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Extracted Knowledge Interpretation in Mining Biological Data : a Survey

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Abstract

This paper discusses different approaches for integrating biological knowledge in gene expression analysis. Indeed we are interested in the fifth step of microarray analysis procedure which focuses on knowledge discovery via interpretation of the microarray results. We present a state of the art of methods for processing this step and we propose a classification in three facets: prior or knowledge-based, standard or expression-based and co-clustering. First we discuss briefly the purpose and usefulness of our classification. Then, following sections give an insight into each facet. We summarize each section with a comparison between remarkable approaches.

Keywords: data mining, knowledge discovery, bioinformatics, microarray, biological sources of information, gene expression, integration.

1 Introduction

Nowadays, one of the main challenges in gene expression technologies is to highlight the main co-expressed¹ and co-annotated² gene groups using at least one of the different sources of biological information [1]. In other words, the issue is the interpretation of microarray results via integration of gene expression profiles with corresponding biological gene annotations extracted from biological databases.

Analyzing microarray data consists in five steps: protocol and image analysis, statistical data treatment, gene selection, gene classification and knowledge discovery via data interpretation [2]. We can see in Figure 1 the goal of the fifth analysis step devoted to interpretation, which is the integration between two domains, the numeric one represented by the gene expression profiles and the knowledge one represented by gene annotations issued from different sources of biological information.

At the beginning of gene expression technologies, researches were focused on the numeric³ side. So, there have been reported ([3, 4, 5, 6, 7, 8]) a variety of data analysis approaches which identify groups of co-expressed genes based only on expression profiles without taking into account biological knowledge. A common characteristic of purely numerical approaches is that they determine gene groups (or clusters) of potential interest. However, they leave to the expert the task of discovering and interpreting biological similarities hidden within these groups. These methods are useful, because they guide the analysis of the co-expressed gene groups. Nevertheless, their results are often incomplete, because they do not include biological considerations based on prior biologists knowledge.

¹Co-expressed gene group: group of genes with a common expression profile.

²Co-annotated gene group: group of genes with the same annotation. A gene annotation is a piece of biological information related to the gene that can be relational, syntactical, functional, etc.

³We understand by numeric part the analysis of the gene expression measures only, disregarding the biological annotations.

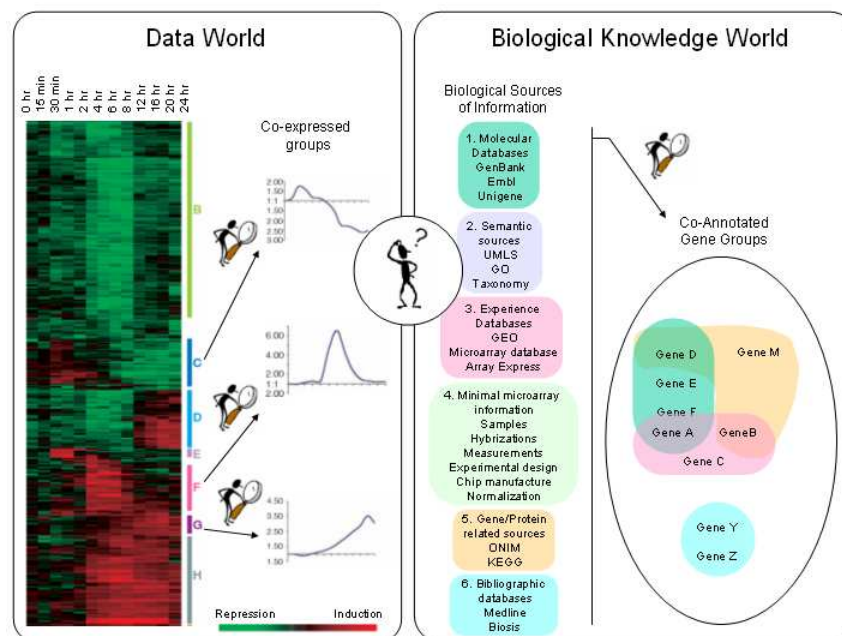


Figure 1: Interpretation of microarray results via integration of gene expression profiles with corresponding sources of biological information

In order to process the interpretation step in an automatic or semi-automatic way, the bioinformatics community is faced to an ever-increasingly volume of sources of biological information on gene annotations. We have classified them into the following six sources of biological information: molecular databases (GenBank, Embl, Unigene, etc.); semantic sources as thesaurus, ontologies, taxonomies or semantic networks (UMLS, GO, Taxonomy, etc.); experience databases (GEO, Arrayexpress, etc.); bibliographic databases (Medline, Biosis, etc.); Gene/protein related specific sources (ONIM, KEGG, etc.); and minimal microarray information as seen in 1. Exploiting these different sources of biological information is quite a complex task so scientists developed several tools for manipulating them or integrate them into more complex databases [9], [10].

This paper presents a complete survey of the different approaches for automatic integration of biological knowledge with gene expression data. A first discussion of these methods is presented by Chuaqui in [11]. Here we present an original classification of the different microarray analysis interpretation approaches.

