Informative gene selection using the Algebraic Connectivity Strength of Point and Scoring Criteria

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This paper presents a novel method that integrates the Algebraic Connectivity Strength of Point (ACSP) and Scoring Criteria to identify genes associated with tumor type. First, for each gene, the ACSP is used to identify reliable expression levels of the gene in all the samples. The informative genes are then selected using Scoring Criteria based on these reliable expression levels. Finally, the Support Vector Machine (SVM) classifier is used to classify the two datasets of gene expression profiling. The results show that the informative genes selected by the proposed method have higher credibility than those selected by Scoring Criteria alone.

tumor, gene expression profiling, Support Vector Machine, Algebraic Connectivity Strength of Point

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Studies based on gene expression profiling have become the mainstay of research on various life phenomena, among which the diagnosis and treatment of cancer is a research focus [1,2].

However, studies based on gene expression profiling still face many challenges, such as background noise, small samples, and the dimensionality curse. To solve these challenges, researchers have proposed many methods in recent decades. Dimension reduction is one of the most commonly used methods. Using the principle of “signal-to-noise ratio (SNR)” method [3], numerous dimension reduction methods have appeared that can be generally divided into two categories: comprehensive property extraction and informative genes selection. In the former category, the dimension can be reduced through linear transformation or non-linear transformation, e.g. Principal Component Analysis (PCA) [4], Nonnegative PCA [5], Independent Component Analysis (ICA) [6], and Nonnegative Matrix Factorization (NMF) [7]. However, the results often lack a reasonable biological

explanation. The methods in the latter category, which can identify informative genes with good biological explanation, are readily accepted by oncologists. These methods include the T -test [8], Revised Feature Score Criterion (RFSC) [9], Relative Entropy (RE) [10], Enrichment Score [11], improved global normalized SNR (improved gn_SNR) [12], and gene regulation probability [13].

Regarding small samples, many methods based on Scoring Criteria, which utilize the mean and variance of samples, can be influenced by outliers caused by abnormal expression of genes or other reasons. It can also lead to low credibility of informative genes. To solve this problem, we propose a novel gene selection method that integrates the ACSP and Scoring Criteria to select informative genes. This method improves the credibility of the selected genes.

1 Materials and methods

1.1 Scoring Criteria

Gene expression profiling can be expressed mathematically

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as a matrix G :

$$G = \begin{bmatrix} g_1 & g_2 & \cdots & g_N \\ value_{11} & value_{12} & \cdots & value_{1N} \\ value_{21} & value_{22} & \cdots & value_{2N} \\ \vdots & \vdots & \ddots & \vdots \\ value_{M1} & value_{M2} & \cdots & value_{MN} \end{bmatrix} \begin{matrix} Y_1 \\ Y_2 \\ \vdots \\ Y_M \end{matrix}, \quad (1)$$

where $value_{ij}$ indicates the expression level of gene g_j in sample Y_i , N is the size of the gene and M is the size of the sample, respectively. Usually $M \ll N$.

There are many methods available for studying Scoring Criteria. This paper mainly focuses on **RFSC** and **RE**:

$$RFSC(g_i) = \frac{1}{2} \left| \frac{\mu_{i,+} - \mu_{i,-}}{\sigma_{i,+} + \sigma_{i,-}} \right| + \frac{1}{2} \ln \frac{\sigma_{i,+}^2 + \sigma_{i,-}^2}{2\sigma_{i,+}\sigma_{i,-}}, \quad (2)$$

$$RE(g_i) = \frac{1}{2} \left(\frac{\sigma_{i,-}^2}{\sigma_{i,+}^2} + \frac{\sigma_{i,+}^2}{\sigma_{i,-}^2} \right) + \frac{1}{2} (\mu_{i,+} - \mu_{i,-})^2, \quad (3)$$

