Gene expression
Dynamically weighted clustering with noise set
Yijing Shen¹, Wei Sun² and Ker-Chau Li¹,3,∗
¹Department of Statistics at University of California, Los Angeles, CA 90095, ²Department of Biostatistics, Genetics, University of North Carolina, NC 27516, USA and ³Institute of Statistical Science, Academia Sinica, Taipei, Taiwan, Republic of China
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ABSTRACT
Motivation: Various clustering methods have been applied to microarray gene expression data for identifying genes with similar expression profiles. As the biological annotation data accumulated, more and more genes have been organized into functional categories. Functionally related genes may be regulated by common cellular signals, thus likely to be co-expressed. Consequently, utilizing the rapidly increasing functional annotation resources such as Gene Ontology (GO) to improve the performance of clustering methods is of great interest. On the opposite side of clustering, there are genes that have distinct expression profiles and do not co-express with other genes. Identification of these scattered genes could enhance the performance of clustering methods.
Results: We developed a new clustering algorithm, Dynamically Weighted Clustering with Noise set (DWCN), which makes use of gene annotation information and allows for a set of scattered genes, the noise set, to be left out of the main clusters. We tested the DWCN method and contrasted its results with those obtained using several common clustering techniques on a simulated dataset as well as on two public datasets: the Stanford yeast cell-cycle gene expression data, and a gene expression dataset for a group of genetically different yeast segregants.
Conclusion: Our method produces clusters with more consistent functional annotations and more coherent expression patterns than existing clustering techniques.
Contact: yshen@stat.ucla.edu
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1 INTRODUCTION
Gene expression variations are expected to provide insights into the cellular roles of the genes in an organism. Clustering analysis methods are useful tools for extracting information from massive microarray gene expression datasets by grouping together genes that share similar expression profiles (Eisen et al., 1998). Such co-expressed genes are likely to be regulated by common cellular signals. The result of clustering analysis can be used to reveal the organization of the genes in different biological processes and to predict the functions of new or poorly annotated genes (Marcotte et al., 1999). There are many clustering methods available: the hierarchial clustering (Eisen et al., 1998; Jain et al., 1999), K-means algorithm (Tavazoie et al., 1999), Self Organization Maps (SOM) (Comrads et al., 2003; Tamayo et al., 1999), mixture model approaches (Ghosh and Zhong, 2005; MacLachlan and Basford, 1988; Yeung et al., 2001) and many others (Cheng et al., 2004; Hastie et al., 2000; Tseng, 2007). To improve the performance of such general purpose clustering methods in microarray studies, some researchers have proposed two additional ideas: (i) allowing for a set of scattered noise genes to remain un-clustered (Hanisch et al., 2002; Pan, 2006; Thalamuthu et al., 2006; Tseng, 2007; Tseng and Wong, 2005); (ii) incorporating the functional annotation information of the genes (Bau et al., 2004; Cheng et al., 2004; Hamisch et al., 2002; Pan, 2006; Segal et al., 2003; Tseng, 2007).
Different strategies have been used to incorporate functional annotations. Pan (2006) used a model-based clustering method by assuming that genes in one Gene Ontology (GO) term have the same prior probability of belonging to one cluster. This may not be optimal because genes sharing the same functional annotation may have different expression patterns. Indeed, by studying the coexpression patterns of protein complexes in yeast for four large scale microarray gene expression databases, Liu et al. (2008) found that except for large protein complexes such as cytoplasmic and mitochondrial ribosomal complexes, proteasome, or ATP synthase, most genes from the same complexes do not have strong correlation. In addition, the method does not allow for scattered noise genes. Tseng (2007) proposed a new clustering method, Penalized and Weighted K-means, which is an extension of K-means that incorporates functional annotations and allows for scattered genes. The loss function of PW-Kmeans is the summation of the weighted dispersions of each clustered gene augmented by penalties for each scattered gene. The weight of each gene is computed based on its minimum distance to any known functional group (e.g. a pathway) where the distance is defined as the average square distance from this gene to all the genes in the group. Intuitively, if one gene is close to one functional group, the weight is small, thus the gene is less likely to be claimed as a scattered gene. Note that all weights are fixed throughout the clustering procedure. The weight assigned to a given gene remains the same no matter which cluster the gene is assigned to. Therefore, while weighting does help identifying the scattered genes, it does not enhance the clustering of genes with similar functions. Furthermore, as discussed above, functionally related
genes are not necessarily co-expressed. Therefore, the weights pre-determined by the average distance to every gene in a functional group may not be appropriate.

