Extreme Value Distribution Based Gene Selection Criteria for Discriminant Microarray Data Analysis Using Logistic Regression

Wentian Li¹,* , Fengzhu Sun², Ivo Grosse³

¹. The Robert S Boas Center for Genomics and Human Genetics, North Shore LIJ Research Institute, 350 Community Drive, Manhasset, NY 11030. USA.

². Department of Biological Sciences, Molecular and Computational Biology Program, University of Southern California, 1042 W. 36th Place, DRB 155, Los Angeles, CA 90089. USA.

³. Institute for Plant Genetics and Crop Plant Research, Corrensstrasse 3, D-06466 Gatersleben, Germany.

* Corresponding author.

To be published in Journal of Computational Biology, Vol.11, Nos.2-3 (2004)
Abstract

One important issue commonly encountered in the analysis of microarray data is to decide which and how many genes should be selected for further studies. For discriminant microarray data analyses based on statistical models, such as the logistic regression models, gene selection can be accomplished by a comparison of the maximum likelihood of the model given the real data, $\hat{L}(D|M)$, and the expected maximum likelihood of the model given an ensemble of surrogate data with randomly permuted label, $\hat{L}(D_0|M)$. Typically, the computational burden for obtaining $\hat{L}(D_0|M)$ is immense, often exceeding the limits of computing available resources by orders of magnitude. Here, we propose an approach that circumvents such heavy computations by mapping the simulation problem to an extreme-value problem. We present the derivation of an asymptotic distribution of the extreme-value as well as its mean, median, and variance. Using this distribution, we propose two gene selection criteria, and we apply them to two microarray datasets and three classification tasks for illustration.

Key words: microarray, gene selection, extreme value distribution, logistic regression
1 Introduction

Discriminant microarray data analysis can be understood as a comparison of the expression levels of samples from one group versus another group, such as disease tissues versus normal tissues, or one subtype of cancer versus another subtype (for a review, see [13]). Discriminant analysis or classification can be carried out on a whole set of genes or on individual genes, and it has become increasingly clear that, for many classification tasks based on microarray data, it is not necessary to consider many genes simultaneously. In many cases it has been shown that a few genes are sufficient for classifying two groups of samples [2, 7, 11, 25, 26, 27, 28, 30, 33, 34, 36, 52]. Usually, even with a very small number of genes being included in a classification, these genes are jointly used in a multivariate fashion. However, in some cases, one or two genes are sufficient for a good classification [30, 44, 52]. This observation led to procedures that examine one gene at a time, rank the gene according to their classification ability, and select only the high-ranking genes for further studies, including new confirmation experiments [4, 42, 45]. Some information could be lost by not considering genes jointly, but focusing on single genes often simplifies the biological interpretation of the results.

Two single-gene classification methods that are often applied to the analysis of microarray data are the fold-change method [6] and the \( t \)-test [41]. As repeatedly pointed out in Refs. [3, 8, 12, 24, 35, 37, 47], the fold-change method is not rigorous from a statistical point of view, because it considers neither the variances nor the sample sizes of the data. For example, a two-fold increase obtained from narrowly distributed data with 1000 samples is statistically more significant than the same increase obtained from broadly distributed data with 10 samples. The \( t \)-test overcomes this shortcoming by including the variance and sample size information. However, the \( t \)-distribution is obtained by assuming that the random variables are sampled from a normal (Gaussian) distribution.

There are alternative discriminant methods that do not rely on the assumption that the random variables are normally distributed. Out of the four linear classification methods – Fisher’s linear discriminant analysis, logistic regression (LR), Rosenblatt’s perceptron, and support vector machine (SVM) – LR and SVM do not rely on this assumption [23], and hence they are more robust when the actual data, including the presence of outliers, are not normally distributed. Another advantage of LR over \( t \)-tests is that \( t \)-tests compare only two group averages, whereas LRs check each individual sample for consistent differential expressions. In the following we focus on LR, which has already been used in discriminant
microarray data analyses [14, 30, 31, 36, 43, 49].

Cross-validation is often used for assessing how accurately a dataset can be classified by a learned model. In cross-validation, a dataset is divided into two parts, where the first part is used for estimating the model parameters, and the second part is used for assessing the classification performance. Due to the splitting of the dataset, not all samples are included in the learning process, which is not optimal for datasets with a small number of samples. On the other hand, if all data points are used in the training process, the error rate of the classifier would be underestimated.

In order to estimate the statistical significance of a learned model, one usually uses resampling methods, such as the bootstrap method (resampling with replacement) or the permutation method (resampling without replacement). Since in this paper only the single-gene LR is used, a significant model implies a significant gene. (This correspondence does not hold for multivariate classifiers due to the possible correlation among genes.) In Ref. [31], likelihoods of single-gene LRs of real datasets are compared to those of the label-permuted datasets, and genes with a likelihood exceeding the likelihood of the top-ranking gene of the permuted data are selected. One problem with actually carrying out permutations as in Ref. [31] is that the calculation of the LR likelihoods for ten-thousands of genes is computationally intensive, and that repeating this calculation for, say, $10^4$ surrogate datasets is prohibitive.

