A Regularized Method for Selecting Nested Groups of Relevant Genes from Microarray Data

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Abstract

Gene expression analysis aims at identifying the genes able to accurately predict biological parameters like, for example, disease subtyping or progression. While accurate prediction can be achieved by means of many different techniques, gene identification, due to gene correlation and the limited number of available samples, is a much more elusive problem. Small changes in the expression values often produce different gene lists, and solutions which are both sparse and stable are difficult to obtain. We propose a two-stage regularization method able to learn linear models characterized by a high prediction performance. By varying a suitable parameter these linear models allow to trade sparsity for the inclusion of correlated genes and to produce gene lists which are almost perfectly nested. Experimental results on synthetic and microarray data confirm the interesting properties of the proposed method and its potential as a starting point for further biological investigations.
1 Introduction

The extraction of relevant biological information from gene expression data – like disease subtype, survival time, or assessment of the gravity of an illness – requires the identification of the list of the genes potentially involved in the process, genes which need to be further scrutinized by cross-checking the available knowledge or through additional quantification methods. In a typical study, the size of the data set is less than a hundred, while the dimensionality of the data may be tens of thousands. As a consequence, feature selection, i.e., the identification of the gene signature actually involved in the process under study, is a formidable task for which classical statistics (designed to deal with large sets of data living in low-dimensional spaces) may not be well suited.

A great amount of supervised learning techniques have been proposed to address the problem of feature selection – see e.g. the recent surveys by Dudoit et al. [2002], Guyon and Elisseeff [2003], Saeys et al. [2007] and Vert [2007], and the references therein. One usually classifies the methods into three categories: filters, wrappers, and embedded methods. Filters implement feature selection through a preprocessing step disconnected from the learning phase. Examples of filters are ranking criteria where standard statistical tests, correlation, and mutual information are used to score each feature [Golub et al., 1999, Weston et al., 2000, Forman, 2003]. Filters are also used as a preprocessing step to reduce the huge dimensionality of feature space. The drawback of these approaches is that the selection of features is performed beforehand and independently of the specific required prediction or classification task. Moreover, the selection is often made on a univariate basis, i.e. neglecting the possible correlations between the features.

On the other hand, in wrappers, the relevance of a feature subset is determined according to prediction performance of a given learning machine ([Guyon et al., 2002, Kohavi and John, 1997, Furlanello et al., 2003] and references therein). However, the exploration of all subsets of a high-dimensional feature space is in general a very demanding task from the computational point of view.
Differently from wrappers and filters, where variable selection and training are two separate processes, *embedded methods* present the advantage of incorporating feature selection within the construction of the classifier or regression model. Besides decision trees [Breiman et al., 1984] and boosting methods such as the popular Adaboost [Freund and Schapire, 1997], an appealing new trend has emerged recently namely the use of penalized methods in genomics or proteomics. These methods consist in the minimization of a well-defined objective function to which a penalty term is added in order to avoid “overfitting”, i.e. to provide some form of “regularization” – or equivalently an implicit reduction of the dimensionality of the feature space. A variety of such methods have been proposed in the recent literature and differ by the choice of the objective function and of the penalty term; for a recent overview, see e.g. [Segal et al., 2003],[Ma and Huang, 2008] and the references therein.

Particularly interesting are penalties which allow to enforce sparsity of the model, namely to perform automatic feature selection by assigning truly zero weights to all but a small number of selected features. The most famous example are the $\ell^1$-type penalties used in the so-called LASSO regression, a name coined by Tibshirani [1996] as an acronym for “Least Absolute Shrinkage and Selection Operator”. The use of LASSO for genomics is also advocated e.g. in the recent papers by Ghosh and Chinnaiyan [2005] and Segal [2006]. However, a drawback of LASSO regression in the presence of groups of correlated features is that the method is not able to identify all members of the group. Under the name “elastic net” Zou and Hastie [2005] have proposed a modification of the LASSO method able to overcome such drawback and to identify groups of correlated genes.

Building on this elastic-net regularization strategy, we propose a two-stage method which produces gene signatures able to effectively address prediction problems from high-throughput data like DNA microarray. In the first stage, the method learns from the available data a minimal set of genes the expressions of which are best suited to accurately predict the biological parameter related to the problem at hand. By selecting the model through the combination of two optimization schemes, elastic net and regularized least
squares, our method leads to a model which, unlike the elastic net alone, is characterized by both sparsity and low bias. In the second stage, by varying a suitable parameter, the method is able to produce models of increasing cardinality by gradually including genes correlated with the set of genes identified in the first stage.

Being formulated as a convex optimization problem, our learning method has a sound mathematical foundation and, moreover, as we will see, the models can be efficiently computed through simple and easy-to-implement algorithms. The method relies on a truly multivariate analysis and, in contrast to the usual gene-by-gene analyses, does not only rank genes on the basis of their differential expression on the samples. The embedded feature selection mechanism takes into account the correlation patterns arising from the organization of genes in cooperating networks.

