5th Annual Cytoscape Retreat & Public Symposium
Academic Medical Center, University of Amsterdam, Netherlands
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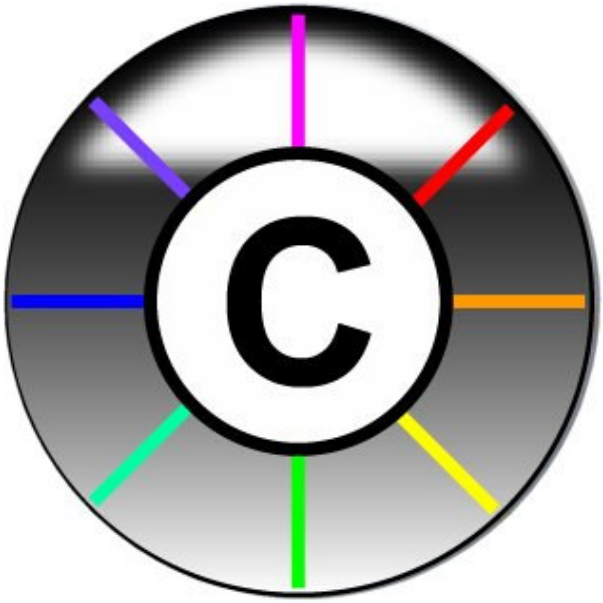
Cytoscape Tutorial

Wednesday 7th November 2007



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Cytoscape:
Biological Network Analysis Training

Schedule of Training

Cytoscape:
Biological Network Analysis Training

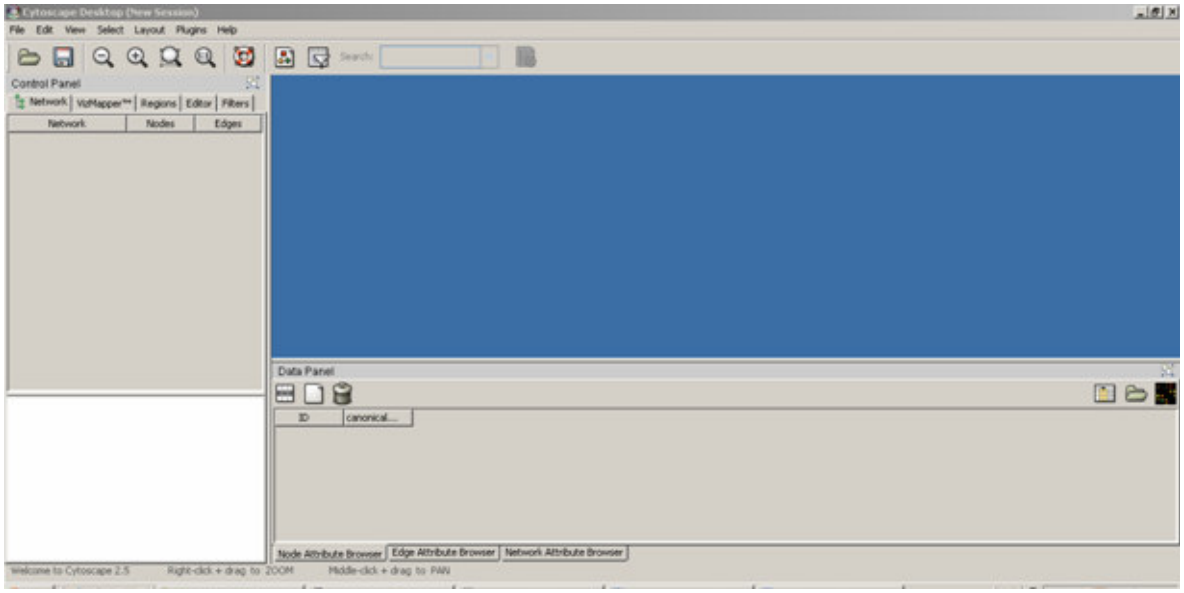
1. Introduction to Cytoscape Software

This section will introduce the Cytoscape user interface and includes central organising metaphors of Cytoscape: a network consists of genes/proteins/metabolites as nodes and interactions represented as links i.e. edges between nodes

First of all we will look at the basic UI of Cytoscape. Then we will load up a network to show all menu features of Cytoscape and some of the core functionality such as layout algorithms and also its plug-ins.

1.1 Cytoscape Layout and User Interface

Launch Cytoscape: You should see a window that looks like this



- At the top of the Cytoscape Desktop window is the toolbar, which contains the command buttons. The name of each command button is shown when the mouse pointer hovers over it.
- In the upper right is the Main Network View window, where network data will be displayed. This region is initially blank.
- At left is the Control Panel (Network Management) Panel. This lists the available networks by name and provides information on the number of nodes and edges.
- Immediately below the Control Panel is the Network Overview Pane
- At lower right is the Data Panel which can be used to display node, edge, and network attribute data

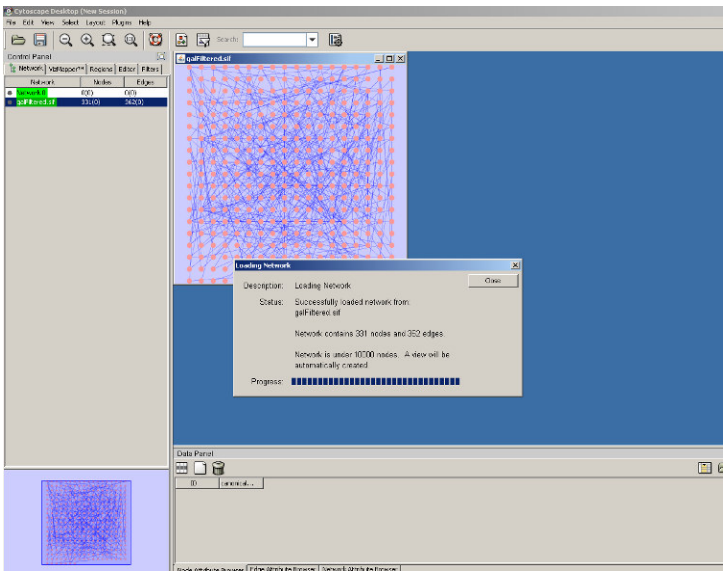
The Network Management and Data browser panels are dockable tabbed panels known as CytoPanels. You can undock any of these panels by clicking on the Float Window control in the upper-right corner of the CytoPanel.

1.2 Loading Networks

We will now load a network to show all features of Cytoscape UI

- Go to **File-> Import -> Network (multiple file types)**
- You should see the Import Network File Dialog
- For Data Source Type select **Local** and then click **Select**
- Open the **sampleData** folder and select **galFiltered.sif** and then click on **open** and then **Import**

You should see the following:



We will now use some of the basic Cytoscape functionality to re-organise and move around the network.

