An ensemble of discriminative local subspaces in microarray data for gene ontology annotations predictions.

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***Abstract*—Genome sequencing has allowed to know almost every gene of many organisms. However, understanding the functions of most genes is still an open problem. In this paper, we present a novel machine learning method to predict functions of unknown genes in base of gene expression data and Gene Ontology annotations. Most function prediction algorithms developed in the past don't exploit the discriminative power of supervised learning. In contrast, our method uses this to find discriminative local subspaces that are suitable to perform gene functional prediction. Cross-validation test are done in artificial and real data and compared with a state-of-the-art method. Preliminary results shows that in overall, our method outperforms the other approach in terms of precision and recall, giving insights in the importance of a good selection of discriminative experiments.**

***Keywords*-gene expression; microarray; gene function prediction; feature selection; gene ontology;**

I. Introduction

During the last decades, it has been possible to access the genome sequence of several species, however, understanding the main functions of most genes is still an open problem. In the biological arena is widely accepted that, in general, genes that share some particular function displays similar pattern of expression [1], [3]–[6]. This suggests the idea of predicting unknown gene functions based on the patterns of expression of genes with known biological functions. This approach has been followed by several previous works [9], [11], [13], [19], [20]. Furthermore, gene expression data has also been enriched with additional data sources, such as protein interactions and phenotype information, among others [8].

In terms of known genes functions, the Gene Ontology (GO) project is a major bioinformatics initiative with the aim of standardizing the representation of gene attributes across species and databases [2]. The GO project has developed three structured controlled vocabularies (ontologies) that describe gene products in terms of their associated biological processes, cellular components, and molecular functions in a species-independent manner. In particular, GO annotations are highly structured by biological process terms with a hierarchical ordering from highly general to more specific gene functions.

In this paper, we use GO terms annotations for genes of Arabidopsis thaliana, a model organism in plant biology. Our goal is to use these known gene functions to predict the functions of unknown genes by comparing their gene expression data. To achieve this, we use the expression of 22 thousand genes under 1701 experimental conditions available from public quality-screened microarray data.

Several previous works [9], [11]–[13], [17], [19], [20] have proposed classification schemes to predict gene functions using microarray data and GO terms. In general, most of these works can be classified under two major approaches. On one hand, discriminative approaches based on supervised algorithms that make use of all the available experimental conditions to establish relations among genes. On other hand, unsupervised schemes based on the so-called biclustering approach that search for subspaces of selective experimental conditions to establish meaningful relations between genes.

The main contribution of our work resides in proposing a new algorithm that takes advantage of the discriminative power of supervised learning, while at the same time explicitly searching for relations among genes in selective subspaces. Our approach is based on an adaptive nearest neighbor scheme that for each gene with a known function establishes a suitable discriminative subspace to perform gene function prediction.

This paper is organized as follows. Section II shows the current state-of-the-art and the main differences with our work. Section III discusses the details of our approach. Section IV shows the results of applying our methodology to the case of synthetic and real datasets. Finally, Section V presents the main conclusions of this work.

II. Related Work

Gene function prediction has been a very active topic in the last decade in the bioinformatics field. Eisen [6] was one of the pioneers in analyzing systematically this kind of data. In his work, he used an average-linkage approach to cluster the budding yeast genes that were co-expressed, using the Pearson correlation coefficient (PCC) as the similarity metric. Interestingly, the clusters grouped genes with known similar functions, leading to the idea of propagating putative functions to the uncharacterized clustered genes. Many other works have used this co-expression idea to try to predict gene function [9], [11]–[13], [17], [19], [20].

In a recent work, Van de Peer et al. [20] developed a method that predicts function based in the enrichment of GO terms in the gene's co-expression neighborhood. This is done by calculating the expression similarities between pairs of genes, using the PCC statistic, and then linking the genes with a PCC higher than a given threshold. The neighborhood of a gene is then determined by the genes linked to it. The statistical significance of the enrichment of each GO term in the gene's neighborhood is evaluated using the hypergeometric distribution, assigning the terms with a $p-value < 0.05$. They applied this method in 129 *A. thaliana* microarray experiments, but obtained overall low recalls $(13-34\%)$. In our work we try to improve this, mainly by using the current functional information, to guide the selection of subsets of experiments where the genes of the same functional category may correlate better. In terms of a pattern recognition problem, this can be seen as a feature selection to improve the similarity of the samples of the same class.

The idea that the gene's co-expression patterns may be hidden in a subset of experimental conditions is not new and lots of biclustering algorithms has been developed recently to try to tackle this (See [14], [15], [18] for reviews of biclustering algorithms). In few words, these methods try to find biclusters, that can be defined as a subset of genes that shows co-expression in a subset of conditions. As a clustering algorithm, this search is done in an unsupervised manner. Thus, to classify new data, an enrichment approach like the one used in [17] or [20] is usually done. However, biologically it is expected that genes with different functions can cooperate or function synchronically in some conditions. Thus, one drawback of the biclustering algorithms is that they can find very general subsets of conditions where genes with different functional roles can be co-expressed. This is specially important considering the great amount and diverse nature of the expression data that is being used and that is continually increasing. Our approach tries to overcome this by doing a supervised search of subsets of conditions (features) that maximize the similarity of genes (samples) of the same function (class) and that at the same time minimize the similarity between the genes with different functions (classes). These are key elements that distinguish our method for the current state-of-the-art.

