ABSTRACT: Microarray technology is highly used in biological research environments due to its ability to monitor the RNA concentration levels. The analysis of the data generated represents a computational challenge due to the characteristics of these data. Clustering techniques are widely applied to create groups of genes that exhibit a similar behavior. Biclustering relaxes the constraints for grouping, allowing genes to be evaluated only under a subset of the conditions. Triclustering appears for the analysis of longitudinal experiments in which the genes are evaluated under certain conditions at several time points. These triclusters provide hidden information in the form of behavior patterns from temporal experiments with microarrays relating subsets of genes, experimental conditions, and time points. We present an evaluation measure for triclusters called Multi Slope Measure, based on the similarity among the angles of the slopes formed by each profile formed by the genes, conditions, and times of the tricluster.

KEYWORDS: triclustering, angular comparison, genetic algorithms, fitness function, microarrays, time series

Introduction

Microarray technology is highly used in biological research environments due to its ability to monitor, for a great gene collection, the RNA concentration levels, thus enabling the study of genetic functions of species. Bioinformatics and data mining have developed a vast number of computational tools that allow us to analyze data obtained using this technology and to find new knowledge that is hidden from human eyesight. One of the most studied approaches is pattern search in gene expression data. The genes exhibiting high correlation among their expression levels could be involved in similar regulatory processes. The relationship between correlation and functionality has been proved in several studies as in the study by D’haeseleer et al.

Clustering techniques are suitable for performing pattern search by creating groups of genes that exhibit similar expression patterns. Traditional clustering algorithms analyze the whole microarray dimensional space grouping genes taking into account all experimental conditions. However, the activity of genes could only appear under a particular set of experimental conditions, exhibiting local patterns. Discovering these local patterns can be key to discover gene pathways, which could be hard to discover in other ways. For this reason, the paradigm of clustering techniques must be modified to methods that allow local pattern discovery in gene expression data. Biclustering addresses this problem by relaxing the conditions and by allowing assessment only under a subset of the conditions of the experiment, and it has proved to be successful in finding gene patterns.

If a third dimension is added to the dataset besides genes and conditions, such as time, clustering and biclustering result insufficient. There is a lot of interest in temporal experiments because they allow an in-depth analysis of molecular processes in which the time evolution is important, for example, cell cycles, development at the molecular level, or evolution of diseases. In this sense, triclustering appears as a technique going one step further by grouping genes under particular conditions and under particular time points, thus being capable of managing three-dimensional (3D) data. Therefore, triclustering is suitable for the analysis of microarray experiments where several samples are taken at different time points. This is of great interest since it allows for a deep analysis of biological processes where temporary development is important.

Both biclustering and triclustering attack NP-hard problems. Therefore, algorithms based on heuristics are well suited to manage this kind of problem. In this sense, defining an appropriate quality measure for triclusters is an important and essential challenge.

In this work, we propose a quality measure called Multi Slope Measure (MSL), which measures the quality of a tricluster based on the similarity among the angles of the slopes formed by each profile formed by the genes, conditions, and times of the tricluster.
We show the results obtained applying the MSL measure embedded in the TriGen Algorithm,37 an algorithm based on an evolutionary heuristic, genetic algorithms. The datasets used are a synthetic dataset and three real experiment datasets: the yeast cell cycle-regulated genes,18 mouse degeneration of retinal cells,14 and human transcription factor oncogene OTX2 silencing effect on D425 medulloblastoma cell line.19

The results have been validated by three different methods. First, by analyzing the correlation among the genes, conditions, and times in each tricluster using two different correlation measures (Pearson20 and Spearman23). Second, by a graphic validation of the patterns extracted based on the graphic representation (see Graphic Representation subsection), and third we have provided functional annotations for the genes extracted from the Gene Ontology (GO) project.22 The results obtained have been compared to two previously defined quality measures, MSR23 and LSL24 showing improvement in the performance of the measure (see Results and Discussion section).

The rest of the article is structured as follows. A review of the latest related works can be found in State of the Art section. Methods section describes the MSL measure as well as a brief description of Triclustering, the graphic representation applied, and the TriGen algorithm. In the Results and Discussion section, we show the results and discussion of applying TriGen to the synthetic and real datasets. The last section shows the conclusions.

State of the Art
This section is to provide a general overview of recent works in the field of gene expression temporal data. In particular, for those works related to the application of triclustering, we focus on the measures applied to evaluate the triclusters.

We first present the authors’ previous contributions to this field. In our study,23 we described MSRJD, an adaptation of the Mean Square Residue (MSR)9 to the 3D space, so that a third factor, time, in this case, can be taken into account. MSRJD measures the homogeneity of a tricluster in the relation of each value of the tricluster, with the average of all genes, average of all conditions, average of all times, average of all genes and conditions, average of all genes and times, average of all conditions and times, and average of all genes, conditions, and times in the tricluster. We also have presented LSL in our recent study,24 which measures the quality of a tricluster based on the similarity among the slopes of the angles formed by the least square lines from each of the profiles formed by the genes, conditions, and times of the tricluster. LSL has obtained better results than MSRJD applied to the same datasets along with the TriGen algorithm.24

Regarding other authors’ contributions, in 2005, Zhao and Zaki25 introduced the triCluster algorithm to extract patterns in 3D gene expression data. They presented a measure to assess triclusters’ quality based on the symmetry property. This allows for very efficient cluster mining since clusters are searched over the dimensions with the least cardinality.

g-triCluster, an extended and generalized version of Zhao and Zaki’s proposal, was published one year later.26 The authors claimed that the symmetry property is not suitable for all patterns present in biological data and proposed the Spearman rank correlation23 as a more appropriate tricluster evaluation measure.

An evolutionary computation proposal was made by Liu et al.27 The fitness function defined is a multiobjective measure that tries to optimize three conflicting objectives: clusters size, homogeneity, and gene-dimension variance of the 3D cluster.

LagMiner was introduced by Xu et al.28 to find time-lagged 3D clusters, what allows in turn to find regulatory relationships among genes. It is based on a novel 3D cluster model called S3 D3 Cluster. They evaluated their triclusters on homogeneity, regulation, minimum gene number, sample subspace size, and time periods length.

Wang et al.29 proposed a new algorithm called ts-cluster basing their definition for coherent triclusters also on finding regulatory relationships among genes. For that purpose, time shifting is also considered among time points in the evaluated triclusters.

A new strategy to mine 3D clusters in real-valued data was introduced by Sim et al.30 The authors defined the Correlated 3D Subspace Clusters (CSCs), where the values in each cluster must have high co-occurrences and those co-occurrences are not by chance. They measure the clusters based on the correlation information measure, which takes into account both prerequisites.

Hu and Bhatnagar presented an approach focusing on the concept of Low-Variance 3-Cluster,31 which obeys the constraint of a low-variance distribution of cell values.

The work by Liu et al.32 was focused on finding Temporal Dependency Association Rules, which relate patterns of behavior among genes. The rules obtained are to represent regulated relations among genes.

Finally, a brief survey on triclustering applied to gene expression time series was published in 2011.13 There are three main features that a triclustering algorithm can perform. According to Mahanta et al.11, these features are temporal coherence that makes reference to the ability of the algorithm to capture the coherence of different genes in a single time point across samples while generating the final triclusters and the ability to find triclusters with nonconsecutive time points and tricluster with a specific type of pattern (shifting, scaling, delayed). g-triCluster,26 Moga3c,27 LagMiner,28 ts-cluster,29 and Temporal Dependency Association Rules32 perform the temporal coherence feature and only TriCluster25 and Moga3c27 perform finding triclusters containing nonconsecutive time points. TriCluster25 finds scaling patterns, LagMiner28 finds shifting and scaling patterns and ts-cluster29
focuses on time-delayed patterns; the rest do not focus on finding a specific type of pattern. Another feature examined is the algorithm type distinguishing between deterministic (Tricluster\textsuperscript{25}, g-triCluster\textsuperscript{26}) and nondeterministic (Moga3c\textsuperscript{27}) approaches.