In this article, we develop a novel weighted clustering method, Dynamically Weighted Clustering with Noise set (DWCN), which allows for the presence of scattered noise genes and makes use of functional annotation data in a different way. Similar to the PW-Kmeans, the loss function we use is also the summation of the weighted dispersions of the clustered gene plus penalties for scattered gene. However, unlike the PW-Kmeans, which uses gene-specific weights, DWCN uses cluster-specific weights: all the genes within one cluster share the same weight. The weight of one cluster represents a penalty term which is inversely proportional to the homogeneity between the cluster and the functional categories. Both the cluster memberships and the weights are iteratively updated in the clustering algorithm.

The remainder of this article is organized as follows. We first describe our method and the datasets used. Then, using both simulated data and real data, we present and compare the results of DWCN with three other clustering algorithms: K-means, tight clustering and PW-Kmeans. We use the GO system to define functional categories throughout our discussion, although our method is general enough to incorporate any functional category information such as pathway annotation.

2 METHODS

A flow chart describing our method is shown in Figure 1. We first initialize a group of clusters by forming tight clusters within each functional group (e.g. GO term) and within the set of remaining genes. Then, we iteratively select a gene and decide whether it should belong to one of the clusters or the noise set. The decision is made by evaluating a loss function which involves cluster-specific weights. The weights are simultaneously updated in order to find the optimal solution, one may compare the loss function for all possible ways of cluster membership reassignment. This, however, is computationally infeasible. To overcome this difficulty, we used simulated annealing to minimize the loss function (Bryan et al., 2006; Chakraborty, 2005).

2.1 Model setup

Let \( C = \{C_1, C_2, \ldots, C_r\} \subset \mathbb{R}^p \) be a set of \( n \) data points in a \( p \)-dimensional space (e.g. expression of \( n \) genes measured in \( p \) conditions). The purpose of our clustering method is to partition these \( n \) genes into \( r \) clusters, \( C_1, C_2, \ldots, C_r \), and a set of scattered noise genes, \( S \). We define the loss function \( W \) as

\[
W(C, r, \lambda) = \sum_{i=1}^{n} \left( \sum_{j=1}^{r} w_{ij}d(x_i, C_j) \right)^{1-\lambda} \left( w_{ij} \right)^{\lambda},
\]

where \( \beta_i \) is 1 if \( x_i \) is a scattered point, and 0 otherwise. Let \( w_{ij} \) be the cluster membership such that \( w_{ij} = 1 \) if \( x_i \in C_j \), and 0 otherwise. The parameter \( \lambda \) is the ‘penalty’ for the scattered genes. It can be viewed as a tuning parameter controlling the size of the noise set because larger \( \lambda \) lead to smaller noise sets. We take the distance \( d(x_i, C_j) \) to be squared \( L_2 \) norm from \( x_i \) to the center of the cluster \( C_j \):

\[
d(x_i, C_j) = ||x_i - \frac{\sum_{k \in C_j} m_{jk} x_k}{|C_j|}||^2,
\]

where \( |C_j| \) is the number of genes in cluster \( C_j \). The ‘weight’ \( w_{ij} \) for cluster \( j \) is defined via the minimum hyper-geometric \( P \)-value, \( g_j \), obtained from testing

the functional enrichment of cluster \( j \) with respect to each of the GO terms. Specifically, let

\[
g_j = \min_{x \in |G_m \setminus C_j|} \left( \frac{|G_m \cap C_j| - y}{|G_m| - y} \right)
\]

where \( G_m \) is the number of genes in the \( m \)-th GO term. Then,

\[
w_j = \begin{cases} P & \text{if } g_j < 0.05 / \kappa \\ 1 & \text{if } g_j > 0.05 \\ b + a \log(g_j) & \text{otherwise} \end{cases}
\]

where \( 0.1 < P < 0.5 \) is a fixed parameter, \( \kappa \) is the number of GO terms, and \( a, b \) are scaling constants, which can be solved for by noting that they are the slope and intercept of the line passing through \( [\log(0.05), 1] \) and \( [\log(0.05/\kappa), P] \). Thus,

\[
a = \frac{1 - P}{\log \kappa}, \quad b = \frac{\log(0.05) + P \log(0.05)}{\log(\kappa)}.
\]