Here, we propose an analytic solution that circumvents these heavy computations. Our approach is based on the observation that we are only interested in the extreme-values in the following sense: in order to define a threshold for gene selection, we compare the maximum likelihood of each gene in the real data with the maximum likelihood of the top-ranking gene in the label-permuted data. Whereas simulation requires the calculation of all single-gene likelihoods in the surrogate data for each permutation, the proposed analytic calculation of the the expected value of the likelihood of the top-ranking gene will be carried out only once.

The extreme-value theory is a well studied topic in statistics [9, 20, 40], with major contributions by Ronald A Fisher, Maurice Frechet, Emil Gumbel, Vilfredo Pareto, Waloddi Weibull, to name just a few. One fundamental assumption often used in deriving an extreme-value distribution is that observations are independent. In our application of the extreme-value distribution, the corresponding assumption is that log likelihood scores of different genes are statistically independent. Clearly, this assumption is violated in most expression data sets, but as we discuss in the Discussion section, there is a simple solution.
to this problem by replacing the number of genes $p$ by the “effective number of genes” $p_{\text{eff}}$.

The topic studied in this paper is closely related to the multiple testing problem. A criterion for claiming statistical significance should be more stringent when many genes are tested than if only one gene is tested, because presumably multiple testings provide more chances to find a significant gene. Traditionally, the Bonferroni correction, which divides the threshold for significance obtained from a single gene by the total number of tests (genes), is used in those cases. Applying extreme-value distribution achieves a similar goal because the largest value among $p$ variables increases with $p$, and this effectively raises the stringency for a gene selection criterion.

2 Methods

2.1 Logistic regression of microarray data

First, we introduce the following notation. Let the samples be indexed by $i$, and let the genes be indexed by $j$. Denote the total number of samples by $N$, the total number of genes by $p$, the expression level by $x$, e.g., $x_{ij} = \log(\text{spot intensity of gene } j \text{ in sample } i)$, and the sample label value by $y$, e.g., $y = 0$ or $y = 1$ for a binary classification problem. Then, the single-gene LR model $M_j$ of gene $j$ is defined by the conditional probabilities of the sample label $y_i$ given the expression levels $x_{ij}$:

$$\Pr(y_i = 1|x_{ij}) = \frac{1}{1 + e^{-a_j - b_j x_{ij}}},$$

for $i = 1, 2, \ldots, N$ and $j = 1, 2, \ldots, p$. Here, $a_j$ and $b_j$ are parameters to be estimated from all samples $i = 1, 2, \ldots, N$. The data-fitting performance of $M_j$ is measured by the maximum likelihood,

$$\hat{L}(D|M_j) = \max_{a_j, b_j} \prod_{i=1}^{N} [\Pr(y_i = 1|x_{ij})]^{y_i} [1 - \Pr(y_i = 1|x_{ij})]^{1-y_i},$$

where $D$ denotes the data. Since a gene is represented by a LR model, selection of genes becomes selection of single-gene LR models with large maximum-likelihoods. Although in a more general context such as multivariate models, model selection is not equivalent to variable (gene) selection, for single-gene models, gene selection and model selection are treated as the same.

2.2 Maximum likelihood for the surrogate data

There are different ways of constructing surrogate datasets. For example, one may sam-
ple the expression levels $x_{ij}$ from a normal distribution, and then assign a label $y_i$ to each sample randomly; or one may start with the available microarray data set, and randomly permute the sample label. If a gene in the microarray data does not differentially express before a permutation, the two ways for generating the surrogate data is the same. However, as pointed out in [38], if a gene is indeed differentially expressed before a permutation, extra variance remains after permutation, and the two methods for generating the surrogate data can be slightly different.

This subtle difference between the two surrogate datasets may affect a $t$-test result, because $t$-test makes certain assumption on the distribution and variance on the data [38]. The extra variance remained in the permuted data violates this assumption. Nevertheless, no such assumption is required for LR. For this reason, we do not make this distinction, and denote by $D_0$ a surrogate dataset with permuted sample labels, whether the original dataset before permutation contains differentially expressed genes or not.

We denote by $\hat{L}(D_0|M_j)$ the maximum likelihood under the single-gene LR model $M_j$. For a particular permutation, we define by

$$ l \equiv \max_j \{\log \hat{L}(D_0|M_j)\} $$

the maximum value of the maximum likelihoods of all genes. Note the two different maximization steps: the first over the parameter values $a_j$ and $b_j$ for a given gene, and the second over all genes $j$. When surrogate dataset $D_0$ is repeatedly generated, those maximum values $l$ vary from realization to realization, and our goal is to characterize the distribution of $l$, e.g. by computing the expected value, the median, or the standard deviation of $l$.