We show that the method gives stable results even in the presence of data set of low cardinality. The two main features of the proposed method are that it provides nested list of genes and that the genes additionally included in the longer lists are correlated with the genes of the shorter lists. Both these properties can be very helpful when analyzing high-throughput data and might shed light on the biological mechanisms under study. As shown by De Mol et al. [2008], instead, the obtained models are asymptotically equivalent in terms of prediction accuracy. The choice of which list is the most appropriate is left to the molecular biologist and ultimately depends on the underlying question and the available prior knowledge.

The paper is organized as follows. In Section 2 we describe the method we propose for extracting nested lists of relevant genes. In Section 3 we analyze the algorithms we developed to solve the main underlying optimization problem and the model selection problem. Experimental results are presented and discussed in Section 4.
2 Our approach

In this section we first set the notation and review some basic concepts of learning theory which are relevant to this research. Then, we present our method and motivate our strategy for model selection.

2.1 Formulation of the problem

We assume we are given a set of \( n \) examples as input/output pairs. We denote the inputs with \( x_i \in \mathcal{X} = \mathbb{R}^p, i = 1, \ldots, n; \) in our case the components of the vector \( x_i \) are the expressions of the \( p \) probe sets synthesized on the chip for each patient \( i \). We note that \( n \) may be about 100 or 1000 times smaller than \( p \). The outputs, or corresponding responses, are denoted with \( y_i \in \mathcal{Y} \) and can be either a discrete class label in classification problems (e.g. discriminating between disease subtypes), or a continuous real variable in regression problems (e.g. a measurement of some biological parameter, survival time, or assessment of the gravity of the illness). The problem we face is to find which of the \( p \) components are needed to predict the response \( y \) as accurately as possible from any given input \( x \). In our case the model cardinality is known to be much smaller than \( p \), though the complexity of gene regulatory networks makes it difficult to determine the number of genes actually involved in the process.

We restrict our attention to linear functions, or equivalently to vectors \( \beta \in \mathbb{R}^p \), modelling the relation between \( x \) and \( y \) as \( y = \beta \cdot x \). For simplicity we assume that both \( x \) and \( y \) have zero mean. As customary in learning theory, the given examples pairs are assumed to be drawn i.i.d. from a fixed but unknown probability density \( p(x, y) \) with \((x, y) \in \mathcal{X} \times \mathcal{Y} \). Therefore, if the risk of predicting \( \beta \cdot x \) instead of \( y \) is measured by \((\beta \cdot x - y)^2 \), the expected risk of a given model \( \beta \), in the least-squares sense, is given by

\[
\mathcal{E}[\beta] = \int_{\mathcal{X} \times \mathcal{Y}} (y - \beta \cdot x)^2 \, p(x, y) \, dx \, dy.
\] (1)
The goal is to determine a sparse model \( \beta^* \), i.e. a model of cardinality much smaller than \( p \) – that is, a vector \( \beta^* \) with only \( k \) entries different from zero (with \( k << p \)) – for which the expected risk, \( \mathcal{E}[\beta^*] \), takes on a small value. We recall that the components of the model vector are called regression coefficients or weights.

### 2.2 Penalized regression methods for learning

The core of the method we propose in this paper is the minimization of the objective function recently proposed by Zou and Hastie [2005] and which we write as

\[
\frac{1}{n} \| Y - X \beta \|^2_2 + \mu \| \beta \|^2_2 + \tau \| \beta \|_1 \quad (2)
\]

with \( X \) the \( n \times p \) matrix such that the entry \( [X]_{ij} \) is \( j \)-th component \( x_{i,j} \) of \( x_i \) and \( Y \) the \( n \times 1 \) vector with \( [Y]_i = y_i \). In order to be consistent with the notation in (1) we subtract to the \( j - th \) component of \( x_i \) the average \( \frac{1}{n} \sum_i^n x_{i,j} \) and to \( y_i \) the average \( \frac{1}{n} \sum_i^n y_i \). In other words, data are recentered with respect to their center of mass. The first term in (2) measures the least-squares discrepancy of the model \( \beta \in \mathbb{R}^p \) on the \( n \) training examples and is the empirical risk – i.e. the empirical counterpart of the expected risk (1).

The second and third terms in (2) enforce uniqueness and numerical stability of the minimizer by penalizing respectively the square of the Euclidean, or \( \ell^2 \)-norm, \( \| \beta \|^2_2 = \sum_{j=1}^p \beta_j^2 \), and the \( \ell^1 \)-norm, \( \| \beta \|_1 = \sum_{j=1}^p |\beta_j| \), of the model vector \( \beta \). The nonnegative parameters \( \mu \) and \( \tau \) are the corresponding regularization parameters. The minimizer \( \beta_{en}(\mu, \tau) \) of (2), called the naïve elastic net by Zou and Hastie [2005], trades closeness to the data with the size of the \( \ell^2 \)- and \( \ell^1 \)-norm of the solution. Before discussing the use of (2) in our approach, let us first summarize the main properties of the two penalized schemes obtained by setting either \( \mu = 0 \) or \( \tau = 0 \) in (2).

model vector $\beta$, measured by its $\ell^2$-norm. Setting $\tau = 0$ in the objective function (2), one gets this $\ell^2$-norm penalized regression. The unique minimizer is then a model vector with typically all entries different from zero. The linear computational schemes arising from this framework are easy to implement and produce numerically stable solutions which, for optimal values of the regularization parameter, lead to accurate predictions. However, they tend to distribute the weights evenly among correlated features and, thus, they are ill-suited for performing feature selection.