The interpretation step may be defined as the result of the integration between gene expression profiles analysis with corresponding gene annotations. This integration process consists in grouping together co-expressed and co-annotated genes. Based on this definition, three research axes may be distinguished: *the prior or knowledge-based axis*, *the standard or expression-based axis* and *the co-clustering axis*. Our classification emphasizes the weight of the integration process scheduling on the final results [12, 13, 14, 15].

Indeed the main criteria underlying the classification we propose is the scheduling of phases which alternatively consider gene measures or gene annotations. In prior or knowledge-based approaches, first the co-annotated gene groups are built and then the gene expression profiles are integrated. In standard or expression-based approaches, first co-expressed gene groups are built and then gene annotations are integrated. Finally, co-clustering approaches integrate co-expressed and co-annotated gene groups at the same time.

This paper is organized in the following way: each section fully explains the corresponding interpretation axis, giving an insight and a comparison of their remarkable approaches. Then, we develop a discussion among the three interpretation axis.

2 Prior or Knowledge-Based Axis

Prior or knowledge-based approaches are based on biological knowledge from the sources of biological information (see Figure 1). Therefore, first they build co-annotated gene groups sharing the same biological annotations. Then, they integrate the expression profiles information for each of the genes classified into co-annotated groups, highlighting those ones which are co-expressed. Later on, the statistical significance of co-annotated and co-expressed gene groups is tested. We give a detail description of this three-step methodology: co-annotated gene groups composition, gene expression profiles integration and significant co-annotated and co-expressed gene groups selection.

2.1 Prior or Knowledge-based Methodology

1. Co-Annotated Gene Groups Composition There exist several ways to build co-annotated gene groups. We present here one structured way of building them. First, we need to choose among different sources of biological information. Each kind of information is stored in a specific format (xml, sql, etc.) and has intrinsic characteristics. In each case, the analysis process needs to deal with each biological source format. Another issue is to choose a nomenclature for each gene identity that has to be coherent with the sources of information and thereafter with the expression data. Next, all the annotations of each gene are to be collected in one or more sources of information. Finally, we gather in a subset of genes that share the same annotation. Thus, we obtain all the co-annotated gene groups as shown in Figure 2.

2. Gene Expression Profiles Integration There are different ways to integrate gene expression profiles with previously built co-annotated gene groups. Here we present one current way to do it. First, expression profiles measures are taken for each gene. Then, a variability measure, as *fold change* or *t - statistic* or *f - score* [16] is used to build a sorted list of gene-ranks based on expression profiles. Finally, this measure is incorporated gene by gene into the co-annotated groups. Thus, we obtain co-annotated gene groups with the expression profiles information within as shown in Figure 2.

3. Selection of the Significant Co-Annotated and Co-Expressed Gene Groups At this stage all co-annotated and co-expressed gene groups are built. The next step is to reveal which of these groups or subgroups are statistically significant. To tackle this issue the most frequent technique is the statistical hypothesis testing. Here, we present the four steps for statistical hypothesis testing:

- a) Formulate the null hypothesis, H_0 ,
 H_0 : *Commonly, that the genes that are co-annotated and co-expressed were expressed together as the result of pure chance.* versus the alternative hypothesis, H_1 ,
 H_1 : *Commonly, that the co-expressed and co-annotated gene groups are found together because of a biological effect combined with a component of chance variation.*
- b) Identify a test statistic: The test is based on a probability distribution that will be used to assess the truth of the null hypothesis.

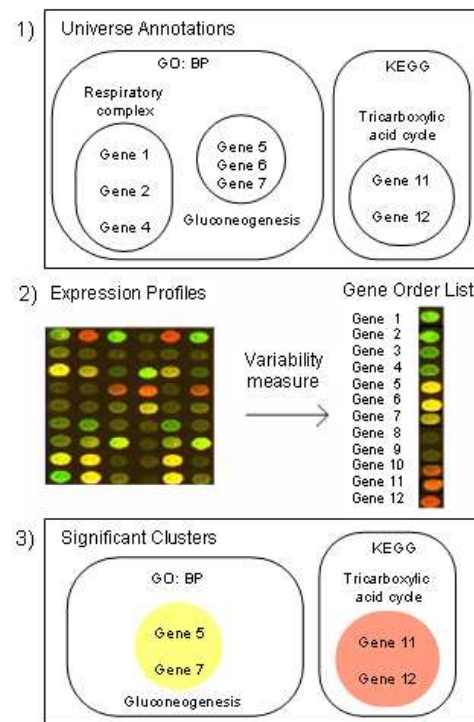


Figure 2: Gene expression profiles integration into previously co-annotated groups

- c) Compute the p – value: The p – value is the probability that a test statistic at least as significant as the one observed would be obtained assuming that the null hypothesis was true.
- d) Compare the p – value: This consists in comparing the p – value to an acceptable significance value α . If p – value $\leq \alpha$ we can consider that the co-annotated and co-expressed gene group is gathered by a biological effect and thus is statistically significant. Consequently, the null hypothesis is ruled out, and the alternative hypothesis is valid.

At the end of the four-step methodology explained before, the prior approaches present the interpretation results as significant co-expressed and co-annotated groups of genes (see third step of Figure 2). The next section will present some of the most remarkable approaches and methods of the prior or knowledge-based axis.