Toward the calculation of the expected value of $l$, we use the Wilks theorem [50], which is “one of the most celebrated folklores in statistics” [15] and is covered by most standard textbooks on mathematical statistics [31 10 16 46 51]. This theorem states that, under very general conditions (which our LR model satisfies), the asymptotic distribution of the 2-log-likelihood ratio – when the data is generated by the null model $M_0$ – is the $\chi^2$ distribution with $df$ degrees of freedom, where $df = d(M_j) - d(M_0)$ is the difference of the number of parameters in models $M$ and $M_0$ [50]. Using our notation, it states that in the $N \to \infty$ limit,

$$ 2 \log \hat{L}(D_0|M_j) = 2 \log \hat{L}(D_0|M_0) + t, $$

where $t$ denotes a random variable sampled from a $\chi^2$ distribution with $df$ degrees of freedom.
We choose the null model $M_0$ to be the same for all genes, i.e., $\Pr(y_i = 1|x_{ij}) = c$ for all $j = 1, 2, \ldots, p$. The maximum likelihood estimate of $c$ is simply the percentage of samples that are labeled as 1, i.e., $\hat{c} \equiv N_1/N$. The maximum likelihood under $M_0$ is

$$L(D_0|M_0) = \hat{c}^{N_1}(1-\hat{c})^{N-N_1},$$

and its logarithm is

$$\log L(D_0|M_0) = -NH$$

where $H$ is the entropy

$$H = -\frac{N_1}{N} \log \frac{N_1}{N} - \frac{N-N_1}{N} \log \frac{N-N_1}{N}.$$ 

Note that $\hat{L}(D|M_0) = \hat{L}(D_0|M_0)$, because the percentage $N_1/N$ of samples with sample label $y = 1$ is the same in $D$ and $D_0$.

Applying the LR model to the surrogate data, we obtain for the best single-gene maximum log-likelihood (in the large sample limit $N \rightarrow \infty$):

$$l = \max_j \left[ \log \hat{L}(D_0|M_j) \right] = \max_j \left[ \log \hat{L}(D_0|M_0) + \frac{t_j}{2} \right]$$

$$= -NH + \frac{1}{2} \max [t_1, t_2, \ldots, t_p],$$

where $t_1, t_2, \ldots, t_p$ are $p$ random variables sampled from a $\chi^2$ distribution with $df$ degrees of freedom. In this example, $M_j$ contains two parameters, and $M_0$ contains one parameter, so $df = 2 - 1 = 1$.

### 2.3 Extreme-value distribution of $\chi^2$-distributed random variables

The extreme-value distribution of normally distributed random variables has been extensively studied (see, e.g., [16]). Gumbel showed [19, 21] that the extreme-value distribution of the $\chi^2$ distributed variables belongs to the same class as that of normally distributed variables, which is now called the standard Gumbel distribution $\exp(-\exp(-(x - a)/b))$. For the case of $\chi^2$ distributed variables, the coefficients $a$ and $b$ are derived in Ref. [22]. Although this extreme-value distribution (of random variables sampled from the $\chi^2$ with one degree of freedom) is known, for the sake of completeness we present here a derivation.

Let $t_1, t_2, \ldots, t_p$ be statistically independent and identically distributed (iid) random values from a $\chi^2$ distribution with one degree of freedom, and define $T_p \equiv \max[t_1, t_2, \ldots, t_p]$. Based on the inequality [5]:

$$\sqrt{\frac{2}{\pi}} \frac{\sqrt{t}}{1+t} e^{-t/2} \leq \Pr(t_i \geq t) \leq \sqrt{\frac{2}{\pi}} \frac{1}{\sqrt{t}} e^{-t/2}$$

(5)
and by defining
\[ c_p \equiv \log \frac{p^2}{\pi \log(p)} \]
one finds that for asymptotically large \((p \to \infty)\), the cumulative distribution of \(v_p = \frac{T_p - c_p}{2}\) converges to the double exponential function:

\[ F_v(x) = \lim_{p \to \infty} F_{v_p}(x) \equiv \lim_{p \to \infty} \Pr \left( \frac{T_p - c_p}{2} \leq x \right) = \exp(-e^{-x}). \quad (6) \]

This result can be derived as follows. For any \(x\), we obtain

\[ \Pr \left( \frac{T_p - c_p}{2} \leq x \right) = \Pr(T_p \leq c_p + 2x) = \prod_{i=1}^{p} [1 - \Pr(t_i > c_p + 2x)]^p = [1 - \Pr(t_i > c_p + 2x)]^p, \]

and from inequality (4), one obtains

\[ \lim_{p \to \infty} p \Pr(t_i > c_p + 2x) = \lim_{p \to \infty} \sqrt{\frac{2 e^{-\log(p)+\log\log(p)+\log(\sqrt{\pi})-x}}{c_p + 2x}} e^{-x} = e^{-x}. \]

Therefore,

\[ \lim_{p \to \infty} \Pr \left( \frac{T_p - c_p}{2} \leq x \right) = \exp(-e^{-x}) \]