- Go to the **Layout -> yFiles -> Organic** to change the layout of the network
- Use the **Network Overview Pane** to move and navigate across the network
- The **Network Management Pane** of the Control displays the number of nodes and edges in your network
- You can **select nodes** by clicking on them with the mouse, or by creating a selection area by dragging with the left mouse button
- Note that the **Node Attribute Browser** on the **Data Panel** lists the information stored on the nodes
- You can also **select edges** by clicking on a few edges; again, note that information about the edges can be viewed on the **Edge Attribute Browser** of the Data Panel

1.3 Cytoscape menus

We will briefly run through all the menus available in Cytoscape.

File
The File menu contains basic file functionality:

- **File → Open** for opening a Cytoscape session file
- **File → New** for creating a new network
- **File → Save** for saving a session file
- **File → Import** for importing data such as networks and attributes
- **File → Export** for exporting data and images.
- **File → Print** allows printing
- **File → Quit** closes all windows of Cytoscape and exits the program

Edit
The Edit menu contains:

- Undo and Redo functions which undo and redo edits made in the Attribute Browser, the Network Editor and the Layout.
- Options for creating and destroying views (graphical representations of a network) and networks
- Options for deleting selected nodes and edges from the current network.
- All deleted nodes and edges can be restored to the network via **Edit → Undo**.
- **Edit → Preferences → Properties** to edit preferences for properties and plugins

View
The View menu allows you to display or hide:

- The network management panel (Control Panel)
- The attribute browser (Data Panel)
- Results Panel
- VizMapper

Select
The Select menu contains:

- Options for selecting nodes and edges
- The **Select → Use Filters** option allows filters to be created for automatic selection of portions of a network whose node or edge attributes meet a filtering criterion (see below for the filters section).

Layout
The Layout menu has an array of features for visually organising the network:

- Rotate, Scale, Align and Distribute are tools for manipulating the network visualisation.
- The bottom section of the menu lists a variety of layout algorithms which automatically lay a network out.

Plugins
The Plugins menu contains options for managing your plugins (install/update/delete) and may have options added by plugins that have already been installed, such as the Agilent Literature Search or Merge Networks.

- Depending on which plugins are loaded, the plugins that you see may be different than what appear here.

Help

- The Help menu allows you to launch the help viewer and browse the table of contents for this manual.
- The "About…" option displays information about the running version of Cytoscape.

1.4 Manipulating Networks

Zooming
Cytoscape provides two mechanisms for zooming:

i) Using the mouse – right click and move the mouse forward and backwards
ii) Using the **zoom buttons** on the toolbar to zoom in and out of the interaction network shown in the current network display. Zoom icons are detailed below:

From Left to Right:

- Zoom Out; Zoom In; Zoom Selected Region; Zoom Out to Display all of Current Network

Panning the network
You can pan the network image by holding down the middle mouse button and moving the mouse in the main network window. You can also pan the image by holding down the left mouse button on the Network Overview panel in the lower left hand of the Cytoscape desktop

Manually rearrange a network
Select the nodes of interest and move them around.

Automatically rearrange a network
There are a variety of layout algorithms which can be found in the Layout Menu

- Select **Layout -> Cytoscape Layouts -> Degree Sorted Circle Layout -> All Nodes**
    - Your network has now been re-arranged and the algorithm partitions the network based on the connectivity of the nodes (i.e. degree)
    - The circles themselves are arranged in a radial tree layout fashion.
    - This algorithm also calculates the degree for each node which cab be viewed in the **Node Attribute Browser** as follows:
        - On the **Data Panel**, select the **Node Attribute Browser** tab, click on **Select Attributes**, select **Degree** then click on any

node, the degree of the node as calculated by the Degree Sorted Circle Layout will be displayed

Try other layout algorithms from the Layout menu.

Rotating and Scaling your network

Finally you can rotate and scale your network within Cytoscape.

- Go to the **Layout Menu -> Rotate**, the Tool Panel will appear at the bottom left of the screen
- Select a few nodes and click on **Rotate Selected Nodes Only** in the Tool Panel, move the slider to 90 degrees to rotate the nodes.
- Note you can also rotate the whole network.
- You can also change the scale of the network (or selected nodes) by clicking on the **Scale** tab in the Tool Panel

2. Constructing and Loading Biological Networks into Cytoscape

In this section we will show the ways in which you can load in your own networks and associated data in to Cytoscape

There are three ways of creating networks in Cytoscape and we will deal with each of these in turn:

- Importing network files.
- Importing text or Excel files.
- Creating an empty network and manually adding nodes and edges.

First of all it's useful to understand the files formats that are used for networks and the associated data.

2.1 Importing network files into Cytoscape

Cytoscape can read network/pathway files written in the following formats:

- Simple interaction file (SIF or .sif format)
- Graph Markup Language (GML or .gml format)
- XGMML (extensible graph markup and modelling language).
- SBML
- BioPAX
- PSI-MI Level 1 and 2.5

The simple interaction format (sif) is convenient for building a graph from a list of interactions. The main disadvantage is that this format does not include any layout information, forcing Cytoscape to re-compute a new layout of the network each time it is loaded.
An example of a sif file format is:

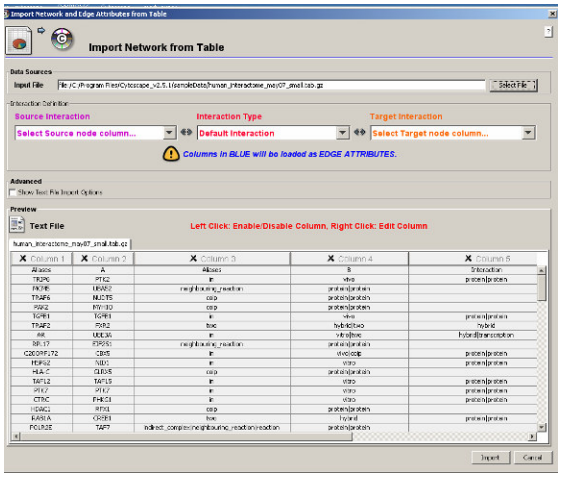
nodeA <relationship type> nodeB
nodeC <relationship type> nodeA

We will open **galFiltered.sif** in Excel to illustrate this

2.2 Importing text or Excel files

Cytoscape can also read file structures are the Delimited Text or Excel

- Go to **File-> Import->Network from Table (Text/MS Excel)**
- You should see the **Import Network from Table** Dialog Box
- In **Data Sources**, click on **Select File**
- Select **human_interactome_may07_small.tab.gz** from the **sampleData** folder and then click on **Open**. You should now see the screen below.