III. Proposed Method

In this section we present DLS, our approach for gene function prediction. We start by describing and defining some important characteristics of the problem. Then, we describe the main steps of our algorithm.

A. Problem Definition

Gene function prediction based on microarray data and GO annotations can be cast as a machine learning based classification problem. As shown in Table I, we have N samples, $\{S_1, S_2, \ldots, S_N\}$, corresponding to N genes of a given organism, whose M features, $\{F_1, F_2, \ldots, F_M\}$, correspond to their expression levels, measured by microarrays under different experimental conditions. So, we have a data matrix $DataMatrix$ of $N \times M$, where $DataMatrix(i, j)$ holds the expression value of gene i under experimental condition j. Each sample belongs to one or more labels or classes $\{T_1, T_2, \ldots, T_{Nc}\}$ that correspond to the respective GO terms annotations. From now on, we will refer to samples and genes, features and experimental conditions, and classes and GO terms, interchangeably.

One relevant property of this type of data is that each sample might belong to multiple classes, as genes can play multiple roles in an organism. To deal with this, we adopted a divide-and-conquer strategy by training a different classifier to each class. In this way, the Nc classes problem is decomposed as Nc binary class problems. For each classification problem the goal is to predict if a new gene can be assigned to a specific functional annotation of interest, based on a training set of genes that have and don't have the given function. From now on, we will refer to the target class samples as $class1$ samples, and to the samples that are not from the class as $class0$ samples.

Another important characteristic is that not all the genes of the same functional category will necessarily share the same expression patterns in the same conditions. Moreover, GO terms are organized hierarchically, so many, more specific functions are embedded within each term, meaning that there can be subclasses within a class. Hence, we focus in finding discriminant local subspaces (DLS) within the genes of the same function. The idea is for each DLS to represent a discriminative pattern found within the $class1$ samples, so that they can be used to classify new instances. Formally, a DLS can be defined as a two-tuple (S_{DLS}, B_{DLS}). Here, $S_{DLS} \in S_1, S_2, ..., S_N$ is a $class1$ training sample and $B_{DLS} \in \Lambda_N$ is a binary vector that defines the feature subset where S_{DLS} is discriminative. Λ_N is the set of all *M*

Table I
GENE EXPRESSION DATA MATRIX REPRESENTATION USED FOR FUNCTION PREDICTION.

		Features					Classes	
		Condition 1	…	Condition j	…	Condition M	GO Terms	Class T_k
Samples	Gene 1	e_{11}	…	e_{1j}	…	e_{1M}	T_{11}, T_{12}, …	1
	…	…	…	…	…	…	…	…
	Gene i	e_{i1}	…	e_{ij}	…	e_{iM}	T_{i1}, T_{12}, …	0
	…	…	…	…	…	…	…	…
	Gene N	e_{N1}	…	e_{Nj}	…	e_{NM}	T_{N1}, T_{N2}, …	0

dimensional binary vectors, excluding the null binary vector.

The main idea of the algorithm is to find a set of DLS that have a high enough correlation with a subset of the known $class1$ samples, but at the same time to have a sufficiently low correlation with the known $class0$ samples, so that they can be distinguished by it, based in their correlations.

B. The Main Algorithm

The method can be divided into three main steps: First we present the intuition behind each step, followed by a detailed explanation is given in the next sections.

Lets emphasize that we are trying to find embedded patterns within subsets of features, that can discriminate the $class1$ samples from the $class0$ samples (see figure 2 in section IV). Thus, the method starts by selecting an initial subset of features for each $class1$ sample of the training data. Selecting the best (most discriminative) subset of features for each $class1$ sample, in a *M* dimensional space, requires evaluating $2^M - 1$ combinations for each training sample. Searching this huge space exhaustively is unpractical. In our case, we adopted an evolutionary search approach. Genetic Algorithms (GA) ([7], [16]) are known for their ability to efficiently find near-optimal global solutions in large hypothesis spaces. We tested sequential approaches to do this, but these kind of greedy techniques tend to fall in local optimums. Furthermore, if a forward selection is used, poorly significant correlations are obtained when few characteristics have been selected. On the other hand, backward selection suffers from the high dimensional calculations it must do at the beginning, when the majority of features are selected. In contrast, the GA can directly search a sufficiently small but significant number of features, that can serve as an initial, first approximation to the global optimum.

In the second step, the ensemble of DLSs that will define the final classifier is determined. With the GA step, we obtained a seed of selected features for each sample. Now, the objective is to decide which of them will be used as DLSs and to refine the subset of features of each. The DLSs can be seen as experts, whose criterion serves to distinguish certain type of $class1$ samples from the $class0$ samples. In this way, this step is the process to discover potentially good experts, and forming their criterion. Each DLS is created iteratively, by searching the most discriminative $class1$ training sample (S_{DLS}) and refining its subset of features (B_{DLS}) in a greedy way, so that its discrimination power is maximized. Then, a neighborhood of the DLS is defined, searching for similar $class1$ samples. These are then excluded as potential DLS samples for the future steps, in order to find samples containing different patterns. Each DLS should be able to classify a subset of the samples of the class. Thus, the final classifier is an ensemble of the (weak) classifiers made by each DLS.