From the asymptotic distribution \(F_v(x)\), we can compute the mean \(E[v]\), the median \(m[v]\), and the standard deviation \(\sigma[v]\):

\[ E[v] = \gamma \]
\[ m[v] = -\log(\log(2)) \quad (7) \]
\[ \sigma^2[v] = \frac{\pi^2}{6}, \quad (8) \]

where \(\gamma \approx 0.5772\) denotes the Euler constant. Hence, we obtain the following asymptotic scaling for the mean, the median, and the standard deviation of \(T_p = c_p + 2v_p\) in the asymptotic limit \(p \to \infty\):

\[ E[T_p] \approx 2 \log(p) - \log(\log(p)) - \log(\pi) + 2\gamma \]
\[ m[T_p] \approx 2 \log(p) - \log(\log(p)) - \log(\pi) - 2 \log(\log(2)) \]
\[ \sigma[T_p] \approx \sqrt{2\pi^2/3} \quad (9) \]

Based on the extreme-value distribution of \(T_p\), we propose the following two gene selection criteria.
2.4 Gene selection based on the E-value of the extreme-value distribution

In the first criterion, which we call the E-criterion, we compare the maximum likelihood of each gene obtained from the real data with the expected value of the maximum likelihood of the top-ranking gene from the surrogate data. This criterion for the likelihood can be easily converted to a criterion for the log-likelihood ratio: for each gene \( j = 1, 2, \ldots, p \), calculate the log-likelihood ratio

\[
t_j \equiv 2 \log \frac{\hat{L}(D|M_j)}{L(D|M_0)} = 2 \log \frac{\hat{L}(D|M_j)}{L(D_0|M_0)} = 2 \log \hat{L}(D|M_j) + 2NH, \tag{10}
\]

order them such that \( t_{(1)} \geq t_{(2)} \geq t_{(3)} \ldots \geq t_{(p)} \), and declare genes \( j = 1, 2, \ldots, J \) as differentially expressed if

\[
t_{(J)} \geq E[T_p] = 2 \log(p) - \log(\log(p)) - \log(\pi) + 2\gamma > t_{(J+1)}. \tag{11}
\]

2.5 Gene selection based on the P-value of the extreme-value distribution

In the second gene selection criterion, which we call the P-criterion, we compare the P-value of the calculated maximum likelihood of each gene obtained from the real data using the distribution of the maximum likelihood of the top-ranking gene from the surrogate data. That is, for each gene \( j = 1, 2, \ldots, p \), calculate the log-likelihood ratio \( t_j \equiv 2 \log \hat{L}(D|M_j) + 2NH \), order them to \( t_{(j)} \), then convert them to \( v_{(j)} \equiv (t_{(j)} - c_p)/2 \). We declare genes \( j = 1, 2, \ldots, J \) as differentially expressed if and only if an upper limit of the P-value for \( v_{(J)} \), \( P_{(J)} = 1 - \exp(-e^{-v_{(J)}}) \), is smaller than the user-specified \( P_0 \), and that of \( v_{(J+1)} \) is larger:

\[
1 - \exp(-e^{-v_{(J)}}) \leq P_0 < 1 - \exp(-e^{-v_{(J+1)}}). \tag{12}
\]

When a small \( P_0 \) is chosen, such as \( P_0 =0.01 \) or \( P_0 =0.001 \), the tail distribution of the extreme-value is used. In the E-criterion, since it is the mean of the extreme-value is chosen, we focus on the middle-range of the extreme value distribution. As a result, the P-criterion is more stringent than the E-criterion, leading to fewer genes selected. This is on the top of the conservative nature of both E- and P-criteria, because even the non-top genes in the real data are compared with the top-maximum-likelihood in the surrogate data.
3 Results

3.1 Confirmation of the extreme-value distribution by numerical simulation

We perform numerical simulations to test if, and to which degree, the asymptotic expressions of the mean \(E[T_p]\), the median \(m[T_p]\), and the standard deviation \(\sigma[T_p]\) are acceptable approximations for finite \(p\) ranging from 1 to 10^5. For each value of \(p\) ranging from 1 to 1.5 \(\times\) 10^5 we generate 10^4 samples of \(p\) random variables sampled from a \(\chi^2\) distribution with 1 degree of freedom. Fig. 1 shows \(E[T_p]\), \(m[T_p]\), and \(\sigma[T_p]\) versus \(\log(p)\), and we find that the asymptotic expressions of \(E[T_p]\) and \(m[T_p]\) agree with the simulation data sufficiently well. The simulations confirm the trend of a linear increase of \(T_p\) with \(\log(p)\) as well as the systematic deviation from this linear trend due to the \(\log\log(p)\) term. The standard deviation \(\sigma[T_p]\) according to Eq.(9) is not a function of \(\log(p)\), and indeed, the simulated values reach a plateau for \(p > 10^3\). Note that the predicted standard deviation \(\sqrt{2\pi^2/3}\) is consistently larger than the simulated standard deviation, and the difference between the two curves becomes smaller as \(p\) increases.

