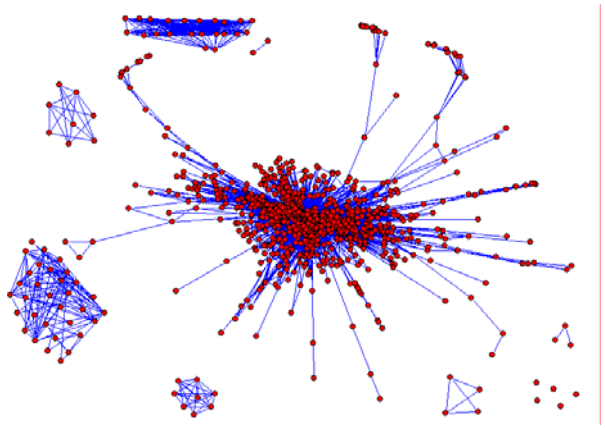

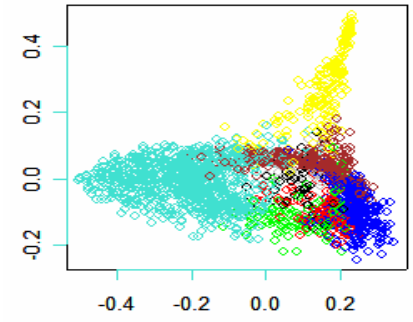

Generalizations of the Topological Overlap Matrix for Module Detection in Gene and Protein Networks

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Mathematical Definition of an Undirected Network

Network=Adjacency Matrix

- A network can be represented by an adjacency matrix, $A=[a_{ij}]$, that encodes whether/how a pair of nodes is connected.
  - A is a symmetric matrix with entries in [0,1]
  - For unweighted network, entries are 1 or 0 depending on whether or not 2 nodes are adjacent (connected)
  - For weighted networks, the adjacency matrix reports the connection strength between gene pairs

Generalized Connectivity

- Gene connectivity = row sum of the adjacency matrix
  - For unweighted networks=number of direct neighbors
  - For weighted networks= sum of connection strengths to other nodes

$$k_i = \sum_j a_{ij}$$

Network Construction

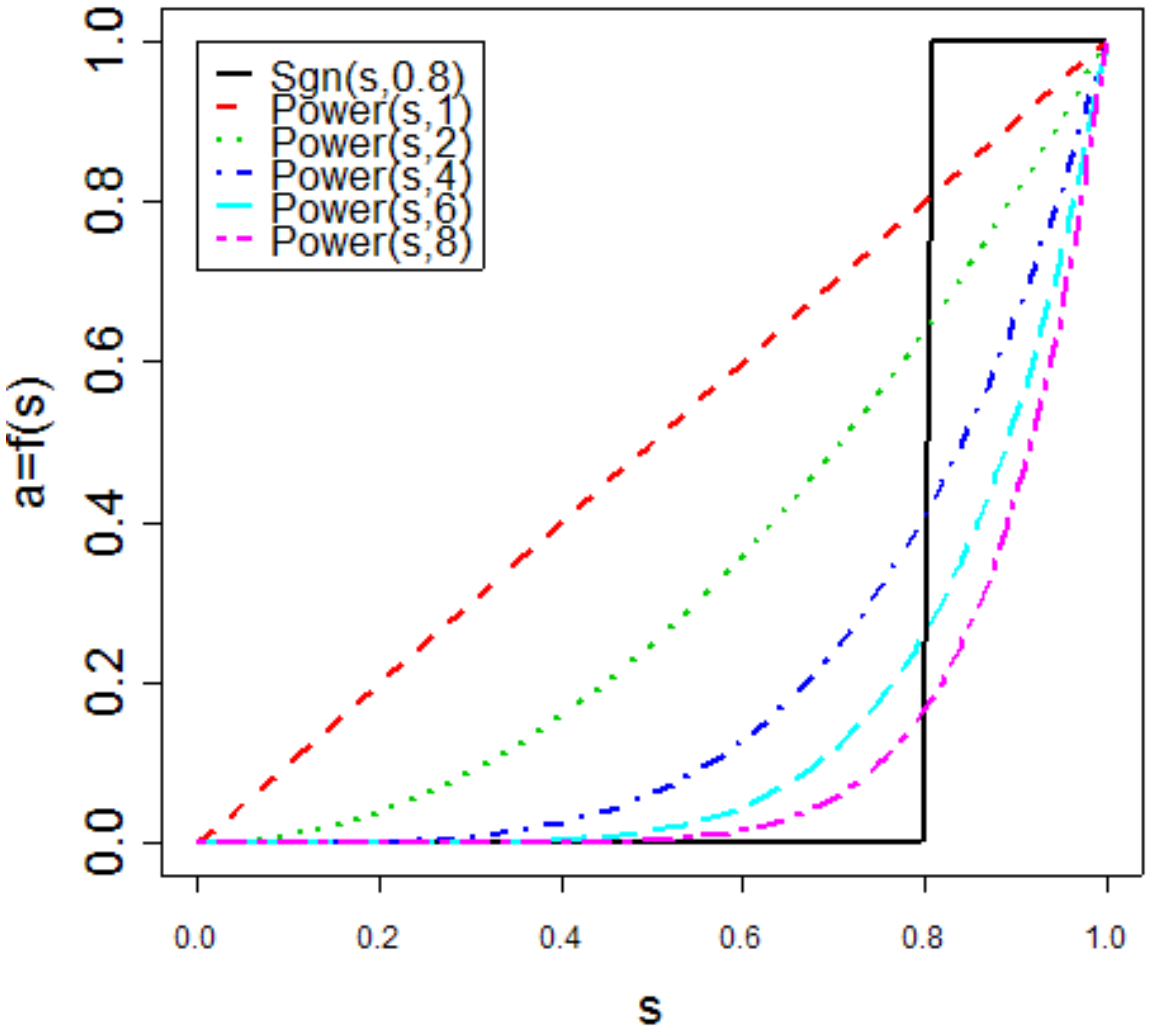
Bin Zhang and Steve Horvath (2005) "A General Framework for Weighted Gene Co-Expression Network Analysis", Statistical Applications in Genetics and Molecular Biology: Vol. 4: No. 1, Article 17.

An adjacency function is used to turn co-expression information into a network

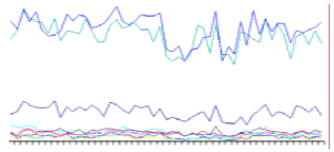
- Measure co-expression by the absolute value of the Pearson correlation
- Define an adjacency matrix by using an adjacency function A(i,j)=AF(cor(x[i],x[j]))
- The adjacency function AF is a monotonic function that maps [0,1] onto [0,1]
- We consider 2 classes of AF
  - Hard Thresholding:
    - step function AF(s)=I(s>tau) with parameter tau
  - Soft thresholding:
    - Power Adjacency function $AF(s)=s^b$ with parameter b
- The choice of the AF parameters determines the properties of the network.