- In the **Advanced** section, select **Show Text File Import Options**
- In the **Delimiter** section, deselect **Space**
- In the **Attribute Names** section, select **Transfer first line attribute names**
- In the **Preview** section click on the columns you want to import: **Aliases A, Aliases B, Interaction Detection Methods, Interactor Types**

The next step is to define the interaction parameters by specifying which columns of data contain the Source Interaction (i.e. source node), Target Interaction (i.e. target node) and Interaction Type in the **Interaction Definition** pane. *If you set the Interaction Type as "Default Interaction", all interactions will be given the value "pp"; this value can be modified in Advanced Options.*

- For the Source Node
  - For the **Source Interaction** dropdown list, select **Column 1**
- For the Target Node
  - For the **Target Interaction** dropdown list Select **Column 2**
- For the Interaction
  - For the **Interaction Type** dropdown list Select **Column 4**
- Note Column 3 ("Interaction detection methods") is highlighted in blue and will be imported as edge attributes.
- Click on **Import**
- In the Network Manager section of the Control Panel a network of 10,176 nodes and 61240 edges is highlighted in red.
- Note that due to the size of the network, it will not be displayed in the Main window.
- *To create the view, right click on the Network name and select **Create View***

2.3 Creating an empty network and manually adding nodes and edges

It is also possible to create new, empty networks that nodes and edges can be manually added to.

- To create an empty network, go to **File → New → Network → Empty Network**
- Select the **Editor** tab from the **Control Panel**
- To add a node to a network, drag and drop a node shape from the palette onto the canvas. Add another node
- To connect two nodes with an edge, drag and drop an arrow shape onto a node on the canvas. This node becomes the source node of the edge.
- Move the cursor and a rubber-banded line will follow the cursor. As the cursor passes over another node, that node is highlighted and the rubber-banded line will snap to a connection point on that second node.
- Click the mouse while over this node and the connection will be established.
- You can delete nodes and edges using the following method
- Select a node and hit the Delete button on your keyboard or by **Edit->Delete selected nodes and edges**

You can also create networks using the following scheme

- Double click on any empty space in the Main Network window and you should see the **Input** dialog box. Type in the following

    - A binds B and then click on OK
    - Repeat above and type in
    - C binds D and then
    - D binds A and then
    - A binds A

You should have a new network of 5 nodes and 4 edges (this information about the number of nodes can be found by clicking the Network tab on the Control Panel)

- You can edit the name of the network by right clicking on the Network name in the Control Panel and selecting Edit Network Title

2.4 Fetching data from external sources using other databases

Agilent Literature Search

- To create an empty network, go to **File** → **New** → **Network** → **Empty Network**
- Under the **Plugins** menu, select **Agilent Literature Search**
- In the **Terms** window, enter **p53**. The term "p53" should appear in the **Query Editor**, and the forward arrow just below should turn blue to indicate it is available.
- Click on the **Play** button to begin searching.
- Under Query Matches, there should appear a numbered list of articles labelled **Results**.
- A slider at the right side of the window allows you to scroll through the list of selected articles
- Each article should be listed along with a URL, and a hyperlink for jumping directly to that URL
- A network should appear in Cytoscape, showing interactions inferred from sentences in the selected articles.
- Right click on the edge between **ctcf** and **ctcfl**
- Select "**Show Sentences from Agilent Literature Search**".
- Click on a URL to access the PubMed article

Linking to external data using LinkOut

LinkOut provides a mechanism to link nodes and edges to external web resources within Cytoscape.

- Select a node of interest,
- Right click on a node and pop up menu with a list of web links will be displayed
- Select **yeast** and then **SGD**

The external links are can be specified and edited by modifying the link out properties found under **Edit** → **Preferences** → **Properties**

- Select the **human_interactome_may07_small.tab** network
- Go to **Edit** → **Preferences** → **Properties**
- Click on Add in the **Properties** section
- Type in *nodelinkouturl.human.BioGrid* as a **Property Name**
- Type in the following for **Property Value**, http://www.thebiogrid.org/search.php?keywords=%ID%&searchbutton=GO&organismid=9606
- Click on OK
- Type in **SERPINA1** in the search box and click ok
- Right click in the selected node, select **human** and then **BioGrid**

2.5 Saving Sessions

You can save your Cytoscape sessions and export visualisations.

To save a session, go to **File->Save**

- Networks, Attributes, Visual Styles and Layouts will be saved as a CYS file

- You can export networks and attributes individually **File->Export->Network as sif File…** or **File->Export->Node Attributes**

You can also export images from Cytoscape:

- **File->Export->Network View as Graphics**

3 Working with Attributes

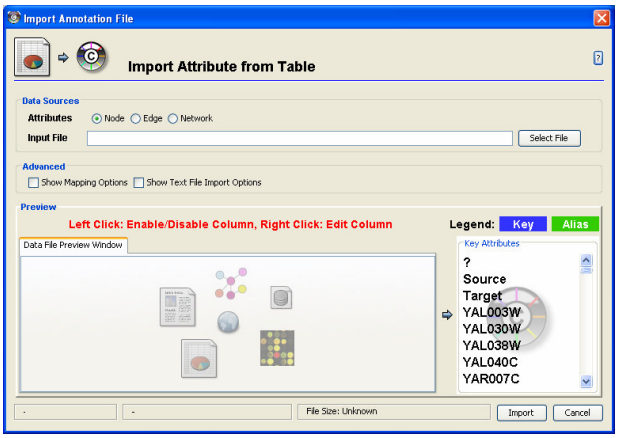
Once you have created a network it is important to overlay relevant data and information. Cytoscape allows you to add information to the nodes, edges and the network as a whole.

3.1 Importing Node Attributes

There are various file formats for node and edge attributes and details can be found in the Help section in Cytoscape.
As of Cytoscape 2.4, importing delimited text and MS Excel attribute data tables is now supported.
The attribute file should contain a *primary key column* and at least one attribute column.
We will look at the node attributes for the **galFiltered** network.
*<Open **galFiltered.nodeAttrTable.xls** as an example>*

To Import Node Attributes

- Select **File** → **Import** → **Attribute from Table (Text/MS Excel)**
- Select one **Node** from the **Attributes** radio buttons. Cytoscape can import node, edge, and network attributes



- To load a local file, click on the Select File button and open the **sampleData** folder, select **galFiltered.nodeAttrTable.xls** and click on **Open**
- *(Optional) If the table is not properly delimited in the preview panel, change the delimiter in the Text File Import Options panel. The default delimiter is the tab. This step is not necessary for Excel Workbooks.*
- By default, the first column is designated as the primary key. Change the key column if necessary.
- In the **Advanced** pane, select "**Show Text File Import Options**"
- In the **Attribute Names** pane, select "**Transfer first line attribute names**"
- In the **Preview** pane click on the columns you want to import (Click them all)
- Double click on the second column to change the from "alias" to "AlternativeName"
- Click on Import

