability of differential expression given η is

$$P(z_i = 1|y_i, \eta) = \frac{p\, p(y_i|z_i = 1, \eta)}{p\, p(y_i|z_i = 1, \eta) + (1 - p)\, p(y_i|z_i = 0, \eta)}$$

Classical Laplace expansions imply that

$$P(z_i = 1|y_i, \eta) = \frac{1}{1 + c_i e^{-J(\hat{\theta}_{0i} - \hat{\theta}_{1i})^2 \tau_i/2} \sqrt{J}} \quad (11)$$

$c_i, \tau_i = O_P(1)$ as J goes to infinity. The constant c_i includes the ratio $(1 - p)/p$. Under suitable regularity conditions this result is uniform in $(\theta_{0i}, \theta_{1i}, \eta)$ over compact sets. In the non-compact case, some conditions on the tails of the priors need to be added. (See, for example, Guihenneuc and Rousseau, 2002.) Therefore, when $|\theta_{0i} - \theta_{1i}|$ is large $p(z_i = 1|y_i, \eta)$ goes to 1 at an exponential rate and thus $P(z_i = 1|y_i)$ is very close to 1 (the error being essentially of the order n^{-1}).

We now use (11) to study the asymptotic behavior of the terminal decision. In particular, we consider $\overline{\mathrm{FDR}}$, $\overline{\mathrm{FD}}$, $\overline{\mathrm{FNR}}$ and $\overline{\mathrm{FN}}$. Let $v_{(1)} \leq \cdots \leq v_{(N)}$ be the ordered posterior probabilities $v_i = P(z_i = 1|y)$ and recall that $\overline{\mathrm{FDR}}(t, y) = \sum_i (1 - v_i)\, I(v_i \geq t)/D$, where $D = \sum_i I(v_i \geq t)$ is the number of discoveries. We will use $N = \sum I(v_i < t)$, $\mathrm{FP} = \sum I(v_i > t) I(z_i = 0)$, $n_1 = \sum I(z_i = 1)$ and $n_0 = n - n_1$ to denote the number of negatives, false positives, and differentially expressed and non-differentially expressed genes, respectively. We will use $A_N, A_{\mathrm{FP}}, A_1$, and A_0 to denote the corresponding sets of genes. The above expansions show that the ordering of v_i is asymptotically linked to the ordering of $|\hat{\theta}_{0i} - \hat{\theta}_{1i}|$, with v_i monotone, increasing in $|\hat{\theta}_{0i} - \hat{\theta}_{1i}|$, with asymptotically

$$v_i \approx 1 - c_i \sqrt{J} \exp[-J(\hat{\theta}_{0i} - \hat{\theta}_{1i})^2 \tau_i/2].$$

The false discovery rate $\overline{\text{FDR}}(t, y)$ as a function of t is a step function taking values in $\{1-v_{(n)},\ ...,\ 1-(v_{(k)}+...+v_{(n)})/(n-k+1),\ ...,\ 1-(v_{(1)}+...+v_{(n)})/n\}$. Similarly, $\overline{\text{FD}}(t, y)$ is a step function with values $\{1-v_{(n)},\ ...,\ 1-v_{(1)}+...+1-v_{(n)})\}$. Both are monotone, decreasing in t. For a large J, the earlier discussion shows that any gene with

$$|\hat{\theta}_{0i} - \hat{\theta}_{1i}| > C\sqrt{\log J}/\sqrt{J} \tag{12}$$

has a posterior probability of $v_i \geq 1 - 1/\sqrt{J}$, when C is large enough, uniformly in θ_{0i}, θ_{1i} belonging to some compact set, and with a large probability. We denote with

$$S = \{i :\ |\hat{\theta}_{0i} - \hat{\theta}_{1i}| < C\sqrt{\log J}/\sqrt{J}\}$$

the set of genes with small $|\hat{\theta}_{0i} - \hat{\theta}_{1i}|$ that violate (12).