Comparing adjacency functions

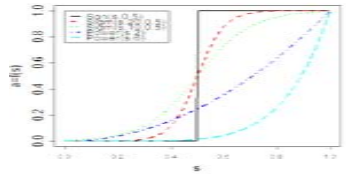
Power Adjancy vs Step Function



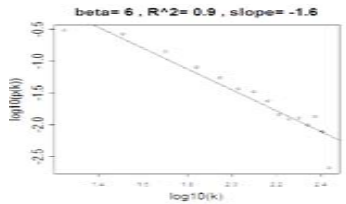
Define a Gene Co-expression Similarity



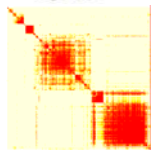
Define a Family of Adjacency Functions



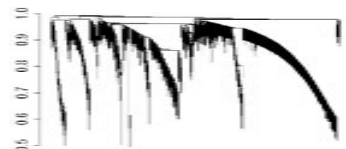
Determine the AF Parameters



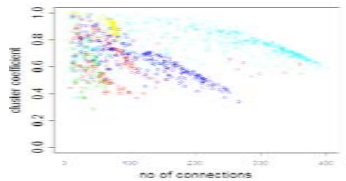
Our focus: **Define a Measure of Node Dissimilarity**



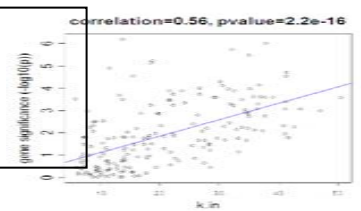
Identify Network Modules (Clustering)



Relate Network Concepts to Each Other



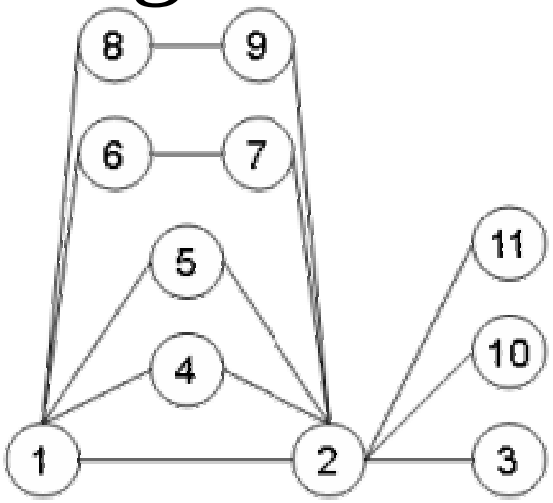
Relate the Network Concepts to External Gene or Sample Information



How to measure distance in a network?

- Graph theoretic answer: Geodesics
  - length of shortest path connecting 2 nodes
- More robust answer: look at shared neighbors
  - Intuition: if 2 people share the same friends they are close in a social network
  - Use the topological overlap measure based distance proposed by Ravasz et al 2002 Science)

Set interpretation of the topological overlap matrix



$$TOM(i,j) = \frac{|N_1(i) \cap N_1(j)| + a_{ij}}{\min(|N_1(i)|, |N_1(j)|) + 1 - a_{ij}}$$

$N_1(i)$ denotes the set of 1-step (i.e. direct) neighbors of node i

| | measures the cardinality

Adding 1-a(i,j) to the denominator prevents it from becoming 0.

Generalizing the topological overlap matrix to 2 step neighborhoods etc

- *Andy M. Yip and SH (2006) The Generalized Topological Overlap Matrix For Detecting Modules in Gene Networks.* *www.genetics.ucla.edu/labs/horvath/GTOM*
- Simply replace the neighborhoods by 2 step neighborhoods in the following formula

$$GTOM\,2(i,j) = \frac{|N_2(i) \cap N_2(j)| + a_{ij}}{\min(|N_2(i)|, |N_2(j)|) + 1 - a_{ij}}$$

where $N_2(i)$denotes the set of nodes within 2 steps of node i

The topological overlap measures interconnectedness

- for an *unweighted* network, one can show that the topological overlap=1 only if the node with fewer links satisfies two conditions:
  - (a) all of its neighbors are also neighbors of the other node, i.e. it is connected to all of the neighbors of the other node and
  - (b) it is linked to the other node.
- In contrast, top. overlap=0 if i and j are unlinked and the two nodes don't have common neighbors.

Topological Overlap leads to a network distance measure (Ravasz et al 2002)

$$TOM_{ij} = \frac{\sum_u a_{iu} a_{uj} + a_{ij}}{\min(k_i, k_j) + 1 - a_{ij}}$$

$$DistTOM_{ij} = 1 - TOM_{ij}$$

- Generalization to unweighted networks is discussed in Zhang and Horvath (2005). Trival since the formula is mathematically meaningful even if the adjacencies are real numbers in [0,1]