**Methods**

In this section, we describe our proposal, the tricluster quality measure called MSL that is based on tricluster’s angular features. We will analyze all MSL principles and fundamentals and how it has been developed.

This section is structured as follows: Triclustering subsection describes the triclustering procedure as an evolution of its well-known predecessor biclustering. In the subsection Graphic Representation, we introduce the graphic representation, which is key for understanding the MSL measure. Then, in the subsection MSL Measure, we analyze the core of our work, the MSL measure. Finally, in the TriGen Algorithm subsection, we briefly describe the TriGen algorithm.

**Triclustering.** Clustering techniques are applied to analyze gene expression data from microarray experiments. The dataset obtained from the experiment, $D$, contains genes and experimental conditions and clustering aims at finding subgroups of genes that share a behavior pattern according to their expression level. Biclustering appears as an evolution of clustering due to its ability to mine subgroups of genes and conditions from the data set $D$, where the genes exhibit highly correlated patterns of behavior under certain experimental conditions.$^9$

Triclustering emerges as an evolution of biclustering, taking into account the temporary evolution of genes under particular experimental conditions. In this way, from a dataset $D$ obtained from a microarray experiment, which contains genes $G$, conditions $C$, and time points $T$, we define triclustering as a technique that finds triclusters $ TRI_{xop}$, from $D$, where a tricluster $ TRI $ is formally defined as $ TRI = G \times C \times T $, where $ G \subseteq G_p, C \subseteq C_p $, and $ T \subseteq T_p $, i.e., a subset of genes that contains information related to the behavior of some genes from dataset $G$ under conditions $C$ at times $T$. Figure 1 shows a tricluster with genes as rows, conditions as columns, and time as depth.

**Graphic representation.** In order to explain the MSL measure, we define the graphic representation of a tricluster $ TRI_{xop} $, with $ x $, $ y $, and $ p $ being either genes $ G $, experimental conditions $ C $, or time points $ T $, so that the $ x $ elements in $ TRI_{xop}$ will be on the X axis and $ y $ elements in $ TRI_{xop} $ will be the outlines represented in as many panels as $ p $ elements in $ TRI_{xop} $ indicates, as can be seen in Figure 2.

To visually analyze the behavior patterns of a tricluster $ TRI $, we always consider three graphical views:

- $ TRI_{yop} (x = G, o = C, p = T) $: one panel for each time, genes on the X axis, the expression levels on the Y axis, and the lines of conditions as the outline.
- $ TRI_{xop} (x = G, o = T, p = C) $: one panel for each condition, genes on the X axis, the expression levels on the Y axis, and the time lines as the outline.
- $ TRI_{op} (x = T, o = G, p = C) $: one panel for each condition, times on the X axis, the expression levels on the Y axis, and the genes as the outline.

With $ TRI_{xop} $ and $ TRI_{yop} $, we can analyze how each gene expression level varies throughout conditions and times, respectively. $ TRI_{xop} $ represents how each gene varies throughout time for each condition.

**MSL measure.** After analyzing the graphic representation of a tricluster, we describe our proposal: the Multi Slope Measure (MSL). MSL measures the differences among the angles formed by every series traced on each of three graphic representations taking into account $ TRI_{xop}, TRI_{yop}, $ and $ TRI_{op} $ (subsection Graphic Representation). MSL takes into account the influence of neighboring time points. We can observe an example of $ TRI_{xop} $ view of $ TRI = G \{g_1, g_2, g_3, g_4\}, C \{c_1, c_2, c_3\}, T \{t_1, t_2, t_3\} $ in Figure 3. We can see how each outline or gene forms a set of angles (two for this particular example) defined by each time point in the X axis for every panel or experimental condition.

To calculate the MSL measure of a tricluster, we first perform the multangular comparison term calculation. The multangular comparison operation of a graphic representation $ xop $ from a tricluster $ TRI $ is defined in Equation 1a. We define $ AC_{multi} $ of a tricluster’s graphic representation $ TRI_{xop} $ as the average of the differences $ \Delta $ of angles vectors $ av_{xop} $ (Equation 1b) of all outlines $ o $ for each panel $ p (H_{av_{xop}} $ in Equation 1c) and its equivalent for the rest of the panels ($ H_{av_{xop}} $ in Equation 1d), with $ N_{av} $ being the number...
of differences made (Equation 1e). An angle vector of an outline $o$ in a panel $p$ is defined as a set of angles that are formed by the outline $o$ taking into account every data point in the X axis (Equation 1f), each outline will have $\text{Number of X axis ticks} - 1$ angles as you can see in Figure 3. The difference $\Delta$ between two angles vector $\text{av}_A$ and $\text{av}_B$ is defined as the average of the $\text{MAX} - \text{MIN}$ (MAX being the maximum and MIN the minimum of two angles $\text{av}_A(i)$ and $\text{av}_B(i)$) of each component (or angle) $i$ of $\text{av}_A$ and $\text{av}_B$ (Equation 1g).

The AC term is based on several operations with $\text{av}_{sp}$ angle vectors. These elements have been obtained based on concept of series (Equation 2a) so that a series $S_{sp}$ of an outline $o$ for a panel $p$ is a set of pair of values from the $x$ axis ($x$) and expression levels ($el$) that form the outline. For each series $S_{sp}$, the alpha angle $\alpha_{xi}$ is calculated as the spin of the arctangent of the slope of the line formed by $(x_i, el_i)$ and $(x_{next(i)}, el_{next(i)})$ points (Equation 2b). The spin operation of an angle showed in Equation 2c is the positive equivalent of this angle if it is negative.

$$\alpha_{xi} = \text{spin} \left[ \arctan \left( \frac{x_{next(i)} - x_i}{el_{next(i)} - el_i} \right) \right]$$  

(2b)
To conclude, the MSL measure of a tricluster TRI (Equation 3) is the average of the angular comparison of the three graphic representations of the triclusters.

Following Figure 3, we show an example of $AC_{multi} (TRI_{gc})$ calculation in Figure 4. First, we arrange the example tricluster so that for each condition (panel) we obtain a table with one row per gene (outline) and one column per time point (X axis). Second, in order to get each $av_{gc}$ (Equation 1f) of angles $\alpha_1$, $\alpha_2$, and $\alpha_3$ vectors, and third, we use Equations 1c and 1d for $V_{mc}$ and $H_{mc}$ calculations, respectively, going through the $av$ set in horizontal and vertical direction. Finally, we use Equation 1a to obtain the $AC_{multi} (TRI_{gc})$ value. We will have to repeat this process twice more, once for each

graphic representation $TRI_{gt}$ and $TRI_{gc}$, to obtain the MSL measure according to Equation 3.

$$MSL (TRI) = \frac{1}{3} [AC_{multi} (TRI_{gt}) + AC_{multi} (TRI_{gc}) + AC_{multi} (TRI_{gc})]$$

**TriGen algorithm.** In this section, we present the TriGen (Triclustering-Genetic based) algorithm,\textsuperscript{17} where the MSL measure has been embedded in order to test its effectiveness. TriGen applies a bio-inspired paradigm of an evolutionary heuristic, genetic algorithms, in such a way that finds a set of triclusters from gene expression datasets where the time is also a component taken into account in the experiment. This method mimics the process of natural selection by creating an initial population of individuals representing solutions that
are crossed and mutated for a number of generations, with the best individuals in the population being finally selected. The MSL measure has been applied as the fitness function to assess the quality of the triclusters or solutions in the population. The flowchart of the TriGen algorithm can be seen in Figure 5. We now define the most important elements of the algorithm such as inputs, outputs, codification of individuals, and genetic operators.