In summary, the loss function is a weighted sum of the within cluster distances plus penalties for the noise set. The weight of one cluster measures the over-representation of genes within this cluster in a group of functional categories. In other words, it is a measure of homogeneity between clusters and functional categories.

2.2 Initialization

First, all annotated genes are clustered using the tight clustering method (Tseng and Wong, 2005) within each GO term. Only the tightest resulting cluster for each GO term is kept, defining the initial cluster configuration \( \{C_j\}_{j=1}^r \) where \( j \) stands for \( j \)-th cluster and \( G \) indicates these clusters are from GO terms. The remaining annotated genes together with the non-annotated
genes define the initial noise set $S^{(0)}$. We refine the initial configuration based on the distance between each gene in $S^{(0)}$ and its nearest cluster. Specifically, let $x_i$ be one gene in $S^{(0)}$, and let $d_i$ be its distance to the nearest cluster. Given two constants $\tau_1$ and $\tau_2$ ($\tau_2 \geq \tau_1$), $x_i$ is assigned to its nearest cluster if $d_i < \tau_1$, and $x_i$ is assigned to a set $T$ if $d_i > \tau_2$. Next we identify tight clusters from $T$, denoted as $\{C_j|i\}$. The number of tight clusters in $T$ is chosen so that it gives the smallest initial loss function. The initial clusters are obtained by combining $\{C_j|i\}$ and $\{C_l|j\}$. The remaining genes in $S^{(0)}$ constitute the initial noise set.

With the initial classification of the cluster genes and the noise genes, the tuning parameter $\alpha$ can be estimated by minimizing the sum of the misclassification rates for the clustered genes and the noise genes. In the case where the GO terms used have few overlapping genes, we estimate the number of clusters $r$ by the total number tight clusters within each GO term and within the un-annotated gene set. When a larger set of GO terms are used, the sizes of GO terms may get smaller, and many genes may belong to multiple GO terms. In such cases, the default initialization of one cluster per GO term may be impractical. We propose to cluster GO terms by hierarchical clustering, and then merge the smaller GO terms by choosing a cutoff of the hierarchical tree. An example is presented in the Results section.

### 2.3 Loss function minimization

Because of the complicated discontinuous, non-linear relationship between the weights $w_i$ and the cluster membership $y_i$, we optimize the loss function by simulated annealing (Bryan et al., 2006; Chakraborty, 2005).

After initialization, we obtain several initial clusters, together with a set of ‘noise’ genes. The initial values for $a_i$ and $b_i$ are defined at the initialization. Next, we screen all the genes. In each iteration, one gene is randomly selected. If it comes from the noise set, we tentatively assign it to the nearest cluster, as determined by $D_i$ (the distance between gene $i$ and cluster $j$) where $D_i = \|x_i - C_j\|$: otherwise, it is tentatively moved to the noise set. After updating the loss function [Equation (1)], the proposed change is always accepted if the loss function decreases, otherwise it is accepted with probability $\exp(-W^{(r+1)} - W^{(r)})/T$, where $T$ is the so-called annealing temperature. In order to achieve convergence, after an initial ‘burn-in’ period, $T$ is slowly decreased at each iteration by $T = T(1+\sigma)$ where $\sigma$ is a constant controlling the annealing schedule (Bryan et al., 2006).

### 2.4 Cluster evaluation

In order to evaluate the final clustering results, we use Rand indexes (Hubert and Arabie, 1985; Rand, 1971) and Weighted Rand indexes (Thalamuthu et al., 2006) to assess the homogeneity between the resulting clusters and the function categories or the true clustering structure. Intuitively, the rand index equations can be explained by the number of agreements between two partitions divided by the total number of agreements and disagreements between two partitions.