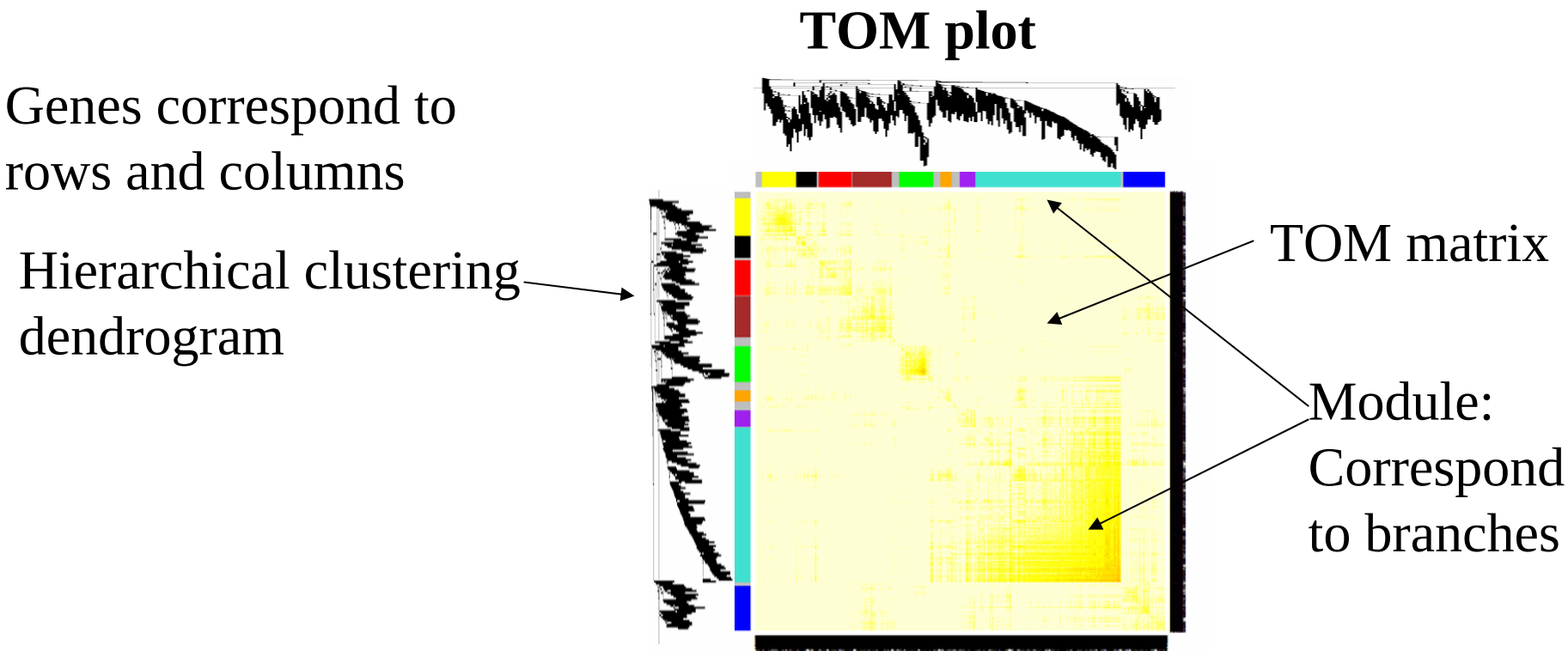
Defining Gene Modules
=sets of tightly co-regulated genes

Module Identification based on the notion of topological overlap

- An important aim of metabolic network analysis is to detect subsets (modules) of nodes that are tightly connected to each other.
- We adopt the definition of Ravasz et al (2002): modules are groups of nodes that have high topological overlap.

Using the TOM matrix to cluster genes

- To group nodes with high topological overlap into modules (clusters), we typically use average linkage hierarchical clustering coupled with the TOM distance measure.
- Once a dendrogram is obtained from a hierarchical clustering method, we choose a height cutoff to arrive at a clustering.
  - Here modules correspond to branches of the dendrogram

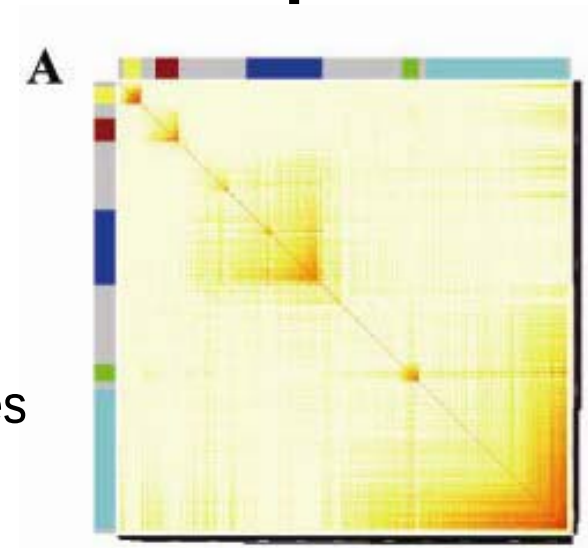


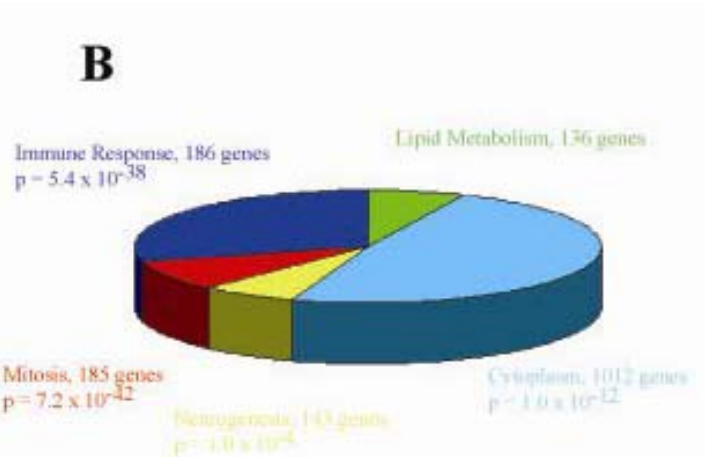
Different Ways of Depicting Gene Modules

Topological Overlap Plot

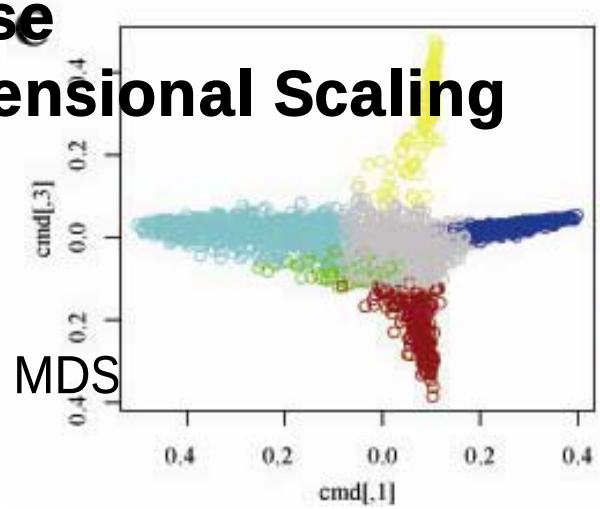
Gene Functions

1) Rows and columns correspond to genes
2) Red boxes along diagonal are modules
3) Color bands=modules