The third global step of the method is the classification itself, using the ensemble created and trained in the previous steps to classify new instances. Here, each DLS or expert gives a score between 0 and 1 to each new instance, that is proportional to its confidence in that the new sample is from the class. As each DLS represents a portion of the $class1$ samples, the output of the global classifier is the maximum confidence score given to each sample by a DLSs.

The following sections provide a detailed explanation of each of the three mentioned steps.

C. GA Feature Selection

In this section we provide the main details behind the implementation of the genetic algorithm for feature selection in the context of our problem. For more general details about GA's see [7], [16]. As we mentioned, the purpose of this GA is to provide an initial seed of discriminative features for each training $class1$ sample. So, the GA receives as input: the $DataMatrix$, the target $class1$ sample index (S_t) for which features will be selected, the number of features to select and other GA related parameters (table II).

Let's start by defining the key elements that compose a GA:

1) Hypothesis space (chromosomes and genes).
2) Fitness function and selection method.
3) Crossover mechanism.
4) Mutation mechanism.
5) Population update policy.

Hypothesis Space: Each hypothesis or chromosomes represents a set of feature indexes. More formally, each chromosome $H_i, i = 1, 2, \ldots, P$, is a subset of fixed length *m* of the *M* features, i.e. $H_i \in \{1, 2, \ldots, M\}, |H_i| = m$, where *P* is the number of chromosomes or population size.

Fitness Function and Selection Method: The fitness function should provide higher scores to the hypotheses whose features gives higher correlations between S_t and his neighboring $class1$ samples and lower correlations between S_t and his neighboring $class0$ samples. Therefore, the fitness score of a given chromosome is calculated as

$$Fitness = \sum_{j=1}^{k} \{SortedCorrs1(j) - SortedCorrs0(j)\}\,, \quad (1)$$

where $SortedCorrs1$ and $SortedCorrs0$ correspond to vectors holding the Pearson Correlation Coefficient between the target sample and each $class1$ and $class0$ samples respectively, sorted in a descending order. As can be seen, only the k higher correlations of each class are used in the fitness function.

The selection of the hypotheses for crossover and direct transition to the next generation is performed by a probabilistic scheme, by selecting one chromosome at a time, with replacement. In each selection, each chromosome H_i has a probability $Pr(H_i)$ of being selected, with:

$$Pr(H_i) = \frac{Fitness(H_i)}{\sum_{j=1}^{P} Fitness(j)}, \quad (2)$$

where $Fitness(H_i)$ is the fitness score (equation 1) of the i-est chromosome.

Crossover Mechanism: Crossover generates new hypothesis by combining two parent hypothesis from the current population. The idea behind crossover is to generate a diverse offspring in order to search the hypothesis space globally. To do this, first each parent is randomly permuted. Then, the first half of the first parent is transmitted to the new chromosome. All the feature already transmitted are discarded from the second parent, to ensure that no repeated indexes can be transmitted. Finally, the first *m/2* indexes that are left in the second parent are selected and transmitted to the new chromosome. This process is repeated, but switching the parents in order to generate a second child chromosome.

Mutation Mechanism: Mutation mechanism provides small random perturbations in the chromosomes, in order to explore the hypothesis space locally. To do this, *Nm* feature indexes are randomly selected from the target chromosome. These are then swapped with other *Nm* feature indexes, selected randomly from a pool with the entire set of feature indexes, but without the ones currently selected in the chromosome. *Nm* is initially set to 4.

Population Update: Each new population is generated as follows. First, 5% of the population chromosomes with highest fitness are added directly to the next generation. This is done in order to preserve the best solutions. Also, a chromosome with the *m* most common features in the current population is kept. The idea behind this can be thought as following the wisdom of the crowd. Next, a *Pc%* of the population is selected using the selection method described before, and submitted to crossover. The offspring of this process is then added to the new population. The remaining $100 - 5 - Pc$% is again selected probabilistically and added directly to the new population. Finally, a *Pm%* of the new population is submitted to mutation, and with this, the new generation is settled. This process is repeated during *Ng* generations (iterations).

To optimize the algorithm, some parameters are refined during the process. Once the $Ng/2$ generation is reached, the crossover population percentage, *Pc*, is updated to $Pc/2$ and the number of mutations per chromosome, *Nm*, is updated to $Nm/2$. Also, once the $3Ng/4$ generation is reached, *Nm* is again updated to $Nm/2$. The idea behind this is to increase global search during the first iterations, when the hypothesis are probably far from the optimum, and increase local search as iterations move to interesting areas of the hypothesis space, where more subtle changes are needed.

