Biological Network Modelling and Pathway Analysis

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Abstract: The search for disease-specific biomarkers for diagnosis, illness monitoring, therapy evaluation, and, prognosis prediction is one of the major challenges in biomedical research. It has long been that diseases are rarely caused by abnormality in a single protein, gene or cell. But by disorder of different processes manifested by intracellular network of interactions between the molecular components in such biological systems.

Despite the popularity of biological network analysis methods and increasing use for identifying genes or of genes) that contribute to diseases and other biological processes, important topological and network information are hardly used in ranking/assessing the relevance of the pathways. Often, gene expression values and confidence score/strength of interactions are not considered when scoring/ranking the resulting pathways. The research presented in this paper focuses on two different, but closely related areas in Bioinformatics: developing new approaches for biological network analysis, and improving the identification of biomarker discovery for disease classification. The inclusion of topological weight and expression level in the calculation of pathways score is expected to facilitate the identification of the pathways that most relevant to pathophysiological processes.

1 RESEARCH PROBLEM

Biological network analysis methods have been incorporated in a number of proprietary and open-source analysis tools, such as GeneNet, and SBEToolbox, in order to link diseases to abnormalities in pathways of proteins and genes (Barabási et al., 2011). However, most adopted biological network analysis methods did not take into account the gene expression values but give increased relevance to the number of differentially expressed genes on the pathways, (see (Garmhausen et al., 2015)). Moreover, proteins interaction confidence scores that reflect the reliability/strength of the interaction are ignored.

This problem is therefore concerned with modelling and effectively using readily available biological network information such as nodes fold change values, edges confidence score, and node degree (number of edges each node has) within a computationally viable framework that facilitates the efficient identification and analysis of essential biological processes such as disease diagnosis.

2 OUTLINE OF OBJECTIVE

Mathematically, molecular interaction networks can be represented as graphs with molecules as nodes and interactions between the molecules as the edges. We aim to investigate the use of graph algorithms to search large-scale molecular network and reveal some significant small sub-networks that are highly expressed under a specific phenotype. Eventually, machine learning could provide mechanisms to identify some disease relevant clusters in interaction network. The main short-term objectives of the research reported in this project relate to three key components:

- Investigating and developing new mathematical tools to improve the analysis of high throughput Omics data with focus on molecular interaction networks.
- Investigating a range of scoring schemes for identifying significant biologically relevant pathways, subnetworks, and clusters.
- Testing the performance and effectiveness of our analysis approaches on more than one database.

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3 BIOLOGICAL NETWORKS ANALYSIS MATERIALS AND CHALLENGES

Over the last two decades, a great deal of effort has been made to archive existing biological knowledge in public databases, and provide tools to access, retrieve, visualize the corresponding biologically relevant knowledge. Indeed, a number of research studies have shown that integrating high-throughput Omics data with prior biological knowledge (Network database) tend to be more informative and give more meaningful analysis of the complex data. The details of all materials that can be used in biological network analysis is given in this section.

3.1 High-throughput Omics Data

Due to the feasibility of accessing and handling gene expression data generated from microarrays, we will use them in this analysis and to test our proposed approaches. However, the same principles can be applied on other type of transcriptional data such as RNA-Seq or other molecular data such as proteins. Gene expression datasets can be accessed and downloaded from public databases such as GEO or Array Express for microarray data.

3.2 Network Databases

Initially, we will focus on protein-protein interaction network (PPI). Later on, the project can be expanded to cover other types of networks. Due to the variety of molecular interaction resources, we are planning to use the PSICQUIC registry service, which enables us to access and integrate networks from different databases. This can be done programmatically using web services provided by some programming environment (e.g. perl) or via some tools such as Cytoscope.