We now show that under all three losses, only genes with small $|\hat{\theta}_{0i} - \hat{\theta}_{1i}|$ are classified as non-differentially expressed, i.e., $A_N \subseteq S$.

Under L_N the argument is straightforward. For all genes satisfying (12) the posterior probability $v_i \approx 1 - 1/\sqrt{J}$ is beyond $t_N = c/(1-c)$ for a sufficiently large J. Thus all genes in A_N satisfy $|\hat{\theta}_{0i} - \hat{\theta}_{1i}| < C\sqrt{\log J/J}$.

To prove the claim under L_{2R} we show that the opposite would violate the constraint on $\overline{\text{FDR}}$. Assume that (12) holds for all $i \in A_D$. Then

$$\overline{\text{FDR}} = 1 - (v_{(n-D+1)} + ... + v_{(n)})/D \leq 1/\sqrt{J}.$$

which is not enough to reach the set level α bound required for L_{2R}. Thus the rejection region A_D has to necessarily include some genes that violate (12). Together with the monotonicity of $|\hat{\theta}_{0i} - \hat{\theta}_{1i}|$ as a function of v_i this proves the claim.

Finally, to show the same for L_{2N}, consider (12) with an even larger C. If $C^2 > 1/\tau_i[\log n - \log(\alpha/2)]$, then $1 - v_{(i)} \leq \alpha/(2n)$ for all genes that satisfy (12) with such C. If only such genes are considered in the rejection region then

$$1 - v_{(k)} + ... + 1 - v_{(N)} \leq \alpha/2,$$

which is not enough to reach the desired bound $\overline{\text{FN}} = \alpha$ under L_{2N}.

We now use (11) and the fact that all negatives have small $|\hat{\theta}_{0i} - \hat{\theta}_{1i}|$ to establish a bound on $\overline{\text{FNR}}$.

$$\overline{\text{FNR}}(t_*, y) = \frac{1}{N}\sum_{j=1}^{N} v_{(j)} = \frac{1}{N}\sum_{j=1}^{N} \frac{1}{1 + c_j\sqrt{J}e^{-J(\hat{\theta}_{0j} - \hat{\theta}_{1j})^2\tau_j/2}(1 + O_P(n^{-1/2})},$$

where $c_j = \sqrt{\tau_j}/\sqrt{2\pi}$ with $\tau_j = i(\theta_{0j})i(\theta_{1j})/(i(\theta_{0j})+i(\theta_{1j}))$ and $i(\theta)$ is the Fisher information associated with the conditional model of X_i (or Y_i) given θ, η, when η is fixed.

If N is large then the sum can be approximated by an integral, with respect to the distribution of $v_{(j)}$ or, equivalently, the distribution of $(\hat{\theta}_{0j}, \hat{\theta}_{1j})$. We split the integral into two parts. With probability w_0 we have $\theta_{0i} = \theta_{1i} \equiv \theta_i$ and with probability w_1 we have $\theta_{0i} \neq \theta_{1i}$. Based on the earlier observation that we only fail to reject the comparison in the case of small estimated differences, we can condition the latter term on $|\hat{\theta}_{0i} - \hat{\theta}_{1i}| < C\sqrt{\log J}/\sqrt{J}$. Let $\sqrt{J}(\hat{\theta}_{0i} - \hat{\theta}_{1i})\sqrt{\tau_j} = \sqrt{J}(\theta_{j0} - \theta_{j1})\sqrt{\tau_j} + \xi_j$, where ξ_j is a standard Gaussian random variable. Let $\Theta_S = \{(\theta_1, \theta_0) : |\theta_1 - \theta_0| < C\sqrt{\log J}/\sqrt{J}\}$ Then,

$$\begin{aligned}
\overline{\mathrm{FNR}}(y, t_{2R}) &\approx w_0 \int_\xi \int_\theta \frac{1}{1 + \sqrt{i(\theta)}/\sqrt{2\pi}(1-p)/pe^{-\xi^2/2}\sqrt{J}} \mathrm{d}p(\xi)\mathrm{d}p(\theta) \\
&+ \quad w_1 \int_\xi \int_{\Theta_S} \frac{1}{1 + c(\theta_0, \theta_1)\sqrt{J}\, e^{-\xi^2/2} e^{-J(\theta_1-\theta_0)^2\tau(\theta_0,\theta_1)/2}} \mathrm{d}p(\xi)\mathrm{d}p(\theta_0, \theta_1).
\end{aligned}$$