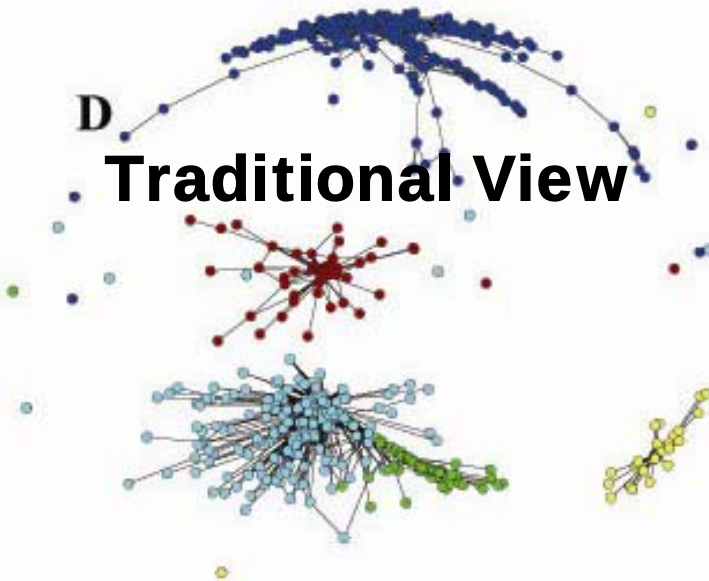




We propose Multi Dimensional Scaling



Traditional View

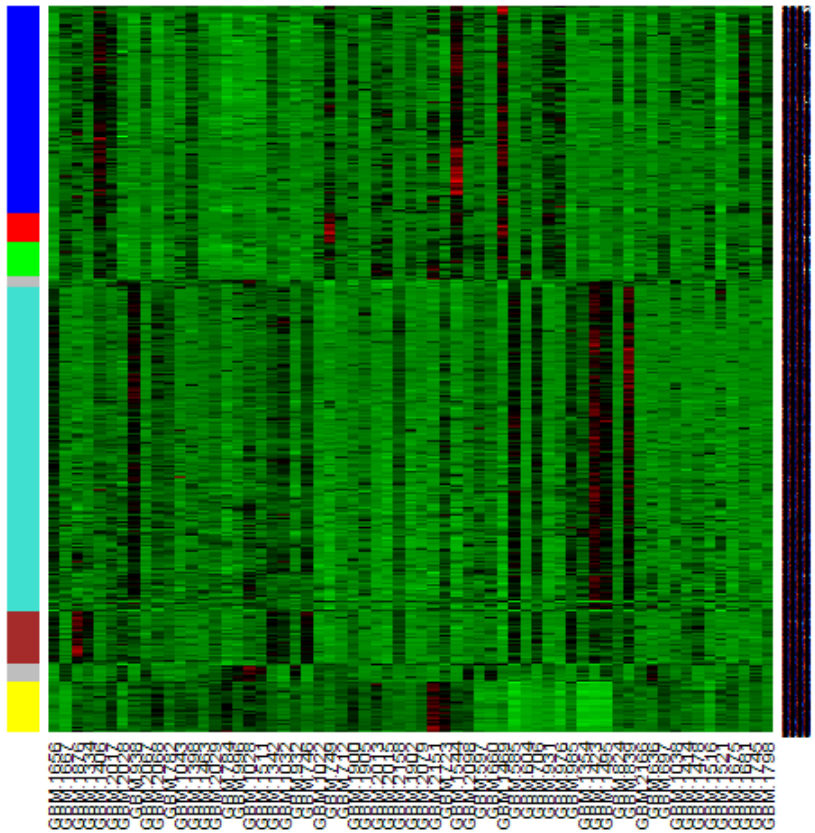


Idea:
Use network distance in MDS

More traditional view of gene co-expression modules

Columns=Brain tissue samples

Rows=Genes
Color band indicates module membership

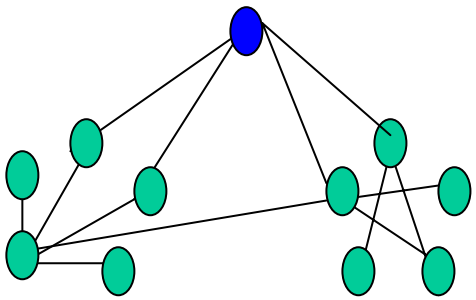


Message: characteristic vertical bands indicate tight co-expression of module genes

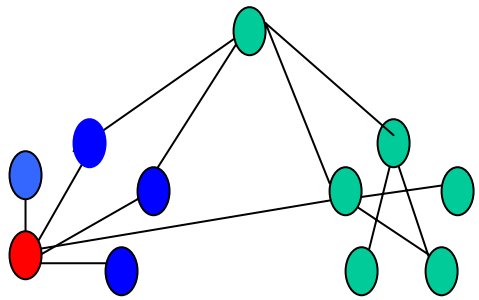
Module-Centric View of Networks

Intra-modular connectivity is biologically and mathematically more meaningful than whole network connectivity

- Whole network connectivity



- Intramodular connectivity



Example: our yeast network example (next slide) that relates intramodular connectivity to knock-out essentiality.
Message: module definition is an important first step towards defining the concept of *intramodular* connectivity.

Yeast network application

M Carlson, B Zhang, Z Fang, PS Mischel, S Horvath, SF Nelson (2006) Gene connectivity, function, and sequence conservation: predictions from modular yeast co-expression networks", BMC Genomics 2006, 7:40

http://www.biomedcentral.com/1471-2164/7/40/

Yeast Data Analysis
Marc Carlson et al (2006)