Most of the available protein protein interaction (PPI) databases such as IntAct, MINT, and BioGRID use a molecular interaction (MI) scoring system which presents a normalized score (S_{MI}) calculating composite score for the interaction based on three different factors can be listed as experimental detection methods (m), the number of publications (p), and interaction types (t). This score is taking value between 0 and 1, and it reflects the reliability of the specific interaction.

$$S_{MI} = \frac{K_p \times S_p(n) + K_m \times S_m(cv) + K_t \times S_t(cv)}{K_p + K_m + K_t} \quad (1)$$

where: K_p Weight factor $K_{[p,m,t]} \in [0-1]$
S_p Publication Score $S_p \in [0-1]$
S_m Method Score $S_m \in [0-1]$
S_t Type Score $S_t \in [0-1]$

For example the database with a score of >0.6 is considered as high-confidence while 0.45-0.6 is considered as medium confidence in the IntAct database, but the users have the option to choice their own threshold to filter the data when they are using the search tool.

3.3 Challenges

Although biological networks can be represented as graphs, different groups of network have different characteristics. For example, graphs can be either directed (e.g. gene-regulatory network, signalling transduction network, and metabolic network) or undirected (e.g. protein-protein interaction network) depending on the nature of interactions between the molecules. Biological networks should be handled carefully and the main challenges can be summarised as follows:

1. The complexity of molecular interaction networks provides a big challenge to any analysis approach due to different node sizes and to complex graphical elements.
2. Some molecules such as proteins can be involved in different cellular functions. Therefore, traditional clustering methods may not be appropriate in the PPI networks because of overlapping clusters should be identified with these kinds of networks.
3. Changes in mRNA levels may not reflect the corresponding changes in protein levels because of the protein degradation or changing in translation.
4. It is very crucial to validate the outcome of our analysis, which can be seen as another source of challenge as it requires sometimes further experiments in follow-up studies.

One of the major restriction in the improvement of biological network methods is the limitation of objective criteria to evaluate the quality of the results. Our resolution is to choose a dataset series that the molecular basis of pathogenesis is well-established in that a powerful and testable outcome providing.

4 STATE OF THE ART

The high dimensionality and heterogeneity of largescale Omics studies (genomics, transcriptomics, proteomics, and metabolomics) are the most researched challenging tasks in biomedical science. Data complexities along with other issues such as the high gene-to-sample ratio in transcriptomics studies make biological network/data analysis a daunting task.

Computational biomedical science has become one of the most attractive research areas for multidisciplinary teams of mathematicians, computer scientists and biomedical scientists, to develop aiding tools that help gain better understanding of such complex data. A wide range of studies has emerged gradually for identifying genes or pathways (groups of genes) that contribute to diseases and other biological processes; we follow them by giving this matter a close attention and studied.

Traditional analysis begins with the preprocessing of intensity values of raw images captured from microarray experiments to extract expression values for a set of probes, performing quality control, filtering and normalizing gene expression data. This is followed by conducting statistical tests for each gene, comparing expression levels in different groups. Finally, genes are sorted in ascending or descending order of their p-values or fold change. The most significant genes are then subjected to clinical conditions or experimental validation and/or used to generate biological hypotheses (Wang et al., 2005; Van't Veer et al., 2002). The outcome of this process, known as molecular biomarkers discovery, offers limited insight due to the fact that genes act in consistent groups rather than alone.

Consequently, the attention shifted toward the identification of a group of genes that interact directly or indirectly in a pathway form with a focus on the topology or the structural information representing interactions between these genes. For example, the number of connected genes and their position to assess their association with complex diseases and to provide more efficient and accurate means for biomarker detection. A number of pathway-based models have been developed over the last several years, some of these methods focus on the topology only in ranking pathways (Vert and Kanehisa, 2003; Gao and Wang, 2007). While, others combine the measurements of expression changes among groups of genes, involved in common pathways, with topology information (Tarca et al., 2009).