here, $\mu_{i,+}$ and $\mu_{i,-}$ represent the mean of the i th gene expression level in “+” category and “-” category, respectively. Similarly, $\sigma_{i,+}$ and $\sigma_{i,-}$ indicate the variance. Each gene has a similar expression level in the gene expression profiling in the same category. Nevertheless, various factors from the environment, equipment and artificial operation etc., will lead to apparent anomalies of the gene expression level in some samples. Thus, the expression levels of the gene in such samples are judged as outliers. Scoring Criteria shows good performance in obtaining informative genes, on the condition that gene expression is not contaminated. However, the mean and variance of gene expression are sensitive to outliers when the above factors exist in gene expression profiling. This undoubtedly influences the true expression and fluctuation level of genes in a given category, and subsequently lowers the credibility of the selected genes. Therefore, these outliers should be excluded for subsequent analysis.

1.2 A novel gene selection method integrating the ACSP and Scoring Criteria

In this paper, for a given gene, all expression levels in the same category will be considered as points on the graph. Connectivity strength is then computed using ACSP between each point and its K neighbor points to determine a set of points that can best represent the true expression level of the gene in the category. Finally, outliers can be excluded for the scored gene using Scoring Criteria.

The ACSP is defined as follows:

Definition 1: Suppose a complete graph $F = \{V(F), E(F)\}$, where $V(F) = \{v_1, v_2, \dots, v_N\}$ is a point set of size N and $E(F) = \{e_{ij}\}$ is an edge set. Edge e_{ij} is given with the

weight w_{ij} , $i, j \in \{1, 2, \dots, N\}$. For each point v_i , calculate the sum of K adjacent edge weights of v_i : Let $\text{Sum}(v_i) = \sum_{j=1}^K w_{i,j}$, $K \leq N$, then $\text{Sum}(v_i)$ is defined as the Algebraic Connectivity Strength of Point (ACSP).

For each gene g_i , the points that are the expression values of g_i in all the samples of a category are constructed as a complete graph. The weight of an edge is defined as follows:

$$w_{ls} = \begin{cases} \frac{1}{\|value_{il} - value_{is}\|} & l \neq s \\ 0 & l = s \end{cases} \quad l, s = 1, 2, \dots, Num, \quad (4)$$

here Num is the number of samples in a category. Each g_i corresponds to a point set, denoted by $Value_i = \{value_{1i}, value_{2i}, \dots, value_{Numi}\}$. When the number of neighboring points $K \approx T \times Num$, where T ($T \in [0, 1]$) is a parameter, calculate $\text{Sum}(value_{ji})$:

$$\text{Sum}(value_{ji}) = \sum_{s=1}^K w_{js} \quad j = 1, 2, \dots, Num, \quad (5)$$

first, the maximum is determined, $\text{Sum}_{\max} = \text{Max}\{\text{Sum}(value_{1i}), \text{Sum}(value_{2i}), \dots, \text{Sum}(value_{Numi})\}$. Then the $value_{ji}$ corresponding to $\text{Sum}_{\max}$ is considered as the center point. The mean and variance of the expression level of gene g_i can be obtained in one category through analyzing $T \times Num$ points adjacent $value_{ji}$ (including $value_{ji}$). The mean and variance of the expression level of gene g_i in the other categories may be obtained in the same way. Finally, gene g_i is scored according to eq. (2) or (3).

2 Experimental procedure

Compared with the dimension, the sample size shows an extreme imbalance in gene expression profiling, i.e., few samples in a high dimensional dataset. Hence, we adopted leave-one-out cross-validation (LOOCV) to train the SVM classifier. LOOCV means randomly selecting one sample as a test sample from the entire dataset with n samples, then the remaining $n-1$ samples are used to train SVM to gain the optimal decision model that determines which class the test sample belongs to. This process does not stop until all samples have been tested separately. Then, the number of test samples that were correctly classified can be determined.