Besides the mean, median, and variance, we also compare the distribution of \(T_p\) for finite \(p\) with the analytically derived distribution for \(p \to \infty\) in order to study to which degree Eq.(6) derived for the asymptotic limit \(p \to \infty\) is an appropriate approximation for finite \(p\) ranging from 10^3 to 10^4. We generate \(p = 6000\) random variables \(t_1, t_2, \ldots, t_p\) sampled from a \(\chi^2\) distribution with one degree of freedom, and we record the maximum value \(T_p \equiv \max[t_1, t_2, \ldots, t_p]\). We repeat this sampling process 10^4 times, and we compare the empirical \(P\)-value, which is the percentage of times the \(v_p = (T_p - c_p)/2\) exceeds a specified value \(x\), to the theoretical \(P\)-value \(1 - F_v(x) = 1 - \exp(-\exp(-x))\). We find that for \(p = 6000\) the two distributions match well.

3.2 Gene selection for microarray datasets

We use two publicly available microarray datasets to illustrate the proposed criteria for deciding how many high-ranking genes should be selected: (i) the leukemia subtype data from the Whitehead Institute [17], and (ii) the colon cancer data from Princeton University [1].

ALL versus AML: Fig. 2(a) shows the rank-ordered distribution of the maximum likelihoods for all single-gene LR models for the discrimination of acute lymphoblastic leukemia (ALL) from acute myeloid leukemia (AML). The sample size is 72, which combines both the training and testing sets, as designated in [17]. The ALL-AML classification
problem is thoroughly discussed in [32], and it is well-known to be a comparatively easy classification problem [30, 33, 36, 44].

According to the E-criterion proposed in Eq. (11), 407 genes are selected. In the converted variable \( v(j) = (t(j) - c_p)/2 \), the E-criterion is equivalent to \( v(j) > \gamma = 0.5772 \). Using the P-criterion proposed in Eq. (12), we obtain that 165 genes are considered to be differentially expressed at the P-value of 0.01 (see Fig. 2(b)). We note in passing that the number of genes selected by both criteria is substantially smaller than 1100, which is the number of genes labeled as “more highly correlated with the AML-ALL class distinction than would be expected by chance,” as reported in [17] using the “neighborhood analysis”.

**T-cell versus B-cell:** As pointed out in [18], the ALL dataset is still a heterogeneous dataset, with sources from B-cells and T-cells being different from each other. Fig. 3(a) shows the rank-ordered distribution of the maximum likelihoods using single-gene LR models for the B-cell versus T-cell classification, with a reduced sample size of 47. The E-criterion declares 114 genes as differentially expressed, and the more conservative P-criterion declares 57 genes as differentially expressed with the P-value of 0.01. These findings are in agreement with the observation in [18] that there are differentially expressed genes in B-cells and T-cells, and also in agreement with another observation in [29] based on cluster analysis.

**Colon cancer versus normal:** Fig. 4(a) shows the rank-ordered distribution of the maximum likelihoods using single-gene LR models for the colon cancer versus normal tissue dataset studied in [1]. This dataset consists of 62 samples, and the data for 2000 genes that have the “highest minimal intensity across the samples” are available from [1]. We find that only 49 and 10 genes are selected by the E-criterion and the P-criterion (at \( P_0 \)-value=0.01), respectively, and one possible explanation why these numbers are small is that the initial number of genes is already restricted to a smaller number of 2000 by some pre-processing method. Another possible explanation is that the classification task in the dataset cannot be accomplished by single-gene models.

4 Discussions and conclusions

The gene selection procedure discussed here circumvents the multiple testing problem by explicitly including the number of genes \( (p) \) in the gene selection criterion. It is an analytic approximations based on the known mathematical theorems concerning (i) the extreme-value distribution of \( \chi^2 \) distributed random variables, and (ii) the asymptotic
distribution of the log-likelihood ratio. The analytical approximation developed in this paper is based on the following assumptions: (1) $N \rightarrow \infty$ so that the distribution of the log-likelihood ratio statistics is the $\chi^2$ distribution; (2) $p \rightarrow \infty$ so that the extreme-value distribution can be applied; (3) the extreme-value is taken from $p$ independent values. In the context of microarray data analysis, these assumptions translate to: (1) the number of microarray samples $N$ is very large; (2) the number of genes $p$ is very large; and (3) the maximum likelihood scores of different genes are statistically independent.

Based on the simulation result presented in Fig. 1, problem (2) may not be a serious problem, since the log($p$) trend, as well as the log(log($p$)) correction, is captured very well by the analytic formula, even when $p$ is small. Besides, for a typical microarray data, the range of $p$ is large, usually beyond a few thousands. It should be mentioned that any asymptotic results (asymptotic for $p$) are not unique in the sense that adding any extra term whose value over $c_p$ tends to zero will also be a valid solution. For example, it can be shown that it is possible to replace $c_p = 2\log(p) - \log(\log(p)) + \log(\pi)$ by $c'_p = 2\log(p) - \log[\log(p) - \log(\sqrt{\log(p) - \log(\sqrt{\pi})}) - \log(\pi)]$. At finite range of $p$’s, however, the difference between different formula can be negligible.