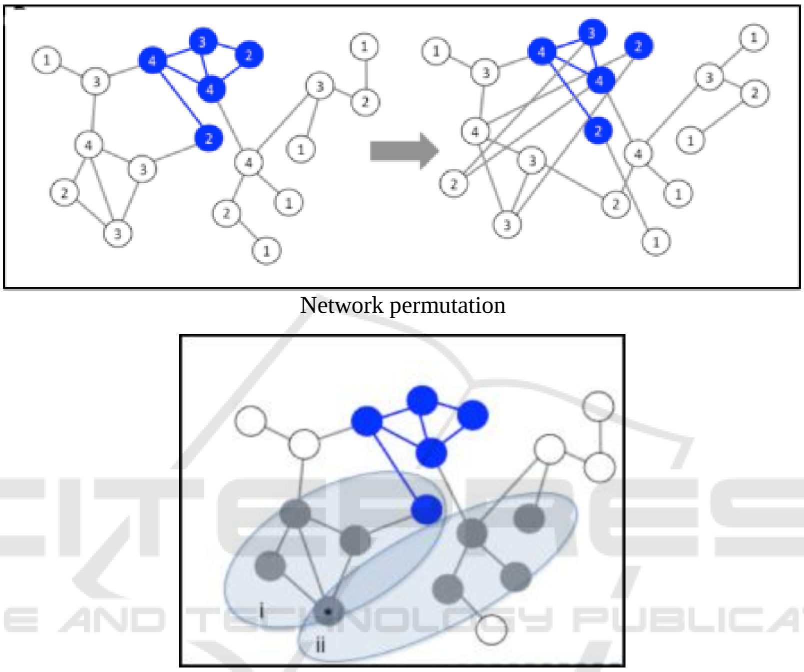
Ibrahim et al. (2012) used fold-change and topology information to identify a new logical meaning of enriching pathway data. It integrates all expressed genes with biological pathways to be ranked using Z-score measurements that combine the total number of expressed genes identified by the microarray with the total number of genes that exceed the fold change and p-value thresholds. Selecting gene groups from highly scored pathways are then used as biomarkers for disease conditions. This Pathway Enrichment and Gene Network Analysis (PEGNA) scheme and its MATLAB implemented tool is an effective approach to pathway-based analysis, which is the initial motivation for our work.

Since the greatest number of human genes has not been linked to a particular pathway and the easy access to large protein networks, pathway-based analysis has been extended to biological network analysis in order to perform meaningful insight into underlying biological processes and biomarker discovery (Chuang et al., 2007). Recent investigations developed a number of novel modelling techniques as well as several MATLAB and Java tools for analysing biological networks.

Konganti et al. (2013) proposed SBEToolbox (Systems Biology and Evolution Toolbox), which is a MATLAB toolbox for biological network analysis that incorporates a wide range of user-controlled functions. The input/output into/from this tool is represented in four different formats: MAT, tabdelimited, Pajeck, and SIF-file. The SBEToolbox offers a collection of network visualization schemes in different layouts such as Tree Ring and Circle, and calculates a variety of centralities and topological metrics like closeness centrality and betweenness centrality. In addition, network's graph can be exported to external programs such as Pajek and Cytoscape for further interpretation and analysis. It adopts three different strategies for clustering nodes into connected sub-networks: ClusterOne, MCL, and MCODE (for more details see (Konganti et al., 2013)). Notably, representing the network interactions information as a sparse adjacency matrix reduces the efficiency of this method.

Taylor et al. (2015) have presented GeneNet toolbox in MATLAB that provides functions that enable users to assess connectivity among sets of genes (seed-genes) within a biological network of their choice. GeneNet offers two methods to determine significant connection between selected genes: Seed Randomization (SR) and Network Permutation (NP). The seed genes should have common attributes for example in a PPI, such as the number of edges each node has, while the NP scheme keeps the seed-genes same and permute the network edges many times, however the network must have sufficient number of permutations (As shown in Fig 1). An empirical P-value can be calculated by comparing the connectivity of direct seed to the random gene-sets connectivity in SR or the connectivity of direct seed in real network to the connectivity of permuted networks in NP.

Garmhausen et al. (2015) proposed a recursive method for implementing Cytoscape plug-in called viPEr (Virtual pathway explorer), restricted by the maximum number of nodes and the numerical values of the nodes (log2fold). It is used to create a focus sub-network by integrating a list of significant genes with the PPI of a specific organism. This plugin offer 3 options:



Network permutation

Seed randomisation

Figure 1: The two main methods implemented in GeneNet Toolbox (this figure is adapted from Taylor et al. (2015)).