1. **Input:** The TriGen algorithm has two input arguments:
   - \( D \): A dataset containing the gene expression values from a microarray experiment containing genes \( D_G \), experimental conditions \( D_C \), and times \( D_T \). Therefore, each cell \([i, j, k]\) from \( D \) where \( i \in D_G, j \in D_C \), and \( k \in D_T \), represents the expression level of the gene \( i \) under the experimental condition \( j \) at time \( k \).
   - \( P \): Set of parameters to execute the algorithm as described in Table 1. These parameters control the number of solutions or triclusters to find \((N)\), the number of generations to execute \((G)\), the number of individuals in the population \((I)\), and the randomness factor they are generated with the initial population \((Ale)\) as well as weights for the selection and mutation operators \((Sel\) and \(Mut))\), weights to control the effect of the MSL measure \((w_f)\), the size of the triclusters \((w_g, w_c, w_t)\), and weights to control the overlap among solutions \((w_{og}, w_{oc}, w_{ot})\).

The parameters have been chosen after an exhaustive experimentation with all possible ranks of values. Each of the parameters has effect on the triclusters found: size, overlapping, exploration versus exploitation of the algorithm, etc. We now describe the effects of each of the parameters. In the execution of TriGen, each parameter is associated to a genetic operator, that is, \(G\) controls the whole evolutionary process so an increase in the number of generations implies a greater number of recombination of individuals. Therefore, an excessive increase in \(G\) may favor exploitation versus exploration in excess and the algorithm may return solutions that fall into a local minimum. \(I\) and \(Ale\) control the initial population creation, and when the number of individuals \(I\) is increased, a larger search space for the solutions is created so that an excessive increase can create a scatter search effect, and therefore, not return good quality

![Figure 5. TriGen algorithm flowchart.](image-url)
We have designed an overlapping-proportionally measure the number of genes, conditions, and times of \( TRI(\{C, T\}, g) \) relative to the dataset’s size \( (|D_{C, T, g}|) \). MSL is a minimizing fitness function, and we have to set 1 minus each amount proportion in order to favor \( TRI \) with greater sizes when \( w_g, w_t, \) or \( w_e \) are increased. The other three members \( R_e(\{TRI, SOL\}) \), \( R_t(\{TRI, SOL\}) \), and \( |TRI|/\left|\{TRI, SOL\}\right| \) measure the repeated genes, conditions, or times elements of \( TRI \) in the set of already found solutions \( SOL(\{R_e, r(\{TRI, SOL\})\}) \) proportionally calculated supposing all of genes, conditions, or times are repeated in \( SOL(\{TRI, C, T|\}) \) in order to favor \( TRI \) with less overlapping when \( w_g, w_t, \) or \( w_e \) are increased. Finally, the main member \( MSL(\{TRI\}) = \frac{2\pi}{w} \) measures \( MSL(\{TRI\}) \) proportionally calculated to its maximum value \( 2\pi \) in order to favor \( TRI \) with smaller \( MSL \) when \( w_e \) is increased. A default configuration for \( w_g, w_t, w_e, w_{R_e}, w_{R_t}, w_{R_g} \), and \( w_{MSL} \) consists in fixing \( w_t \) to 0.8 and distributing 0.2 among \( w_g, w_e, w_{R_g}, w_{R_t}, \) and \( w_{R_e} \).

For the fitness function, we have:

\[
FF(\{TRI\}) = \frac{1}{w_g, w_t, w_e, w_{R_e}, w_{R_t}, w_{R_g}} \left[ \frac{w_e}{MSL(\{TRI\})} + w_g * \frac{1 - \left| TRI_C \right|}{\left| D_C \right|} + w_t * \left( \frac{1 - \left| TRI_T \right|}{\left| D_T \right|} \right) + w_g * \left( \frac{1 - \left| TRI| \right.}{\left| D_T \right|} \right) + w_{R_e} * \frac{R_e(\{TRI, SOL\})}{\left| TRI| \right.\left| SOL\right|} + w_{R_t} * \frac{R_t(\{TRI, SOL\})}{\left| TRI| \right.\left| SOL\right|} + w_{R_g} * \frac{R_g(\{TRI, SOL\})}{\left| TRI| \right.\left| SOL\right|} \right]
\]
c. **Selection:** Three groups of individuals are randomly selected sorted from lowest to highest according to the fitness function, and then a random selection from the three groups is made. The Se parameter indicates how many of these individuals will pass to the next generation. The rest of the individuals until completing the next population (\( I - \#\text{Selected individuals} \)) will be created based on the crossover operator.

d. **Crossover:** To complete the next generation, we create new individuals with this operator as follows: two individuals (parents, \( A \) and \( B \)) are combined to create two new individuals (offsprings, \( \text{child1} \) and \( \text{child2} \)). The parents are randomly chosen. Their genetic materials are combined by a random one-point cross in the genes \( TRIG_A \), conditions \( TRIG_C \), and time \( TRIG_T \) and mixing the coordinates in both children.\(^{17}\)

e. **Mutation:** An individual can be mutated according to a probability of mutation, \( \text{Mut} \). The mutation probability is verified for every individual, and if it is satisfactory, one out of nine possible actions is taken. These actions are add a new random gene to \( TRIG_A \), add a new condition to \( TRIG_C \), or add a new time point to \( TRIG_T \), by removing a random gene, condition, or time, or by changing a random gene or condition for another that is randomly chosen. The election of these actions is also random. For the case of addition of a new gene, condition, or time, the operator checks whether the new member is already in the individual or not.

### Results and Discussion

In this section, we show the results obtained by application of MSL as a fitness function embedded in the TriGen algorithm\(^{17}\) (see the subsection TriGen Algorithm).

MSL has been applied to four different datasets: one synthetically generated dataset and three real datasets. The real datasets are obtained from experiments with the yeast cell cycle (Saccharomyces cerevisiae),\(^{18}\) an experiment with mice (Mus musculus) called GDS4510\(^{14}\) and data from experiments with humans (Homo sapiens) called GDS4472.\(^{19}\) The last two data sets have been retrieved from Gene Expression Omnibus,\(^{20}\) a database repository of high-throughput gene expression data. All biological experiments examine the behavior of genes under conditions at certain times.

For the analysis of the triclusters obtained as the result of the experiments, we have developed a three-step process based on providing information related to correlation among expression values, the graphic properties of the representation of the values, and biological validation.

The correlation validation is based on the Pearson and Filon\(^{20}\) and Spearman\(^{21}\) coefficients. For every tricluster, we calculate the average of the correlation coefficients between each combination of gene, condition, and time for all genes. For instance, for a tricluster with four genes \([1, 4, 8, 10]\), two conditions \([3 \text{ and } 7]\), and three times \([1, 3 \text{ and } 5]\), we provide the Pearsons and Spearman’s correlation coefficient average for values at the eight possible combinations, each having three time points: \( V_{g1, c3, t7} \), \( V_{g1, c3, t7} \), \( V_{g4, c3, t7} \), \( V_{g4, c3, t7} \), \( V_{g8, c3, t7} \), \( V_{g8, c3, t7} \), \( V_{g10, c3, t7} \), and \( V_{g10, c3, t7} \).

The graphic properties of the representation are shown as described in the subsection Graphic Representation as a way to visually check how the gene patterns behave.