3.2 Viewing Attributes

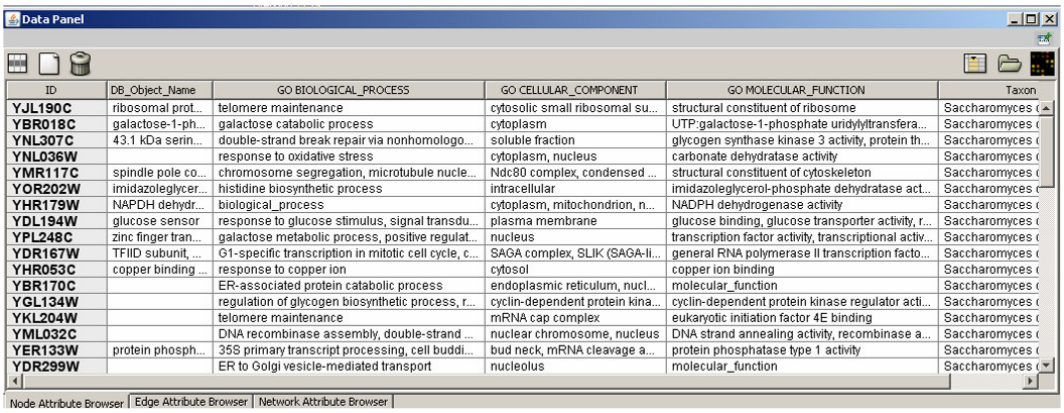
The node attributes can be viewed in the Node Attribute Browser of the Data Panel. To swap between nodes, edges or network browsers click on the appropriate tabs on the Data Panel

To view the node attributes you have just loaded in, click on the **Select Attributes** button in the **Node Attribute Browser**

Select the following Node Attributes by clicking in check boxes

- DB_Object_Name
- GO Biological Process
- GO Molecular Function
- GO Cellular Component
- Taxon

Select any nodes on the network and the attributes will be displayed in the Node Attribute Browser of the Data Panel:



ID	DB_Object_Name	GO BIOLOGICAL_PROCESS	GO CELLULAR_COMPONENT	GO MOLECULAR_FUNCTION	Taxon
YJL190C	ribosomal prot...	telomere maintenance	cytosolic small ribosomal su...	structural constituent of ribosome	Saccharomyces
YBR018C	galactose-1-ph...	galactose catabolic process	cytoplasm	UTP:galactose-1-phosphate uridylyltransfera...	Saccharomyces
YNL307C	43.1 kDa serin...	double-strand break repair via nonhomologo...	soluble fraction	glycogen synthase kinase 3 activity, protein th...	Saccharomyces
YNL036W		response to oxidative stress	cytoplasm, nucleus	carbonate dehydratase activity	Saccharomyces
YMR117C	spindle pole co...	chromosome segregation, microtubule nucle...	Ndc80 complex, condensed ...	structural constituent of cytoskeleton	Saccharomyces
YOR202W	imidazoleglycer...	histidine biosynthetic process	intracellular	imidazoleglycerol-phosphate dehydratase act...	Saccharomyces
YHR179W	NAPDH dehydr...	biological_process	cytoplasm, mitochondrion, n...	NADPH dehydrogenase activity	Saccharomyces
YDL194W	glucose sensor	response to glucose stimulus, signal transdu...	plasma membrane	glucose binding, glucose transporter activity, r...	Saccharomyces
YPL248C	zinc finger tran...	galactose metabolic process, positive regulat...	nucleus	transcription factor activity, transcriptional activ...	Saccharomyces
YDR167W	TFIID subunit, ...	G1-specific transcription in mitotic cell cycle, c...	SAGA complex, SLIK (SAGA-li...	general RNA polymerase II transcription facto...	Saccharomyces
YHR053C	copper binding ...	response to copper ion	cytosol	copper ion binding	Saccharomyces
YBR170C		ER-associated protein catabolic process	endoplasmic reticulum, nucl...	molecular_function	Saccharomyces
YGL134W		regulation of glycogen biosynthetic process, r...	cyclin-dependent protein kina...	cyclin-dependent protein kinase regulator acti...	Saccharomyces
YKL204W		telomere maintenance	mRNA cap complex	eukaryotic initiation factor 4E binding	Saccharomyces
YML032C		DNA recombinase assembly, double-strand ...	nuclear chromosome, nucleus	DNA strand annealing activity, recombinase a...	Saccharomyces
YER133W	protein phosph...	35S primary transcript processing, cell buddi...	bud neck, mRNA cleavage a...	protein phosphatase type 1 activity	Saccharomyces
YDR299W		ER to Golgi vesicle-mediated transport	nucleolus	molecular_function	Saccharomyces

- The right-click menu on the Attribute Browser has several functions, such as exporting attribute information to spreadsheet applications.
- For example, use the right-click menu to Select All and then Copy the data, and then paste it into a spreadsheet application
- You can also link out to external resources by right clicking a cell in the table

3.3 Deleting Attributes

To delete attributes from the browser (**and CYTOSCAPE!!**) click on the **Delete Attribute Button**

Select the attributes you want to delete and then click on **Delete**

3.4 Editing Attributes

You can also edit attributes by double clicking on the appropriate cell in the Node Attribute Browser

3.5 Importing Ontologies

Note you can also import Ontology and Annotation by doing the following:
File-> Import-> Ontology and Annotation

4 Working with Data

It is not only useful to add information to networks as we have just done. In addition to normal node and edge attribute data, Cytoscape also supports importing gene expression data (and any other molecular state data).

Network-based analysis of High Throughput data is a very useful tool in systems biology and many other disciplines and this section will cover the integration of HTP data with biological networks in order to perform powerful visualisation and analyse in a biological context.

PLEASE NOTE: DNA microarray data will be used as an example in this section of the tutorial however other types of data can be used

Gene expression data are imported using a different file format than normal attributes; however, the resulting attributes are not treated differently by Cytoscape. Gene expression data (like attribute data) can be loaded at any time, but are (generally) only relevant once a network has been loaded.

4.1 File format

The file format for importing molecular state data (or any other data) consists of a header and a number of space- or tab-delimited fields, one line per node, with the following format:

Identifier [CommonName] value1 value2 ... valueN [pval1 pval2 ... pvalN]

Let's take a look at a Gene Expression file to get an idea of the format

- Brackets [ ] indicate fields that are optional.
- The first field is a primary key that identifies which Cytoscape node the data refers to.
    - In the simplest case, this is the gene name - exactly as it appears on the network generated by Cytoscape (***NOTE*** it is case sensitive!).
    - Alternatively, this can be some node attribute that identifies the node uniquely, such as a probeset identifier for commercial microarrays.
- The next field is an optional common name. It is not used by Cytoscape, and is provided strictly for the user's convenience.
- The next set of columns represent expression values, one per experiment. These can be either absolute expression values or fold-change ratios. Each experiment is identified by its experiment name, given in the first line.
- Optionally, significance measures such as P values may be provided.
    - If you are using significance measures, then your expression file should contain them in a second set of columns after the expression values. The column names for the expression significance measures need to match those of the expression values **exactly**.

Expression data files commonly have the file extensions ".mrna" or ".pvals", and these file extensions are recognised by Cytoscape when browsing for data files.