- A to B: Finds all paths of length N between two selected nodes. The search is stopped when the maximum number of steps is exceeded or the target node is reached, then a path is stored and scored using formula 2. The sub-network is created from all the nodes in stored paths

$$Score = \frac{\text{Number of Differentially Regulated Nodes} \in \text{path}}{\text{path length}^2} \quad (2)$$

Note that the gene expression value is ignored so no difference between highly expressed and not so highly expressed genes as long as they pass a threshold.

- **Connecting in Batch:** Finds all paths of length N between two groups of nodes, by calculating all paths between all members of a starter list and a target list by using the (A to B) search.
- **Environment Search:** Creates a sub-network of all outgoing length N paths from a given node. Pathways that have two consecutive nodes are not differentially expressed, will be discounted.

This plugin has achieved some success in exploring enrichment sub-networks by allowing for one unregulated node in the resulting paths, the outcome may restrict the accuracy of the results.

Notably, the research in the field of biological systems is not restricted to develop some software tools for visualizing and analysing biological networks, several efforts have been carried out to extract a rich information from these interactions networks While some traditional methods ignored non-differentially expressed genes (NDEGs), Zhang et al. (2014) developed a mathematical model to identify edge biomarker based on differentially correlated expression between two genes, which is measured by Pearson correlation coefficient, and demonstrated that the NGEDs can contribute to classifying different phenotypes samples.

Genes are first sorted by the descending order of their standard deviation values (SD) and excluding 20%, the lowest SD genes in normal or disease groups. The P-values are calculated for the filtered genes and genes with P-value >0.6 are chosen as statistically non-differentially expressed genes. Then the Pearsons correlation coefficient (PCC) are computed for all possible pairs of NDEGs in normal and diseased samples; the pairs of genes which have $|PCC| > 0.9$ are selected as differently correlated gene pairs (DCPs). Each DCP pair of genes are then transformed to two coupled edge features for the normal and the diseased groups to be analysed by machine Learning. The Fishers discriminant score is used to rank the edge features decreasingly and get the top score edge, then the wrapper method applied to select the edge-biomarkers from the chosen set to improve the prediction.

A key limitation of correlation networks research is that edges are constructed using expression correlation, with no background network information, i.e. edges represent potential coexpression or functional association among molecules rather than physical interaction.

This is similar to the weighted correlation network analysis (WGCNA) or functional interaction with STRING database.

In this paper, we propose an expansion of the approach developed by Garmhausen et al. (2015), discussed above, by incorporating the actual gene expression values as well as interaction information.

5 MICROARRAY DATA PREPROCESSING AND ANALYSIS

In this section, we present a brief overview of DNA microarray technology with focus on the basic principles of microarray techniques. We illustrate the preprocessing of microarray raw data in order to produce reliable gene expressions, for the diabetes mellitus disorder as a case study. We shall demonstrate our analytical approach by first importing PPI network from public database, input the network as a MATLAB variable, and using an appropriate network search algorithm to search for significant pathways, then ranking these pathways by a new innovative score formula that reflects our approach.