2.2 Remarkable Prior or Knowledge-based Approaches

We present here four representative approaches: GSEA [17], iGA [18], PAGE [19] and CGGA [20]. In the following we describe each of them and emphasize some parameters particularly: the source of biological information, the profiles expression measure, the expression variability measure, the hypothesis testing parameters and details (type of test, test statistic, distribution, corrections etc.).

1. Gene Set Enrichment Analysis, GSEA

This approach [17] proposes a statistical method designed to detect coordinated changes in expression profiles of pre-defined groups of co-annotated genes. This method is born from

the need of interpreting metabolic pathways results, where a group of genes is supposed to move together along the pathway.

In the first step, it builds a priori defined gene sets using specific sources of information which are the NetAffX and GenMapp metabolic pathways databases.

In the second step, it takes the Signal to Noise Ratio (SNR) to measure the expression profiles of each gene within the co-annotated group. Then it builds a sorted list of genes for each of the co-annotated groups.

Third, it uses a non-parametric statistic: enrichment score, ES , (based in a Kolmogorov-Smirnoff normalized statistic) for hypothesis testing. It takes as null hypothesis:

H_0 : The rank ordering of genes is random with regard of the sample.

Then, it assesses the statistical significance of the maximal ES by running a set of permutations among the samples. Finally, it compares the $max ES$ with a threshold α , obtaining the significant co-expressed and co-annotated gene groups.

2. Parametric Analysis of Gene Set Enrichment, PAGE

This approach [19] detects co-expressed genes within a priori co-annotated groups of genes like GSEA, but it implements a parametric method.

In first step, it builds a priori defined gene sets from Gene Ontology (GO)⁴, NetAffX⁵ and GenMapp⁶ metabolic databases.

In second step, it takes the *fold change* to measure the expression profiles of each gene within the co-annotated group. Then, it builds a z -score from the corresponding *fold change* of the two comparative groups (normal versus non normal) as variability expression measure.

Third, it uses the z - score as parametric test statistic. Then, it uses the central limit theorem [21] to argue that when the sampling size of a co-annotated group is large enough, it would have a normal distribution. Using the null hypothesis:

H_0 : The z - score within the groups has a standard normal distribution.

Thus, if the size of the co-annotated gene groups is not big enough to reach normality, then it would be significantly co-expressed.

3. Iterative Group Analysis, iGA

This approach [18] finds co-expressed gene groups within a priori functionally enriched groups, sharing the same functional annotation.

In a first step, it builds a priori functionally enriched groups of genes from Gene Ontology (GO) or other sources of biological information.

In a second step, it uses the *fold change* gene expression measure to build a complete sorted list of genes. Then, it generates a reduced sorted list specific to the functionally enriched group.

In a third step, it calculates iteratively the probability of change for each functionally enriched group (based in the cumulative hypergeometric distribution). It states the null hypothesis:

H_0 : The top x genes are associated by chance within the functionally enriched group.

Then, it assesses the statistical significance of each group comparing the probability of change p - value against a user-determined α value.

4. Co-expressed Gene Group Analysis, CGGA

This approach [20] automatically finds co-expressed and co-annotated gene groups.

⁴<http://www.geneontology.org>

⁵<http://www.affymetrix.com/analysis>

⁶<http://www.genmapp.org>

In a first step, it builds a priori defined gene groups from a source of biological information for instance Gene Ontology (GO) and KEGG⁷.

In a second step, it uses the *fold change* as a gene expression measure. Then, it composes the *f-score* from the corresponding gene's *fold change*. Using the *f-score* on each gene it builds a sorted list of gene ranks. Then, it generates a reduced list of gene ranks specific to the co-annotated enriched group.

In a third step, it states the null hypothesis:

$$H_0 : x \text{ genes from a co-annotated gene group (or subgroup) are co-expressed by chance.}$$

A hypergeometric distribution and *p-value* calculated from the cumulative distribution is assumed. This *p-value* is compared against α to reveal all the significant co-expressed and co-annotated gene groups, including all the possible subgroups.

2.3 Comparison between Prior or Knowledge-based Approaches

Table I presents the brief summary of the four prior approaches described in last section. For each approach the four following parameters are presented: sources of biological information used, expression profile measure, variability expression measure and hypothesis testing details (test statistic, distribution and particular characteristics).

First of all, the four approaches are concerned by metabolic pathways within biological processes, but they use different sources of information: iGA, PAGE and CGGA uses Gene Ontology and GSEA uses manual metabolic annotations, GENMAPP and NetAffx. CGGA is the only one which uses KEGG database combined with Gene Ontology.

For expression profiles parameters, GSEA is the only one which choice is the SNR measure while the others opted for the *fold change* measure. PAGE and CGGA use respectively *z-score* and *f-score* variability measures to detect the changes in gene expression profiles.

For hypothesis testing, GSEA is the only one which uses a non parametric method based on a maximal ES statistic and sampling to calculate the *p-value*. In the contrary, PAGE (normal distribution), CGGA (hypergeometric distribution) and iGA (hypergeometric distribution) chose a parametric approach. iGA chose a hypothesis proof based in the most over-expressed or under-expressed genes (in the rank list) of a co-annotated group, while CGGA searches all the possible co-expressed subgroups within a co-annotated group (the internal sub-group position in the group does not matter).