**LASSO regression** [Tibshirani, 1996] avoids overfitting by enforcing sparsity, i.e. by favoring model vectors with only a small number of entries different from zero. This scheme is equivalent to $\ell^1$-norm penalized regression and is obtained by setting $\mu = 0$ in the objective function (2). In applications in which the solution is known to depend on a relatively small number of features, LASSO appears to be quite appropriate. The minimizer is known to be unique (except for very special configurations of the inputs) and stable with respect to noise in the output data $y_i$. However, small changes in the components of the input data $x_i$ lead to a different feature selection, typically with no appreciable change in the overall expected risk (or accuracy in the performance) of the obtained model. Consequently, when the inputs are affected by noise or the number of examples is small compared to the number of features, the selection of the components of the model vector $\beta$ might be driven by random fluctuations.

Empirical evidence [Zou and Hastie, 2005] indicates that the naïve elastic net produces stable solutions, exhibits an interesting grouping effect by selecting correlated features (due to the presence of the $\ell^2$-norm term), but suffers from a quite severe solution bias (due to the shrinkage phenomenon induced by the $\ell^1$-norm term). Moreover, good generalization performances are reported only for large values of the $\mu$ parameter, case in which the obtained solution is very similar to ridge regression. In order to contrast bias and enhance the ability of $\ell^1$-norm of promoting sparse solutions, Zou and Hastie [2005] proposed to rescale the coefficients and introduced what they called the elastic net. We rely on the naïve elastic net for our method but, in order to overcome its limitations, we
explore a different direction and propose an alternative remedy.

### 2.3 A two-stage method

Our method consists of two stages. In stage I we obtain a model with minimal cardinality and small bias by selecting the model through the coupling of two optimization procedures. In the first optimization procedure we perform gene selection by minimizing (2) for a small value of the $\ell^2$-norm parameter $\mu$. The second optimization is a regularized least squares and consists in minimizing

$$
\frac{1}{n} \left\| Y - \tilde{X}\tilde{\beta} \right\|_2^2 + \lambda \left\| \tilde{\beta} \right\|_2^2,
$$

(3)

in which $\tilde{\beta}$ and $\tilde{X}$ represent respectively the weights vector $\beta$ and the input matrix $X$ restricted to the genes selected by the first procedure. The cross-validation protocol which we employ for model selection yields relatively large values of $\tau$ and very small values of $\lambda$. Consequently, for the optimal parameter pair, the solution obtained from the first optimization selects a small number of genes (typically characterized by a severe bias) while the regularized least-squares minimization (3) restricted to the selected genes returns a model capable of more accurate predictions.

In Stage II we aim at gradually increasing the model cardinality by including genes correlated to the minimal set identified in stage I. This result is achieved by running the two optimization procedures of the previous stage for increasing values of $\mu$, while keeping $\lambda$ and $\tau$ fixed to the optimal values obtained through the cross-validation protocol mentioned above. The resulting one-parameter family of solutions, $\{\beta_\mu|\mu \geq 0\}$, yields lists of relevant genes of increasing cardinality. The experimental results reported in Subsection 3.2 show that the obtained lists, for increasing values of $\mu$, are almost perfectly nested.

Our findings are in line with a recent work [De Mol et al., 2008] in which the minimization of (2) is shown to yield consistent estimators of the linear model for $n \to \infty$. This means that we can find suitable sequences of parameters $\tau_n$ and $\mu_n = \epsilon \tau_n$, tending to
zero as $n \to \infty$, such that we have, in probability,

$$
\mathcal{E}[\beta(\mu_n, \tau_n)] \to \inf \mathcal{E} \quad \text{for} \quad n \to \infty.
$$

Notice that consistency, which is obtained for any value of the parameter $\epsilon$ controlling the degree of correlation, implies Bayes consistency, namely that the misclassification error of $\beta(\mu_n, \tau_n)$, $R[\beta]$, converges to the Bayes risk $R^*$, since (Bartlett et al. [2006])

$$
(R[\beta] - R^*) \leq \sqrt{\mathcal{E}[\beta] - \inf \mathcal{E}}.
$$

2.4 The need for a second optimization procedure

Leng et al. [2006] have shown that if the prediction accuracy is used as a criterion to choose the tuning parameter, $\ell^1$-norm penalized regression methods (like LASSO) provide consistent estimates in terms of prediction accuracy but not necessarily in terms of variable selection. With the following toy example we provide empirical evidence of the fact that very good prediction accuracy and correct variable selection can be both achieved by coupling the $\ell^1$-norm penalized regression method with a second optimization step restricted to the selected variables.

We consider a linear regression model $y = x \cdot \beta^* + \epsilon$ in which the samples $x$ and the model $\beta^*$ belong to $\mathbb{R}^{1000}$, $y \in \mathbb{R}$, and $\epsilon$ represents the noise. The training and the validation sets are built by randomly drawing 50 and 1000 samples, respectively, from a uniform distribution between $[-1, 1]$ for each of the 1000 components. The true model $\beta^*$ has only the first three components different from 0 while the noise is sampled from a zero-mean Gaussian distribution with standard deviation $\sigma = 0.5$.