D. DLS Ensemble Creation

```
Input: $Sclass1$: The $class1$ training samples.
    $Sclass0$: The $class0$ training samples.
    $Fseed$: the feature indexes used as initial seed.
Output: $DLSs$: the set of DLS's
    $\{DLS_1, DLS_2, ..., DLS_i, ..., DLS_n\}$,
    where $n$ is the number of DLSs found.
 1: $Sleft \leftarrow$ indexes of all the $class1$ samples.
 2: $i \leftarrow 1$.
 3: repeat
 4:     $S_{DLS,i} \leftarrow$ Use the corresponding $Fseed$ features to select the most discriminative sample from $Sleft$.
 5:     $B_{DLS,i} \leftarrow$ Refine the set of features of $S_{DLS,i}$ using a Stepwise Selection method starting with the features in $Fseed(S_{DLS,i})$.
 6:     $DLS_i \leftarrow (S_{DLS,i}, B_{DLS,i})$
 7:     $Corrs1 \leftarrow$ Compute the correlations between $S_{DLS,i}$ and each $class1$ sample in the subspace defined by $B_{DLS,i}$.
 8:     $Corrs0 \leftarrow$ Compute the correlations between $S_{DLS,i}$ and each $class0$ training sample in the subspace defined by $B_{DLS,i}$.
 9:     $CorrTh \leftarrow$ Use $corrs1$ and $corrs0$ to select the minimum correlation where the proportion of $class1$ samples above that threshold is at least $0.5$ ($50\%$).
10:     $Snbors \leftarrow$ Select form $corrs1$ the $class1$ samples that have correlation above $CorrTh$.
11:     Delete from $Sleft$ the samples in $Snbors$.
12:     $i \leftarrow +1$.
13: until $Sleft$ is $empty$.
```

Figure 1. Pseudo-code for the DLS ensemble creation.

Now we provide details about the second main part of our method (figure 1). First, a variable (*Sleft*) is created to hold the remaining potential $class1$ samples that could be selected as a DLS. Initially, all $class1$ samples are available, so all its indexes are assigned to *Sleft*.

Then, the discrimination power of each $class1$ sample is evaluated, in his corresponding seed of features, and the most discriminative is selected as a DLS. The function used to evaluate the discrimination of each sample is similar, but not equal, to the fitness function used in the GA. The main difference is that now the number of neighbors is important, because it is desired for the potential sample to have the greatest number of $class1$ neighboring samples. Furthermore, we applied a linear transformation to the correlations of the class1 and class0 samples in order to have maximum discrimination in the ranges that are expected. Thus, the designed function to evaluate a sample is

$$\begin{aligned} Score = \; & \textstyle\sum_{j=1}^{n_{t1}} minMax(corrs1(j), 0.7, 1.0) \\ & - \textstyle\sum_{j=1}^{n_{t0}} minMax(corrs0(j), 0.5, 0.8), \end{aligned} \quad (3)$$

where $corrs1$ and $corrs0$ are the correlations between the evaluated sample and the $class1$ and $class0$ samples respectively. The function *minMax(X,a,b)* linearly transforms the values of vector *X* that are between *a* and *b* to values between 0 and 1. Furthermore, all values below *a* are given a value of 0 and all value above *b* are given a value of 1.

Once a suitable discriminative sample is found, its seed of features is refined, in order to maximize its discrimination power as much as possible. This is done with a greedy, stepwise feature selection. Since the GA returns a seed of *m* features and we don't know if the optimum number of features is lower or greater than *m*, using forward or backward selection could incorrectly bias the search. Hence, we do not force the algorithm to add or delete features. Instead, the method evaluates each already selected feature for deletion and each unselected features for addition, and the best scoring option is taken. In other words, in each iteration, the algorithm will either add or delete the feature that rises the score the most. For this porpoise we use the scoring function defined in equation 3.

With the final set of features (B_{DLS}) of the discriminative sample (S_{DLS}) selected, a complete DLS is determined. Now, we expect that this DLS will be able to represent a certain kind of pattern shared by some $class1$ samples, that we call its neighborhood. In order to discover DLSs that can represent and discriminate other $class1$ samples, the neighboring samples of the current DLS are eliminated from the possible DLS candidates list ($Sleft$).

To decide which $class1$ training samples will be considered as part of the neighborhood, a correlation threshold is determined, so that all the $class1$ samples with a correlation with S_DLS above the threshold are considered neighboring genes. This threshold is determined as the minimum correlation needed to get a precision of 0.5 within the neighboring samples. This conservative choice was made because we are aware that in real, complex, noisy data, like the gene expression data, it will be impossible in some cases to avoid having some $class0$ samples with high correlations, and therefore, inside the neighborhood. If we wouldn't consider this, there could be a huge impact in the size of the neighborhood and we could have a lot of redundant DLSs. Furthermore, as will be seen in the artificial data tests (section IV), this works well even if the neighborhoods are well defined.

Finally, once the samples from the cluster are selected and removed from the *Sleft* variable, the process is repeated (steps 4 to 11 in figure 1) until no samples are left in *Sleft* to be evaluated.

E. Classification

Here we give the details of how to use those trained DLSs to classify new instances. As mentioned earlier, each DLS represents a pattern, that can be used to discriminate certain type of $class1$ samples from the $class0$ samples. This discrimination is given in the sense that the DLS has high correlations with some $class1$ samples but low correlations with the $class0$ samples. Thus, a minimum correlation threshold could be used in order to classify new instances. But, as mentioned earlier, real data is not that simple and it isn't always clear what correlation threshold to use: setting it too high could translate in a poor recall and setting it too low could translate in a poor precision. Moreover, not all the DLS's have the same degree of discrimination and, therefore, there should be different degrees of confidence to the classifications they do.