3 Standard or Expression-based Approach

This axis is called standard because it follows the more frequent procedure for microarray data analysis, which consists of five steps: image analysis, statistical data treatment, genes selection, genes classification and results interpretation via biological knowledge integration. This axis has been used since the beginning of microarray technology with encouraging interpretation results [4], [5] and [3]. Thereafter, it has been used as the reference methodology in microarray data analysis. Expression-based approaches start by building gene groups or clusters of genes sharing similar expression profiles. Then, they integrate the biological annotations of each gene contained inside the expression cluster, building co-expressed and co-annotated subsets of genes. Later on, the statistical significance of co-expressed and co-annotated gene groups is tested. In the following section, we explain in detail this three-step methodology: gene expression profiles classification, biological annotations integration and significant co-expressed and co-annotated gene groups selection.

⁷<http://www.genome.jp/kegg>

Approach	Biological Source of Information	Expression Profile Measure	Variability Expression Measure	Hypothesis Testing Details
GSEA (Mootha et al. 2003)	Manual Annotations, NetAffx and GENMAPP	SNR (Signal to Noise Ratio)	Mean Expression Difference	One-tailed test. Test statistic: Maximal ES. Non-parametric distribution.
iGA (Breitling et al. 2004)	GO	Fold Change	Fold Change	One-tailed test. Modified Fisher's exact statistic: The most over or Under expressed Genes in a group. Hypergeometric distribution.
PAGE (Kim et al. 2005)	GO	Fold Change	z-score	One-tailed test. z-score statistic. Normal distribution.
CGGA (Martinez et al. 2006)	GO: (MP, BP, CC) and KEGG	Fold Change	F-score	One-tailed test. Modified Fisher's exact statistic: All over or under expressed genes in a group. Hypergeometric distribution. Binomial distribution for N large. Bonferroni Correction.

TABLE I
COMPARISON BETWEEN FOUR KNOWLEDGE-BASED INTEGRATION APPROACHES

3.1 Standard or Expression-based Methodology

1. Gene Expression Profiles Classification There exist several methods for classifying gene expression profiles from cleaned microarray data, i.e. data matrix of thousands of genes measured in tens of biological conditions. Various supervised methods and non supervised methods tackled the gene classification issue. Between the most common methods, we can mention: hierarchical clustering, k-means, Diana, Agnes, Fanny [22], model-based clustering [23] support vector machines SVM, self organizing maps (SOM), and even association rules (see more details in [24]).

The target of these methods is to classify genes into clusters sharing similar gene expression profiles as shown in the first step of Figure 3.

2. Biological Annotations Integration Once clusters of genes are built by similar expression levels, each gene annotation is extracted from sources of biological information. As in prior axis, this step deal with different formats of information. A list of annotations is composed for each gene, and then all the annotations are integrated into the clusters of genes (previously built by co-expression profiles). Thus, subsets of co-annotated and co-expressed gene groups are built within each cluster. Figure 3 illustrates this process: three clusters of similar expression profiles are first built, and then all the individual gene annotations are collected to be incorporated in each cluster. For example in the first under-expressed green group we have found three subsets of co-annotated genes. These subsets are respiratory complex: Gene E and Gene D, gluconeogenesis: Gene G and Y and tricarboxylic acid cycle Gene E and Gene T. We can observe intersections of genes within the under-expressed cluster because of the different annotations that each gene may have. Thus, we obtain all the co-annotated gene groups.

3. Selection of the Significant Co-Annotated and Co-Expressed Gene Groups At this stage all the co-expressed and co-annotated gene groups are built and the issue is to reveal

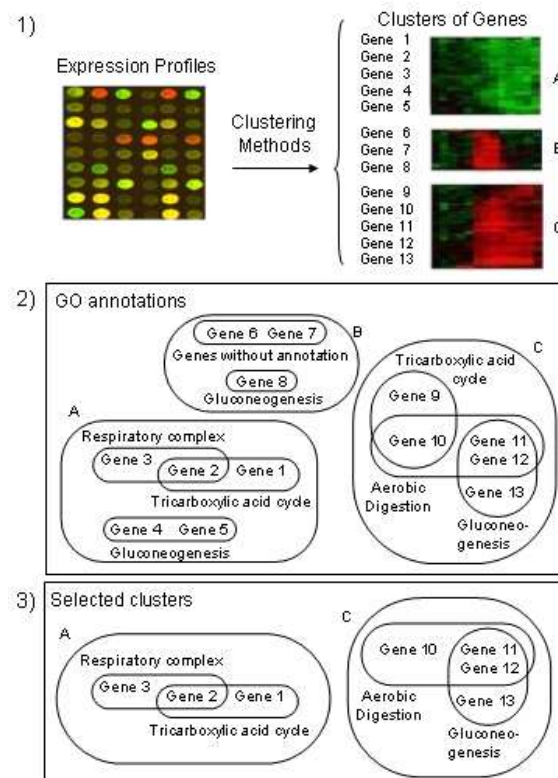


Figure 3: Interpretation of microarray results via integration of gene expression profiles with corresponding sources of biological information

which of these groups or the possible subgroups are statistically significant. The most current technique in use is the statistical hypothesis testing (see Figure 3).