A few versions of Rand indexes are used. Rand, [Equation (4)] computes the similarity between GO and clustering partitions including the un-annotated set as one of the GO term, and the noise set as one of the clusters. It treats noise genes and clustered genes with equal importance and thus it favors methods without scattered genes. Rand excludes all noise and un-annotated genes, and it is biased against methods with a noise set (Thalamuthu et al., 2006). RandW defined in Equation (5), is a weighted average between the two measures, which is used for validating the simulation by comparing the similarity of the true partitions and the clustering results.

\[
\text{Rand}(G, r) = \frac{\sum_{i=1}^{G} \sum_{j=1}^{r} \frac{Y_{ij} \times Y_{ij}}{\left|Y_{ij}\right| \times \left|Y_{ij}\right|}}{\left|Y_{ij}\right| \times \left|Y_{ij}\right|}.
\]

\[
\text{RandW}(G, r) = a \times \text{Rand}(G, r) + (1-a) \times \text{Rand}(G, r),
\]

where $Y_{ij}$ is the number of GO terms, $G$ indexes the un-annotated set, $r$ is the number of clusters, $r-1$ indexes the scattered set, and $Y_{ij}$ represents the number of genes belonging to the $i$-th GO term and $j$-th cluster.

The weighted index is simply a linear combination of Rand$_1$ and Rand$_2$ weighted by

\[
\text{RandW}(G, r) = a \times \text{Rand}(G, r) + (1-a) \times \text{Rand}(G, r),
\]

where $u = \max(Y_{ij} \times Y_{ij} - Y_{ij} + 1)/Y_{ij}$.

In the absence of the knowledge about true clusters (e.g. for the real datasets), we evaluate the concordance between clustering results and GO terms using another Rand index, Rand$_2$, which treats each gene in the noise term that comes from a GO term as an individual cluster.

In addition, we also employ a cross-validation approach to evaluate how well the functions of the un-annotated genes were predicted. Specifically, the GO annotations of a subset of genes were deleted prior to clustering. After clustering, the accuracy rate is computed based on how well the testing genes are grouped into the GO terms.

Moreover, we also measure the concordance of the GO terms and the clustering results by quantifying the clustering homogeneity within each GO term through its entropy.

\[
e_i = \sum_{j=1}^{r} p_j \log \frac{1}{p_j},
\]

where $r$ is the number of clusters, and $p_j$ are defined as $p_j = Y_{ij}/m_i$, with $m_i$ being the total number of non-scattered genes which belong to the $i$th GO term. For the purpose of consistently comparing different clustering results, entropies of each GO term are pooled together. The total entropy of each clustering method is represented by

\[
E = \sum_{i=1}^{G} e_i.
\]

The higher the entropy is, the lower the homogeneity.

Because our method used the GO data as prior information to form clusters, evaluation measures such as the entropy for assessing the homogeneity of the clusters with respect to GO terms can introduce some bias. Thus the reported better performance of our method under GO term enrichment related criteria are anticipated.

This also raises the concern of ‘overfitting’—namely, our method may fine-tune the clusters to better capture the GO term structure to the extent of over-compromising the quality of clusters. However, as we shall demonstrate by cluster tightness and size criteria, the clusters obtained from our method are indeed tighter and no smaller than the clusters obtained from other methods. Thus our method produces better clusters in terms of quality of the clusters while achieving the aim of better GO term enrichment.

### 3 RESULTS

#### 3.1 Simulation

We first assess the performance of our method using a simulated dataset of sample size $m=50$. Consider $w = 10$ clusters corresponding to 10 hypothetical GO terms. We first generated the centers $(\mu_l, l=1, \ldots, 10)$ of the clusters from normal distribution $N(0, \sigma I_m)$, where $I_m$ is an identity matrix of size $m \times m$. Subsequently, for each cluster, expression profiles of 50 genes were generated from $N(\mu_l, \sigma^2 I_m)$. Separately, 500 ‘noisy’ gene expression profiles were generated from the uniform distribution, $U(0, -2, 2)$, making a total of 1000 genes for the simulation. The genes were ‘annotated’ so that 25 out of 50 genes in each cluster and 10 ‘noisy’ genes were assigned to each GO term/category, i.e. 35 genes per GO term. The remaining 650 genes
were considered to be un-annotated. More details of the simulation are described in Simulation section in Supplementary Material. The true cluster membership is shown in Figure 2a. The 1000 simulated gene expression profiles are ordered in such a way that the first 500 genes belong to clusters and the rest of them are in the noise set. Figure 2b–e describe the results from tight clustering, traditional K-means, PW-Kmeans and DWCN, respectively. Tight clustering only finds five out of the ‘requested’ 10 clusters. Because K-means does not allow scattered genes, it is unable to find the correct clusters. PW-Kmeans finds 10 clusters and one noise set; the improvement from K-means is slight. In contrast, DWCN adequately distinguishes most of the noise and forms more correct clusters (see Fig. 2e).