For simplicity, we set $\mu = \lambda = 0$ and compare the results obtained with

- (a) $\ell^1$-norm penalized regression (LASSO) alone, and
- (b) $\ell^1$-norm penalized regression followed by ordinary least squares on the selected
Tab. 1

Table 1:

The error curves in Figure 1 show how procedure (b) allows to reach a lower minimum than (a). The validation error is taken to be the mean-square error. The minimum is clearly reached for a larger value of \( \tau \), i.e. when the \( \ell^1 \)-norm penalized regression algorithm selects a lower number of features.

Fig. 1

Figure 1:

Let us now compare the estimators \( \beta^a \) and \( \beta^b \) obtained, respectively, through (a) with \( \tau = \tau^a \) and through (b) with \( \tau = \tau^b \) (these parameters minimize the corresponding validation error). In Table 1 we report the first three components of \( \beta^*, \beta^a, \beta^b \). The last column \( \beta^c \), represents the output of \( \ell^1 \)-norm penalized regression with \( \tau = \tau^b \). From Table 1, we can see that both \( \beta^a \) and \( \beta^b \) approximate well the relevant components of \( \beta^* \). However, while \( \beta^b \) correctly selects the model, \( \beta^a \) has many non-zero components besides the first three. This is due to the \( \ell^1 \)-norm penalized regression which, in order to reduce bias, induces an optimal choice of \( \tau \) smaller than the one needed to correctly identify the model. This can also be seen from the fact that, whereas the \( \ell^1 \)-norm penalized regression followed by ordinary least squares selects the correct features (the model \( \beta^b \) has only the first three components different from zero) and returns almost perfect feature weights, the estimator \( \beta^c \) – obtained for \( \tau = \tau^b \) – underestimates all three coefficients. We can thus conclude that the model selection obtained by coupling the two optimization procedures appears to be more effective.
3 Algorithmic aspects

In this section we discuss several algorithmic aspects of our work. We first present an iterative algorithm for estimating the elastic net solution, and then describe the details of our procedure for model selection which, even for data sets of small size, requires a very large number of optimization cycles. Finally, we provide empirical evidence of the correctness of a procedure capable of obtaining almost exactly the same lists of genes with a large reduction of computing time.

3.1 Damped iterative thresholding

In order to minimize (2), we use an algorithm which generalizes the following Landweber or gradient-descent iterative procedure [Engl et al., 1996], known to converge to a minimizer of the unpenalized least-squares objective function $\Lambda(\beta) = \frac{1}{n} ||Y - X\beta||^2_2$:

$$
\beta^{(l+1)} = \beta^{(l)} + \frac{1}{C}[X^TY - X^TX\beta^{(l)}]; \quad l = 0, 1, \ldots
$$

(4)

where $X^T$ denotes the transpose of $X$ and the constant $2C$ is a strict upper bound for the spectral norm of the matrix $X^TX : \|X^TX\| < 2C$.

Inspired by the iterative thresholding algorithm proposed by Daubechies et al. [2004] for pure $\ell^1$-norm penalized regression, we propose a double modification of Landweber algorithm which provably converges to the minimizer of (2). The first modification amounts to applying a soft-thresholding operator $S_{\alpha/C}$ at each iteration. The soft-thresholding operator $S_{\alpha}$ acts on a vector component-wise as follows

$$
[S_{\alpha}(\beta)]_j = \begin{cases} 
(|\beta_j| - \alpha/2) \text{sign}(\beta_j) & \text{if } |\beta_j| \geq \alpha/2 \\
0 & \text{if } |\beta_j| < \alpha/2.
\end{cases}
$$

(5)

This operation enforces the sparsity of the regression coefficients in the sense that all coefficients below the threshold $\alpha/2$ are set to zero. The second modification is a simple
multiplication which leads to the following damped iterative thresholding scheme:

\[
\beta_{en}^{(l+1)} = \frac{1}{1+\tau \mu} S_{\tau \mu} (\beta_{en}^{(l)} + \frac{1}{C} [X^T Y - X^T X \beta_{en}^{(l)}]).
\] (6)

We recover the cases of ridge regression and damped Landweber iteration for \(\tau = 0\), whereas pure \(\ell^1\)-regularization and the iterative thresholding scheme considered in Daubechies et al. [2004] correspond to the special case \(\mu = 0\). For \(\tau = \mu = 0\), we get the original Landweber iteration (4). The convergence of (6) – for \(\mu > 0\) and any initial vector \(\beta_{en}^{(0)}\) – to the minimizer of (2) is a straightforward consequence of Banach’s fixed point theorem for contractive mappings (see De Mol et al. [2008] for an extensive discussion of the properties of this algorithm in a broader setting).