Afterward, this full three-step methodology the expression-based approaches present the interpretation results as significant co-expressed and co-annotated groups of genes.

The next section presents some of the most representative approaches and methods of the expression-based axis. Since these approaches are quite numerous, we have classified them according their main source of biological information. Thus, we have the following classification: minimal information approaches, ontology approaches and bibliographic source approaches.

3.2 Expression-based Semantic Approaches

Expression-based semantic approaches integrate fundamentally semantic annotations (contained in ontologies, thesaurus, semantic networks etc.) into co-expressed gene groups. Nowadays, semantic sources of biological information i.e. structured and controlled vocabularies are one of the best available sources of information to analyze microarray data in order to discover meaningful rules and patterns [1].

Actually, expression-based semantic approaches are widely exploited. In this section we present seven among them: FunSpec [25], OntoExpress [26], Quality Tool [27], EASE [28], THEA [29], Graph Theoretic Modeling [12] and GENERATOR [30]. Each approach uses Gene Ontology (GO) as source of biological annotation, sometimes combined with another gene/protein related specific sources as MIPS, KEGG, Pfam, Smart, etc. or molecular database as Embl, SwissProt⁸, etc.

During last years, GO has been chosen preferably over other sources of information, because of its non ambiguous and comprehensible structure. That is the reason of the recent explosion of many more expression-based GO approaches. Among these approaches, we can cite the integration tools which integrate gene expression data with GO as GoMiner [31], FatiGO [32], Gostat [33], GoToolbox [34], GFINDER [35], CLENCH [36], BINGO [37], etc. This up to date GO compendium⁹ gives more integration methods, GO searching tools, GO browsing tools and related GO tools.

In the next section, we describe seven remarkable expression-based semantic solutions.

3.3 Remarkable Expression-based Semantic Approaches

1. FunSpec: web-based cluster interpreter

This approach [25] proposes a statistical evaluation of groups of co-expressed genes and proteins with respect to existing annotations.

It takes as input clusters of genes previously built by similarity in expression. Then it searches for all gene and protein annotations in four biological sources of information: Gene Ontology (GO), Munich Information Center for Protein Sequences (MIPS)¹⁰, Nucleotide sequence database (EMBL)¹¹, Protein families of alignments and HMMs (Pfam)¹². It builds all the subsets of co-annotated and co-expressed gene and protein groups within each cluster. It makes the selection of the significant subsets (really functionally enriched) via hypothesis testing. It states the null hypothesis:

H_0 : A functionally enriched group of genes is associated by chance within the cluster of co-expressed genes.

⁸<http://www.ebi.uniprot.org>

⁹<http://www.geneontology.org/GO.tools.shtml>

¹⁰<http://mips.gsf.de>

¹¹<http://www.ebi.ac.uk/embl>

¹²<http://www.sanger.ac.uk/Software/Pfam>

This one-tailed hypothesis is solved on the basis of an hypergeometric distribution and using a $p - value$ calculated from the cumulative distribution as in Fisher's exact test [38]. A Bonferroni correction is applied to compensate for multiple testing. Finally, it assesses the statistical significance of each group comparing the $p - value$ against a user-determined α value (more details in [39]).

2. *Onto-Express: Global functional profiling of gene expression*

This approach [26] proposes several statistical evaluations of co-expressed gene groups with respect to GO existing annotations. It takes as input clusters of genes previously built by similarity in expression. In a second step, it takes all the existing GO annotations included in three ontologies, molecular function, cellular component and biological process. Then, it builds all the subsets of co-annotated and co-expressed gene groups within each cluster.

In a third step, it makes the selection of the significant subsets rejecting the null hypothesis:

H_0 : A GO annotated group of genes is associated by chance within the cluster of co-expressed genes.

This one-tailed hypothesis is solved using a probability distribution and using a $p - value$ calculated from the cumulative distribution. Finally, it assesses the statistical significance of each group comparing the $p - value$ against a user-determined α value. Onto-Express gives the following test options: binomial distribution [21] (when the number of genes is very large), Fisher's exact test [40] (when the number of genes is not too important), and χ^2 test for equality of proportions [41].

3. *Quality Tool: judging the quality of gene expression-based clustering methods*

This approach [27] proposes a measure for testing the quality of clusters of gene expression profiles based on mutual information between cluster membership and known gene annotations. In a first step, it takes clusters of co-expressed genes. In a second step, it takes all the existing GO annotations included in the three ontologies: molecular function, cellular component and biological process. Then, it builds a wide matrix of GO attributes for all genes containing 1 if the gene matches the attribute and 0 if not. It builds a contingency table for each cluster-attribute pair, from which it computes cluster-attribute entropy and mutual information [42]. In a third step, it compares this measure with clusters grouped by chance from the same microarray experiments, to check if they are better than random clusters.

This approach uses the same one-tailed hypothesis as seen before (Onto-Express and FunSpec), but it supposes a normal distribution and uses $z - score$ statistic for calculations. Finally, it obtains co-expressed and co-annotated significant groups of genes.