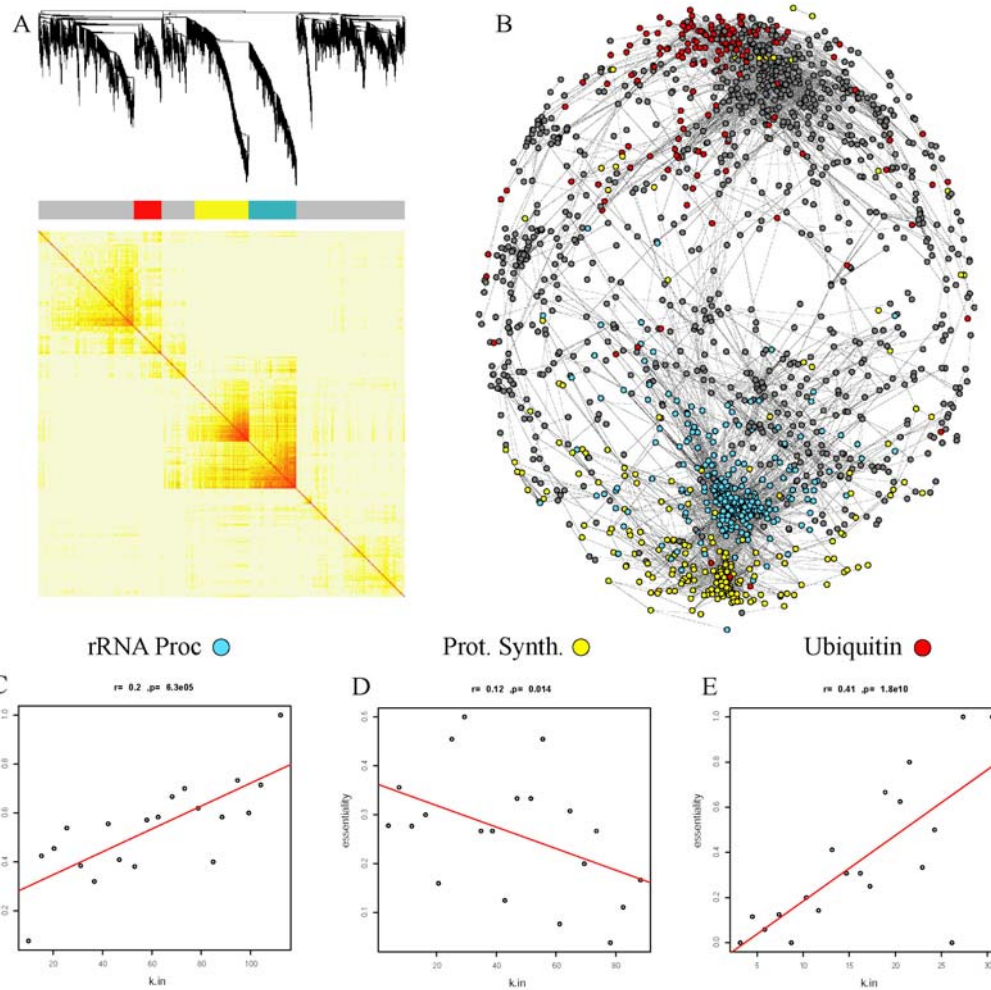
Within Module Analysis

Findings

1) The intramodular connectivities are related to gene essentiality:

- in the turquoise module there is a positive relationship.
- In the yellow module there
- may be an inverse relationship.

2) Modules are highly preserved across different data sets



Topological Overlap Matrix for Multiple Nodes

Ai Li and SH

Topological Overlap Measure for 2 Nodes

The topological overlap matrix can be re - written as follows

$$TOM_{ij} = \frac{\sum_{u \neq i,j} a_{iu} a_{ju} + a_{ij}}{\min(\sum_{u \neq i,j} a_{iu}, \sum_{u \neq i,j} a_{ju}) + 1}$$

One can easily prove that if $0 \leq a_{ij} \leq 1$ then

$0 \leq TOM_{ijk} \leq 1$

The topological overlap of two nodes reflects their similarity in terms of the commonality of the nodes they connect to.

Topological Overlap Matrix for 3 nodes

$$MTOM_{ijk} = \frac{\sum_{u \neq i,j,k} a_{iu} a_{ju} a_{ku} + a_{ij} + a_{ik} + a_{kj}}{\min\left(\sum_{u \neq i,j,k} a_{iu} a_{ju}, \sum_{u \neq i,j,k} a_{iu} a_{ku}, \sum_{u \neq i,j,k} a_{ju} a_{ku} \right) + 3}$$

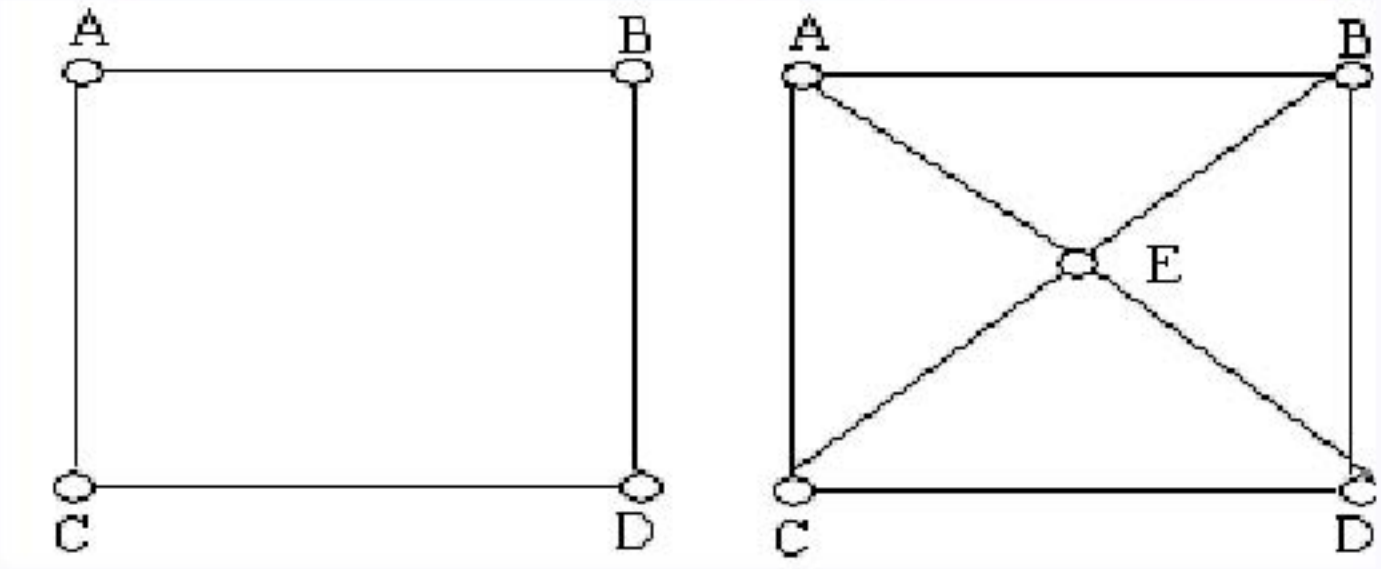
One can easily prove that if $0 \leq a_{ij} \leq 1$ then

$0 \leq MTOM_{ijk} \leq 1$

Topological Overlap Matrix for 4 nodes

$$MTOM_{ijkl} = \frac{\sum_{u \neq i,j,k,l} a_{iu}a_{ju}a_{ku}a_{lu} + a_{ij} + a_{ik} + a_{il} + a_{jk} + a_{jl} + a_{kl}}{\min\left(\sum_{u \neq i,j,k,l} a_{iu}a_{ju}a_{ku}, \sum_{u \neq i,j,k,l} a_{iu}a_{ju}a_{lu}, \sum_{u \neq i,j,k,l} a_{iu}a_{ku}a_{lu}, \sum_{u \neq i,j,k,l} a_{ju}a_{ku}a_{lu}\right) + \binom{4}{2}}$$

Topological Overlap for 4 nodes A,B,C,D



$$\frac{0+4}{0+6} = 0.667$$

All share the node E, MTOM increases

$$\frac{1+4}{1+6} = 0.714$$

Using the multinode topological measure for neighborhood analysis

Many biological questions can be interpreted as neighborhood analysis

Abstract definition: Find the network neighborhood of an initial (seed) set of highly interconnected nodes.