Fig. 2

Figure 2:

Since the proposed scheme is iterative we need to define a stopping rule. We first tried to use a fixed tolerance \(\delta\), letting the iterations stop if \(|\beta_k^{(l+1)} - \beta_k^{(l)}|_2 \leq \delta |\beta_k^{(l)}|_2\), for all \(k\). However, after extensive experimentation on toy examples and real data, we empirically observed that a tolerance depending on the number of iterations is more efficient and equally easy to implement. As shown in Figure 2, if the algorithm stops when the relative change of each coefficient \(\beta_k^{(l)}\) is smaller than a tolerance \(\delta = 0.1/l\), with \(l\) the number of performed iterations, the support of the selected features appears to be stabilized.

3.2 Model selection procedure

In all of the performed experiments the training sets were recentered as described in Subsection 2.2 and the test sample (either of the validation or of the test set) was recentered with respect to the center of mass obtained from the training set.

The data set is initially divided in training and test set. The training set is further partitioned in \(k\) subsamples \(X_1, \ldots, X_k\) with \(k\) depending on the cardinality of the training
set. In Stage I, for each subsample $X_i$, a classifier is first built using as training set the remaining $k - 1$ subsamples with $\tau$ and $\lambda$ ranging on a grid in the parameters space, and then validated on $X_i$. Each classifier is built by minimizing the objective functions (2) and (3) with the current values of $\tau$ and $\lambda$ and a fixed small value for $\mu$ (typically $\mu = 10^{-6}$). For each parameter pair the validation error is estimated as the average error over the $k$ subsamples. Finally the optimal parameter pair, $(\tau_{opt}, \lambda_{opt})$, is selected as the minimizer of the validation error.

In Stage II a family of classifiers is built on the entire training set with $\tau = \tau_{opt}$, $\lambda = \lambda_{opt}$ and for $m$ increasing values of $\mu$. Along with a test error each classifier returns a list of variables indexed by the value of $\mu$. A pseudo-code version of this procedure is summarized in the box below.
Given

-\((X, Y)\) training set, and \((X^{\text{test}}, Y^{\text{test}})\) test set

-\\{\((X_1, Y_1), \ldots, (X_k, Y_k)\)\} partition of \((X, Y)\)

-\(\mu_0 < \mu_1 < \cdots < \mu_{m-1}\)

Stage I

-let \(\mu = \mu_0, (\tau_t, \lambda_l)_{t \in T, l \in L}\) a grid in parameter space

-for \(t \in T\) and \(l \in L\)

\[
\text{for } i = 1 \text{ to } k \text{ let }
X_i^{tr} := X_1, \ldots X_{i-1}, X_{i+1}, \ldots, X_k
\]
\[
Y_i^{tr} := Y_1, \ldots Y_{i-1}, Y_{i+1}, \ldots, Y_k
\]
\[
\beta(t, l, i) := \text{classifier built on } (X_i^{tr}, Y_i^{tr}) \text{ for } \tau = \tau_t, \mu = \mu_0, \text{ and } \lambda = \lambda_l
\]
\[
\text{Err}(t, l, i) := \text{error made by } \beta(t, l, i) \text{ on } (X_i, Y_i)
\]

\[
\overline{\text{Err}}(t, l) := \frac{1}{k} \sum_{i=1}^{k} \text{Err}(t, l, i)
\]

end

Stage II

-find \((\tau_{opt}, \lambda_{opt})\) minimizing \(\overline{\text{Err}}(t, l)\)

-for \(i = 0 \text{ to } m - 1 \) let

\[
\beta_{\mu_i}^* := \text{classifier built on } (X, Y) \text{ for } \tau = \tau_{opt}, \mu = \mu_i, \text{ and } \lambda = \lambda_{opt}
\]
\[
\text{Err}_{i}^{\text{test}} := \text{error made by } \beta_{\mu_i}^* \text{ on } (X^{\text{test}}, Y^{\text{test}})
\]

end

For small values of \(\mu\), the solution of the damped iterative thresholding scheme (6) – first step for the construction of each classifier \(\beta(t, l, i)\) in Stage I – requires a very large number of iterations. Consequently, the procedure for determining the optimal values of \(\tau\) and \(\lambda\) in Stage I, procedure which must be repeated \(k \times |T| \times |L|\) times with \(\mu = 10^{-6}\), is quite time-consuming. In order to speed up the entire process, we explored
a different approach in which, for each value of $\tau$ and $\lambda$, a series of damped iterative thresholding schemes are run for 10 decreasing values of $\mu$ ($\mu_1 = 10^{-3}, ..., \mu_{10} = 10^{-6}$), the $i$-th scheme being restricted to the variables selected in the $(i-1)$-th scheme with $i = 2, ..., 10$. Extensive experiments on synthetic and real data indicate that the features selected through this alternative approach are almost always the same as those obtained with the procedure described in the original scheme, but with about a 100-fold reduction in computing time. For example Table 2 shows the results we obtained on three data sets of patient microarrays which we analyze in Subsection 4.3. As it can easily be verified by inspection of Table 2 the group of genes selected with the parameter pair $(\tau_{opt}, \mu)$ is almost perfectly enclosed in the group selected with the parameter pair $(\tau_{opt}, \bar{\mu})$ for $\bar{\mu} > \mu$. Therefore, we decided to implement our procedure with the optimization described above. Notice that, by doing so, the nesting of the obtained lists is always perfect, since the optimization for $\mu = \mu_i$ is restricted to the variables selected for $\mu = \mu_{i-1}$. 