So, confidence scores are given to each new instance by each DLS, based on the correlations that the DLS has with the training set and with the new instance. Intuitively, the are two factors that should influence the score. First, the proportion of $class1$ and $class0$ training samples that have correlations with the DLS that are "similar" to the one that has the new sample. The higher is the proportion of $class1$ samples, the higher the score. For example, if a new instance has a correlation of 0.85 with a certain DLS, but there are lots of $class0$ samples with correlations (with the DLS) around $0.8 - 0.9$, then there is not enough evidence to say that the new instance is from $class1$, so a low confidence should be given. The second factor that should influence the confidence is how "similar" are the correlations. The closer the $class1$ sample correlations are from the new instance correlation, the better, and the opposite is expected from the $class0$ samples.

One natural way to measure this would be to use a window of a fixed width, centered in the correlation of the new instance, and counting the number of $class1$ and $class0$ training samples that have correlations that fall inside the window. Each training sample then would vote with a weight proportional to its distance to the center of the window. Thus, the confidence of the new instance, S_new, could be:

$$ConfScore(S_{new}) \quad = \quad \frac{w_1}{w_0 + w_1}, \tag{4}$$

where

$$\begin{aligned} w_0 &= \sum_{i=1}^{n_{t0}} w(corrs1(i), corr(S_{new}), width) \\ w_1 &= \sum_{i=1}^{n_{t1}} w(corrs1(i), corr(S_{new}), width), \end{aligned}$$

where $corrs1(i)$ and $corrs0(i)$ hold the correlations of the *i-est* $class1$ and $class0$ samples with the DLS sample, respectively, $corr(S_{new})$ is the correlation of the new instance with the DLS sample, and *w* is the window function

$$w(x, y, width) = \begin{cases} |x-y|, & |x-y| \leq width) \\ 0, & |x-y| > width. \end{cases} \tag{5}$$

The problem with this score is that the window function is binary, so all the training samples that fall outside the window do not have any influence in the score. Thus, we

Table II
PARAMETERS USED IN THE TESTS BY THE DLS ALGORITHM.

Part I	#Features	#Generations	#Chromosomes	Pc	Pm	k
	50	300	100	0.6	0.5	5
Part II	Precision	Part III	Width			
	0.5		0.1			

decided to use a continuous version of the window function. The idea is for all the training samples to have an influence, but that this influence drops rapidly as the distance of the training samples grows. To capture this, we used a Gaussian kernel function as the *w* function, defined as

$$w = N(x, y, width) = \frac{1}{width\sqrt{2\pi}} \exp\left(-\frac{(x-y)^2}{2\ width^2}\right), \quad (6)$$

where *N(x,y,width)* is the value of the normal probability density function, centered in *y*, with standard deviation *width* and evaluated in *x*. So, the Gaussian is centered in the correlation of the new instance, and measures each training sample with a weight that decreases with its distance from the center. The *width* parameter defines the width of the area in which there will be more influence. We thought that a reasonable width should be 0.1.

Now, each DLS uses this confidence score to evaluate each new instance. The final output of the classification algorithm is then the maximum confidence score given to each new instance. This confidence output is much richer for the user than a simply binary output, because it gives information about the credibility of the classification.

IV. EXPERIMENTAL RESULTS AND DISCUSSION

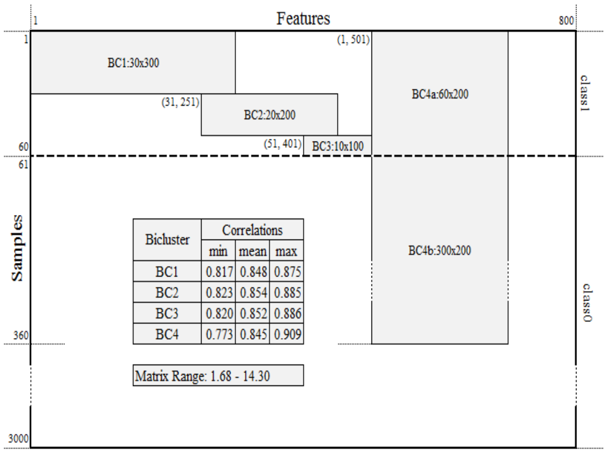
The proposed method was implemented in Matlab and evaluated with synthetic and real data. Given the complex and noisy nature of biological data, the initial stage of evaluation uses a synthetic dataset with known discriminative local subspaces. Also, we are currently doing analysis with real gene expression data and some preliminary results are shown in this section.

To compare the performance of our approach, we selected a state-of-the-art method [20], that uses a nearest neighbors enrichment method to predict gene function from an expression network (See section II for more details). From now on, we will refer to this method as the NNE (Nearest Neighbors Enrichment) method.

The parameters used (table II) for our method were not exhaustively fine-tuned, but were chosen based on tests performed in synthetic data. We are currently working in some details of the GA implementation, so a more exhaustive tune of the parameters will be done after this process.