4. *Identifying biological themes within lists of genes*

This approach [28] provides a friendly interface for quick annotation of genes within a cluster, giving a selection method for co-expressed and co-annotated gene groups. In a first step, it takes clusters of co-expressed genes (previously made by classification algorithms). In a second step it takes the available gene annotations from GO, KEGG, Swiss-Prot, PFAM, SMART. Then, it builds all the subsets of co-annotated and co-expressed gene groups within each cluster. In a third step, it shows the statistically significant co-expressed and co-annotated gene groups.

This approach uses the same one tailed hypothesis testing assumptions: null hypothesis, hypergeometric distribution, fisher's exact test, $p - value$ and α as used in Onto-Express and FunSpec. The only difference is the use of an alternative statistic named *ease - score*, which is a conservative adjustment that weights statistical significance in favor of co-annotated groups supported by more genes.

5. *THEA: Tools for high-throughput experiments analysis*

This approach [29] proposes a set of tools designed for manipulating microarray results obtained by hierarchical clustering trees. It integrates gene annotations from biological sources of information and evaluates co-expressed and co-annotated groups of genes.

It takes as input clusters of genes obtained by a hierarchical clustering algorithm. Then, it queries a database in order to obtain all the possible gene annotations from the ontologies in GO on biological process, molecular function and cellular component. Then, it shows all the possible subsets of co-annotated and co-expressed gene groups within each cluster. It displays graphically the statistical evaluation of the co-expressed and co-annotated gene groups. This approach uses the same one tailed hypothesis: H_0 , Fisher's exact test, p - value and α set of values as used in Onto-Express and FunSpec.

6. Graph-theoretic modeling

This approach [12] extracts common GO annotations of the genes within a cluster of co-expressed genes through the modified structure of gene ontology called GO tree.

In a first step, it takes as input clusters of co-expressed genes obtained with any clustering technique. In a second step, it annotates all genes in a cluster with GO terms, taking into account the hierarchical nature of GO. It proposes a quantitative measure for estimating how well gene clusters of expression profiles are gathered together along with known GO categories. This measure is based in a graphical distance between nodes in the directed acyclic graph (DAG) of GO. In a third step, it compares this quantitative measure with the same measure taken from random clusters to see if it is better or not. Thus, it obtains co-expressed and co-annotated significant groups of genes.

7. GENERATOR: Theme discovery from gene lists for identification and viewing of multiple functional groups

This approach [30] takes co-expressed gene groups and it splits them into homogeneous co-annotated significant groups within each group.

In a first step, it takes co-expressed gene groups. In a second step, it takes all GO annotations (studying each GO ontology separately) for each gene group. Then, it runs a clustering algorithm based in a Non-negative Matrix Factorization (NMF) to create a k-means (begins with $k=2$) partition of co-annotated groups within each gene group. This process is repeated, applying k-means algorithm (increasing each time the number of k clusters) and building a non-nested hierarchical clustering tree. At each step, it tests for significant co-expressed and co-annotated groups. For this purpose, it uses one-sided test hypothesis with the same assumptions: null hypothesis: H_0 , hypergeometric distribution, fisher's exact test, p - value and α as used in Onto-Express.

3.4 Expression-based Bibliographic Approach

Nowadays bibliographic databases represent one of the richest update sources of biological information. This type of information, however, is under-exploited by researchers because of the highly unstructured free-format characteristics of the published information and because of its overwhelming volume. The main challenges coming up with bibliographic databases integration are to manage interactions with textual sources (abstracts, articles etc.) and to resolve syntactical problems that appears in biological language like synonyms or ambiguities. At the moment, some text mining methods and tools have been developed for manipulate this kind of biological textual information. Among these methods we can mention Suiseki [43] which focuses on the extraction and visualization of protein interactions, MedMinder [44] takes advantage of GeneCards¹³ as a knowledge source and offers gene information related to specific keywords, XplorMed [45] which presents specified gene-information through user interaction, EDGAR [46] which extracts information about drugs and genes relevant to cancer from the biomedical literature, GIS [47] which retrieves and analyzes gene-related information from PubMed¹⁴ abstracts. These methods are useful as stand-alone applications but they do not integrate gene expression profiles.

¹³<http://www.genecards.org>

¹⁴<http://www.pubmed.gov>

Approach	Biological Source of Information	Hypothesis-testing Type and Statistics	Hypothesis-testing Distribution and details	Distinctive Characteristic
FunSpec (Robinson et al. 2002)	GO, MIPS, EMBL and Pfam	One-tailed test Fisher's exact statistic	Hypergeometric Bonferroni Correction	Online integration of 4 different sources of biological information
OntoExpress (Draghici et al. 2002)	GO (MP, BP and CC)	One-tailed test Fisher's exact statistic χ^2	Binomial Hypergeometric χ^2	Choice of 3 different statistical methods
Quality Tool (Gibbons et al. 2002)	GO (MP, BP and CC)	One-tailed test z-score	Normal	Measure based in cluster-attribute Entropy and mutual information
EASE (Hosack et al. 2003)	GO, KEGG, Pfam, Smart, and SwissProt	One-tailed test Fisher's exact statistic	Hypergeometric EASE correction	Friendly interface for quick gene annotation
THEA (Pasquier et al. 2004)	GO (MP, BP and CC)	One-tailed test Fisher's exact statistic	Hypergeometric Binomial Bonferroni Correction	Friendly interface for quick annotation and cluster's analysis
Graph Theoretic Modeling (Sung 2004)	GO (MP, BP and CC)	One-tailed test Average PD statistic	Non-Parametric	Graphical method who proposes an Average statistic for cluster's significance
GENE-RATOR (Pehkonen et al. 2005)	GO (MP, BP and CC)	One-tailed test Fisher's exact statistic	Hypergeometric	Non-negative matrix factorization to create k-means partition. Results presented as a non-nested hierarchical tree
Annotation-Tool (Masys et al. 2001)	Medline (abstracts), Mesh (key-words), UMLS	One-tailed test Estimated likelihood Vs. Observed likelihood	Semi-Parametric: Empirical Likelihood	Hierarchical groups of co-annotated groups within co-expressed clusters

