Dear Editor,

Enclosed please find our substantially revised manuscript “MGRFE: multilayer recursive feature elimination based on embedded genetic algorithm for cancer classification”. In this revised manuscript, we have carefully addressed all the concerns by the reviewers. We greatly appreciate the Referee’s comments on our manuscript. The following is our point-by-point response to each comment of the reviewers. Furthermore, I would like to take this opportunity to thank you for handling the review of our manuscript and provide us the chance to modify our manuscript again.

Our responses to the review comments are in blue.

Yours sincerely,

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**Response to Editor Comments**

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Editor Comments

Associate Editor

Comments to the Author:

The manuscript was reviewed by the original reviewers.

Although Reviewer 1 is satisfied with the revised version, Reviewer 2 gives very critical comments.

Therefore, I recommend the authors to revise the manuscript with taking all comments into account.

Since I understand that giving theoretical justification is difficult,

it is enough to give some discussions.

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**Response**: Thanks very much for providing us this valuable opportunity to revise our manuscript again. We have carefully revised the previous manuscript considering all the review comments. The point-to-point response to each comment of the reviewer 2 in the detail are provided as follows.

**Response to Comments of Reviewer 2**

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Reviewer: 2

Recommendation: Reject

Comments:

The selection of highly informative genes in cancer patients is a standard problem with many techniques in existence. The paper presents yet another approach based on an embedded genetic algorithm. In my previous review I had raised a number of queries, which have essentially been dismissed by the authors in their revised version. My queries have NOT been addressed satisfactorily.

My original comment 3 is that there is no validation on an independent data set. The authors state in their rebuttal that "Thus, the currently published gene selection algorithms on microarrays are commonly validated within each microarray benchmark dataset."

I am sorry to say that this is incorrect. I have published several papers in computational cancer biology, and ALL of them had validations on independent data sets. I am not persuaded by the authors' argument.

"For microarray benchmark datasets about same disease, the features and sample classes are usually different. Different microarray datasets usually have different gene features for the gene probes vary among different microarray analysis platform. For example, on the leukemia related datasets of Leuk and MLL used in this study, the gene probes are very different for generating from different microarray platforms."

This is PRECISELY the reason why validation on an independent data set is so crucial. It is true that two different databases of the same form of cancer may have different genes under study. The way to handle this is to study only those genes that are common to both databases. One can also convert microarray values to Z-scores by subtracting the sample mean and dividing by the sample variance. The authors don't even try to do this.

"Thus, the currently published gene selection algorithms on microarrays are commonly validated within each microarray benchmark dataset."

This is not correct. The authors are simply trying to justify whey they did not do any validation on an independent dataset.

If they have managed to do cross-validation on another dataset for leukemia, then that should be in the main paper, not in the supplementary material.

My comment 4 was that their method lacked theoretical justification and compared it to SVM-RFE. Here again the authors simply explain away my objection. They say that their GA (genetic algorithm) works faster than that of Kar et al. That was not my point at all.

In short, I believe that the authors have not adequately addressed my previous comments. Without either theoretical justification or validation on independent datasets, there is very little merit in the paper.

**Response:**

Thank you very much for your constructive and valuable comments. We also do appreciate your patient and detailed explanation on the issue that we did not understand well, which not only provide us the great help in this process of our revision, but also in our future research. As you said, in the previous revision, we indeed did not addressed your queries well due to our incorrect understanding. In this revision, we have supplemented more experiments and revised our manuscript again. We sincerely hope you can provide us another chance to review our revision.

**# Response** for previous comment 3:

According to your suggestion, we have totally added 10-group cross-validation experiments on independent datasets (Please see **Table 1**), the later seven validation datasets are collected from GEO data repository. For each experiment, firstly, the selected gene probe features by MGRFE from the first dataset were transformed into the official gene symbols; secondly, the obtained gene symbols were transformed into corresponding gene probe Ids in the second dataset; thirdly, a kind of classifier were used to perform 10 times *k*-fold cross validation using the samples and selected gene probe features on the second dataset; and fourthly, the performance of three different classifiers, Naive Bayes (NB), Support Vector Machine (SVM) and Random Forest (RF), on each validation dataset were recorded. Particularly, no feature mapping between Gas1 and Gas2 for they are generated simultaneously and have identical feature set [1].