Finally, for the biological validation, we will show the GO terms\(^{22}\) related to the triclusters. We present a GO analysis table in which we include the most representative terms extracted by the Ontologizer software,\(^{23}\) each term associated to a \( P \)-value that denotes the relevance level of the term. In this type of studies, \( P \)-values are considered as relevant below 0.05 and are better when closer to 0. Regarding the GO project, it is a major bioinformatics initiative with the aim of standardizing the representation of gene and gene product attributes across species and databases. The project provides an ontology of terms for describing gene product characteristics and gene product annotation data. The ontology covers three domains: cellular component, the parts of a cell or its extracellular environment; molecular function, the elemental activities of a gene product at the molecular level, such as binding or catalysis; and biological process, operations, or sets of molecular events with a defined beginning and end, pertinent to the functioning of integrated living units: cells, tissues, organs, and organisms.

We have compared the results obtained to those from Gutiérrez-Avilés and Rubio-Escudero,\(^{23}\) where the fitness function was the MSL\(^{14}\) measure and also to the results in Gutiérrez-Avilés and Rubio-Escudero,\(^{24}\) where the fitness function was the LSL measure. The comparison has been made in terms of correlation and GO analysis. For each real experiment, we have compared the maximum, minimum, and mean Pearson's and Spearman's correlation index and the maximum, minimum, and mean \( P \)-value for each solution considered.

All experiments were executed on a multiprocessor machine with 64 processors, Intel Xeon E7-4820 2.00 GHz with 8-GB RAM memory. We have used Java to implement TriGen algorithm (and other ad hoc developments) and an R framework to create graphics and get dataset resources from GEO.\(^{33}\)

We now analyze the results obtained in each of the four experiments.

**Synthetic experiments.** Synthetic data are widely used not only for testing the performance of microarray analyzing techniques\(^{24}\) but also in more general data mining publications.\(^{35}\) It has the advantage that the process that generated the data is well known and so one is able to judge the success or failure of the algorithm.\(^{16}\)

In this work, we have used an application designed by ourselves to generate the synthetic data used in this experiment. We have executed the TriGen algorithm with the MSL.
measure over a synthetic dataset composed of 4,000 genes, 30 experimental conditions, and 20 time points whose expression levels were randomly generated by a cryptographic secure standard library Math3 provided by Apache Commons. In this dataset, we inserted 10 triclusters composed of 150 genes, 6 experimental conditions, and 4 time points, whose expression levels form a constant behavior pattern. These triclusters are located in random positions in the dataset.

To see the behavior of the MSL measure applied along with TriGen and also with the aim of analyzing the effect of the value of the parameters in the solutions, we have made executions setting N to 200 and varying other control parameters as follows: G ε [100, 200], I ε [50, 100], Sel ε [0.5], Mut ε [0.2, 0.3], Ale ε [0.3, 0.5], w ε [0.03, 0.05, 0], w ε [0, 0.01], w ε [0.04, 0.05], w ε [0, 0.03], and w ε [0, 0.03] (see Input in the TriGen Algorithm subsection for a detailed description of these parameters). The algorithm has been capable of finding between 94% and 100% of the inserted triclusters. There were no false positives or false negatives found. The application of the MSR3D along with the TriGen algorithm in the study by Gutiérrez-Avilés and Rubio-Escudero was capable of finding 91% to 95% and LSL24 obtained a matching ratio between 93% and 97% of the triclusters, so we can see in slight improvement when applying the MSL measure.

Yeast elutriation experiments. For this experiment, we have applied the TriGen algorithm with MSL measure to the yeast (Saccharomyces cerevisiae) cell cycle problem, specifically the Elutriation experiment. The yeast cell cycle analysis project’s goal is to identify all genes whose mRNA levels are regulated by the cell cycle. The resources used are public and available in http://genome-www.stanford.edu/cellcycle/. Data have been normalized as part of the preprocessing. We have created a dataset Delta3D from the elutriation experiment with 7,744 genes, 13 experimental conditions, and 14 time points. Experimental conditions correspond to different statistical measures of the Cy3 and Cy5 channels while time points represent different moments of taking measures from 0 to 390 minutes.

The parameter configuration used for this experiment is shown in Table 2. We set G and I values in order to obtain a default exploration of the solution space and Ale, Sel, and Mut provide us with a high random factor in population generation, low elitism in the next generation promotion, and high mutation factor, respectively. We favor solutions with a high number of genes setting w to 0.05 and solutions with high variability in genes, conditions, and times thanks to w, w, and w, set to 0.05. These configurations have been obtained as a result of a deep experimental study on the Delta3D dataset.

Regarding the correlation analysis, we can observe in Table 3 show Pearson and Spearman’s values vary between [0.95, 0.97] and [0.98, 1], respectively, which implies a high correlation between genes series for every experimental condition through time points. These high values confirm us that the quality of the triclusters obtained from these experiments is very high in terms of correlation.

We can see the graphic representation of tricluster TRI11 in Figure 6. Only one out of the 20 triclusters obtained has been represented for legibility reasons. We can observe how TRI11
with 200 genes, 2 conditions, and 3 time points shows a coherent pattern through time points for CH1I and CH1B experimental conditions (TRI_{11} view, Fig. 6C). TRI_{11} (Fig. 6A) and TRI_{10} (Fig. 6B) show how conditions vary for 0 minutes, 330 minutes, and 390 minutes for all genes and how times vary for CH1I and CH1B for all genes, respectively. In Figure 6A we can see that each of the two conditions vary in almost the same way for every time point. This can also be observed in Figure 6B but according to time variation for every experimental condition.

Finally, regarding the experiment biological analysis, we can see GO results of the biological annotations related to the genes selected in TRI_{11} (Table 4). We can see how three related biological processes have been annotated with TRI_{11} genes, these are cellular amide catabolic process (GO:0043605), allantoin metabolic process (GO:0000255), and catabolic (GO:0000256) process. These biological processes are related to a more general one called cellular amide metabolic process (GO:0043603) in which individual cells carry out the chemical reactions and pathways involving an amide. We can observe how two molecular functions as RNA-DNA hybrid ribonuclease activity (GO:0004523) and endonuclease activity with either ribo- or deoxyribonucleic (GO:0016893) have been annotated for TRI_{11} as well. Both groups of terms, biological processes, and molecular functions are related with the metabolic process of the cell. All terms annotated have a high level of statistical significance denoted by P-values in the [1.98E-09, 1.01E-03] interval.

We can observe in Table 5 a comparison among MSR_{3D} LSL, and MSL measures according to the published Elutriation results published in the studies by Gutiérrez-Avilés and Rubio-Escudero^{23,24} Regarding correlation indexes, a clear improvement of MSL in relation to MSR_{3D} and LSL can be observed as well as an improvement in terms of the P-value.

Mouse GDS4510 experiments. In this experiment, we have used a dataset obtained from the GEO^{31} with accession code GDS4510 whose title is rd1 model of retinal degeneration: time course. In this biological experiment, the degeneration of retinal cells in different individuals of home mice (Mus musculus) is analyzed over 4 days just after birth, specifically on days

with 200 genes, 2 conditions, and 3 time points shows a coherent pattern through time points for CH1I and CH1B experimental conditions (TRI_{11} view, Fig. 6C). TRI_{11} (Fig. 6A) and TRI_{10} (Fig. 6B) show how conditions vary for 0 minutes, 330 minutes, and 390 minutes for all genes and how times vary for CH1I and CH1B for all genes, respectively. In Figure 6A we can see that each of the two conditions vary in almost the same way for every time point. This can also be observed in Figure 6B but according to time variation for every experimental condition.