TABLE II
EXPRESSION-BASED APPROACHES

We define expression-based bibliographic approaches as methods that integrates at least one of the bibliographic databases (Medline, Biosis, MeSH, etc.) annotations into co-expressed gene groups. Only a small number of approaches have integrated this kind of biological information into co-expressed gene groups. Masys et al. [48] proposed to use keyword hierarchies to interpret gene expression patterns for integrating bibliographic databases.

In a first step, his method proposes to take as input clusters of genes grouped by similarity in expression (previously built by any of the supervised or non supervised methods). Second, it searches for gene indexing terms contained in some PubMed articles. Then, it translates these indexing terms to MeSH¹⁵ “keywords” terms. Later, it combines the UMLS¹⁶ knowledge, the enzyme code nomenclature and MeSH terms to build hierarchical groups of genes classified by annotation. Third, it makes the selection of the significant groups of co-annotated genes in each co-expressed cluster. For this purpose, it states:

H_0 : Keyword would appear at or above the observed frequency by chance in a group of keywords of the same size within the cluster of co-expressed genes.

This hypothesis test is solved by comparing the observed versus the expected frequency of each keyword retrieved in association with a set of genes and a p – value estimate of the likelihood under the null hypothesis. Finally, it obtains co-expressed and co-annotated significant groups of genes.

3.5 Comparison between several Expression-based Approaches

Table II presents a brief summary of eight expression-based approaches. The comparison is based on four characteristics: the source of biological information, the hypothesis-testing type and statistics, the hypothesis-testing distribution and a distinctive characteristic.

All the approaches appear in chronological order, the first one integrates bibliographic sources of information i.e. Medline abstracts and the seven others integrate semantic sources of information principally GO, sometimes combined with another gene/protein related specific sources as MIPS, KEGG, Pfam, Smart, etc. or molecular database as Embl, SwissProt, etc.

Concerning selecting co-expressed and co-annotated gene groups all the approaches have chosen a one-tailed test. FunSpec, OntoExpress, EASE, THEA and Generator have opted for Fisher’s exact statistic, and their statistical evaluation methods have small variations. FunSpec, THEA, EASE, Generator have used the typical fisher’s test with hypergeometric distribution. The first two of these have chosen bonferroni correction against multi-testing problem and EASE has used an ease-score correction against the over-representation weight given in bigger gene groups by Fisher’s test. Only two approaches Graph Theoretic Modeling and AnnotationTool have chose non-parametric and semi-parametric statistical evaluation models respectively.

The last column in Table II contains an important distinctive feature. For example GENERATOR uses a particular method based on k – means that builds a non-nested hierarchical tree, as final result.

4 Co-Clustering Axis

From the beginning of gene expression technologies, clustering algorithms were focused on grouping gene expression profiles with biological conditions [16]. Sources of biological information and well structured ontologies as GO and KEGG particularly, are constantly grow-

¹⁵<http://www.nlm.nih.gov/mesh>

¹⁶<http://umlsks.nlm.nih.gov>

ing in quantity and quality and have opened the interpretation challenge of grouping heterogeneous data as numeric gene expression profiles and textual gene annotations. Co-clustering approaches focus their effort to answer this challenge. Each co-clustering approach has its specific parameters: biological source of information, clustering method and integration algorithm. They generally follow a three-step methodology described in the following.

New co-clustering integration approaches are currently one of the interpretation challenges in gene expression technologies. At the moment, few co-clustering approaches have been reported since the principal barrier is the difficulty to build clustering methods fitting heterogeneous sources of information. Among the co-clustering approaches we can cite Co-Cluster [15] and Bicluster [14] described in subsection remarkable co-clustering methods.

4.1 Co-Clustering Methodology

In a first step, they state two different measures: one measure to manipulate gene expression profiles and the other one for gene annotations in an independent manner.

In a second step, they apply an integration criterion (merging function, graphical function etc.) within the co-clustering algorithm for building the co-expressed and co-annotated gene groups simultaneously.

They select the significant co-expressed and co-annotated gene groups. In the last step, most recent solutions [49], [50], [51], [52], [53], [54] and [55] test the quality of the final clusters.

4.2 Remarkable Co-Clustering Methods

1. Co-cluster: Co-clustering of biological networks and gene expression data

This approach [15] constructs a merging distance function which combines information from gene expression data and metabolic networks, computing a joint clustering of co-expressed genes and vertices (annotations from KEGG database) of the network.