In fact, the training problem of the SVM classifier is a convex quadratic programming problem, which can be transformed into seeking the optimal solution of the following objective function.

$$\text{Max:} \quad \sum_{i=1}^n a_i + \frac{1}{2} \sum_{j=1}^n a_i a_j y_i y_j K(x_i, x_j), \quad (6)$$

$$\text{Subject to:} \quad \begin{cases} 0 \leq a_i \leq C & i = 0, 1, \dots, n \\ \sum_{i=1}^n y_i a_i = 0 \end{cases}, \quad (7)$$

where a_i is the Lagrange Multiplier, $y_i \in \{1, -1\}$ is a category label of sample x_i , and C is the upper boundary of the Lagrange Multiplier. The decision function,

$$f(x) = \text{sign} \left(\sum_{i=1}^n a_i y_i K(x_i, x) + b \right),$$

is then used to determine which category sample x belongs to. Here b is a constant and $K(x_i, x) = \exp(-\|x - x_i\|^2 / \sigma^2)$.

In this paper, the parameter: $C=200$ and $\sigma=7$ from a leukemia experiment ($\sigma=3$ in the diffuse large B cell lymphoma (DLBCL) experiment), were determined when the number of the test samples which were correctly classified was the largest. The experimental steps are as follows:

Step 1: In a given category C_k , for gene g_i , calculate the mean and variance in two categories using the ACSP, respectively. Gene g_i is scored according to the Scoring Criteria by eqs. (2) and (3).

Step 2: Similar to **Step 1**, all the genes will be scored and ranked in a descending order.

Step 3: Select the first L genes as the informative genes subset, and perform LOOCV experiments using SVM.

Step 4: Locate subsequently informative genes based on the experimental results. To assist oncologists to prevent and treat cancer, provide the location of these genes.

3 Results and analysis

This paper used two groups of public datasets: a Leukemia dataset and the DLBCL dataset. The leukemia dataset consists of 52 samples with 12564 genes in each sample. The samples include two subtypes: 24 Acute Lymphoblastic Leukemia (ALL) samples and 28 Acute Myeloid Leukemia (AML) samples (<http://www.broadinstitute.org/cgi-bin/cancer/datasets.cgi>). DLBCL comprises 19 follicular lymphoma (FL) samples and 58 DLBCL samples with each sample containing 5469 genes (<http://www.gems-system.org/>). The description of all the genes and more information is available in the corresponding websites. The two datasets were normalized in this paper.

3.1 Leukemia dataset

From the perspective of a small-scale analysis of genes, the classification accuracy of ACSP+RFSC is significantly higher than that of RFSC alone when $L < 20$ (Figure 1). Under the condition of $L=3$, the classification accuracy of this method achieves 98.8%, while classification accuracy of RFSC alone is less than 20%. This is probably because several of the most important genes, which had been contaminated, are selected by RFSC as informative genes. Nevertheless, this can be avoided by ACSP+RFSC.

In Figure 2, gene No. 3634 obtained by ACSP+RFSC has higher expression levels in the ALL category than in the

Figure 1 Classification results of the Leukemia dataset with ACSP+RFSC (☆) and RFSC (▽), respectively.

Figure 2 Expression levels of gene No. 3634 with highest scores by ACSP+RFSC in all the samples (sample label: 1–24, ALL samples; 25–52, AML samples).

AML category, and almost all expression levels with large fluctuations are greater than 0 in the ALL category. On the other hand, the expression values are close to -1 and the expression level is stable in the AML category. Apparently, gene No. 3634 is a very good discriminant factor to determine which category a sample belongs to. However, we noticed that 23 expression levels of gene No. 282 in the AML category are similar to those in the ALL category (Figure 3). Only five distinctly different expression levels as outliers lead to the mean and variance of the expression level of gene No. 282 being seriously affected. Therefore, gene No. 282 may be assumed to be a factor with no discriminatory ability.

The influence of these outliers can be avoided when neighboring points $K \approx T \times \text{Num}$ (AML: $22.4 = 0.8 \times 28$, if $T=0.8$), such that the five outliers are excluded. The mean and variance obtained can better reflect the real situation of gene expression and improve the credibility of the genes as informative genes.