Finally, regarding the experiment biological analysis, we can see GO results of the biological annotations related to the genes selected in TRI_{11} (Table 4). We can see how three related biological processes have been annotated with TRI_{11} genes, these are cellular amide catabolic process (GO:0043605), allantoin metabolic process (GO:0000255), and catabolic (GO:0000256) process. These biological processes are related to a more general one called cellular amide metabolic process (GO:0043603) in which individual cells carry out the chemical reactions and pathways involving an amide. We can observe how two molecular functions as RNA-DNA hybrid ribonuclease activity (GO:0004523) and endonuclease activity with either ribo- or deoxyribonucleic (GO:0016893) have been annotated for TRI_{11} as well. Both groups of terms, biological processes, and molecular functions are related with the metabolic process of the cell. All terms annotated have a high level of statistical significance denoted by P-values in the [1.98E-09, 1.01E-03] interval.

We can observe in Table 5 a comparison among MSR_{3D} LSL, and MSL measures according to the published Elutriation results published in the studies by Gutiérrez-Avilés and Rubio-Escudero^{23,24} Regarding correlation indexes, a clear improvement of MSL in relation to MSR_{3D} and LSL can be observed as well as an improvement in terms of the P-value.

Mouse GDS4510 experiments. In this experiment, we have used a dataset obtained from the GEO^{31} with accession code GDS4510 whose title is rd1 model of retinal degeneration: time course. In this biological experiment, the degeneration of retinal cells in different individuals of home mice (Mus musculus) is analyzed over 4 days just after birth, specifically on days

The table below lists the GO analysis for the tricluster TRI_{11} found in the yeast cell cycle dataset.

### Table 4: GO analysis for tricluster TRI_{11} found in the yeast cell cycle dataset.

| ID          | NAME                                      | P-VALUE  |
|-------------|-------------------------------------------|----------|
| GO:0043605  | Cellular amide catabolic process           | 1.98E-09 |
| GO:0000255  | Allantoin metabolic process               | 1.17E-08 |
| GO:0000256  | Allantoin catabolic process               | 1.17E-08 |
| GO:0004523  | RNA-DNA hybrid ribonuclease activity      | 4.72E-05 |
| GO:0016893  | Endonuclease activity, active with either ribo- or deoxyribo nucleic acids and producing 5-phosphomonoesters | 7.50E-05 |
| GO:0043603  | cellular amide metabolic process          | 1.46E-04 |
| GO:0006144  | purine nucleobase metabolic process       | 3.91E-04 |
| GO:0044419  | interspecies interaction between organisms | 4.03E-04 |
| GO:0009112  | nucleobase metabolic process              | 6.08E-04 |
| GO:0016891  | endonuclease activity, producing 5-phosphomonoesters | 1.01E-03 |

The table below compares MSR_{3D}, LSL, and MSL yeast cell cycle results.

### Table 5: Comparison of MSR_{3D}, LSL, and MSL yeast cell cycle results.

|            | MSR_{3D} | LSL | MSL |
|------------|----------|-----|-----|
| Max Pearson| 1.04 × 10^{-2} | 7.53 × 10^{-6} | 1.01 × 10^{-3} |
| Min Pearson| 1.97 × 10^{-3} | 4.35 × 10^{-6} | 1.98 × 10^{-9} |
| Mean Pearson| 5.68 × 10^{-3} | 4.29 × 10^{-5} | 2.68 × 10^{-4} |
2, 4, 6, and 8. Data have been normalized as part of the preprocessing. We have formed our input dataset $DGDS4510_{3D}$ with 22,690 genes, 8 experimental conditions (one for each individual involved in the biological experiment), and 4 time points. Each of the replicates has been treated as an independent condition.

The parameter configuration used for this experiment is shown in Table 6. We set $G$ to a default value and $I$ is increased in order to expand the exploration of the solution space, $Ale$, $Sel$, and $Mut$ have been set to 0.5 in order to get a medium rate of randomness factor, elitism, and mutation rates, respectively. With this size and overlapping control, we use TriGen to find solutions with a high number and variability of time points. This configuration has been obtained as a result of an extensive experimental study over $DGDS4510_{3D}$ dataset.

According to Table 7, we can see how Pearson and Spearman’s values vary between [0.54, 0.96] and [0.56, 0.9], respectively. Pearsons values fall in the [0.8, 0.9] interval are predominant in most of the solutions as well as Spearman’s ones. This fact implies a high correlation between gene series for every experimental condition through time points in most of the solutions of this experiment.

In Figure 7 we show three graphic representations of tricluster $TRI_{10}$ composed by 50 genes, 2 experimental conditions, and 4 time points. In the $TRI_{10}$ graphic representation, we can see how all genes form an almost perfectly coherent pattern through the four time points for all experimental conditions.

### Table 6. TriGen algorithm control parameters for mouse GDS4510 dataset.

| PARAMETER | VALUES |
|-----------|--------|
| $N$       | 20     |
| $G$       | 150    |
| $I$       | 500    |
| $Ale$     | 0.5    |
| $Sel$     | 0.5    |
| $Mut$     | 0.5    |
| $w_t$     | 0.8    |
| $w_g$     | 0      |
| $w_c$     | 0      |
| $w_t$     | 0.1    |
| $w_o_g$   | 0      |
| $w_o_c$   | 0      |
| $w_o_t$   | 0.1    |

### Table 7. Correlation results for tricluster mouse GDS4510 dataset.

| $TRI_{10}$ | PEARSON | SPEARMAN |
|------------|---------|----------|
| $TRI_1$    | 0.93    | 0.9      |
| $TRI_2$    | 0.58    | 0.57     |
| $TRI_3$    | 0.92    | 0.89     |
| $TRI_4$    | 0.65    | 0.67     |
| $TRI_5$    | 0.61    | 0.65     |
| $TRI_6$    | 0.53    | 0.54     |
| $TRI_7$    | 0.87    | 0.59     |
| $TRI_8$    | 0.53    | 0.63     |
| $TRI_9$    | 0.63    | 0.65     |
| $TRI_{10}$ | 0.6     | 0.56     |
| $TRI_{11}$ | 0.95    | 0.9      |
| $TRI_{12}$ | 0.89    | 0.85     |
| $TRI_{13}$ | 0.93    | 0.89     |
| $TRI_{14}$ | 0.95    | 0.89     |
| $TRI_{15}$ | 0.95    | 0.9      |
| $TRI_{16}$ | 0.56    | 0.62     |
| $TRI_{17}$ | 0.93    | 0.87     |
| $TRI_{18}$ | 0.92    | 0.85     |
| $TRI_{19}$ | 0.94    | 0.89     |
| $TRI_{20}$ | 0.94    | 0.89     |

Figure 7. $TRI_{10}$ graphic representations from mouse GDS4510 results. (A) Sample curves, (B) time curves, (C) gene curves.
conditions. This fact is reinforced when we observe $TRI_{10}$ (Fig. 7A) and $TRI_{p}$ (Fig. 7B) graphic representations in which variation of expression levels in sample and time perspectives show coherent patterns.

We can see the biological validity of $TRI_{10}$ in Table 8. It reflects very good results with $P$-values in the $[7.92E-30, 7.52E-07]$ interval. Terms like olfactory receptor activity (GO:0004984), which is the molecular function that denotes the transmission of the signal from both sides of cellular membrane in order to initiate a change in cell activity due to detection of smell, has been annotated close to sensory perception of chemical stimulus (GO:0007606) and detection of chemical stimulus involved in sensory perception of smell (GO:0050911), which have a very low $P$-value, are biological processes that have olfactory receptor activity as a part of them. We can see how pheromone receptor activity (GO:0016503), that involves the effects of pheromone in cell activity, is related with G-protein–coupled receptor signaling pathway (GO:0007186) since it is a biological process whose pheromone receptor activity is part of this aforementioned term through G-protein–coupled receptor activity (GO:0004930) term that has been annotated as well.