- Examples
  - A) Drosophila protein-protein interaction network: Find the neighborhood of a set of essential proteins. Hypothesis: it should be enriched with essential proteins as well→ predicting knock out effect
  - B) Consider survival time as an idealized gene expression profile. Find the neighborhood of genes in the corresponding gene co-expression network. Hypothesis: it should be enriched with prognostic genes that are associated with survival time→ variable selection
  - C) Yeast protein network: Find the neighborhood of a set of cell-cycle related genes. Hypothesis: it should be enriched with other cell-cycle related genes→ useful for annotation

Recursive and non-recursive approaches for defining an MTOM neighborhood of size S

Recursive approach

- Input a seed of starting nodes and the neighborhood size S
- For each node outside of the current neighborhood compute its MTOM value with the current version of the neighborhood.
- Add the node with highest MTOM value to the neighborhood.
- Repeat b) and c) until the neighborhood size is reached.

Advantage: results in neighborhoods with high MTOM values

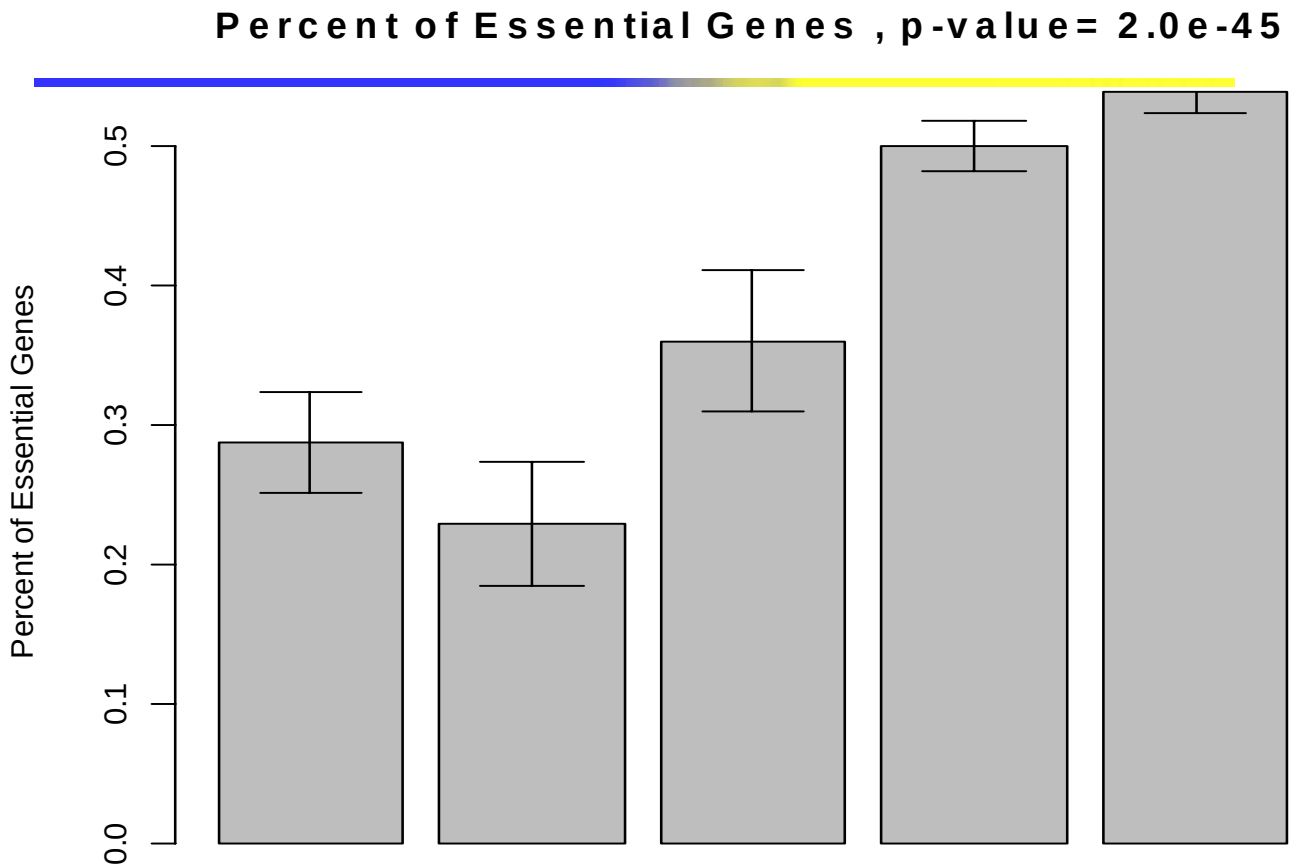
Disadvantage: computationally intensive.

Non-recursive approach: carry out step b) and select a neighborhood based on the highest MTOM values with the seed

Fly protein-protein network analysis (BioGrid Data)

Goal: study the neighborhood of highly connected essential genes
Record the proportion of genes that remain essential as a function of different seed genes