Problem (3) can be handled by introducing an “effective number of genes” $p_{eff}$. For example, if two genes have identical expression profiles, they lead to the identical maximum-likelihood scores, and the number of genes should be reduced by one, i.e., $p_{eff} = p - 1$. In cDNA arrays, several probes may consist of ESTs originated from the same gene, so these probes will give highly correlated expression profiles. Since the exact degree of correlation is usually unknown, one must estimate the total number of redundant probes, and subtract them from $p$ to obtain $p_{eff}$. As $p_{eff} < p$, and $c_{p_{eff}} < c_p$, the effect of a gene-gene correlations is to relax the gene selection criterion and hence more genes are selected. Interestingly, a few recent publications show that gene-specific test scores are almost independent [39, 48]. As a result, the problem (3) may not be a serious problem for real data.

When the multiple testing is considered in a $t$-test, the gene selection criterion becomes more stringent with more number of genes. It is the same situation for the E-criterion and P-criterion. Both E- and P-criterion are conservative in the sense that the $j$-th ranked gene is compared to the top-ranked classification performer, instead of the $j$-th ranked one, in the surrogate data. If the E- and P-criterion are compared to each other, we find that for small values of $P_0$, such as $P_0 = 0.01$ or $P_0 = 0.001$, the P-criterion is more stringent than the E-criterion. It is because the E-criterion uses the average of the extreme-value
distribution whereas the P-criterion uses the tail area of the distribution.

The conservative nature of the E- and P-criterion yields a side effect that fewer number of genes are selected than some other gene selection criteria. This may be a positive or negative side effect, depending on the goal of the data analyst. Selecting many genes as differentially expressed increases the risk of declaring non-differentially expressed genes as differentially expressed, and selecting only a few genes increases the risk of missing differentially expressed genes. In the framework of hypothesis testing, one can reduce the type-I error (the number of false positives) at the cost of increasing the type-II error (the number of false negatives). A too stringent gene criterion reduces the number of false positives in the set of selected genes at the cost of missing potentially meaningful genes. Whether or not a good balance is reached in the E- and P-criterion can only be judged by future applications of these to real data.

**Acknowledgments**

We thank Yaning Yang, Stephan Beirer, Hanspeter Herzel, Dirk Holste, Armin Schmitt, and Stefan Posch for valuable discussions, Wei Pan for recommending references, and NIH (N01-AR12256), NSF (0241102), and BMBF for financial support.

**References**

[1] U Alon, N Barkai, DA Notterman, K Gish, S Ybarra, D Mack, AJ Levine (1999), “Broad patterns of gene expression revealed by clustering analysis of tumor and normal colon tissues probed by oligonucleotide arrays”, *Proceedings of National Academy of Sciences*, 96(12):6745-6750.

[2] A Antoniadis, S Lambert-Lacroix, F Leblanc (2003), “Effective dimension reduction methods for tumor classification using gene expression data”, *Bioinformatics*, 19:563-570.

[3] P Baldi, AD Long (2001), “A Bayesian framework for the analysis of microarray expression data: regularized t-test and statistical inference of gene changes”, *Bioinformatics*, 17(6):509-519.

[4] P Broberg (2003), “Statistical methods for ranking differentially expressed genes”, *Genome Biology*, 4:R41.
[5] G Casella, RL Berger (2002), *Statistical Inference*, 2nd edition (Duxbury). Chapter 10.

[6] Y Chen, E Dougherty, ML Bittner (1997), “Ratio-based decisions and the quantitative analysis of cDNA micro-array images”, *Journal of Biomedical Optics*, 2:364-374.

[7] F Chiaromonte, J Martinelli (2002), “Dimension reduction strategies for analyzing global gene expression data with a response”, *Mathematical Biosciences*, 176(1):123-144.

[8] JM Claverie (1999), “Computational methods for the identification of differential and coordinated gene expression”, *Human Molecular Genetics*, 8:1821-1832.

[9] S Coles (2001), *An Introduction to Statistical Modelling of Extreme Values* (Springer-Verlag).

[10] DR Cox, DV Hinkley (1974), *Theoretical Statistics* (Chapman & Hall). Chapter 9.

[11] CHQ Ding (2003), “Unsupervised feature selection via two-way ordering in gene expression analysis”, *Bioinformatics*, 19:1259-1266.

[12] S Draghici (2002), “Statistical intelligence: effective analysis of high-density microarray data”, *Drug Discovery Today*, 7(11):S55-S63.

[13] S Dudoit, J Fridlyand, TP Speed (2002), “Comparison of discrimination methods for the classification of tumors using gene expression data”, *Journal of the American Statistical Association*, 97(457):77-87.