We used the Rand index to compare the clustering results and the underlying true clusters (Table 1, panel A). DWCN outperforms K-means clustering, PW-Kmeans and tight clustering based on Rand1 [Equation (4)], Rand2 and the weighted Rand index [Equation (5)].

3.2 Yeast cell-cycle data

We tested the performance of DWCN on the yeast cell-cycle dataset (Spellman, 1998). A full description and complete datasets are publicly available at http://cellcycle-www.standford.edu. Overall, the dataset consists of 5878 genes on 73 experimental conditions, missing values imputed by the K-Nearest Neighbor (KNN) method (Troyanskaya et al., 2001).

3.2.1 GO slim

We used GO slim terms of biological process as the functional categories, and only concentrate on the GO terms of size 20–300. Altogether 2789 out of 5878 genes were annotated using 27 GO terms. The number of clusters \( r \) is set to 30, which comes from the number of GO terms plus the three tight clusters generated from the noise set. A heat map of the expression profiles for the three additional clusters was shown in Supplementary Figure 2.

Figure 3a shows distributions of the prediction accuracy rates from the cross-validation study where 20% of the GO term prior annotations are removed. Across 10 validation sets, DWCN achieves significantly higher accuracy rates than PW-Kmeans.

Finally, we measure the ‘tightness’ of the clusters produced by different methods using the within-cluster mean squared distances. As shown in Supplementary Figure 3d, the sizes of the DWCN clusters are not smaller than those of clusters produced by tight clustering and PW-Kmeans, yet the DWCN clusters are ‘tighter’ (Supplementary Fig. 3a).
Table 1. Rand indices comparisons on simulation

| Panel A                  | Rand index 1 | Rand index 2 | Weighted rand |
|-------------------------|--------------|--------------|---------------|
| DWCN                    | 0.53         | 0.72         | 0.59          |
| K-means                 | 0.10         | 0.47         | 0.29          |
| Tight                   | 0.25         | 0.35         | 0.29          |
| PWK-means               | 0.37         | 0.23         | 0.32          |

| Panel B                  | Rand index 3 | Total entropy | No. of over-represented GO terms (P < 0.01) |
|-------------------------|--------------|---------------|------------------------------------------|
| DWCN                    | 0.136        | 57.72         | 27                                       |
| R-DWCN                  | 0.026        | 83.47         | 7                                        |
| K-means                 | 0.06         | 69.57         | 18                                       |
| Tight                   | 0.95 × 10⁻⁵  | 109.95        | 12                                       |
| PWK-means               | 0.004        | 85.3          | 1                                        |

| Panel C                  | Rand index 3 | Total entropy | No. of over-represented GO terms (P < 0.01) |
|-------------------------|--------------|---------------|------------------------------------------|
| DWCN                    | 0.23         | 71.33         | 32                                       |
| K-means                 | 0.026        | 87.46         | 21                                       |
| Tight                   | 0.01         | 76.7          | 18                                       |
| PWK-means               | 0.0019       | 101.83        | 0                                        |

| Panel D                  | Rand index 3 | Total entropy | No. of over-represented GO terms (P < 0.01) |
|-------------------------|--------------|---------------|------------------------------------------|
| DWCN                    | 0.14         | 47.4          | 27                                       |
| K-means                 | 0.049        | 55.63         | 19                                       |
| Tight                   | 0.013        | 53.56         | 14                                       |
| PWK-means               | 0.008        | 83.05         | 2                                        |

Panel A: Comparisons made between DWCN, K-means, tight clustering and PWK-means algorithms. Higher indices imply better consistency between the identified clusters and the underlying tree clusters. Panels B and C: Evaluation of the clusters identified from yeast cell-cycle data. Rand index 3, total entropy and total number of over-represented GO terms comparisons between DWCN, K-means, tight clustering and PWK-means by using (Panel B) 27 GO slim terms and (Panel C) 214 GO terms as functional categories correspondingly. Panel D: Same evaluation scheme used as (Panel B and C) for the clusters identified from yeast segregants data using 27 GO slim terms.