A. Synthetic Data

A synthetic data set was created simulating the expected patterns in the real data (figure 2). To do this, a matrix



Bicluster	Correlations		
	min	mean	max
BC1	0.817	0.848	0.875
BC2	0.823	0.854	0.885
BC3	0.820	0.852	0.886
BC4	0.773	0.845	0.909

Figure 2. Characteristics of the artificial database used for testing. There are 4 biclusters embedded in the matrix, but only biclusters 1, 2 and 3 are discriminative, because, contrarily to bicluster 4, they are embedded only within class1 samples.

of 3060×800 random values was created. Of the 3060 samples, the first 60 were chosen to be $class1$ samples, and the next 3000 to be $class0$ samples. The expected pattern are represented by a subspace correlation cluster (bicluster). A big bicluster was created so that its samples had a high internal pairwise correlation within its set of features. Then, four partitions of it were embedded within the matrix. Each partition can be considered as a bicluster by its own ($BC1$ to $BC4$) and they represent the different patterns within the samples.

The configuration in which these biclusters were embedded was done so that each $class1$ sample belonged to two bicluster. However, only one of them was made to be discriminative. So, for each sample, there a three kind of features: features that don't belong to a bicluster, features that belong to a non-discriminative bicluster and features that belong to a discriminative bicluster. The features that are discriminative belong to the biclusters embedded only within $class1$ samples (BC1, BC2 and BC3 in figure 2). Contrarily, the non-discriminative features belong to a big bicluster embedded within both $class1$ and $class0$ samples (BC4 in the figure 2). More specific details of the synthetic data created are shown in figure 2.

To construct the bicluster, there are several types of mathematical models [14]. We used a *coherent values* model to create them, because they are relatively simple to construct and are a good representation of the co-expression patterns expected in real data. In this model each cell of a given bicluster must satisfy the following criteria:

$$a_{i,j} = \mu + \alpha_i + \beta_j \ + N(0, \sigma), \quad (7)$$

where μ is a base common value of the entire bicluster, α_i is the common value adjustment for row *i* and β_j is the common value adjustment for column *j*. $N(0, \sigma)$ is an

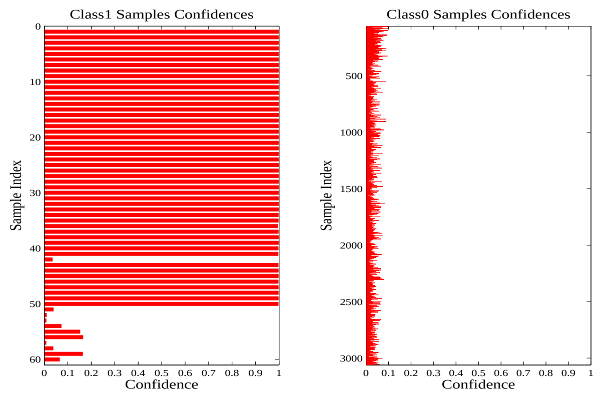


Figure 3. Confidence scores obtained by the $class1$ (left) and $class0$ (right) samples from the DLS ensemble trained with the entire artificial data set.

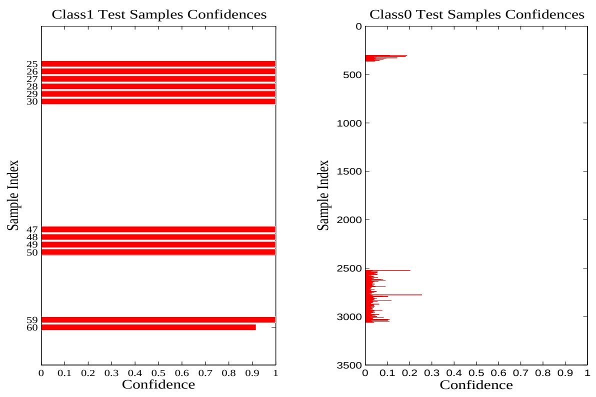


Figure 4. Confidence scores obtained by the $class1$ (left) and $class0$ (right) held-out test samples from the DLS ensemble trained with the remaining 80% of the artificial data set.

aleatory number produced with a normal distribution with standard deviation σ. This term was added to the standard model to introduce some noise to the clusters and make them more real. Otherwise, the pairwise correlations of each sample of the bicluster would be one. The σ used was 0.5 and with this, we obtained a bicluster with a minimum correlation of ~ 0.81, a mean correlation of ~ 0.85 and a maximum correlation of ~ 0.88.

To test if the algorithm is capable to find the discriminative subspaces we first trained a DLS ensemble with the entire data set and then used it to classify the same data. The ideal result would be for the method to find a few DLSs (close to 3) to represent the class1 samples, and with them, give high confidence scores to the remaining $class1$ samples (samples 1 to 60) and low confidences to the $class0$ samples (samples 61 to 3060). After doing this, the method found 8 DLSs. The maximum confidences given to each $class1$ and $class0$ sample are shown in figure 3. Of the 60 $class1$ samples, 49 obtained confidences very close to 1 and none of the $class0$ samples obtained confidences above 0.1, including the first 300 samples that were part of BC4. This shows that the method was robust in finding only subspaces that were discriminative, but that it wasn't able to find all of them.