Regarding the comparison with $MSR_{1D}$ and $LSL$ measures (see Table 9), we can see that $MSL$ performs slightly better than $MSR_{1D}$ and very similar to $LSL$, which performs exceptionally well for this particular experiment, in terms of correlation indexes. In terms of GO analysis, we can see a marked improvement of $MSL$ against $MSR_{1D}$ and the minimum $P$-value of the $LSL$ measures.

**Human GDS4472 experiments.** For this experiment, the $DGSDA4472_{1D}$ dataset has been built from source data obtained from GEO under code GDS4472 titled *Transcription factor oncogene OTX2 silencing effect on D425 medulloblastoma cell line: time course.* In this experiment, the effect of doxycycline on medulloblastoma cancerous cells at six time points after induction for 0, 8, 16, 24, 48, and 96 hours has been analyzed. Data have been normalized as part of the preprocessing. $DGSDA4472_{1D}$ is composed of 54,675 genes, 4 conditions (one for each individual involved), and 6 time points (one per hour).

We can see the parameter configuration for TriGen used for this experiment in Table 10. We want to expand the search space setting $G$ to 700 and $I$ to 500 due to the size of the input dataset, we increase the random factor setting $S$ to 0.2 and $M$ to 0.9, respectively. Regarding size control, we can see how we want triclusters with a balance between a high number of genes, $\omega_0$ to 0.1, and a high number of conditions and times, $\omega_1$ to 0.045 and $\omega_2$ to 0.045, respectively. The overlapping control follows the same proportion with $\omega_3$ set to 0.01, $\omega_4$ set to 0.045, and $\omega_5$ set to 0.045 in terms of variability of genes, experimental conditions, and time points of the solutions.

As regards the correlation analysis, we can observe in Table 11 how Pearson and Spearman’s values vary between [0.47, 0.96] and [0.45, 1], respectively, and most of the

| ID     | Name                                           | P-Value |
|--------|------------------------------------------------|---------|
| GO:0007606 | Sensory perception of chemical stimulus             | 7.92E-30 |
| GO:0004984 | Olfactory receptor activity                        | 4.98E-25 |
| GO:0050911 | Detection of chemical stimulus involved in sensory perception of smell | 4.98E-25 |
| GO:0050907 | Detection of chemical stimulus in sensory perception | 1.17E-24 |
| GO:0007186 | G-protein coupled receptor signaling pathway       | 7.72E-23 |
| GO:0007608 | Sensory perception of smell                        | 3.62E-22 |
| GO:0005933 | Detection of chemical stimulus                     | 7.82E-22 |
| GO:0050906 | Detection of stimulus involved in sensory perception | 1.43E-20 |
| GO:0004888 | Transmembrane signaling receptor activity          | 2.52E-20 |
| GO:0038023 | Signaling receptor activity                        | 5.35E-19 |
| GO:0007600 | Sensory perception                                | 7.33E-18 |
| GO:0004872 | Receptor activity                                 | 7.21E-17 |
| GO:0051606 | Detection of stimulus                              | 1.90E-16 |
| GO:0050877 | Neurological system process                        | 5.97E-15 |
| GO:0004871 | Signal transducer activity                         | 6.91E-15 |
| GO:0060089 | Molecular transducer activity                      | 1.48E-13 |
| GO:0004930 | G-protein–coupled receptor activity                | 4.69E-13 |
| GO:0003008 | System process                                    | 1.07E-11 |
| GO:0007166 | Cell surface receptor signaling pathway             | 4.10E-11 |
| GO:0016503 | Pheromone receptor activity                        | 1.15E-09 |
| GO:0019236 | Response to pheromone                              | 4.42E-09 |
| GO:0005550 | Pheromone binding                                 | 5.88E-09 |
| GO:0055549 | Odorant binding                                   | 1.59E-08 |
| GO:0042221 | Response to chemical                              | 1.51E-07 |
| GO:0016021 | Integral component of membrane                     | 3.76E-07 |
| GO:0031224 | Intrinsic component of membrane                    | 7.52E-07 |

**Table 9.** Comparison of $MSR_{1D}$, $LSL$ and $MSL$ GDS4510 results.

|          | $MSR_{1D}$ | $LSL$ | $MSL$ |
|----------|------------|-------|-------|
| Max Pearson | 1         | 1     | 0.95  |
| Min Pearson | 0.52      | 0.64  | 0.54  |
| Mean Pearson | 0.91      | 0.89  | 0.78  |
| Max Spearman | 1         | 1     | 0.9   |
| Min Spearman | 0.5       | 0.6   | 0.56  |
| Mean Spearman | 0.91      | 0.89  | 0.77  |
| Max P-value | $7.34 \times 10^{-4}$ | $7.40 \times 10^{-8}$ | $7.52 \times 10^{-7}$ |
| Min P-value | $1.53 \times 10^{-6}$ | $8.79 \times 10^{-21}$ | $7.92 \times 10^{-30}$ |
| Mean P-value | $3.33 \times 10^{-4}$ | $8.02 \times 10^{-9}$ | $5.02 \times 10^{-8}$ |
solutions found have both index above 0.9; therefore, this is a good experiment in terms of correlation.

According to graphic representation, we show the tricluster $TRI_{19}$ in Figure 8, which has 40 genes, 4 experimental conditions, and 5 time points. We can see a coherent behavior pattern for all genes through five time points for every experimental condition in the $TRI_{tgc}$ graphic representation (Fig. 8C). In the $TRI_{gct}$ graphic representation (Fig. 8A), we can see a homogeneity variation of expression levels of all genes for all experimental conditions at each time point. We can also see this fact in $TRI_{gtc}$ graphic representation (Fig. 8B).

We can see the biological analysis of $TRI_{19}$ in Table 12 in which there is a set of terms annotated with $P$-value in $[3.33E-60, 5.99E-48]$ interval that is a very low rate of $P$-value; therefore, they are good biological results. We pay attention to term GO:0006614 named SRP-dependent cotranslational protein targeting to membrane that describes the targeting of proteins to a membrane that occurs during translation and it is related to another two biological processes annotated as well: cotranslational protein targeting to membrane (GO:0006613) and protein targeting to ER (GO:0045047). We also underline the GO:0019083 term named viral transcription that describes the process by which a viral genome is transcribed within the host cell that is closely related with viral gene expression (GO:0019080) biological process since the transcription process is part of the gene expression one. Finally, we can see how nuclear-transcribed mRNA catabolic

**Table 10.** TriGen algorithm control parameters for human GDS4472 dataset.

| PARAMETER | VALUES |
|-----------|--------|
| $N$       | 20     |
| $G$       | 700    |
| $i$       | 500    |
| $Ale$     | 0.8    |
| $Sel$     | 0.2    |
| $Mut$     | 0.9    |
| $w_r$     | 0.8    |
| $w_g$     | 0.01   |
| $w_s$     | 0.045  |
| $w_t$     | 0.045  |
| $w_o_r$   | 0.01   |
| $w_o_s$   | 0.045  |
| $w_o_t$   | 0.045  |