Drosophila, protein-protein network

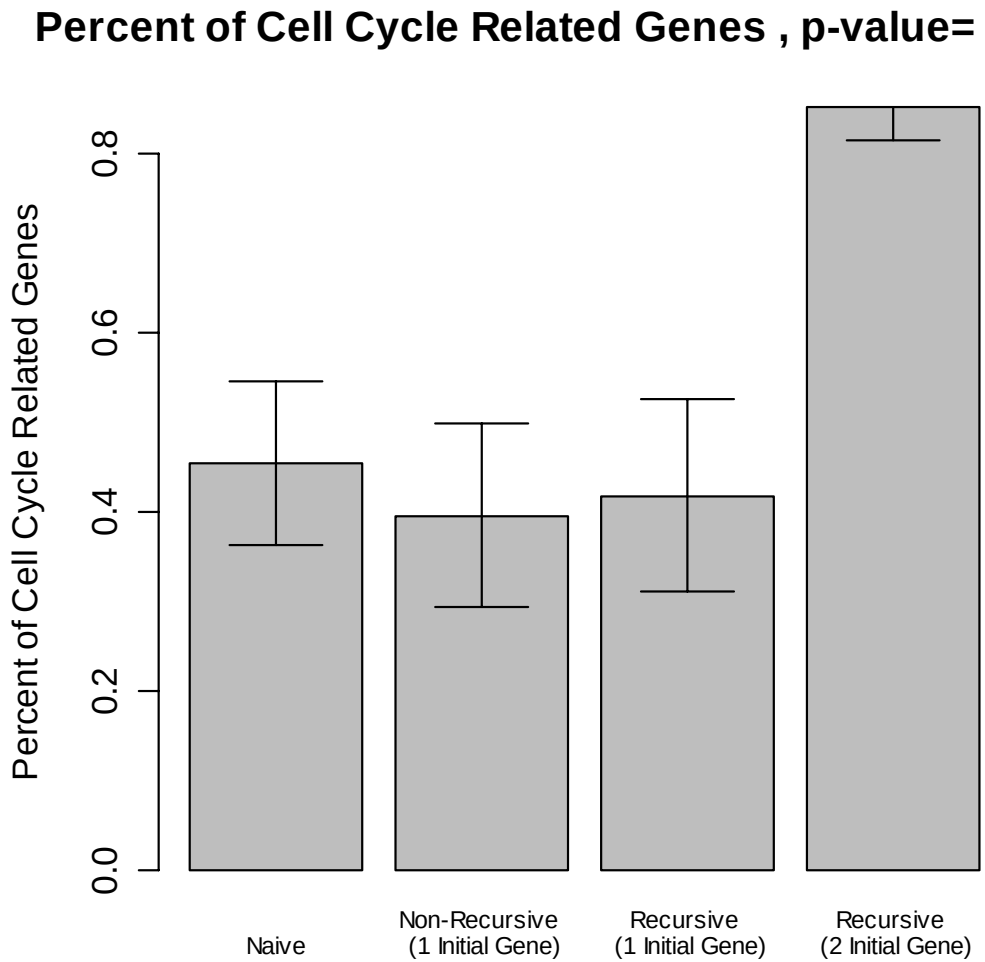


Using 3 genes as seed of recursive MTOM analysis leads to neighborhoods with the highest percentage of essential genes

Applications: Yeast Protein-Protein Network, cell cycle gene prediction

- This network is build up based on the yeast protein-protein interaction (PPI)from the Munich Information Center for Protein Sequence(MIPS)
- 3858 proteins with 7196 pair-wise physical interactions
- 101 cell cycle related genes found in Kyoto Encyclopedia of Genes and Genome (KEGG).
- We considered each of the 101 cell cycle genes as initial protein.
- Neighborhood size= 10
- MTOM based recursive approach and other approaches

Yeast Protein-Protein Network: Neighborhood analysis for predicting cell cycle related genes.



Recursive MTOM analysis with 2 initial genes works best

Brain Cancer Network Application I:

Finding the neighborhood of 5 cancer genes in a brain cancer gene co-expression network

- A major advantage of the MTOM approach is that it allows one to input more than 1 probe set as initial neighborhood.
- In this application, we were interested in finding the neighborhood of five highly correlated cell mitosis related cancer genes: TOP2A, Rac1, TPX2, EZH2 and KIF14.
- Neighborhood size = 20
- Out of 20 probes 13 are cancer related.

Brain Cancer Network Application II: Finding the Neighborhood of a Clinical Outcome (Survival Time)

- Recursive MTOM based neighborhood of size S=20 of the patient survival time (TTS)
- Result: highly enriched in cancer- and neuron related genes:
  - ***11*** probe sets are related to neuron cells
  - ***10*** probe sets are related to cancers.

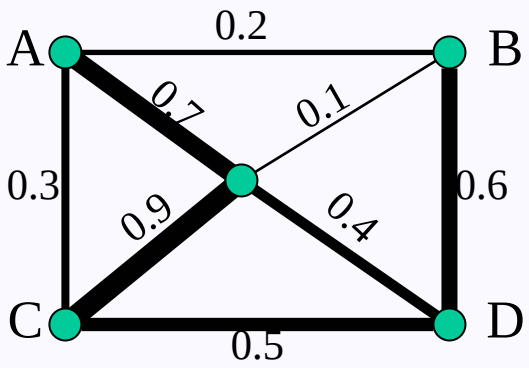
- A standard approach which simply selects a neighborhood on the basis of the absolute values of the correlations between gene expression profile and survival time, leads to a neighborhood with fewer cancer- and neuron related genes. only 4 probe sets are related to neuron cells and 6 probe sets are related to cancer.

Using local permutations to determine the neighborhood size

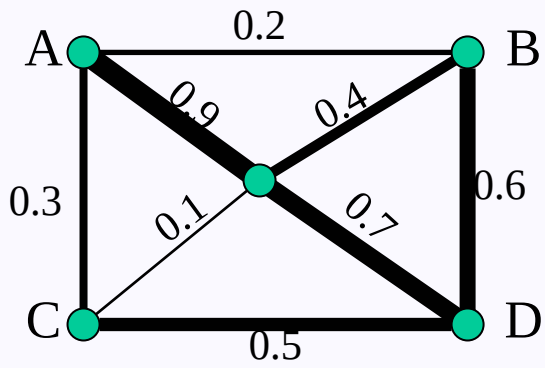
Local Permutation to Choose a Neighborhood Size

- The permutation test is based on comparing the MTOM values of the observed network neighborhood to those of permuted versions of the network.
- *Global (whole network) permutations*: it can noise up the module structure of the network and thus leads to very large neighborhoods comprised of possibly thousands of nodes.
- *Local Permutation*: for a given node in the initial neighborhood set, it permutes the adjacencies with all other nodes while keeping the remaining adjacencies intact.