Table 2:
4 Results and discussion

In this Section we report and discuss the results we obtained by running our method on both synthetic and real data. Real-data experiments encompass the analysis of both highly purified cell lines grown in laboratories and samples from patients’ tissues.

4.1 A toy problem

We first applied our method on a toy example generated according to scenario (d) in Zou and Hastie [2005], where the relevant features are known in advance. The problem is close to real gene expression data conditions in that it encompasses both dependence on more than one variable and intra-variables correlation, though in a setting of much lower dimensionality.

We consider a set of $n = 100$ toy-patients. To each patient $i$ we associate a 40-dimensional vector $x_i$ built as follows. We divide the 40 components in four groups. Group $G_1$ consists of the first five components $x_{i,1}, \ldots, x_{i,5}$ which are obtained by randomly drawing a number $Z_1$ from a zero-mean Gaussian distribution with $\sigma = 1$ and adding to it a noise term $\epsilon_j, j = 1, \ldots, 5$, randomly drawn from a zero-mean Gaussian distribution with $\sigma = 0.01$. For each $x_{i,j}$ we thus have

$$x_{i,j} = Z_1 + \epsilon_j, \quad \text{for } j = 1, \ldots, 5.$$ 

The second and third five components, belonging to the groups $G_2$ and $G_3$ respectively, are built similarly and read

$$x_{i,j} = Z_2 + \epsilon_j, \quad \text{for } j = 6, \ldots, 10$$

$$x_{i,j} = Z_3 + \epsilon_j, \quad \text{for } j = 11, \ldots, 15.$$ 

The fourth group $G_4$ consists of the remaining 25 components randomly drawn from a
zero-mean Gaussian distribution with $\sigma = 1$. By construction each of the groups $G_1$, $G_2$, and $G_3$ consists of five equivalent variables and the true model (of all possible models the one with largest cardinality) is written as

$$\beta = (1, 1, \ldots, 1, 0, 0, \ldots, 0).$$

For small values of $\mu$ the method is thus expected to select one variable from each of the $G_1$, $G_2$, and $G_3$ groups, while for increasing values of $\mu$ it should also include the remaining 12 variables. All the variables of the group $G_4$, instead, should be discarded independently of $\mu$.

We first run stage I of the method by setting $\mu = 0$ (i.e. perform a LASSO regression) and repeat the experiment over 50 data sets. Each data set was split in training and validation set and the parameters $\tau^*$ and $\lambda^*$ were chosen as the ones minimizing the error on the validation set. The method selects a correct model (one variable from each of the three groups $G_1$, $G_2$, and $G_3$) about 60% of the times and a slightly redundant set (one extra variable from any of the three groups) about 20% of the times.

We then run stage II with $\mu = 1000 \cdot \tau^*$. The frequency histogram of the number of selected features is shown in Figure 3, left. As expected, the histogram is peaked in correspondence of 15, the maximum number of relevant variables. The fact that the ratio between the number of the relevant features selected by the model and the number of features selected by the model is peaked around 1 (see Figure 3, right) confirms that most of the times the selected features belong to the correct model.
4.2 Cell-culture microarray data

We now analyze the RAS data set used in Bild et al. [2006] and available on line at http://data.genome.duke.edu/oncogene.php. In Bild et al. [2006] human primary mammary epithelial cell cultures (HMECs) were used to develop a series of pathway signatures related to different oncogenes. In short, cells were infected with different adenoviruses for eighteen hours and signatures were extracted as the set of genes most correlated to the classification of HMEC samples into oncogene-activated versus control. In order to test our method for gene selection, we applied our protocol on a subset of 20 HMEC samples, comprising 10 controls and 10 samples infected with adenovirus expressing activated H-Ras, thus extracting an alternative pathway signature for RAS oncogene. The classification task concerned with this data set is trivial since most classification algorithms can easily discriminate between the two classes. In this case, however, we are not interested in the classification performance on an independent test set, but in the selection of relevant gene lists and in their hierarchical structure. We thus apply our method to the RAS data and report the heat maps of the selected gene lists in Figure 4. For \( \mu = 0 \), the method extracts a minimal set consisting of two probe sets of RAP1A (Figure 4, left), a gene belonging to the RAS oncogene family, whereas for increasing values of \( \mu \), the method selects perfectly nested larger sets of genes (probe sets) correlated or anti-correlated with the first two, but with lower fold change. In Figure 4, middle and right, we show the results obtained for \( \mu = 0.05 \) and \( \mu = 0.5 \) respectively (corresponding to 15 and 144 genes).