[14] PH Eilers, JM Boer, GJ van Ommen, HC van Houwelingen (2001), “Classification of microarray data with penalized logistic regression”, *Proceedings of SPIE*, 4266:187-198.

[15] J Fan, HN Hung, WH Wong (2000), “Geometric understanding of likelihood ratio statistics”, *Journal of American Statistical Association*, 95:836-841.

[16] TS Ferguson (1996), *A Course in Large Sample Theory* (Chapman & Hall). Chapter 22.

[17] TR Golub, DK Sonim, P Tamayo, C Huard, M Gassenbeek, JP Mesirov, H Coller, ML Loh, JR Downing, MA Caligiuri, CD Bloomfield, ES Lander (1999), “Molecular classification of cancer: class discovery and class prediction by gene expression monitoring”, *Science*, 286:531-536.
[18] G Grant, E Manduchi, C Stoeckert Jr. (2002), “Using non-parametric methods in the context of multiple testing to determine differentially expressed genes”, in Methods of Microarray Data Analysis: Papers from CAMDA’00, eds. SM Lin, KF Johnson (Kluwer Academic), pp.37-55.

[19] EJ Gumbel (1954), Statistical Theory of Extreme Values and Some Practical Applications (National Bureau of Standards Applied Mathematics Series 33) (US Government Printing Office).

[20] EJ Gumbel (1960), Statistics of Extremes (Columbia University Press).

[21] EJ Gumbel, J Lieblein (1954), “Some applications of extreme-value methods”, American Statistician, 8(5):14-17.

[22] SS Gupta (1960), “Order statistics from the gamma distribution”, Technometrics, 2:243-262.

[23] T Hastie, R Tibshirani, J Friedman (2001), The Elements of Statistical Learning: Data mining, inference, and prediction (Springer).

[24] T Ideker, V Thorsson, AF Siegel, LE Hood (2000), “Testing for differentially-expressed genes by maximum-likelihood analysis of microarray data”, Journal of Computational Biology, 7(6):805-817.

[25] J Jaeger, R Sengupta, WL Ruzzo (2003), “Improved gene selection for classification of microarrays”, Pacific Symposium on Biocomputing, 8:53-64.

[26] KE Lee, N Sha, ER Dougherty, M Vannucci, BK Mallick (2003), “Gene selection: a Bayesian variable selection approach”, Bioinformatics, 19:90-97.

[27] H Li, F Hong (2001), “Cluster-Rasch models for microarray gene expression data”, Genome Biology, 2(8):research0031.

[28] J Li, H Liu, JR Downing, AEJ Yeoh, L Wong (2003), “Simple rules underlying gene expression profiles of more than six subtypes of acute lymphoblastic leukemia (ALL) patients”, Bioinformatics, 19:71-78.

[29] L Li, LG Pedersen, TA Darden, CR Weinberg (2002), “Computational analysis of leukemia microarray expression data using the GA/KNN method”, in Methods of Microarray Data Analysis: Papers from CAMDA’00, eds. SM Lin, KF Johnson (Kluwer Academic), pp.81-95.
[30] W Li, Y Yang (2002), “How many genes are needed for a discriminant microarray data analysis?”, in *Methods of Microarray Data Analysis: Papers from CAMDA’00*, eds. SM Lin, KF Johnson (Kluwer Academic), pp. 137-150.

[31] W Li, Y Yang (2002), “Zipf’s law in importance of genes for cancer classification using microarray data”, *Journal of Theoretical Biology*, 219:539-551.

[32] SM Lin, KF Johnson, eds. (2002), *Methods of Microarray Data Analysis: Papers from CAMDA’00* (Kluwer Academic).

[33] J Lu, S Hardy, WL Tao, S Muse, B Weir, S Spruill (2002), “Classical statistical approaches to molecular classification of cancer from gene expression profiling”, in *Methods of Microarray Data Analysis: Papers from CAMDA’00*, eds. SM Lin, KF Johnson (Kluwer Academic), pp. 97-107.

[34] F Model, P Adorjan, A Olek, C Piepenbrock (2001), “Feature selection for DNA methylation based cancer classification”, *Bioinformatics*, 17(Suppl 1):S157-S164.

[35] DM Mutch, A Berger, R Mansourian, A Rytz, MA Roberts (2002), “The limit fold change model: a practical approach for selecting differentially expressed genes from microarray data”, *BMC Bioinformatics*, 3:17.

[36] DV Nguyen, DM Rocke (2002), “Tumor classification by partial squares using microarray gene expression data”, *Bioinformatics*, 18:39-50.

[37] W Pan (2002), “A comparative review of statistical methods for discovering differentially expressed genes in replicated microarray experiments”, *Bioinformatics*, 18(4):546-554.

[38] W Pan (2003), “On the use of permutation in and the performance of a class of nonparametric methods to detect differential gene expression”, *Bioinformatics*, 19(11):1333-1340.