4.2 Loading Expression Data

We will now load the expression data for the **galFiltered** network:

- Go to **File** → **Import** → **Attribute/Expression Matrix....**
- The **Import an Attribute/Expression Matrix** will be displayed
- In the field labelled "Please select an attribute or expression matrix file...", click on the Select button
- Select **galExpData.pvals** the sampleData folder and click on **Open**
    - The identifiers used in this file are the same ones used in the network file sampleData/galFiltered.sif, so you do not need to touch the field labelled "Assign values to nodes using..."
- Click on Import and details of the expression data will be displayed

That's it! You've now integrated your experimental data with your network.

In the next section we will learn how to us the visualisation and filtering tools in Cytoscape to start to use this data for network-based analysis

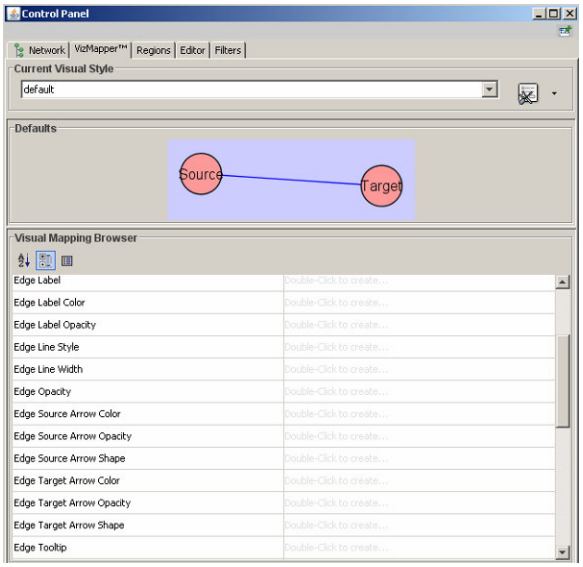
5 Customising Visualisation of Networks

Customising the way you visualise and manipulate networks is a key function of Cytoscape. This is achieved through the use of the VizMapper tool and in this section we will co through this functionality in some detail.

Following on from the previous sections, we will use the networks and data that have already been loaded in but remember, this will work with whatever data you are interested in.

5.1 The basics of VizMapper in Cytoscape

To launch the VizMapper, either select the VizMapper tab on the Control Panel or click on the VizMapper icon at the top of the tool bar



There are 3 components in the VizMapper UI:

Current Visual style

This panel allows you to create/delete/view/switch between different visual styles using the Current Visual Style options.

Defaults Appearance Style

Clicking on the section labelled "Defaults" on the Main Panel will bring up an editor, which allows users to visually edit the default appearance of nodes and edges for the selected visual style.

Visual Mapping Browser

Visual Mapping Browser at the bottom displays the mapping details for a given visual style and is used to edit these details as well.

5.2 Different types of Visual Mapping in Cytoscape

Before we start creating our own visual styles there are a few concepts that need to be understood.

The Cytoscape VizMapper uses three core concepts:

- A **visual attribute** is any visual setting that can be applied to your network. For example, you can change all nodes from circles to squares by changing the node shape visual attribute.
- A **network attribute** is any data attribute associated with a node or an edge. For example, each edge in a network may be associated with a label, such as "pd" (protein-DNA interactions), or "pp" (protein-protein interactions).
- A **visual mapper** maps network attributes to visual attributes. For example, a visual mapper can map all protein-DNA interactions to the colour blue, and all protein-protein interactions to the colour red

What can we visualise?
Cytoscape allows a wide variety of visual attributes to be controlled details of which can be found in the Cytoscape User Manual. Examples include:

Nodes

- Node Colour
- Node Opacity
- Node Border Colour
- Node Border Opacity
- Node Shape

Edges

- Edge Source and Target Arrow Colour
- Edge Source and Target Arrow Opacity
- Edge Label: the text label for each edge.
- Edge Label Colour
- Edge Label Opacity
- Edge Font: edge label font and size.

Global Visual Properties

- Background Colour
- Selected Node Colour
- Selected Edge Colour

The types of Visual Mappers
For each visual attribute, you can specify a default value or define a dynamic visual mapping. Cytoscape currently supports three different types of visual mappers:

1. Pass-through Mapper
   The values of network attributes are passed directly through to visual attributes. A pass-through mapper is only **used to specify node/edge labels**. For example, a pass-through mapper can label all nodes with their common gene names.
2. Discrete Mapper
   Discrete network attributes are mapped to discrete visual attributes. For example, a **discrete mapper can map all protein-protein interactions to the colour blue**.
3. Continuous Mapper
   Continuous graph (usually **numerical) attributes are mapped to visual attributes**.

5.3 VizMapper Examples

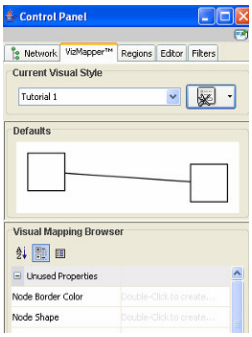
The best way to learn about the VizMapper is to try to use it on some real examples. In this tutorial we will look at a few examples

- ***Example 1: Creating a Basic Visual Style and Setting Default Values***
- ***Example 2: Creating a New Visual Style with a Discrete Mapper***
- ***Example 3: Visualizing Expression Data on a Network***
- ***Example 4: How to Use Utilities for Discrete Mappers***

5.3.1 Example 1 – Creating a basic visual style and setting default values

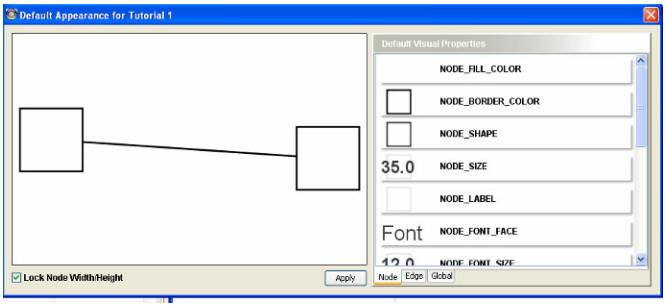
We will continue to use **galFiltered.sif**

- Ensure that **galFiltered** is selected in the Control Panel
- Open the VizMapper
- In the **Current Visual Style** section, create a new visual style by clicking the **Options** button  and selecting "**Create new Visual Style**"
- Enter a name for your new visual style when prompted, click on **OK**
- You will see an empty visual style in the VizMapper Main Panel, as shown below.

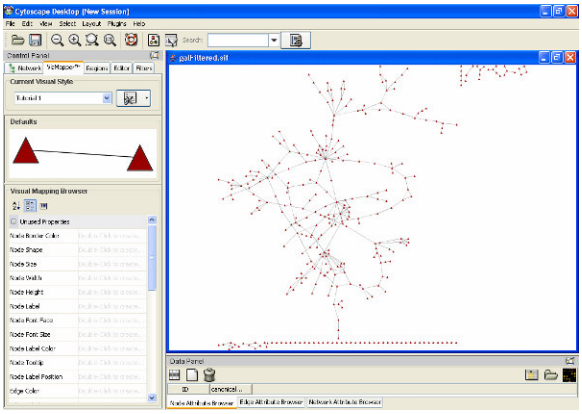


Since no mapping is set up yet, all visual attributes are listed in the **Unused Properties** category of the **Visual Mapping Browser**.