**Table 11.** Correlation results for tricluster human GDS4472 dataset.

| $TRI_{sol}$ | PEARSON    | SPEARMAN   |
|-------------|------------|------------|
| $TRI_1$     | 0.96683677 | 1          |
| $TRI_2$     | 0.48750048 | 0.47676471 |
| $TRI_3$     | 0.75202621 | 0.79373957 |
| $TRI_4$     | 0.95392167 | 1          |
| $TRI_5$     | 0.94749964 | 1          |
| $TRI_6$     | 0.74349646 | 0.80536153 |
| $TRI_7$     | 0.47685516 | 0.46931529 |
| $TRI_8$     | 0.95370964 | 1          |
| $TRI_9$     | 0.95102082 | 1          |
| $TRI_{10}$  | 0.94672151 | 1          |
| $TRI_{11}$  | 0.48325216 | 0.47366678 |
| $TRI_{12}$  | 0.72992209 | 0.80331134 |
| $TRI_{13}$  | 0.95754954 | 1          |
| $TRI_{14}$  | 0.95225892 | 1          |
| $TRI_{15}$  | 0.95622999 | 1          |
| $TRI_{16}$  | 0.95113522 | 1          |
| $TRI_{17}$  | 0.95365099 | 1          |
| $TRI_{18}$  | 0.47363796 | 0.45628145 |
| $TRI_{19}$  | 0.95549408 | 1          |

![Figure 8. $TRI_{19}$ graphic representations from human GDS4472 results. (A) Sample curves, (B) time curves, (C) gene curves.](image-url)
process, nonsense-mediated decay, GO:0000184 term, is annotated beside nuclear-transcribed mRNA catabolic process (GO:0000956) and mRNA catabolic process (GO:0006402) whose biological processes define the first one.

Regarding to the comparison between MSL and the other two measures, MSR$_{3D}$ and LSL (Table 13) we can see an improvement of MSL from MSR$_{3D}$ and LSL in terms of maximum correlation indexes, this fact is less perceptible in terms of minimum and mean correlation indexes. We can also appreciate a high improvement of MSL over MSR$_{3D}$ and LSL in terms of three considered aspects of $P$-value. Therefore, we can affirm that MSL globally improves the other two measures.

### Conclusion

In this work, we have presented a new evaluation measure for triclusters, MSL, which measures the homogeneity among genes, conditions, and times in a tricluster. We have analyzed how this measure has been formulated in detail and we have also applied it as a part of TriGen algorithm, which is our triclustering approach to mine triclusters from microarray experiments involving time, in order to assess the quality of the measure.

We have applied MSL embedded in the TriGen algorithm in four datasets: synthetically generated data, data from experiments with the yeast cell cycle (Saccharomyces cerevisiae) obtained from the Stanford University, and two datasets retrieved from Gene Expression Omnibus (H. sapiens) and an experiment with mice (Mus musculus) and an experiment with humans (H. sapiens). All experiments examine the behavior of genes under conditions at certain times. The results obtained in real experiments have been validated by a three-way analysis: first analyzing the correlation among the genes, conditions, and times in each tricluster using two different correlation measures: Pearson and Spearman, second graphical analysis and finally providing functional annotations for the genes extracted from the GO project. We have provided a comparison among MSL and another two developed measures MSR$_{3D}$ and LSL as well, showing that MSL performs better in terms of correlation coefficient and functional annotations.

Summarizing, regarding synthetic experimental results, we can observe how MSL combined with TriGen has been capable to extract from 94% to 100% of the triclusters. Regarding the real dataset results, they are also successful, with correlation values close to 1, good graphical representations in which you can distinguish very clear behavior pattern and GO validation with high levels of significance for the terms extracted (P-values smaller than 0.05 and very specific terms).

MSL is a tricluster evaluation measure created to assess the quality of triclusters extracted from temporal experiments with microarrays, but it can be used in other biologically related fields, for instance combining expression data with gene regulation information by means of substituting the time dimension by ChIP-chip data representing transcription factor–gene interactions what can provide us with regulatory network information. This proposal can also be applied to mine RNA-seq data repositories. Triclustering can also be applied to not biologically related fields, for instance, the seismic zonification of areas at risk of undergoing an earthquake. In this case, the third component does

### Table 12. GO analysis for tricluster TRI$_{15}$ found in the human GDS4472 dataset.

| ID          | NAME                                                                 | $P$-VALUE |
|-------------|----------------------------------------------------------------------|-----------|
| GO:0006614  | SRP-dependent cotranslational protein targeting to membrane         | 3.33E-60  |
| GO:0006613  | Cotranslational protein targeting to membrane                        | 6.24E-60  |
| GO:0045047  | Protein targeting to ER                                              | 1.15E-59  |
| GO:0022626  | Cytosolic ribosome                                                  | 2.83E-59  |
| GO:0072599  | Establishment of protein localization to endoplasmic reticulum      | 3.80E-59  |
| GO:0000184  | Nuclear-transcribed mRNA catabolic process, nonsense-mediated decay | 1.19E-58  |
| GO:0070972  | Protein localization to endoplasmic reticulum                       | 4.72E-57  |
| GO:0003735  | Structural constituent of ribosome                                   | 1.91E-55  |
| GO:0044391  | Ribosomal subunit                                                   | 8.37E-55  |
| GO:0006415  | Translational termination                                           | 3.29E-53  |
| GO:0006612  | Protein targeting to membrane                                       | 8.04E-53  |
| GO:0000956  | Nuclear-transcribed mRNA catabolic process                          | 3.17E-52  |
| GO:0019083  | Viral transcription                                                 | 1.18E-51  |
| GO:0006402  | mRNA catabolic process                                              | 2.22E-51  |
| GO:0044033  | Multi-organism metabolic process                                    | 5.60E-51  |
| GO:0019080  | Viral gene expression                                               | 5.60E-51  |
| GO:0044445  | Cytosolic part                                                      | 2.46E-50  |
| GO:0005840  | Ribosome                                                            | 1.00E-49  |
| GO:0006401  | RNA catabolic process                                               | 1.32E-49  |
| GO:0006413  | Translational initiation                                            | 5.99E-48  |

### Table 13. GO analysis for tricluster TRI$_{15}$ found in the human GDS4472 dataset.

| MSR$_{3D}$ | LSL | MSL |
|------------|-----|-----|
| Max Pearson| 0.95 | 0.83 | 0.96 |
| Min Pearson| 0.8  | 0.76 | 0.47 |
| Mean Pearson| 0.89 | 0.8  | 0.8  |
| Max Spearman| 0.95 | 0.81 | 1    |
| Min Spearman| 0.8  | 0.74 | 0.45 |
| Mean Spearman| 0.89 | 0.78 | 0.83 |
| Max $P$-value| $3.30 \times 10^{-3}$ | $6.9 \times 10^{-32}$ | $5.99 \times 10^{-48}$ |
| Min $P$-value| $6.48 \times 10^{-4}$ | $1.15 \times 10^{-44}$ | $3.33 \times 10^{-60}$ |
| Mean $P$-value| $1.64 \times 10^{-3}$ | $7.88 \times 10^{-33}$ | $3.1 \times 10^{-49}$ |
not identify time points but features associated to every pair of geographical coordinates of the area under study.

The next step in our researching work is to gather all aspects of tricluster experimental evaluation presented in Results and Discussion section, that is correlation, graphic representation, and biological validation, in one measure, and thus, improving the experimental workflow of triclustering.

**Author Contributions**

Conceived and designed the experiments: DG-A, CR-E. Analyzed the data: DG-A. Wrote the first draft of the manuscript: DG-A. Contributed to the writing of the manuscript: DG-A, CR-E. Agree with manuscript results and conclusions: DG-A, CR-E. Jointly developed the structure and arguments for the paper: DG-A, CR-E. Both authors reviewed and approved of the final manuscript.