As can be seen in figure 3, all the samples from BC1 were correctly found. This is the easiest bicluster to find, because it has the biggest number of discriminative features. In fact, 29 of the 30 samples of BC1 were found by the first DLS. Also, 19 of the 20 samples from BC2 (samples 31 to 50) were correctly found by the fourth discovered DLS. The two DLSs discovered before (DLSs two and three) belong to BC2 too, but in some way, they managed to find discriminative subspaces with samples from BC1, giving low scores to the rest of the samples. Something similar happened with the DLSs five to eight, that belong to BC3. This is the most difficult bicluster to find, because only 100 of the 800 features are discriminative and only 10 samples belong to it.

From this results it can be seen that the method can find with great precision DLSs. However, when the proportion of discriminative features is too low (like in BC3), we found that the GA has trouble to find a good seed of discriminative features. We are currently working in doing some improvements to this part of the method. Also, it is interesting that the method found some DLSs from BC2 and BC3 that are very connected with samples from BC1, a pattern that was not explicitly inserted. This means that it is likely to find aleatory correlation patterns and, as they are aleatory, they could be perfectly found between $class0$ and $class1$ samples, for example, by a biclustering method. This gives more importance to the discriminative nature of the search, in order to avoid those aleatory patterns between $class0$ and $class1$ samples.

To test the classification and generalization capabilities of the method, we did a cross-validation test, where a 20% of the samples of each class (1 and 0) were held-out for testing and the remaining 80% was used for training. In this process we were careful to select a 20% of each bicluster for the test set, because a random approach could have not selected any samples from some bicluster or, although improvably, all of them from another. The results obtained in the $class1$ and $class0$ test samples are shown in figure 4. The training results obtained are not shown, but it's worth mentioning that this time the algorithm found DLSs for all the three biclusters, in spite of the less training samples. This can be seen in the results of the test data (figure 4) in that all the test samples, including the BC3 samples, got high confidence scores. This significant change in the training search can happen due to the stochastic nature of the GA algorithm that this time found a better seed. This also reinforces that further refinements must be made to the GA in order to obtain a better stability and convergence of the features seeds. In spite of this, the classification in the held-out test samples was perfect, showing the generalization power of the algorithm, that in this case, used only 5 DLSs, corresponding to 5 $class1$ samples, to perfectly represent and classify the remaining $class1$ samples.

B. Real Data

The tests in the artificial data provided good insights about the performance of the method in a controlled environment. However, real gene expression data can be far more complex and thus, cross-validation tests in real data are needed to asses the performance and utility of the method in terms of gene function predictability.

The data used for this purpose consists of $20,858$ samples (gene expression profiles) and 773 features (microarray experiments). This data was obtained after the pre-processing of the original microarray data, that consists of 1701 Affymetrix ATH1 microarray slides acquired from the NASC's International Affymetrix Service (http://affymetrix.arabidopsis.info/). The data was normalized with the RMA method [10] using R's Bioconductor package and the replicates of the same experiments were averaged, resulting in a final set of 773 microarray experiments (features). Each microarray has $22,810$ probes to measure gene expression. Nevertheless, some probes were filtered out, either because they hybridize to more than one gene or because there is another probe that hybridize the same gene, resulting in a set of $20,858$ unique probe-gene pairs (samples).

To be able to do the cross-validation test in this data, we needed to create a gold-standard from the information retrieved from GO. Although the GO database gives information about which genes perform a given function, it doesn't give certain and explicit information about what genes doesn't perform that function. In other words, the genes not annotated with a certain term are probably genes that doesn't perform that function. However, many of them could be genes that it's still unknown that they perform that function. So, it's difficult to know which genes to include as $class0$ samples in the gold-standard. If we decided to include all the genes not annotated with a given term as $class0$ samples, then we probably would be including some unknown $class1$ samples as $class0$ samples. The biggest problem is that this 'hidden' samples could introduce a lot of noise to the method, because they will probably share the correlation patterns from the known $class1$ samples, and therefore, they could hide the discriminative subspaces. So, when constructing the training gold-standard for a term T_k from GO, we decided to include a gene as a $class0$ sample only if it is not annotated with the term T_k and if it is annotated with at least one term $T_i, i \neq k$ in which there are no genes annotated with T_k. The fact that there are no known genes that share the terms T_i and T_k gives more confidence in that this functions are not complementary and in consequence, that the genes participating in the function T_i will not participate in function T_k.

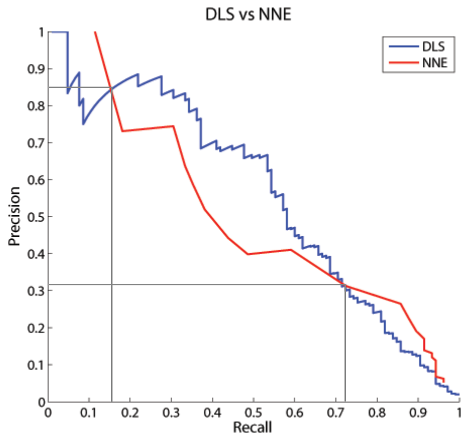


Figure 5. Comparison of the results obtained by DLS and NNE.