5.1 Microarray Technology

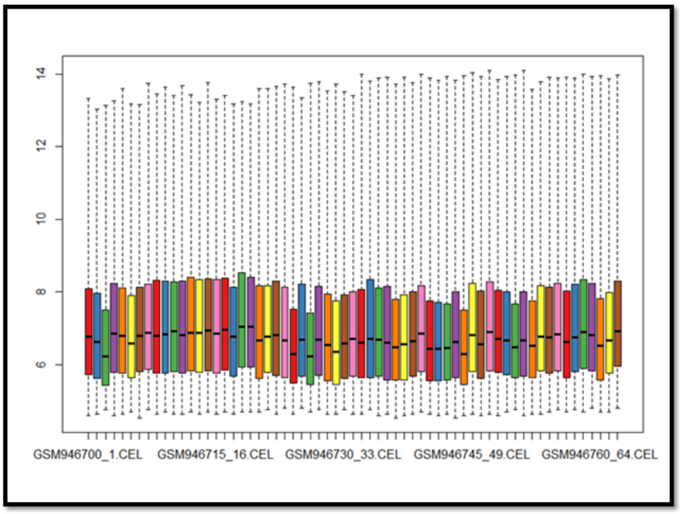
The second half of the 1990s had witnessed the appearance of the novel technology of DNA microarray. Microarray is defined as thousands of spots each representing an identified sequenced genes in a regular arrangement printed on a chip made of glass or silicon at fixed grids.

Depending on the length of printed DNA fragments, the technique used to spot the DNA on the slide/chip and also the generated images. Microarrays come in two commercial types that are cDNA and Oligonucleotide arrays; cDNA is a complementary DNA spot on a glass surface by using highspeed robot. While, Oligonucleotide is a short DNA oligonucleotides; represent single or family of gene are spotted onto a solid support using photolithographic masks and photo labile protecting groups. For more details, the reader is referred to (Pollack, 2007; Singh and Kumar, 2013).

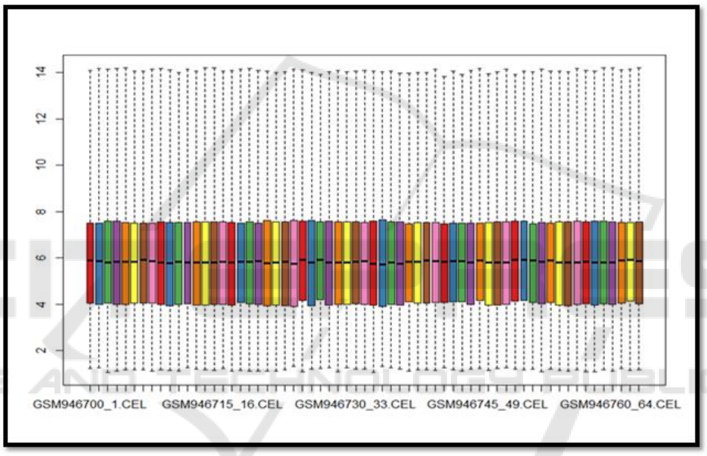
A microarray technology can be divided into three main steps: sample preparation, probes labelling and hybridization and finally, image scanning and data analysis. For our research we focus on the last two steps when fluorescence intensities are collected to produce two independent tagged image file format (TIFF) for each channel. The quantity of each transcript represented on the chip can be calculated by measuring the intensity of the spot on the image.

5.1.1 Case Study

Diabetes mellitus (DM) is a common serious metabolic disease that results from different risk factors such as a genetic predisposition, inheritance environment interaction along with other sedentary lifestyle and obesity. Increased hunger, and increased thirst, lipids, impaired carbohydrates, protein metabolism, high blood sugar that either result of insulin action or insufficient insulin production or both are the most important symptoms of diabetes mellitus. There are two major types of diabetes, insulindependent diabetes mellitus (type 1) caused by pancreas failure to produce sufficient insulin, and noninsulin-dependent diabetes mellitus (type 2) that starts with insulin resistance when body cells fail to respond to insulin properly (Ozougwu et al., 2013; Wu et al.,2014; Lind et al., 2015). Our objective is to analyse the molecular mechanism and



Unnormalised intensity values



Normalised intensity values

Figure 2: The effects of the RMA normalisation.

identify some biomarkers for type 2 diabetes mellitus (T2DM) using a dataset created by Taneera et al. (2012) who compared gene expression levels in mRNA isolated from human pancreatic islets taken from 63 donors (9 with type 2 diabetes (T2DM) and 54 non-diabetic). The data were hybridised to Robust Multi-array Analysis (RMA) and Affymetrix Human Gene 1.0 ST to normalise the expression values before uploading to the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE38642). Notably, this data has been used in other articles for different purposes. For example Cui et al. (2016) used expression profile of GSE38642 microarray to analyse the pathogenesis of T2DM through constructed differential expression network of T2DM signature genes. These genes have been screened by Affy package (R/Bioconductor) of R language into three steps background adjustment, quantile normalization and finally summarization, and logarithmic transformation. Differentially expressed genes (DEGs) analysis were applied using Multiple Linear Regression Limma, with a threshold of $P<0.001$ which identified 59 up- and 88 down-regulated genes (total 147 DEGs).