From the obtained results we can see that the method selects nested groups of genes which appear to be relevant to the RAS status, nicely sorted by their differential expressions. The two minimal probe sets are not part of the RAS signature defined in [Bild et al., 2006], whereas in the larger gene lists about 80% of the genes overlap with those found in [Bild et al., 2006] (12 out of 15 and 112 out of 144).
4.3 Patient-tissue microarray data

Finally, we carried out experiments on data sets relative to three diseases: leukemia, lung cancer and prostate cancer. These gene expression data sets are available on line and concern classification problems. The first one is the Golub data set [Golub et al., 1999] (http://www.broad.mit.edu/cgi-bin/cancer/datasets.cgi) which comprises expressions of 7129 probe sets for 72 patients (samples) divided in two classes according to the diagnosis of Acute Myeloid Leukemia (AML) or Acute Lymphoblastic Leukemia (ALL). The lung cancer data set [Gordon et al., 2002] (http://www.chestsurg.org) consists of 181 samples with 12533 probe sets and each patients is either affected by malignant pleural mesothelioma (MPM) or adenocarcinoma (ADCA). Finally, the prostate data set [Singh et al., 2002] (http://www-genome.wi.mit.edu/mpr/prostate) consists again of 12533 probe sets for 102 samples, tumor or normal tissue. In all cases, the vector $Y$ is formed by labels $+1$ or $-1$ distinguishing the two classes.

We carried out our experiments through leave-one-out (LOO) cross-validation on the Leukemia data (given the small number of available samples), and 10-fold cross-validation on both the Lung and Prostate Cancer data. A first indicator of the effectiveness of the proposed method is the stability of the various gene lists obtained in the training phase. In Figure 5, 6, and 7 we report the number of selected genes versus the selection frequency for different values of the parameter $\mu$. By inspecting Figures 5, 6, and 7 one sees that the produced gene lists are remarkably stable. For increasing values of $\mu$ the number of genes appearing in all of the lists ranges from about $1/3$ to about $1/2$ of the average number of genes, while the number of genes appearing in at least 50% of the lists is very close to the average.
The accuracy of the method on the three data sets (which should remain the same for the different values of $\mu$) is illustrated in Table 3. By inspection we can see that for each disease and different values of $\mu$ (column A) the model cardinality from top to bottom increases (column B) while the prediction accuracy on the test set (column B) remains quite stable. For each disease in column B errors are reported for the two classes separately. The rightmost column C gives the percentage of samples which have to be rejected for both classes in order to reach 100% classification rate.

The rejection region corresponding to $\mu = 0$ for the three diseases is depicted in Figure 8. The solid line gives the decision boundary, while the dashed lines mark the rejection region needed to reach the perfect score. No rejection region is needed for the Leukemia study (Figure 8, left), a one-sided rejection region for the lung cancer study (Figure 8, middle) and a wider two-sided rejection region for the prostate cancer case (Figure 8, right).

An improvement in prediction accuracy is not the aim of the proposed method. However, it is interesting to notice that the proposed method reaches performances which are at least as good as and often better than those reported in the original studies. In the leukemia original paper [Golub et al., 1999], a 50-genes classifier is built which scored 100% on the test set, though only 29 of the 34 test samples corresponded to strong prediction (i.e. prediction with a high confidence level). The prediction accuracy of our method ranges from 91% to 100%. As for the lung cancer data analysis in Gordon et al. [2002], different classifiers were reported with prediction accuracy ranging from 91% to
99%, to be compared with the 99% achieved with our algorithm. In the end, for the prostate cancer data set, in Singh et al. [2002] – after gene ranking with variation of a signal-to-noise metric – a $k$-NN algorithm obtained a prediction accuracy ranging from 82.9% to 95.7% depending on the number of genes used (4 or 6); with our method the accuracy ranges from 92% to 96%.

Where available (leukemia and lung cancer), we have compared the gene lists we obtained with the lists produced by other methods. The results show partial superposition (depending on $\mu$) as well as important differences. The difference between our results and the ones reported in the original papers is not surprising given the multivariate flavor of our selection procedure. Ultimately, only biological validation can assess the actual relevance of the gene lists obtained by different methods.

5 Conclusion

In this paper we have proposed and analyzed a two-stage method able to select nested groups of relevant genes from microarray data. The first stage establishes a minimal subset of genes relevant to the classification or regression task under investigation. The second stage produces a one-parameter family of groups of genes, showing a remarkable nesting property and similar performance in terms of classification/prediction tasks. In several problems the ability of returning nested list of relevant genes is a key to establish the biological significance of the obtained results and is often regarded as the most precious information for further investigation based on biological knowledge and subsequent
In both stages the method consists of an initial step in which a certain amount of genes is selected through the minimization of the objective function (2) by means of a convergent damped iterative thresholding algorithm and of a second step in which the weights of the selected genes are refined through ridge regression.

In the first stage, the $\ell^1$ parameter $\tau$ and the regularization parameter $\lambda$ of the subsequent ridge regression are estimated from the data by cross-validation, while the $\ell^2$ parameter $\mu$ is set to a small value. This leads to a solution characterized by a minimal subset of genes. In the second stage, the $\ell^1$ and the ridge parameter are kept fixed to their estimated optimal value and a one-parameter family of solutions is generated for increasing values of the $\ell^2$ parameter $\mu$. In the proposed scheme the role of this $\ell^2$ parameter can be thought of as a way of controlling the trade-off between sparsity and correlation in the solution vector.

The results which we obtained on several data sets, including cell-culture and patient-tissue microarray data confirm the potential of our approach.
Acknowledgments

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Author Disclosure Statement

No competing financial interests exist.
References


Table 1: Relevant components of a linear model in $\mathbb{R}^{1000}$ in a toy problem estimated by different regression techniques.