To edit default values, open the **Default Appearance Editor** by clicking on the **Defaults graphics window** (shown below) in the VizMapper Main Panel.



- The next step is to change the default node shape.
- To set the default node shape to triangles, click **Node Shape** in the **Default Visual Properties** list. A list of available node shapes will be shown.
- Click on the **Triangle** icon and then click the **Apply** button.
- The Default Appearance Editor will be automatically updated.
- The next step is to change the node colour
- Click on **Node_Fill_Color** in the **Default Visual Properties** list, the colour selection panel will appear. Select **red**, then **OK** and then **Apply**
- The end result is that all nodes are red triangles
- Other default values can be edited by clicking on visual attribute names on the list.
- To change default edge properties, select the **Edge** tab on the **Default Visual Properties** section
- To change default global properties, select the **Global** tab on the **Default Visual Properties** section



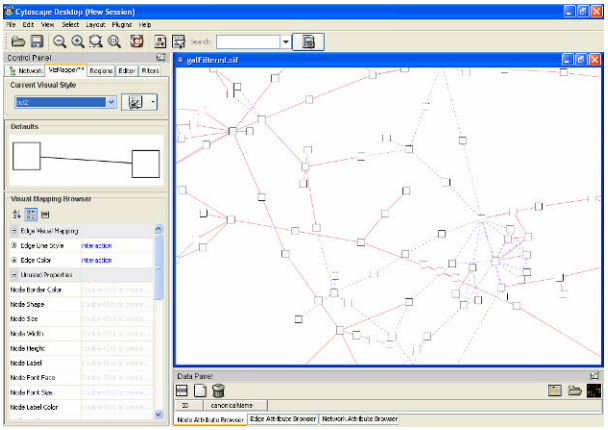
5.3.2 Example 2: Creating a New Visual Style with a Discrete Mapper

The following tutorial demonstrates how to create a new visual style using a discrete mapper. Recall that discrete network attributes are mapped to discrete visual attributes. The goal is to draw protein-DNA interactions as dashed blue lines, and protein-protein interactions as solid red lines.

Using **galFiltered.sif**

- Open the VizMapper
- In the **Current Visual Style** section, create a new visual style by clicking the **Options** button 
- Select Create new visual style
- Then enter a name for your new visual style when prompted, click on **OK**
- Next step is to choose a visual attribute
- Since we want to change the edge colour, double click the **Edge Colour** entry listed in **Visual Mapping Browser**. Edge Colour will now appear at the top of the list, under the Edge Visual Mapping category
- The next step is to choose a **network attribute**
- Click on the cell to the right of the Edge Colour entry and select **interaction** from the dropdown list that appears
- The next step is to specify the type of mapping
- Click on the cell next to the **Mapping Type** entry and select **Discrete Mapper** from the dropdown list
- All available attribute values for interaction will be displayed
- The next step is to set the mapping relationship
- Click the empty cell next to **pd** (protein-DNA interactions).
- On the right side of the cell, **...** and **X** buttons will appear
- Click on the **...** button. A popup window will appear; select **blue** and the change will immediately appear on the network window.
- Repeat above steps for **pp** (protein-protein interactions) but select **red** as the edge colour

Can you also specify that your protein-protein interactions should be solid and the protein DNA interactions will be dashed?

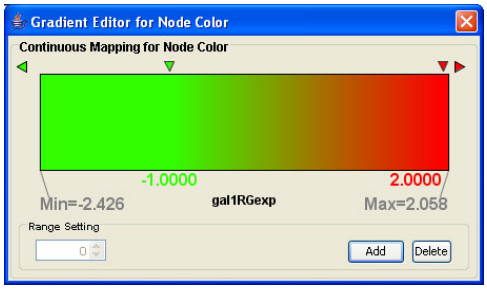


5.3.3 Example 3: Visualizing Expression Data on a Network (Using continuous mapper)

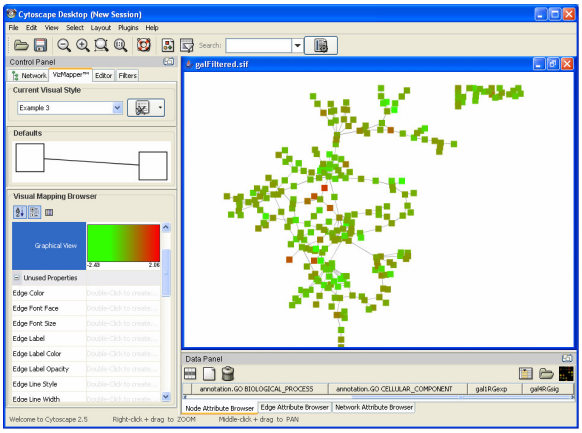
The following tutorial demonstrates how to create a new visual style using a continuous mapper.
The goal is to superimpose gene expression data onto a network and display gene expression values along a colour gradient.

Using **galFiltered.sif**

- (Load in **galFiltered.sif** and the associated expression data set: **galFiltered.pvals**)
- (Ensure **galFiltered** is selected in the Control Panel)
- Open the VizMapper
- In the **Current Visual Style** section, create a new visual style by clicking the **Options** button 
- Select **Create new visual style**
- Then enter a name for your new visual style when prompted, click on **OK**
- Next step is to choose a visual attribute
- Since we want to change the node colour, double click the **Node Colour** entry listed in **Visual Mapping Browser**. Node Colour will now appear at the top of the list, under the Node Visual Mapping category
- The next step is to choose a **network attribute**
- Click on the cell to the right of the Node Colour entry and select **gal1Rexp** from the dropdown list that appears
- The next step is to specify the type of mapping
- Click on the cell next to the **Mapping Type** entry and select **Continuous Mapper** from the dropdown list
- The next step is to define the points where the colours will change
- Click on **Graphical View** in the **Node Visual Mapping** category. The Gradient Editor for Node Colour will appear
- Click the **Add** button twice to create two data points, which will show up as overlapping triangles at the right of the scale.
- Click and drag one point to -1, or type the value in the Range Setting box. Set the second point to 2.
- The next step is to define the colours between points.
- Double-click on the leftmost triangle (facing left) and a colour palette will appear. Choose a **shade of green** and click OK.
- Make the triangle at -1 a **shade of green**.
- Double-click on the triangle set at 2, and set its colour to red.
- Set the rightmost triangle to Red.
- The end result is shown below



The colour gradients will immediately appear in the network window. All nodes with a gal1RGexp value less than –1 will be set to green, and all nodes with a gal1RGExp value greater than 2 will red. Additionally, all values between –1 and 2 will be painted with a green to red colour gradient.