We used the Affy package (R/Bioconductor) of the R language to download the transcription profile GSE38642 from GEO. Then the raw data (CEL files) produced by the Affymetrix software and contain an estimated intensity values of the probe were preprocessed in three steps: (1) Remove background effects to adjust observed intensities and remove possible noise from the optical detection system; (2) Normalize intensity values across the 63 arrays that may be caused by variations related to laboratory conditions and hybridization reactions;

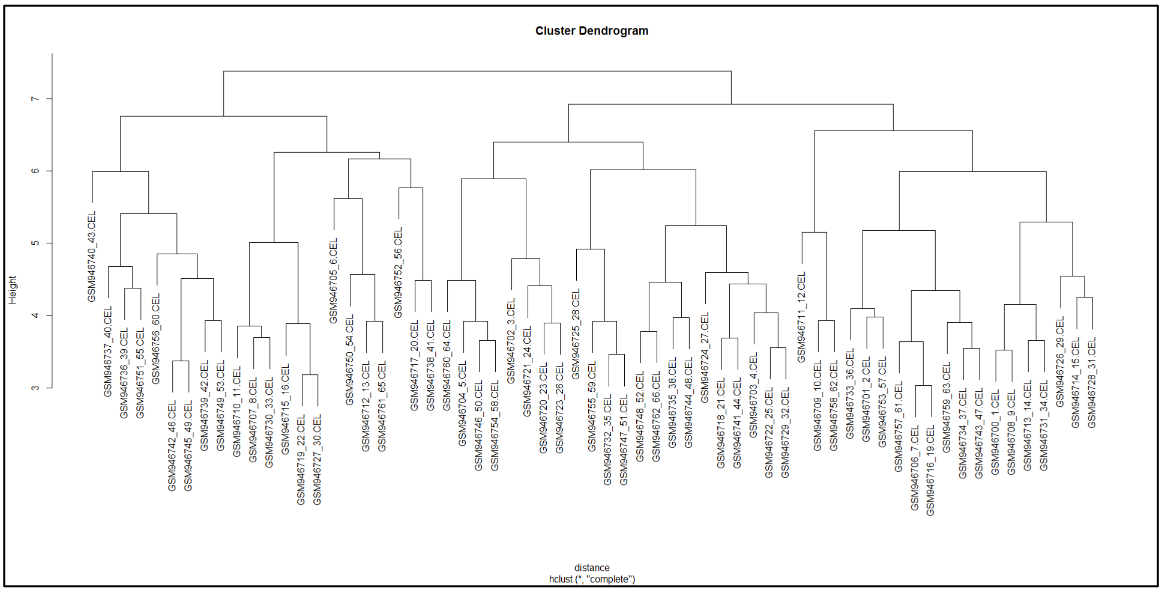


Figure 3: The relationships between the samples.

and (3) Summarize the normalized intensities into one quantity that estimates the rate of the proportional amount to the RNA transcript. All steps used the oligo::rma package, and figure 2 shows the output.

The intensities from all chips have brought into similar distribution characteristics. The samples were hierarchically clustered by tissue type and the relationships between the clusters are shown in figure 3. Prior to the differential gene expression analysing, we used the nsFilter to remove uninformative data, such as low intensity, empty and bad quality spots as well as genes that have a low variance or uniformly expressed. This resulted in the removal of 16161 probe sets. A design matrix was reconstructed with each row and each column corresponding to an array and a coefficient that used to describe the mRNA sources in the experiment respectively. The expression data were fitted to the multiple linear regression model which specified by the design matrix using lmFit() in Limma package, and Empirical Bayes statistical methods (eBayes ()) were applied for DEGs analysis. Then, a matrix with gene level has obtained from a matrix with probe level based on annotation files. The biomaRt package have been used to map Ensembl Gene and Uniprot Swissprot accessions to the 7572 differentially expressed genes symbols that we assessed their significance.