To further illustrate the advantages of ACSP+RFSC, 50 genes selected are shown (Figures 4 and 5). There are

Figure 3 Expression levels of gene No. 282 with highest scores by RFSC in all the samples (sample label: 1–24, ALL samples; 25–52, AML samples).

significant blocks in Figure 4 showing that the expression levels of genes in ALL samples are obviously less than those in AML samples (genes labeled 1–12), and expression levels of genes labeled 13–50 in ALL samples are greater than those in AML samples. However, using RFSC alone, most of the genes are not discriminant factors, except genes labeled as 3, 6, 34, and 36 (Figure 5). Therefore, the performance using RFSC alone is poor.

3.2 DLBCL dataset

The DLBCL dataset was tested to evaluate the proposed method (Figure 6). The classification accuracy of ACSP+RFSC is higher than that of RFSC when the number of informative genes is 30 to 150. The highest classification accuracy is 97.40% in the right part of Figure 6. The method is superior to RFSC with respect to selecting a few of the most important genes.

We designed the method ACSP+RE to analyze the two datasets (Figure 7) and the problem of informative genes redundancy was briefly considered. The redundancy is judged according to the classification accuracy and it gradually

Figure 4 Expression levels of 50 informative genes with ACSP+RFSC (sample label: 1–24, ALL sample; 25–52, AML sample).

Figure 5 Expression levels of 50 informative genes with RFSC (sample label: 1–24, ALL sample; 25–52, AML sample).

Figure 6 Classification results of the DLBCL dataset with ACSP+RFSC (☆) and RFSC (▽), respectively.

Figure 7 Classification results of the Leukemia and DLBCL datasets by ACSP+RE (☆) and RE (▽), respectively ((a) Leukemia; (b) DLBCL).

increases with increasing numbers of selected informative genes. The classification accuracy reaches its highest value when the number of informative genes reaches a certain number. Any further increase the number of informative

genes causes the classification accuracy to plateau, or even decline. This implies that genes selected later have produced redundant information and therefore represent noisy genes.

The above analysis shows that the informative genes obtained by the proposed method have a higher reliability. Finally, according to these experimental results, this paper has provided some genes associated with tumor type to biomedical experts, as shown in Table 1.

4 Conclusions

The study shows that the expression level of a gene, which is impacted by outliers, is only a small proportion in a given category. The expression level of the gene will deviate from the real situation if Scoring Criteria, which does not exclude these outliers, is used alone. On the other hand, the experimental results of the Leukemia and DLBCL datasets indicate that the developed ACSP+RE method can exclude the

impact of these outliers and the real expression level of the gene can be reflected better, without analyzing these outliers. Future work will concentrate on exploring the numbers informative genes in relation to different types of tumors and the redundancy problem of informative genes.

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Table 1 The genes associated with tumor type obtained by the ACSP+RE method from the Leukemia and DLBCL dataset, respectively^{a)}

Leukemia dataset		DLBCL dataset	
Label	Feature	Label	Feature
9832	40282_s_at	4588	V00594_s_at
8585	36638_at	52	AB002409_at
1119	34168_at	5136	X65965_s_at
3634	39318_at	1600	M57710_at
5627	36021_at	5133	X06700_s_at
12418	266_s_at	3124	X01060_at
3277	38242_at	478	D79997_at
11607	1077_at	4323	D13666_s_at
8869	37403_at	3867	Y08374_rna1_at
3399	38604_at	3564	X76534_at
6278	37988_at	4767	M14328_s_at
11603	1096_g_at	3127	X02152_at
9005	38017_at	1055	L17131_rna1_at

a) The first column and third column "Label" correspond to the location of genes in G (eq. (1)). The column "Feature" indicates the real location from which the detailed information concerning the gene can be obtained from the corresponding websites (<http://www.broadinstitute.org/cgi-bin/cancer/datasets.cgi>; <http://www.gems-system.org/>).

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