5.3.4 Example 4 – How to Use Utilities for Discrete Mappers

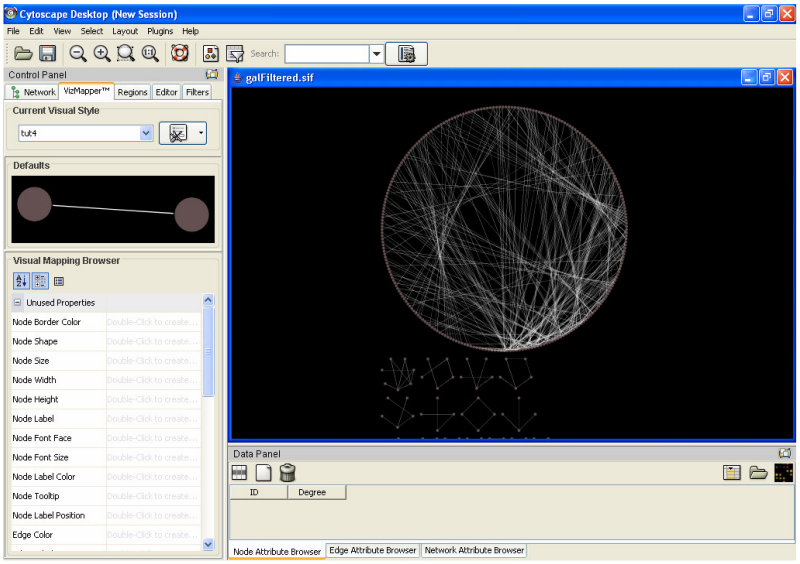
The following tutorial demonstrates new features in Cytoscape 2.5. The new VizMapper user interface has some utilities to help users editing discrete mappings. The goal of this section is learning how to set and adjust values for discrete mappings automatically.

Using **galFiltered.sif**

- (Load in **galFiltered.sif**)
- (Ensure **galFiltered** is selected in the Control Panel)
- Apply the Degree Sorted Circle Layout to the network: From the main menu, select **Layout** → **Cytoscape Layouts** → **Degree Sorted Circle Layout**. This layout algorithm sort nodes in a circle by degree of the nodes. Degrees will be stored as node attribute names Degree after you applied this algorithm. You can check this in the **Node Attribute Browser**
- Open the VizMapper
- In the **Current Visual Style** section, create a new visual style by clicking the **Options** button 

The next step is to change the default appearance to:

- Node Opacity: 100
- Edge Colour: White
- Background Colour: Black

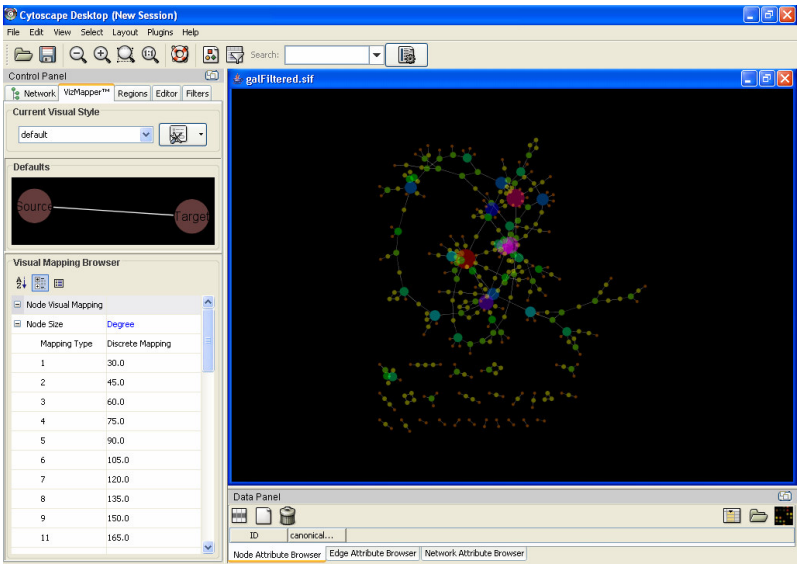


The next step is to create a *Discrete Node Colour Mapping*

- Since we want to change the node colour, double click the **Node Colour** entry listed in **Visual Mapping Browser**. Node Colour will now appear at the top of the list, under the Node Visual Mapping category
- The next step is to choose a **network attribute**, select **Degree** as controlling attribute.
- Click on the cell next to the **Mapping Type** entry and select **Discrete Mapper** from the dropdown list
- All available attribute values for **Degree** will be displayed
- Right click on **Discrete Mapping** and Select **Generate discrete values → Rainbow 1**. It generates different colours for different attribute values as shown below

The next step is to create a *Discrete Node Size Mapping*

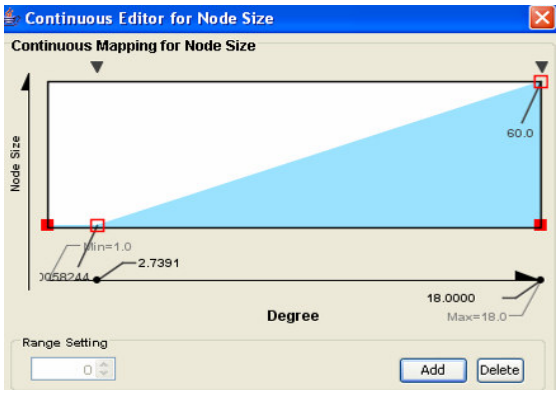
- Double click the **Node Size** entry listed in **Visual Mapping Browser**
- The next step is to choose a **network attribute**, select **Degree** as controlling attribute.
- Click on the cell next to the **Mapping Type** entry and select **Discrete Mapper** from the dropdown list
- All available attribute values for **Degree** will be displayed
- Right click on **Discrete Mapping** Select Generate discrete values → Series.
- Type *30* for the first value and click OK. Enter *15* for increment.
- Apply Force-Directed layout (**Layout->Cytoscape Layouts-> Force-Directed Layout**). Final view of the window looks like the following.

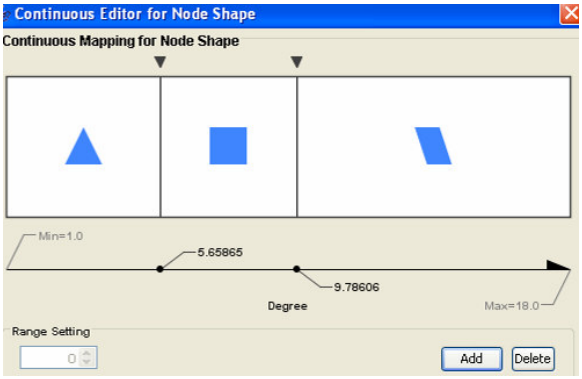


5.4 Other aspects of visual styles

Continuous-Continuous Editor
Continuous-Continuous Editor is for creating mapping between numerical attributes and numerical visual properties (size/opacity). To change the value assigned on Y-drag the red squares or double click on the squares to directly type exact value.

Continuous-Discrete Editor is used to create mapping from numerical attribute values to discrete visual properties, such as font, shape, or line style. To edit a value for specific region, double click on the icon on the track.