In a first step, it computes two distances: a network distance obtained from the proximity of enzymes in the metabolic pathway network beneath undirected graph form, and a gene expression distance obtained from Pearson correlation coefficients of expression matrix [56].

In a second step, it builds a merging function that consists in a mapping that relates genes to enzymes nodes in the undirected graph. Then, it applies hierarchical average linkage clustering algorithm using the merged (enzyme-gene) distance.

Finally, it evaluates the significant co-expressed and co-annotated clusters using the silhouette coefficient [57]. This quality cluster method determines the number of optimal clusters in a hierarchical dendrogram.

2. Bi-cluster: Gene Ontology friendly bi-clustering of expression profiles

This approach [58] directly incorporates Gene Ontology information into the gene expression clustering process, using Smart Hierarchical Tendency Preserving clustering algorithm (SHTP). HTP is a bi-clustering algorithm capable of discovering gene expression patterns embedded in only a subset of conditions. It becomes "Smart" when it integrates the GO functional annotations.

In a first step, it calculates two trees, the Tendency Preserving (TP) Cluster tree obtained from gene expression matrix (rank measures) and the Gene Ontology tree decomposition obtained from GO gene annotations.

In a second step, it builds a hierarchical structure by mapping the TP cluster tree onto GO Hierarchy.

While applying HTP clustering algorithm, the GO annotations tree is useful for two purposes: assessing functional enrichments of a cluster (using one-tailed Fisher's test as shown in OntoExpress) and selecting the subset of conditions critical to a function category (building the α threshold). Finally, the subset of co-expressed genes contained in the subset of the

Approach	Biological Source of Information	Gene Expression Profiles Measure and Gene Matrix Distance	Co-clustering Details	Co-expressed and Co-annotated gene group Selection Details
Co-Cluster (Hanisch et al. 2003)	KEGG	Fold Change Pearson Correlation distance	Hierarchical Average Linkage	Silhouette Coefficient
GO Bi-clustering (Liu J. et al. 2004)	GO: (MP, BP, CC) and KEGG	Fold Change Rank between conditions	SHTP: Smart Hierarchical Tendency preserving	One-tailed Fisher's test Alfa threshold construction

TABLE III
CO-CLUSTERING INTEGRATION APPROACHES

GO annotations tree becomes the selected significant group of co-annotated and co-expressed genes by tendency.

4.3 Comparison between Co-clustering Approaches

Table III presents a brief summary of the two co-clustering approaches explained in last subsection. It is based on four parameters: source of biological information, expression profile measure, co-clustering details, and co-expressed and co-annotated gene groups selection details as seen in Table III.

Both approaches select well-structured ontologies: KEGG database in Co-Cluster and GO for Bi-Cluster. These ontologies have a graph-based representation that allows the clustering algorithm to integrate gene expression profiles with gene database annotations.

For manipulating gene expression measures, both methods use *fold change* expression measures. Nevertheless, co-cluster chooses Pearson's correlation coefficient as gene to gene distance and bi-cluster chooses a gene tendency measure based in the gene-rank between biological conditions.

Concerning to co-clustering details, both co-cluster and bi-cluster have chosen a hierarchical clustering method. However, co-cluster has opted for typical hierarchical average linkage algorithm and bi-cluster has developed the Smart Hierarchical Tendency preserving (SHTP) algorithm.

Related to gene group selection, co-cluster uses the silhouette coefficient for determining the quality of the clusters built (selecting the significant ones). In the other hand, bi-cluster states for a selection in two different stages.

First it uses standard one-tailed Fisher's test for calculate the *p-value* for the co-annotated and co-expressed gene groups and then it builds a particular α threshold for each of them. Finally, as seen in the previous approaches, it compares *p-value* against α to select or not the co-expressed and co-annotated gene group.

5 Discussion

The bioinformatics community has developed many approaches to tackle the interpretation microarray challenge, we classify them in three different interpretation axes: prior, standard and co-clustering. The important intrinsic characteristics of each axis have been developed before.

Standard or expression-based approaches give importance to gene expression profiles. However, microarray history has revealed intrinsic errors in microarray measures and proto-

cols that increase during the whole microarray analysis process. Thus, the expression-based interpretation results can be severely biased [13], [14].

On the other hand prior or knowledge-based approaches give importance to biological knowledge. Nevertheless, all sources of biological information fix many integration constraints: the database format or structure, the weak quantity of annotated genes or the availability of maintaining up to date and well revised annotations for instance. Consequently, the knowledge-based interpretation results can be poor or somewhat quite small in relation to the whole studied biological process.

Co-clustering approaches represent the best compromise in terms of integration, giving the same weight to expression profiles and biological knowledge. But, they have to deal with the algorithmic issue of integrating these two elements at the same time. However, they are often forced to give more weight to one of these elements. In the last section above, we have seen two examples: co-cluster algorithm gives more weight to knowledge, and expression profiles were used to guide the clustering analysis while hand bi-cluster algorithm gives more weight to tendency in expression profiles and GO annotations are used to guide the clustering analysis.

Indeed, the improvement of microarray data quality, microarray process analysis and the completion of biological information sources should make the interpretations results more independent on the interpretation axis.

As long as there is not enough reliability on these main elements, the choice of the interpretation approach remains of crucial importance for the final interpretation results.

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