Simple calculations imply that the above quantities are of the order $\sqrt{\log J/J}$, when N is large.

Moreover, $\overline{\mathrm{FN}} = N\,\overline{\mathrm{FNR}}$. We now prove that $n/N = O_P(1)$ with a high probability under all three losses.

We start with the argument for L_{2R}. Under the assumed sampling model $n_0 \approx p \cdot n$ genes satisfy $\theta_{0j} = \theta_{1j}$. If $N/n \to 0$, then a large proportion of genes satisfying $\theta_{0j} = \theta_{1j}$ would have posterior probabilities $v_j > t_{2R}$. Recall that A_{FP} is the set of false positives. This would imply that

$$\begin{aligned}
\overline{\mathrm{FDR}} &\geq \frac{1}{n} \sum_{i \in A_{FP}} (1 - v_i) \\
&= \frac{1}{n} \sum_{i \in A_{FP}} \frac{c_j \sqrt{J} e^{-J(\hat{\theta}_{0i} - \hat{\theta}_{1i})^2 \tau_j/2}}{1 + c_j \sqrt{J} e^{-J(\hat{\theta}_{0i} - \hat{\theta}_{1i})^2 \tau_j/2}} (1 + O_P(n^{-1/2})) \\
&\geq \frac{p}{2} \int_\theta \int_\xi \frac{\sqrt{J} c(\theta) e^{-\xi^2/2}}{1 + \sqrt{J} c(\theta) e^{-\xi^2/2}} \mathrm{d}\xi \mathrm{d}p(\theta) (1 + O_P(n^{-1/2}),
\end{aligned}$$

when n is large enough, with a high probability. The last inequality is true since under the assumption $N/n \to 0$ eventually more than $N/2 \approx np/2$ genes would be in A_{FP}. As J goes to infinity, the above term goes to $p/2$. This is a contradiction if $\alpha < p/2$, and we thus conclude that $n/N = O_P(1)$.

Under L_{2N} we use an analogous argument for $\overline{\mathrm{FD}}$. The right-hand side in the first two

(in-)equalities above remains unchanged, except for removing the leading $1/n$ factor. We conclude that $\overline{\text{FD}} \geq np/2$ and thus have a contradiction for $\alpha < np/2$.

Finally, under L_N, $t_N = c/(c+1)$, so $v_j \leq t_N \Leftrightarrow$

$$1 \leq c\, c_j e^{-J(\hat{\theta}_{0i}-\hat{\theta}_{1i})^2\tau_j/2}\sqrt{J}(1 + O_P(n^{-1/2}).$$

The number of genes $v_j \leq t_N$ is large with a high probability. Indeed, if $\theta_{0i} = \theta_{1i}$,

$$P\left[1 > c\, c_j e^{-J(\hat{\theta}_{0i}-\hat{\theta}_{1i})^2\tau_j/2}\sqrt{J}\right] = O(J^{-1/2})$$

by Chebychev's inequality. Recall that FP is the number of genes satisfying $\theta_{0i} = \theta_{1i}$ and $v_i > t_N$. Then, FP is a binomial random variable $\text{B}in(n_0, p_J)$, with $p_J = O_P(J^{-1/2})$ and where n_0 is the number of genes with $\theta_{0i} = \theta_{1i}$. Thus with a probability of $1 - e^{-c_1\sqrt{J}}$, for some positive constant c_1, $n_1 \leq c_2 n$, with $c_2 < 1$.

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