3.3 Yeast segregant data

We tested our method on another large-scale gene expression data coming from 112 yeast segregants in a cross between two parental strains BY and RM (Brem and Kruglyak, 2005; Brem et al., 2005). We use this dataset to test the robustness of DWCN. The same 27 GO slim terms is used as functional categories. The number of clusters is chosen to be 29 (27 GO related and two tight clusters). We applied the all mentioned clustering methods on this dataset. Again, we are able to demonstrate the superiority of DWCN. The results are shown in Table 1 (Panel D), Supplementary Figure 3b and c.

4 DISCUSSION

In this article, we proposed a novel clustering method, DWCN, to identify clusters of genes with both coherent expression profiles and similar biological functions. Our method exploits the known biological functions when evaluating cluster-specific weights in the loss function. The weights are updated according to the cluster memberships so that if the genes within one cluster share similar biological functions, the cluster is assigned a smaller weight in the loss function. This construction allows the weights to refine clusters under more meaningful biological contexts. At the same time, the un-annotated or ungrouped genes can be partitioned into one of the existing clusters, or simply left un-clustered.

In the initialization of our method while constructing tight cluster within each GO term, one gene is allowed to be assigned to multiple clusters. However, during the iterative updates of our algorithm, one gene is only assigned to one GO term. This restriction is necessary; otherwise, the loss function will increase. A possible extension is to adjust for the number of clusters it belongs. This is a direction worth further research.

As demonstrated in the ‘Results’ section, our method can generate clusters that are both biologically coherent and statistically tight. Importantly, we have shown that the clusters obtained from our method are tighter and no smaller than the clusters obtained from other methods. In other words, our method produces better clusters in terms of their sizes and tightness, which is unrelated with GO term information. Several parameters used by the algorithm i.e. the number of clusters, and penalization constant for noise genes, can be fine tuned to meet specific needs in application. We have derived some guidelines for parameter setting. Our results verified that the clusters generated by DWCN are more likely to overlap with biological categories as compared with other clustering methods. Several criteria of evaluating these comparisons were employed. Our method requires an initial cluster assignment to start the iteration. We employ the tight clustering method together with biological annotation to obtain the initial clusters. If no gene function annotations are available, DWCN is equivalent to the tight clustering method. In cases where prior functional categories are small and with significant overlaps (e.g. GO terms from the bottom of the GO
hierarchy), we propose to merge them by hierarchical clustering followed by cutting the tree at an appropriate height.

The recurrent issue about the optimal number of clusters is a topic we have not fully explored in this report. We used hierarchical clustering followed by dynamic tree cut to select a certain number of GO terms, which guides the clustering procedure. However, as one referee suggested, sometimes rigorous and data-driven criteria may be preferable. Methods such as prediction strength (Tibshirani et al., 2001) or prediction based resampling method (Dudoit and Fridlyand, 2002) could be tested. In addition, methods based on stability criteria (Bertoni and Valentini, 2007, 2008; Ho, 1998; Smelkin and Ghosh, 2003) could also be explored.

The proposed clustering method is not restricted to clustering genes. The same idea can be extended for clustering conditions such as clustering tumor samples. Gene expression profiles have been widely used to discriminate different sub-types, or clinical outcomes of human cancers (Furey et al., 2000; Ghosh, 2002; Golub et al., 1999; Shipp et al., 2002; Singh et al., 2002) In a dataset where the class labels for some cancer patients are missing or incomplete, we can use the known labels to generate 'function categories', and apply our clustering algorithm so that patients having similar expression profiles can be clustered consistently with their class labels.

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