Managing Visual Styles
- All Cytoscape Visual Style settings are initially loaded from a default file called vizmap.props that cannot be altered by users.
- When users make changes to the visual properties, a vizmap.props file is saved in the session file.
- This means that assuming you save your session; you will not lose your visual properties. No other vizmap.props files are saved during normal operation

Saving Visual Styles
- Visual styles are automatically saved with the session they were created in. Before Cytoscape exits, you will be prompted to make sure you save the session before quitting.
- It is also possible to save your visual styles in a file separate from the session file. To do this, navigate to the File → Export → Vizmap Property File menu option and save the properties as a file. This feature can be used to share visual styles with other users.

Importing visual Styles
- To import existing visual styles, navigate to the File → Import → Vizmap Property File menu option and select a vizmap.props file.
- Imported properties will supplement existing properties or override existing properties if the properties have the same name.

6 Filtering and Searching Networks

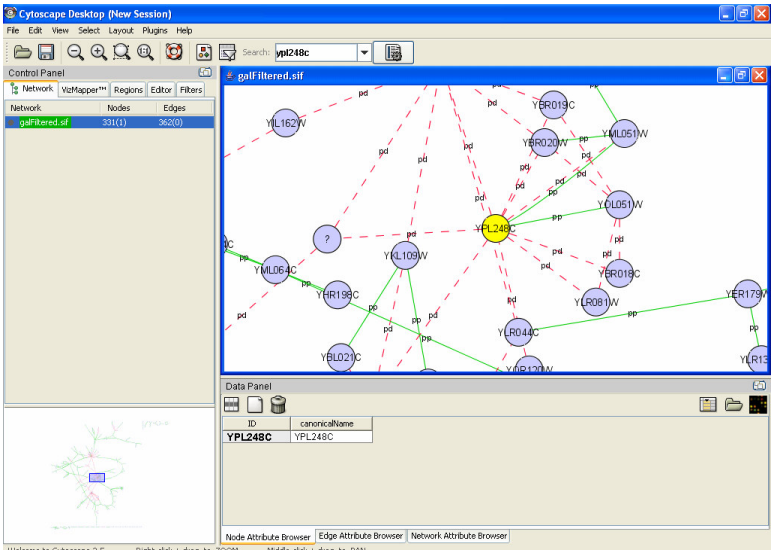
Searching and filtering networks is another powerful feature of Cytoscape. In this section we will cover how to go about navigating your networks

6.1 Quick Find

Cytoscape includes a Quick Find feature, which enables you to quickly find nodes and edges.

Using Quick Find is very simple.
Using **galFiltered.sif**

- (Load in **galFiltered.sif**)
- (Ensure **galFiltered** is selected in the Control Panel)
- Type **YPL248C** in the search box which will automatically display a list of all matching nodes
- Hit the returned key and the matching node within the network will be highlighted and zoomed in



Configuring Quick Find
Quick Find works by creating an internal index of all nodes within the network. By default, Cytoscape indexes all nodes by the node identifier. However, you can configure Quick Find to index nodes or edges, and you can set any attribute as the index.

- For example, to index the network based on cellular location, click the Quick Find configuration button 

- In the **Select Index Type**, select **Index Nodes**
- Select **GO_Cellular_Component** from the dropdown list of the **Search on Attribute** section and then click on **Index Network**
- Type **Cytoplasm** in the search box
- The resulting nodes are highlighted in the network

Dynamic Filtering via Quick Find
If you choose to index on a numerical attribute, the Quick Find search box changes to a dynamic slider for quick filtering.

- To index the network based on expression, click the Quick Find configuration button 
- In the **Select Index Type**, select **Index Nodes**
- Select **gal1RGexp** from the dropdown list of the **Search on Attribute** section and then click on **Index Network**
- A range slider will appear in the search box
- Move the slider and the resulting nodes are highlighted in the network

6.2 Filters

Filters allow you to quickly select multiple nodes or edges of interest by comparing node and edge attributes loaded onto Cytoscape networks to properties you specify.

For example, you can select all the nodes whose name contains a specific pattern, or whose numeric attribute value falls within a certain range. Cytoscape provides filters as a core plugin.

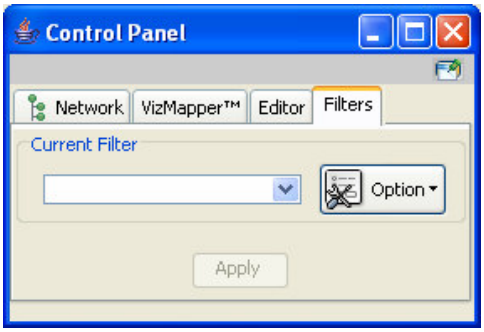
Users can perform complex selection by defining basic filters (selection based on a single attribute) and compound or Boolean filters (combining basic filters for selection based on multiple attributes).

There are two types of filters
- **Newly designed** which allows users to filter on attributes
- **Old filters** which allows users to filter on attributes and topology

Filtering on Attributes

Using New Filters

- Click on the filter icon on the tool bar 
- Alternatively click on the **Filters** tab on the **Control Panel**



The next step is to define a filter (numerical)

- Click on the Filter Option button, select **Create New Filter** and enter a name for your filter
- Select **gal4RGexp** from the **Attribute** dropdown list of the **Filter Definition** section
- Click on **Add** and a slider should appear
- Set the slider to -1 to 0 and then click on **Apply**
- The resulting nodes will be highlighted in the network
- (You can work with the resulting nodes on a separate network, go to **File-> New-> Network-> From selected nodes, all edges**)

You can also combine filters

- Select **gal4RGexp** from the **Attribute** dropdown list of the **Filter Definition** section
- Click on Add and et the slider to 0 to 2
- Click on the + sign in the **Advanced** section and select **AND** in the **Relation** section
- Click on apply
- (You can work with the resulting nodes on a separate network, **go to File-> New-> Network-> From selected nodes, all edges**)

Filtering on Topology

<To filter on topology you will need to use to Old filters (Select->Use Old Filters)>

6.3 Finding Complexes

Complexes are a special type of module: they are a group of proteins that interact to form one single piece of cellular machinery, such as the ribosome or the spliceosome.

This section illustrates a couple of methods for determining complexes.

MCODE

The first is the MCODE method, which follows the principle that highly-connected regions of the network are often complexes.

Using **galFiltered sif**

- Launch MCODE (**Plugins-> MCODE-> Start MCODE**) and the MCODE Plugin tab will appear in the Control Panel
- In the **Find Clusters** section, select **In Whole Network**
- Click on the **Analyze** button
- The results are shown in the **Results Panel**
- Select a sub-network from the **MCODE Cluster Browser**
- The nodes of the subnetwork will be highlighted in the network
- (You can work with the resulting nodes on a separate network, go to **File-> New-> Network-> From selected nodes and edges**)
- The highest scoring subnetwork shows the peroxisome complex