Local Permutation to Choose a Neighborhood Size



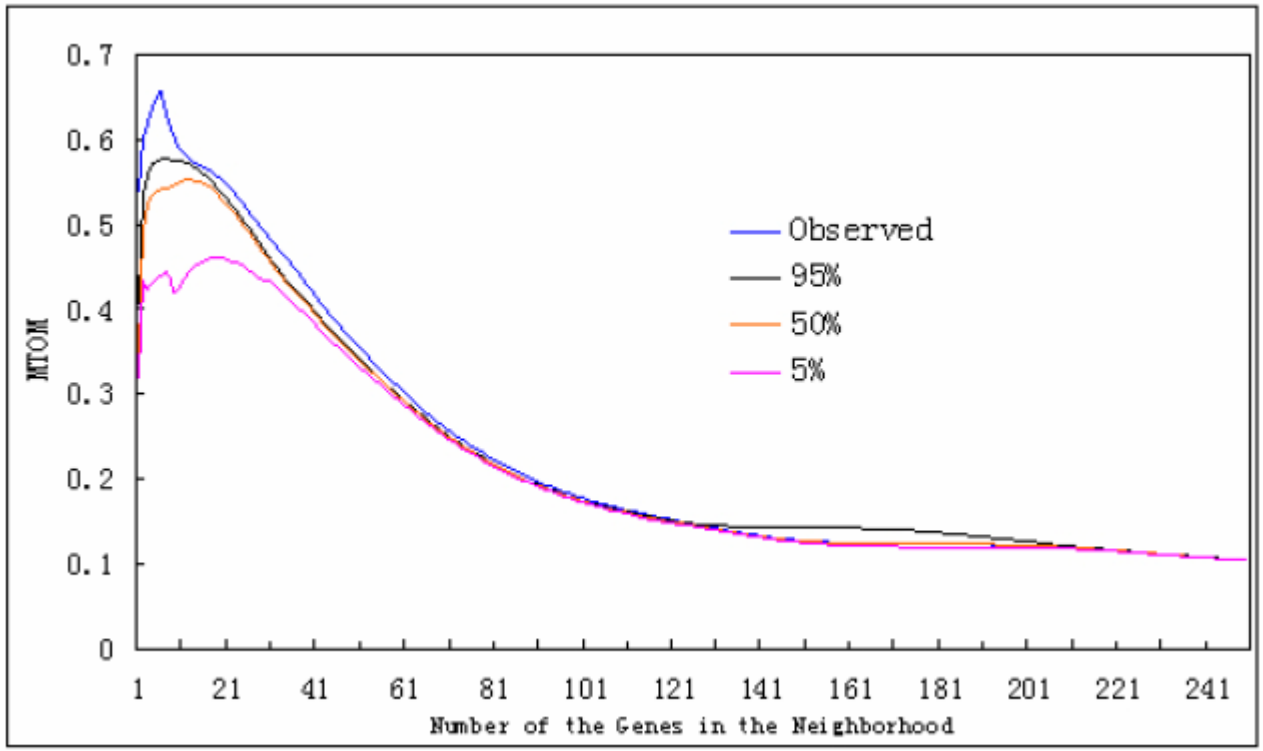
Original Weighted Network



One Possible Local Permutation

Local permutation also works for unweighted network

Local Permutation to Choose a Neighborhood Size



Comparing the MTOM values of the observed network with locally permuted versions to determine the neighborhood size *S*

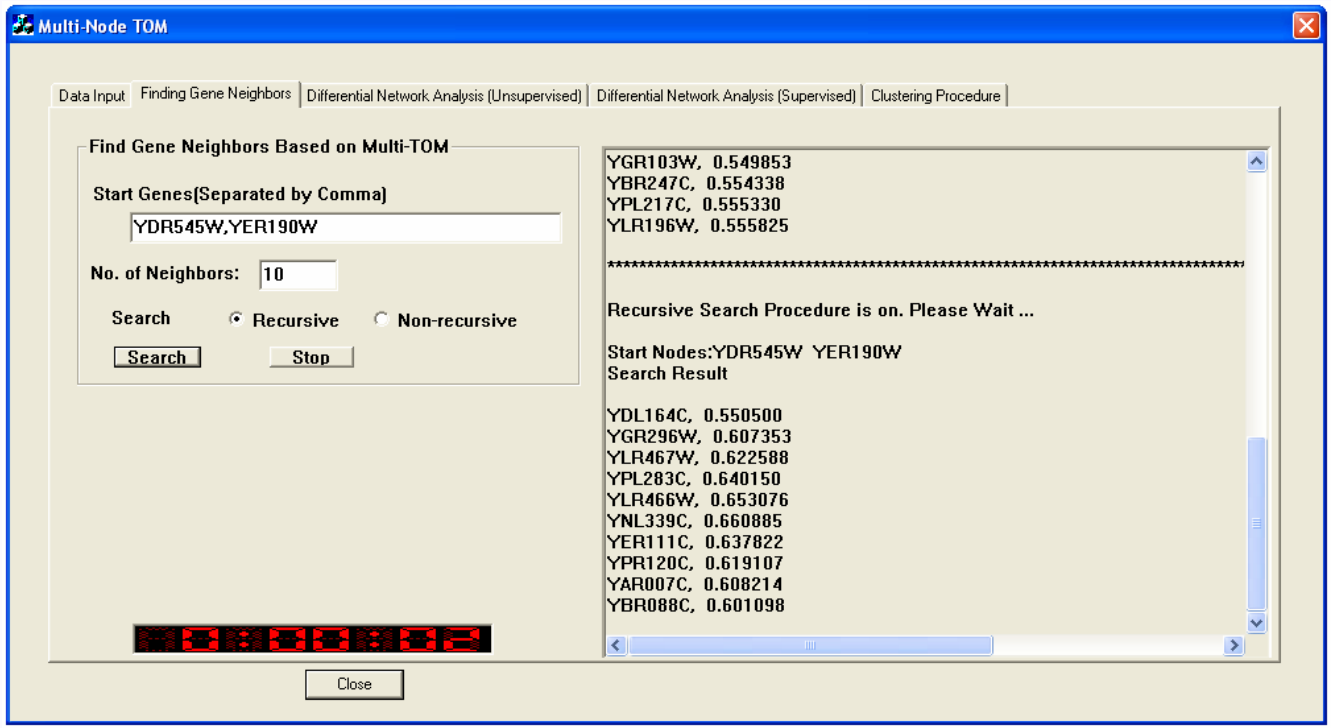
Discussion

- In several applications, we find that the topological overlap matrix leads to biologically meaningful results.
- Since it considers shared neighbors, it tends to be more robust to spurious connections.
- We described how to use the multinode TOM in network neighborhood analysis and provide empirical evidence that it produces biologically meaningful results.
- In our real data applications we find that the results are particularly good if
  - More than 1 node is used in the initial seed set
  - Highly connected hub nodes are used as seed
  - The nodes in the seed have high topological overlap themselves.
- MTOM can also be used in conjunction with a suitable clustering analysis to define modules.

MTOM Software Availability

Visual C++ implementation of the multinode TOM software can be found here

http://www.genetics.ucla.edu/labs/horvath/MTOM/



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- Andy Yip, Assistant Prof, Math, Singapore

Brain Cancer/Yeast

- Marc Carlson, Postdoc, UCLA
- Paul Mischel, Prof, UCLA
- Stan Nelson, Prof, UCLA

Webpages and References

- This talk and relevant R code

Ai Li, Steve Horvath (2006) The Multi-Point Topological Overlap Matrix for Gene Neighborhood Analysis. Proceedings Volume Gene Networks: Theory and Application Workshop at BIOCOMP'06, Las Vegas

  - http://www.genetics.ucla.edu/labs/horvath/MTOM/

- Main Network Webpage

www.genetics.ucla.edu/labs/horvath/CoexpressionNetwork/

- *Bin Zhang and Steve Horvath (2005) "A General Framework for Weighted Gene Co-Expression Network Analysis", Statistical Applications in Genetics and Molecular Biology: Vol. 4: No. 1, Article 17.*

www.bepress.com/sagmb/vol4/iss1/art17

- *MRJ Carlson, B Zhang, Z Fang, PS Mischel, S Horvath, SF Nelson, Gene connectivity, function, and sequence conservation: predictions from modular yeast co-expression networks", BMC Genomics 2006, 7:40 (3 March 2006).*

http://www.biomedcentral.com/1471-2164/7/40/