6 METHODOLOGY

Having reviewed above the recent biological network analysis methods, our approach attempts to achieve computational efficiency and address the challenges raised above by appropriate incorporation of nodes fold change values, edges confidence score, and nodes degrees. The PPI network was obtained from the open-access IntAct, MINT, Mentha, and HPRD databases to provide molecular interaction data with rich annotation. These databases use UniProt as its main identifier type. The Cytoscape Plugin BridgeDB have been used to map the listed target proteins (UniProt ids) to Entrez IDs. The fold change values from the microarray experiment are assigned to the network nodes to be incorporated into our new pathway scoring formula introduced later. Then, the network is downloaded by using Cytoscape in XML file format, and the information were read into MATLAB. After removing redundancies, a total of 339016 unique PPI pairs have saved along with their confidence scores as MAT-file. The file consists of two cell string vectors stored all the edges and nodes information in the network respectively. For efficiency, the adjacency list were used to represent the interactions information as a two-dimensional array, one for each node label and another contains the labels of the other nodes, which is connected to it by an edge, see Figure 4.

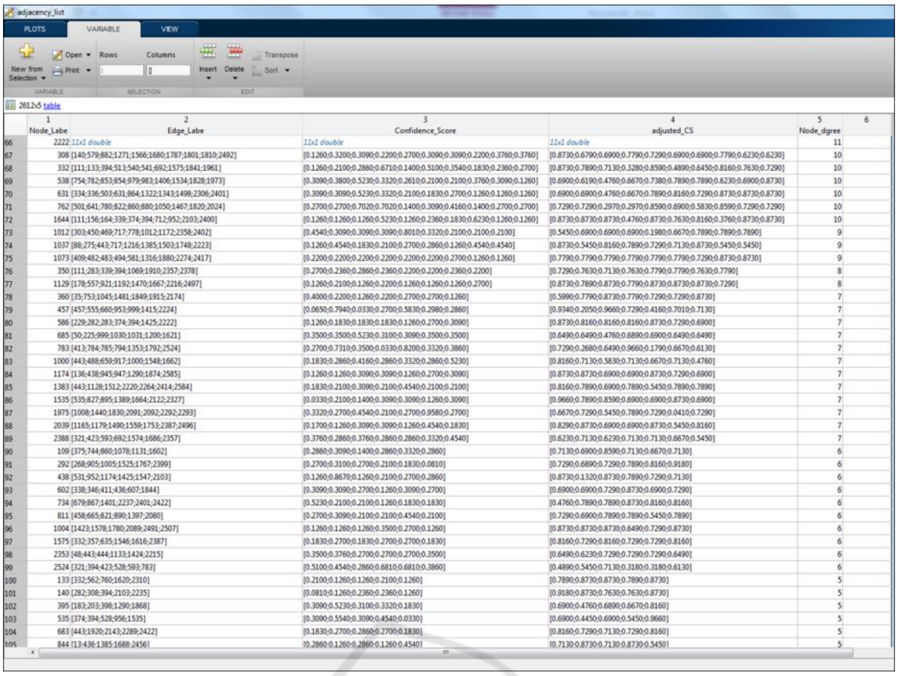


Figure 4: The network representation.

Next, we exploited Graph Theory to identify certain clusters from the big network. For that reason, we implemented one of a well-known network searching technique known as the Dijkstras algorithm. The Dijkstras algorithm recursively scans all nodes in a network starting from a special node, called the root, and creates a spanning tree of a connected subnetwork consisting of all shortest paths from the root. The link-distance of the path refers to the total of costs labelling its edges. In our case the root is chosen to be the highest degree node ni which forms the initial tree with no edges. At each subsequent step, the algorithm searches for a node that can connect to the root, so far constructed tree as long as the cost of connecting it creates the shortest path and does not create acycle. Once such a node is found it will be added to the current tree together with its connecting edge. The search stops when all the nodes of the specific subnetwork have been connected to the tree. (For more details see (Newman, 2010)).