Below **Table** **1** becomes the TABLE 7 in the revised manuscript.

**Table 1**. Independent validation of selected gene features by MGRFE with 10-time *k*-fold cross validation.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Feature From / #Features | Feature Tested / #Features | #Samples | Classifier | *Sn* | *Sp* | *Acc* | *Avc* | *MCC* | *AUC* |
| Leuk / 2 | MLL / 4 | 52 | NB | **0.963** | **0.955** | **0.961** | **0.959** | **0.929** | **0.993** |
| SVM | 0.935 | 0.887 | 0.913 | 0.911 | 0.844 | 0.975 |
| RF | 0.960 | **0.955** | 0.959 | 0.958 | 0.925 | 0.977 |
| Gas1 / 2 | Gas2 / 3 | 124 | NB | **0.968** | **0.966** | **0.967** | **0.967** | **0.937** | **0.993** |
| SVM | 0.952 | **0.982** | **0.967** | **0.967** | **0.937** | 0.992 |
| RF | 0.957 | 0.931 | 0.944 | 0.944 | 0.895 | 0.987 |
| Gas2 / 3 | Gas1 / 2 | 144 | NB | **0.949** | 0.968 | **0.958** | **0.958** | **0.920** | **0.975** |
| SVM | 0.941 | **0.972** | 0.956 | 0.956 | 0.916 | 0.970 |
| RF | 0.936 | 0.958 | 0.947 | 0.947 | 0.900 | 0.974 |
| DLBCL / 3 | GSE56315 / 7 | 88 | NB | **1.000** | **1.000** | **1.000** | **1.000** | **1.000** | **1.000** |
| SVM | **1.000** | **1.000** | **1.000** | **1.000** | **1.000** | **1.000** |
| RF | **1.000** | **1.000** | **1.000** | **1.000** | **1.000** | **1.000** |
| Prostate / 4 | GSE8511 / 5 | 41 | NB | **0.884** | **0.852** | **0.870** | **0.868** | **0.753** | **0.935** |
| SVM | **0.900** | 0.665 | 0.806 | 0.783 | 0.582 | 0.900 |
| RF | 0.868 | 0.752 | 0.822 | 0.810 | 0.646 | 0.917 |
| Gastric / 3 | GSE2685 / 1 | 30 | NB | 0.919 | **0.650** | **0.846** | **0.785** | **0.584** | 0.861 |
| SVM | **0.990** | 0.440 | 0.843 | 0.715 | 0.464 | **0.865** |
| RF | 0.862 | 0.500 | 0.765 | 0.681 | 0.365 | 0.686 |
| Gastric / 3 | GSE66229 / 7 | 400 | NB | 0.903 | **0.896** | 0.902 | 0.900 | 0.764 | 0.961 |
| SVM | **0.955** | 0.864 | 0.932 | 0.909 | 0.823 | **0.971** |
| RF | 0.950 | 0.894 | **0.936** | **0.922** | **0.835** | **0.971** |
| Adenoma / 1 | GSE8514 / 3 | 15 | NB | 0.900 | 0.800 | 0.867 | 0.850 | 0.700 | **0.960** |
| SVM | 0.900 | 0.500 | 0.767 | 0.700 | 0.400 | 0.920 |
| RF | **0.910** | **0.820** | **0.880** | **0.865** | **0.730** | 0.950 |
| Colon / 6 | GSE44076 / 23 | 148 | NB | **0.988** | 0.950 | **0.976** | **0.969** | **0.948** | 0.996 |
| SVM | 0.969 | 0.952 | 0.963 | 0.961 | 0.924 | 0.995 |
| RF | 0.977 | **0.960** | 0.972 | **0.969** | 0.940 | **0.998** |
| ALL1 / 1 | GSE2604 / 4 | 14 | NB | **1.000** | **1.000** | **1.000** | **1.000** | **1.000** | **1.000** |
| SVM | **1.000** | **1.000** | **1.000** | **1.000** | **1.000** | **1.000** |
| RF | **1.000** | **1.000** | **1.000** | **1.000** | **1.000** | **1.000** |