[39] W Pan, J Lin, C Le (2003), “A mixture model approach to detecting differentially expressed genes with microarray data”, *Functional & Integrative Genomics*, 3:117-124.

[40] SI Resnick (1987), *Extreme Values, Regular Variation, and Point Processes* (Springer-Verlag).

[41] RR Sokal, FJ Rohlf (1995), *Biometry*, 3rd edition (Freeman).
[42] M Sanchez-Carbayo, N Socci, JJ Lozano, W Li, T Belbin, M Preystowski, A Ortiz, G Childs, C Cordon-Cardo (2003), “Gene discovery in bladder cancer progression using cDNA microarrays”, American Journal of Pathology, 163:505-516.

[43] SK Shevade, SS Keerth (2002), “A simple and efficient algorithm for gene selection using sparse logistic regression”, Technical Report CD-02-22, Control Division, Department of Mechanical Engineering, National University of Singapore.

[44] JN Siedow (2001), “Making sense of microarrays” (meeting report), Genome Biology, 2(2):reports4003.

[45] GK Smyth, YH Yang, T Speed (2003), “Statistical issues in cDNA microarray data analysis”, in Functional Genomics: Methods and Protocols, eds. MJ Brownstein and AB Khodursky (Methods in Molecular Biology Series, Vol 224) (Humana Press), pp.111-136.

[46] A Sturt, JK Ord, S Arnold, M Kendall (1999), Kendall’s Advanced Theory of Statistics: Vol 2A: Classical Inference and and the Linear Model, 6th edition (Edward Arnold). Chapter 22.

[47] JG Thomas, JM Olson, SJ Tapscott, LP Zhao (2001), “An efficient and robust statistical modeling approach to discover differentially expressed genes using genomic expression profiles”, Genome Research, 11:1227-1236.

[48] R Tibshirani, T Hastie, B Narasimhan, G Chu (2003), “Class prediction by nearest shrunken centroids, with applications to DNA microarrays”, Statistical Science, 18:104-117.

[49] LJ van’t Veer, H Dai, MJ van de Vijver, YD He, AAM Hart, M Mao, JL Peterse, K van der Kooy, MJ Marton, AT Witteveen, GJ Schreiber, RM Kerkhoven, C Roberts, PS Linsley, R Bernards, SH Friend (2002), “Gene expression profiling predicts clinical outcome of breast cancer”, Nature, 415:530-536.

[50] SS Wilks (1938), “The large sample distribution of the likelihood ratio for testing composite hypothesis”, Annals of Mathematical Statistics, 9:60-62.

[51] SS Wilks (1962), Mathematical Statistics (Wiley).

[52] M Xiong, WJ Li, J Zhao, L Jin, E Boerwinkle (2001), “Feature (gene) selection in gene expression-based tumor classification”, Molecular Genetics and Metabolism, 73(3):239-247.
Figure 1: Numerical simulation of the extreme-values $T_p = \max\{t_1, t_2, \ldots, t_p\}$ of $p$ random variables $t_1, t_2, \ldots, t_p$ sampled from the $\chi^2$ distribution with 1 degree of freedom. The mean $E[T_p]$ (solid dots), the median $m[T_p]$ (triangles), and the standard deviation $\sigma[T_p]$ (crosses) are plotted against $\log(p)$ for $p$ ranging from 1 to $1.5 \times 10^5$. The analytic results of the mean, median, and standard deviation by Eq.(9), which are exact for asymptotic $p$, are shown in solid lines. For asymptotically large $p$, both the mean and the median of $T_p$ increase with $p$ as $\sim 2 \log(p) - \log(\log(p))$. A linear regression line fitting the mean of $T_p$ is displayed in dashed line: $E[T_p] \approx -1.14 + 1.89 \log(p)$ (the fitting range of $p$ is from $10^3$ to $1.5 \times 10^5$). The horizontal solid line is the standard deviation of $\sqrt{2\pi^2/3} \approx 2.56$. 
Figure 2: (a) Rank-ordered log-likelihood ratios for ALL versus AML dataset: $t(j) \ (j = 1, 2, \ldots p)$ defined in Eq.(10). (b) Rank-ordered P-values for the same dataset: $P(j) = 1 - \exp(-\exp(-v(j)))$, where $v(j) = (t(j) - c_p)/2$. In (a) E-criterion declares 407 genes as differentially expressed, and in (b) the more conservative P-criterion declares 165 genes as differentially expressed.
Figure 3: (a) Rank-ordered log-likelihood ratios for T-cell versus B-cell dataset. (b) Rank-ordered P-values for the same dataset. In (a) the E-criterion declares 114 genes as differentially expressed, and in (b) the more conservative P-criterion declares 57 genes as differentially expressed.
Figure 4: (a) Rank-ordered log-likelihood ratios for colon versus normal dataset. (b) Rank-ordered P-values for the same dataset. In (a) the E-criterion declares 49 genes as differentially expressed, and in (b) the more conservative P-criterion declares 10 genes as differentially expressed.