As a preliminary test of the predictive power of the algorithm, a 10-fold cross-validation test was done in the gold-standard created for the term 'Photosynthesis' (GO:0015979), that is in the 4th level of the 'Biological Process' sub-ontology. We used this term for our first analysis because it has been reported [20] that these genes show correlation (co-expression) patterns. Besides, there are 127 genes annotated with this term and 105 of them are in our data set, which is a high enough number to find significant patterns, but low enough to do the tests in a reasonable time. The $class0$ samples were selected with the criteria explained above, resulting in a set of 5342 genes.

The NNE method, as a nearest neighbors method, is designed to do a leave-one-out classification. This is, to classify a sample, it uses all the rest of the available samples to determine the nearest neighbors. The total number of $class1$ and $class0$ samples is an important factor in this method, because they can alter the hypergeometric p-value estimation to determine enrichment. So, contrarily to our method, to test the NNE method we used the entire gold-standard set as input, in order to avoid harming its performance. Also, to compute the p-values, we used the number of samples from the gold standard as the total number of samples given to the hypergeometric distribution, as we think is the correct way to do it. Furthermore, we evaluated using the real total number of genes and obtained worst overall results (data not shown).

To compare the results of the two methods, a recall vs precision curve (figure 5) was constructed. This is very straightforward in our method, because of its continuous confidence output. To construct the curve we just applied different thresholds to the output and registered the corresponding recalls and precisions. However, the NNE output is binary, so, in order to obtain different grades of recall and precision, we had to run the method several times with different correlation cutoffs ranging from 0 to 1.

As can be seen in figure 5, the results of both methods are far from perfect, but are in fact good results considering the complex nature of the data and that there are almost 50 times more $class0$ samples than $class1$ samples. Comparing both results, it can be seen that our method got better results in almost all the recall and precision space, whose limits are marked in the figure with grey lines. The NNE method got a little better results in the extreme cases, but those are not really very useful. For example, at a recall of 0.1, the NNE and DLS methods got precisions of 1.0 and 0.8 respectively, but at that low recalls is very difficult to predict new genes. In contrast, for example, our method obtained a precision of 0.68 at a recall of 0.52, much better than the precision of 0.40 obtained by the NNE method at the same recall.

Despite this, we expected to get a better advantage by using a discriminative subspace approach, specially in terms of recall, because we expect that some patterns will be hidden within subspaces of the data. There are several possible reasons of why we are not achieving this. The first and more obvious is the somewhat mediocre performance of the GA seen in the artificial data. So, this results could get better if we improve the GA mechanism and tune the parameters. Another reason could be for the real data to have very few samples in some clusters, making it very difficult to find a generalizable DLS in each of the 10 folds. In this context, it is important to notice that the NNE method had an advantage in using the leave-one-out strategy compared with the 10-fold cross-validation used to test the DLS method, because it uses all the information to classify each instance and those clusters with few samples are always present. Unfortunately, it is practically impossible to use a leave-one-out strategy with our method, because we would need to train it once for each test sample.

Another aspect to mention, is that the similar results obtained by the NNE and the DLS methods in this GO term, could indicate that the genes of the photosynthesis process that share co-expression patterns, do it across a very wide range of experimental conditions, and thus, a subspace approach is not needed to find them. This makes some sense, because photosynthesis is a very global and constitutive process in the plant. Thus, we could get a more marked gain in other more specific processes. Nevertheless, this test demonstrates that the method can do functional predictions that are superior or at least as good as a state-of-the-art method, and serves to continue our work for further improvements of the method.

V. Conclusions

In this paper we presented a novel algorithm to enhance the functional prediction of genes in large sets of microarray data. The main contribution of our work resides in taking advantage of the discriminative power of supervised learning to explicitly search for relations among genes in selective subspaces. To our knowledge, this is the only method to date that uses a supervised approach to select discriminative subsets of features in microarray data to predict gene function.

The results obtained in real data, although preliminary, shows a significant improvement over a recent work in terms of precision and recall. Although more tests in different GO terms must be done, the results obtained so far are encouraging to continue this direction of work. On one hand, the overall better recall increases our believe in that co-expression patterns can be hidden in a subset of conditions, that regular methods that uses all conditions may be missing. On the other hand, the overall better precisions increases our believe in that the addition of a discriminative selection of subset is important to decrease false positives.

The experiments done in the artificial data show that, in most cases, the algorithm accomplishes the task of finding discriminative local subspaces and that they can be used for an accurate prediction. Theoretically, this shows the power of instance based approaches like nearest neighbors when are combined with a good selection of discriminative features. Also, it shows that the global and stochastic search of genetic algorithms and the greedy refinements of sequential feature selection can be a good combination for feature selection.

However, the results showed that there's still room for improvement, as some of the discriminative subspaces couldn't be found, mainly because the implemented GA wasn't able to find a good seed of features. We are currently working in improvements for this. One possibility we are evaluating is the addition of a backward selection heuristic before the GA, to discard clearly useless features. This would help to make smaller the huge hypothesis space that must traverse de GA in the search for useful features and may provide a faster and more robust convergence. After this, we expect to have good foundations to train our method with all the currently known functional GO annotations, and be able to predict novel biological functions to genes with vague or no functional GO annotations.

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