<table>
<thead>
<tr>
<th></th>
<th>$\beta^*$</th>
<th>$\beta^a$</th>
<th>$\beta^b$</th>
<th>$\beta^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.6449</td>
<td>0.5667</td>
<td>0.6705</td>
<td>0.3912</td>
</tr>
<tr>
<td>2</td>
<td>0.8180</td>
<td>0.7389</td>
<td>0.8106</td>
<td>0.6388</td>
</tr>
<tr>
<td>3</td>
<td>0.6602</td>
<td>0.5785</td>
<td>0.6794</td>
<td>0.4210</td>
</tr>
</tbody>
</table>

From left to right: the true weights of the only three non-zero components of the model ($\beta^*$), the corresponding weights obtained with $\ell^1$-norm penalized regression with $\tau = \tau^a$ ($\beta^a$), with $\ell^1$-norm penalized regression followed by ordinary least squares with $\tau = \tau^b$ ($\beta^b$), and with $\ell^1$-norm penalized regression with $\tau = \tau^b$ ($\beta^c$).
<table>
<thead>
<tr>
<th>Leukemia</th>
<th></th>
<th>Lung C.</th>
<th></th>
<th>Prostate C.</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu$</td>
<td>A</td>
<td>B</td>
<td>$\mu$</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>0</td>
<td>30</td>
<td>100%</td>
<td>0</td>
<td>37</td>
<td>97%</td>
</tr>
<tr>
<td>$6 \cdot 10^{-6}$</td>
<td>35</td>
<td>100%</td>
<td>$2 \cdot 10^{-7}$</td>
<td>36</td>
<td>83%</td>
</tr>
<tr>
<td>$10^{-5}$</td>
<td>38</td>
<td>100%</td>
<td>$3 \cdot 10^{-6}$</td>
<td>54</td>
<td>94%</td>
</tr>
<tr>
<td>$3 \cdot 10^{-5}$</td>
<td>50</td>
<td>100%</td>
<td>$9 \cdot 10^{-6}$</td>
<td>79</td>
<td>99%</td>
</tr>
<tr>
<td>$5 \cdot 10^{-5}$</td>
<td>57</td>
<td>100%</td>
<td>$10^{-5}$</td>
<td>98</td>
<td>98%</td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>77</td>
<td>100%</td>
<td>$3 \cdot 10^{-5}$</td>
<td>152</td>
<td>100%</td>
</tr>
<tr>
<td>$2 \cdot 10^{-4}$</td>
<td>119</td>
<td>100%</td>
<td>$5 \cdot 10^{-5}$</td>
<td>182</td>
<td>100%</td>
</tr>
<tr>
<td>$4 \cdot 10^{-4}$</td>
<td>144</td>
<td></td>
<td>$7 \cdot 10^{-5}$</td>
<td>218</td>
<td></td>
</tr>
</tbody>
</table>

The leftmost column contains the values of the parameter $\mu$, while for each disease column A contains the number of selected genes and B the percentage of genes present in the gene set selected with the next larger value of $\mu$ and with $\tau$ fixed at its optimal value.
Table 3: Prediction accuracy of the proposed method on microarray data sets.

<table>
<thead>
<tr>
<th>Leukemia ($n_{test} = 34$)</th>
<th>Lung Cancer ($n_{test} = 90$)</th>
<th>Prostate Cancer ($n_{test} = 51$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu$</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>0</td>
<td>28 (0,0) (0%, 0%)</td>
<td>0</td>
</tr>
<tr>
<td>6$\cdot$10$^{-6}$</td>
<td>32 (0,1) (5%, 0%)</td>
<td>2$\cdot$10$^{-7}$</td>
</tr>
<tr>
<td>1$\cdot$10$^{-5}$</td>
<td>34 (0,2) (9%, 0%)</td>
<td>3$\cdot$10$^{-6}$</td>
</tr>
<tr>
<td>3$\cdot$10$^{-5}$</td>
<td>40 (0,2) (18%, 0%)</td>
<td>9$\cdot$10$^{-6}$</td>
</tr>
<tr>
<td>5$\cdot$10$^{-5}$</td>
<td>50 (0,3) (39%, 0%)</td>
<td>10$^{-5}$</td>
</tr>
<tr>
<td>1$\cdot$10$^{-4}$</td>
<td>71 (1,1) (20%,14%)</td>
<td>3$\cdot$10$^{-5}$</td>
</tr>
<tr>
<td>2$\cdot$10$^{-4}$</td>
<td>108 (1,2) (14%, 8%)</td>
<td>5$\cdot$10$^{-5}$</td>
</tr>
<tr>
<td>4$\cdot$10$^{-4}$</td>
<td>135 (1,2) (14%,15%)</td>
<td>7$\cdot$10$^{-5}$</td>
</tr>
</tbody>
</table>

For each of the three diseases the first column contains the values of the parameter $\mu$, the column A the number of selected genes, the column B the number of misclassified samples for the two original classes respectively, and the column C the percentage of samples to be rejected in each predicted class in order to obtain 100% classification rate. The two classes are (ALL, AML) for Leukemia, (MPM,ADCA) for Lung Cancer, and (normal, tumor) for Prostate Cancer.