On the tested datasets with sample number greater than 50, 10-time 10-fold cross validation were performed with different random seeds. Meanwhile, 10-time 5-fold cross validation were performed on datasets with samples less than 50.

The latter seven validation datasets are retrieved from GEO and named as their GEO accessions.

NB, SVM and RF represent Naive Bayes, Support Vector Machine and Random Forest classifiers, respectively.

The bold face values denote the highest performance achieved by the three classifiers.

The extra pre-processing on below four GEO datasets should be explained.

1. Dataset GSE8511 has three kinds of samples: “Benign Prostate”, “Local Prostate Cancer” and “Metastatic Prostate Cancer”. The latter two kinds of samples are combined together as “Prostate Cancer” samples in validation.
2. Dataset GSE44076 has three kinds of samples: “Mucosa sample from healthy Normal donor”, “Normal paired sample from patient” and “Tumor sample from patient”. The first two kinds of samples are combined together as “Normal” samples in validation.
3. On dataset GSE8511, there are total seven gene probe features mapped from Prostate dataset, but two of them contain Null values and have been abandoned. Thus, only five gene features are used on GSE8511.
4. On dataset GSE2604, there are 36 samples in total, but the 22 samples with Null values have been removed. Thus, only 14 samples are used on GSE2604.

On tested datasets GSE56315 and GSE2604 with gene features from DLBCL and ALL1 respectively, NB, SVM and RF classifiers all achieved 1.0 cross validation accuracy in each test. In particular, there are only 14 samples totally on GSE2604, which means the classifiers were trained on merely about 10 samples in each 5-fold cross validation. Thus, the selected unique gene *CD3D is* one ideal discrimination for B-cell acute lymphoblastic leukemia (ALL) and T-cell ALL. On GSE2685, there is only one gene probe Id mapped from the selected gene *LIFR* on Gastric dataset, and no mapping items for selected genes of *GATA6-AS1* and *HHIP*. Meanwhile, the sample number of GSE2685 is merely 30. But NB and SVM still achieved acceptable cross validation accuracies over 0.8. Except three tested datasets of GSE8511, GSE2685 and GSE8514 with samples less than 50, the prediction accuracies of three classifiers are above 0.9 on all other seven datasets in cross validation. The independent validation results proved that the selected genes features by MGRFE in each dataset have strong association with the disease phenotype and can be selected as the candidates for biomarkers.

**# Response** for previous comment 4:

We are sorry not to address the comment 4 very well during the previous response and modification. In this revision, we try to discuss and compare in depth the SVM-RFE and GA-RFE from experiment to theory.

To provide a fair comparison between GA-RFE and SVM-RFE, comprehensive experiments were performed on all the 17 binary classification datasets and below details have been considered.