Since, Dijkstras algorithm has taken the smallest edge weight into account during its search for the shortest paths across a subnetwork. We used the edges confidence score after adjusting the values according the formula 3:

$$adjusted\ value\ =\ Max(CS) - CS_i \qquad (3)$$

where Max(CS) is the maximum confidence score over all the network. To ensure selecting the high confidence interaction between the proteins, once the all shortest paths tree is constructed we revert to the original edge confidence scores. As we are interested in investigating the topological relationships between the proteins and counting the number of differential expression genes. We created a score for each node by classifying their fold change values known as *Score** which can be defined as follow:

Table 1: Fold change classification.

FC	*Score**
0.6-0.8	1
0.8-1.0	2
1-1.2	3
1.2-1.4	4
1.4-1.6	5
1.6-1.8	6
….	…

Finally, we count the number of nodes on the Dijkstra shortest paths and calculate their confidence score by the following formula:

$$Path\ Score = \frac{\sum Score^*}{Max(FC).Pathlength^2} \qquad (4)$$

After running the Dijkstras algorithm we reveal all outgoing paths of length N, from the selected node, scored in three different categories: path length, path confidence score, and the score calculated by formula 4. By combining the path confidence scores and path score listed above in a weighted sum and taking into account the path length, we can easily filter those pathways to get the most significant and highly scored ones. Next we plan to merge the top scoring pathways in order to identify biomarkers that are strongly correlated with diabetes mellitus type 2. Finally, we should assess effectiveness of those biomarkers, by measuring sensitivity and classify accuracy criteria, we intend to use cross validation and Support Vector Machine methods for that purpose. In addition we will investigate the outcomes by literature mining in order to prove their relevance to the studied phenotype.

7 EXPECTED OUTCOME

The most important applications for biological network modelling and pathway analysis are biomarkers identification. In comparison to traditional biomarkers discovery approaches which handle genes/proteins individually. We argue that integrating molecular data with prior biological knowledge (i.e. pathways, Gene ontology (GO), biological networks) will improve our understanding of the underlying disease and provide us with more accurate biomarkers for disease classification. Moreover, grouping genes into clusters based on similarity of their expression profiles can help annotate or predict the function of some unknown genes or proteins with unknown functions.

8 STAGE OF THE RESEARCH

At present, we have developed a new approach to reveal some sub-networks and scoring them by own formula. This is part of our attempt to identify some biomarkers for diabetes mellitus type 2. After assessing its success, we will implement this approach to different public datasets. Furthermore, we will seek to further tune our scoring system and implement a tool to analyse biological networks to overcome the limitations of existing analysis tools through considering a number of factors such as edges confidence score, nodes fold change values and nodes degree. The implementation should enable biomedical researchers to:

- Import and merge molecular interaction networks from different repositories.
- Upload a list of genes/proteins with their expression/fold change values and map them onto the large merged network.
- Use the proposed analysis approaches according to own requirements and assess their outcome in different textual and visual manners.

The next immediate work will be to test the perform-ance of our approach with the case study as detailed at the end of the methodology section. We shall also go beyond the case study by constructing a correlation network for a PCR data conducted in Buckingham Institute for Translational Medicine to examine the effectof interleukin-1β (IL-1β) on the expression of 84 cytokine and chemokine genes. In this experiment, Alomar et al. (2015) focused on measuring the effect of interleukin-1b on each single gene and ignored the relation between them which may lead to inhibited or exhibited the expression of some those genes. We are interested in identifying a subset of differentially correlated molecular pairs, known as edge-biomarkers, and hope to propose a new approach to representing edges between those genes using Pearson correlation coefficient and one of machine learning technique for feature selection.

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