**REFERENCES**

1. Brown PO, Botstein D. Exploring the new world of the genome with DNA microarrays. *Nat Genet.* 1999;21:33–7.
2. Bajcsy P, Han J, Liu L, Yang J. Advanced Information and Knowledge Processing: Data Mining in Bioinformatics. Part I. Survey of biodata analysis from a data mining perspective. *Data Min Bioinf.* 2005;1:9–39.
3. Quackenbush J. Computational analysis of microarray data. *Nat Rev Genet.* 2001;2(6):418–27.
4. Tan MP, Smith EN, Broach JR, Floudas CA. Microarray data mining: a novel optimization-based approach to uncover biologically coherent structures. *BMC Bioinformatics.* 2008;9(3):268.
5. D’haeseleer P, Liang S, Somogyi R. Genetic network inference: from co-expression clusters to reverse engineering. *Bioinformatics (Oxford, England).* 2000;16(8):707–26.
6. Rubio-Escudero C, Martínez-Álvarez F, Romero-Záliz R, Zwir I. Classification of gene expression profiles: comparison of K-means and expectation-maximization algorithms. In: Proceedings of the 8th International Conference on Hybrid Intelligent Systems. Publisher: IEEE, Barcelona. 2008:831–6.
7. Hartigan JA. Direct clustering of a data matrix. *J Am Stat Assoc.* 1972;67(337):123–9.
8. Ben-Dor A, Chor B, Karp R, Yakhini Z. Discovering local structure in gene expression data: the order-preserving submatrix problem. In: Proceedings of the 6th International Conference on Computational Biology. Publisher: ACM, New York. 2002:49–57.
9. Cheng Y, Church GM. Biclustering of expression data. *ISMB.* 2000;8:93–103.
10. Madeira SC, Oliveira AL. Biclustering algorithms for biological data analysis: a survey. *IEEE/ACM Trans Comput Biol Bioinform.* 2004;1(1):24–45.
11. Pontes B, Divina F, Giráldez R, Aguilar-Ruiz JS. Improved biclustering on expression data through overlapping control. *IJCC.* 2010;3(2):293–309.
12. Bar-Joseph Z. Analyzing time series gene expression data. *BMC Bioinformatics (Oxford, England).* 2004;20(16):2493–503.
13. Mahanta P, Ahmed HA, Bhattacharyya DK, Kalita JK. Triclustering in gene expression data analysis: a selected survey. In: Proceedings of IEEE 2011 2nd National Conference on Emerging Trends and Applications in Computer Science, Publisher: IEEE, Shillong. 2011:1–6.
14. Dickinson VM, Richmond AM, Abu-Iqaba A, et al. A role for prenylated ras acceptor 1 in vertebrate photoreceptor development. *BMC Neurosci.* 2012;13:152.
15. Tanay A, Sharan R, Shamir R. Discovering statistically significant biclusters in gene expression data. *Bioinformatics.* 2002;18(S1):S136–44.
16. Divina F, Pontes B, Giráldez R, Aguilar-Ruiz JS. An effective measure for assessing the quality of biclusters. *Comput Biol Med.* 2012;42(2):245–56.
17. Gutiérrez-Avildes D, Rubio-Escudero C, Martínez-Álvarez F, Riquelme JC. TriGen: a genetic algorithm to mine triclusters in temporal gene expression data. *Bioinformatics.* 2014;30(12):152–43.
18. Spellman PT, Sherlock G, Zhang MQ, et al. Comprehensive identification of cell cycle-regulated genes of the yeast *Saccharomyces cerevisiae* by microarray hybridization. *Mol Cell Biol.* 1998;9(12):3273–97.
19. Buitj H, Hassel NE, Zwijnenburg DA, et al. opt2 directly activates cell cycle genes and inhibits differentiation in medulloblastoma cells. *Int J Cancer.* 2012;131(2):E21–32.
20. Pearson K, Filon LNG. Mathematical contributions to the theory of evolution. IV. On the probable errors of frequency constants and on the influence of random selection on variation and correlation. *Philos Trans R Soc A.* 1899;191:345–56. Shanghai.
21. Spearman C. Correlation calculated from faulty data. *Br J Psychol.* 1910;3(3):271–95.
22. Ashburner M, Ball CA, Blake JA, et al. Gene Ontology: tool for the unification of biology. The Gene Ontology consortium. *Nat Genet.* 2000;25(1):25–9.
23. Gutiérrez-Avildes D, Rubio-Escudero C. Mining 3D patterns from gene expression temporal data: a new tricluster evaluation measure. *Sci World J.* 2014;2014:1–16.
24. Gutiérrez-Avildes D, Rubio-Escudero C. LSL: a new measure to evaluate triclusters. In: IEEE International Conference on Bioinformatics and Biomedicine, IEEE, Belfast 2014:10–7.
25. Zhao L, Zaki MJ. tCluster: an effective algorithm for mining coherent clusters in 3D microarray data. In: Proceedings of the 2005 ACM SIGMOD international conference on Management of data –SIGMOD ’05. New York, NY, USA: ACM Press, 2005:69(12):42–53.
26. Jiang H, Zhou S, Guan J, Zheng Y. tFRICLUSTER: A more general and effective 3D clustering algorithm for gene-sample-time microarray data. In: BioDM, Springer Berlin Heidelberg, Singapore. 2006:48–59. [No. 60373019].
27. Liu J, Li Z, Hu X, Chen Y. Multi-objective evolutionary algorithm for mining 3D clusters in gene-sample-time microarray data. In: 2008 IEEE International Conference on Granular Computing, no. 6057365. IEEE, Hangzhou. 2008:442–7.
28. Xu X, Lu Y, Tan K-L, Tung AKH. Finding time-lagged 3D clusters. In: 2009 IEEE 25th International Conference on Data Engineering, IEEE, Shanghai.
29. Wang G, Yin L, Zhao Y, Mao K. Efficiently mining time-delayed gene expression patterns. *IEEE Trans Syst Man Cybern B.* 2010(40)(2):400–11.
30. Sim K, Aung Z, Golpakrishnan V. Discovering correlated subspace clusters in 3D continuous-valued data. In: 2010 IEEE International Conference on Data Mining, IEEE, Sydney, NSW 2010:471–7.
31. Hu Z, Bhattacharji R. Algorithm for discovering low-variance 3-clusters from real-valued datasets. In: Data Mining (ICDM), 2010 IEEE 10th International Conference on, IEEE, Sydney, NSW 2010:236–45.
32. Liu Y-C, Lee C-H, Chen W-C, Shin J, Hsu H-H, Tseng VS. A novel method for mining temporally dependent association rules in three-dimensional microarray datasets. In: Computer Symposium (ICS), 2010 International, IEEE, Tainan 2010:759–64.
33. Barrett T, Wilhite SE, Ledoux P, et al. NCBI GEO: archive for functional genomics data sets – update. *Nucleic Acids Res.* 2013;41(Database issue):D991–5.
34. Bauer S, Grossmann S, Vingron M, Robinson PN. Ontologizer 2.0—a multi-functional tool for GO term enrichment analysis and data exploration. *Bioinformatics (Oxford, England).* 2008;24(14):1650–1.
35. Pargas RF, Harrold MJ, Peck RP. Test-data generation using genetic algorithms. *J Softw Test Verif Rel.* 1999;6:263–82.
36. Mendez P. GEPA: a software package for modelling the dynamics, steady states and control of biochemical and other systems. *Bioinformatics.* 1993;9(5):563–71.
37. Commons A. The apache commons mathematics library. 2015. Available at: commons.apache.org/proper/commons-maths/.
38. Morales-Esteban A, Martínez-Álvarez F, Reyes J. Earthquake prediction in seismogenic areas of the Iberian peninsula based on computational intelligence. *Tectonophysics.* 2013;593:121–34.