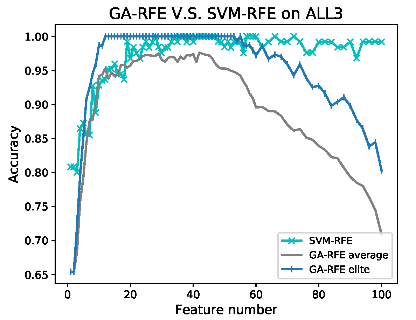
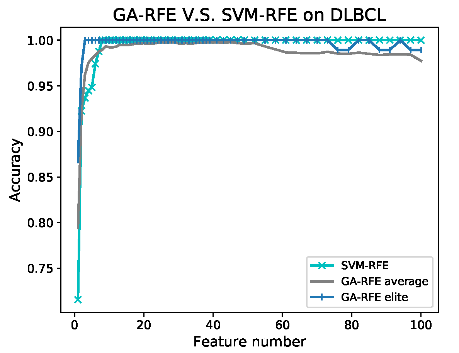
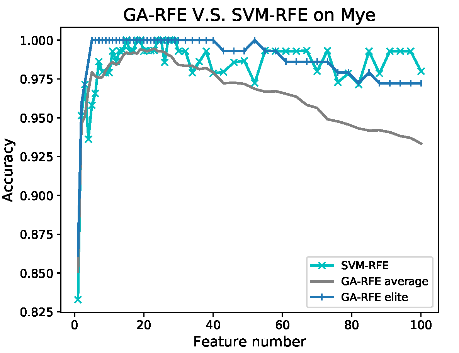
1. Isabelle Guyon [2] pointed out that the feature normalization in pre-processing is of great importance to SVM-RFE. For each feature, we have subtracted its mean and then divided the result by its standard deviation as suggested. As a result, the feature scales are comparable within a dataset.
2. Both GA-RFE and SVM-RFE use SVM model with linear kernel as the embedded classifier in the whole process. The penalty parameter *C* is set as 100 as in the original paper.
3. Feature filter process are performed on each datasets to provide GA-RFE and SVM-RFE with same initial high quality features.
4. Only one GA-RFE process is used to do comparison with SVM-RFE on each dataset. The multi-layer iteration manner is abandoned here for fairness.

**Table 2**. Both GA-RFE and SVM-RFE can achieve 100% 5-fold cross validation accuracies on 17 binary classification datasets. But GA-RFE used more compact gene subsets with smaller sizes.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Dataset | GA-RFE | | SVM-RFE | |
| #Genes | Accuracy | #Genes | Accuracy |
| DLBCL | 3 | 1.0 | 8 | 1.0 |
| Pros | 6 | 1.0 | 11 | 1.0 |
| Colon | 5 | 1.0 | 5 | 1.0 |
| Leuk | 2 | 1.0 | 2 | 1.0 |
| Mye | 11 | 1.0 | 18 | 1.0 |
| ALL1 | 1 | 1.0 | 2 | 1.0 |
| ALL2 | 10 | 1.0 | 17 | 1.0 |
| ALL3 | 12 | 1.0 | 28 | 1.0 |
| ALL4 | 5 | 1.0 | 12 | 1.0 |
| CNS | 6 | 1.0 | 8 | 1.0 |
| Lym | 3 | 1.0 | 3 | 1.0 |
| Adeno | 1 | 1.0 | 1 | 1.0 |
| Gas | 3 | 1.0 | 4 | 1.0 |
| Gas1 | 5 | 1.0 | 15 | 1.0 |
| Gas2 | 2 | 1.0 | 4 | 1.0 |
| T1D | 14 | 1.0 | 21 | 1.0 |
| Stroke | 3 | 1.0 | 6 | 1.0 |

**Figure 1**. Sizes of selected gene subsets by GA-RFE and SVM-RFE for achieving 100% cross validation accuracy on 17 datasets.

The performance of GA-RFE and SVM-RFE on 17 datasets are recorded in **Table 2**. Both GA-RFE and SVM-RFE could achieve 100% 5-fold cross validation accuracies on all these datasets. **Figure 1** provides the histogram graph of the used feature number by these two methods for better visual illustration. On 14 datasets, GA-RFE could find more compact feature subsets to achieve the same performance as SVM-RFE did.

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**Figure 2**. Performance comparison of GA-RFE and SVM-RFE with varying feature number. The accuracy is calculated in 5-fold cross validation. These two methods have been provided with an initial feature set containing 500 genes generated by feature filter process on each dataset. Then, GA-RFE begin its evolution from some randomly sampled feature subsets 100 in length to save time. SVM-RFE starts with the exactly provided feature set.

To better analyze the reason why GA-RFE is more effective than SVM-RFE in finding the minimal discriminatory feature subset, we plot their dynamic performance on three datasets where GA-RFE could achieve obvious smaller feature subsets than SVM-RFE. **Figure 2** shows the performance of GA-RFE and SVM-RFE in their iterations with feature size ranging from 1 to 100 on datasets DLBCL, Mye and ALL3. SVM-RFE begins its iteration from the initial 500 features provided by the filter process. GA-RFE starts the iteration with randomly sampled feature subsets 100 in length. From the three sub-plots in **Figure 2**, it could be noticed that GA-RFE need a convergence process to find some elites in population with 100% accuracies. For example, on ALL3 and Mye, the average performance in population (GA-RFE average) and the performance of population elites (GA-RFE elite) are constantly increased in the process of reducing the feature range from 100 to ~50. In fact, in this process, the GA evolution number is set very small (1 or 2) and RFE step is set relatively large (3 or 2) for each feature length to save time. But when some GA elites find 100% accuracy feature subsets, this best performance is constantly kept for a long feature range. The performance of SVM-RFE is relatively stable when the feature size is larger than its finally selected feature subset. But when the feature range is smaller than its finally selected feature subset, the performance usually decline obviously. GA-RFE is more robust than SVM-RFE for the elites in GA population could maintain 100% accuracy to smaller gene subset.

SVM-RFE by Isabelle Guyon [2] followed the Structural Risk Minimization (SRM) principal and has been widely recognized as the classical feature selection method on microarray data and other related problems. Its key procedure can be briefly described as follows.

1. Train a SVM classifier with linear kernel using current feature set.

A weight vector **w** and a bias value **b** are learnt in optimizing the loss function. The obtained decision function is D(**x**) = **w**·**x** + **b**, where **x** is an input train sample.

1. Compute the ranking criterion for all features. The weight of *i*-th feature is (**w*i***)2.
2. Remove the feature with smallest ranking weight. The removed feature is added to the head of a feature ranking list, an empty list at the start.
3. Repeat steps a~c until the feature set is empty.
4. Output the feature ranking list.

SVM-RFE has below remarkable advantages compared to its previous methods.

1. The mutual information between features are considered in SVM training. This avoids the implicit orthogonality assumption of features in simple feature filter methods.
2. The selecting of useful samples (support vectors of SVM model) and useful features could be connected together.

Meanwhile, it should be noted that there are also some potential shortcomings of SVM-RFE.

1. Its greedy feature elimination manner makes it not very robust in achieving the minimal discriminatory feature subset. The (**w*i***)2 is only the estimation for the influence of a feature on the loss function. When a feature with currently lowest (**w*i***)2 is removed, it don’t have chance to be considered again.
2. No validation of feature subset. From beginning to end, the SVM model is trained on the same training dataset and the weight vector **w** is used for computing feature importance, without any independent test or validation.
3. The classifier models that can be used are limited. Only the classifiers that can infer feature importance could be considered for replacing SVM in the SVM-RFE framework.
4. Though SVM-RFE is fast, the time cost is still high when the feature range is very large. SVM-RFE is a sequentially executed algorithm in essence. When there are tens of thousands of features in dataset, it is too time consuming to train same number of SVM models sequentially to generate the feature ranking.

The original idea of our algorithm comes from combining the RFE framework with swarm intelligence method of GA to build a more robust and flexible feature selection method. The feature filter process is used to quickly reduce the feature range. The multi-layer iteration manner is designed to help improve stability. As a result, the former 4 potential shortcomings of SVM-RFE could be eased or improved.

1. The population of feature subsets in GA could bring about more robustness. When a feature is removed from one feature subset, it could still exist in other feature subsets. The population evolution of GA is more robust and fault-tolerant than the feature refining process on a single feature subset in original SVM-RFE.
2. For each feature subset, cross validation could be performed to evaluate its performance. The generation and evaluation processes of feature subsets are separated in GA-RFE.
3. All kinds of classifiers could be embedded in our method to get their most suitable feature subsets.
4. Two filter methods, *t*-test and MIC, are employed to quickly generate an obviously reduced feature subset with high quality feature candidates. The later RFE process are performed based on the limited candidate features. Thus, time cost is notably reduced compared with direct RFE process on initial feature set.

In our algorithm, GA is employed to generate different feature subsets. As a kind of bio-inspired algorithm, the applications of GA on various problems are far ahead of its theoretical researches. There are two basic theoretical analysis ways for GA: Schema Theorem and Markov chain. The Schema Theorem formalized by Holland is a mile stone in theory analysis for GA [3]. Simply stated, he try to prove that in the evolution process of a canonical GA, the good schemas with performance higher than the average performance in population are exponentially increased in expectation. However, there are several shortcomings in his proof which lead to more modern approaches [4-6].

For the information of GA population in the next generation usually only rely on the population in the current generation, Markov chain could be used naturally to model the behavior of GA evolution.

Goldberg and Segrest present a finite Markov chain analysis to a single-locus, binary-coded finite population GA [7]. Eiben et al. employed Markov chain to prove that GA with elitism preservation mechanism has global convergence ability [8]. The elitism preservation mechanism has been adopted in the design of GA-RFE.

As you have pointed out, the swarm intelligence based optimization methods like GA have some well-known shortcomings, including the lack of precise theoretical analysis, sensitive to parameters and time cost problem. But on the other hand, it should also be noticed that the evolution calculation based method is one of the main branches for gene selection on microarray. Many related leading methods have been constantly published in recent years and still cause high concerns [9-13]. This is because these swarm intelligence based methods have their own advantages in this feature selection problem. The feature combinations in microarray is exponential correlated with the feature number, thus make this problem NP-hard. But swarm intelligence based methods are widely known for their effectiveness in solving many NP-hard and complex optimization problems.

Re-think the motivation for identifying compact discriminatory gene features in the microarray, the below two points should be noticed.

1. Structural risk minimization. Structural risk minimization is an inductive principle for model selection used for learning from finite training data sets. It describes a tradeoff between the empirical error in training data and hypothesis space complexity of a learning model. On microarray data, there are usually several thousand to tens of thousands of gene features but only dozens or hundreds of samples. Thus, the features used by the prediction model must be limited to control the model complexity. By selecting relatively small number of gene features, the learnt model could avoid the overfitting problem and have better generalization ability on unseen data. In Recursive Feature Elimination process, the number of gene features is reduced step by step, thus the corresponding learnt models are arranged in order of decreasing complexity. In minimizing both the empirical error and capacity of a model, the idea of SRM is clearly embodied.
2. Finding disease related genes and potential biomarkers. The selected minimal discriminatory gene subset has high correlation with the disease phenotype on microarray data. Thus, they are biomarker candidates for the specific disease and may provide researchers with insights into the genetic nature of the disease and mechanism behind it. Therefore, the discriminatory genes are well worth of further biological analysis.

By introducing GA into the RFE framework, our designed algorithm is fault-tolerant in feature elimination and flexible in classifier selection and feature subset evaluation. GA-RFE is robust and effective in finding the minimal discriminatory feature subset. The independent validation experiments proved that the selected gene features have good generalization performance, thus could be regarded as biomarker candidates for corresponding disease. The shortcomings of our algorithm including lack of precise theoretical analysis and kind of time consuming.

In doing the comparison experiments between GA-RFE and SVM-RFE, we find that the combination of feature normalization process and SVM model with linear kernel is more effective than our previously used Gauss Naive Bayes. The performance records in **Table 2** are higher than our previous performance records on 17 binary datasets in manuscript. Updating these records means that all the selected gene features on these datasets need to be updated simultaneously, thus many cascaded analysis and experiments, like independent validation, need to be checked and performed again. We plan to update these records and all the affected table and figure results in our next revision.

We do appreciate your rigorous academic style and responsible attitude for review work. Your comments have obviously enhanced our algorithm and manuscript. We sincerely hope that you can provide us another chance to improve our manuscript.

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