Cytoscape 2.0 Manual

1. Introduction

Cytoscape is an open-community software project for integrating biomolecular interaction networks with high-throughput expression data and other molecular states into a unified conceptual framework. Although applicable to any system of molecular components and interactions, Cytoscape is most powerful when used in conjunction with large databases of protein-protein, protein-DNA, and genetic interactions that are increasingly available for humans and model organisms. A software "Core" provides basic functionality to layout and query the network; to visually integrate the network with expression profiles, phenotypes, and other molecular states; and to link the network to databases of functional annotations. The Core is extensible through a straightforward plug-in architecture, allowing rapid development of additional computational analyses and features. **The central organizing metaphor of Cytoscape is a network graph, with genes, proteins, and molecules represented as nodes and interactions represented as links, i.e. edges, between nodes.**

Development

Cytoscape is a collaborative project between the Institute for Systems Biology (Dr. Benno Schwikowski), the University of California San Diego (Dr. Trey Ideker), and Memorial Sloan-Kettering Cancer Center (Dr. Chris Sander).

Schwikowski Lab: http://www.systemsbiology.org/personal/benno
Ideker Lab: http://www-bioeng.ucsd.edu/faculty/area/ideker_lab/
Sander Lab: http://www.cbio.mskcc.org/

License

Cytoscape is protected under the LGPL (Lesser Gnu Public License). The License is included as an appendix to this manual, but can also be found online:
http://www.gnu.org/copyleft/lesser.txt

2. Launching Cytoscape

Currently, Cytoscape runs under Java on Linux, Windows, and Mac OS X. Although Cytoscape handles arbitrary types and sizes of interaction network, it is most powerful when used in conjunction with large interaction data sets such as are currently available for species such as the yeast S. cerevisiae.

(1) Download and unpack the distribution. Cytoscape is distributed as a compressed archive (tar.gz or zip) containing the following files and directories:

`cytoscape.jar`	Main Cytoscape application (Java archive)
`cytoscape.props`	User-configurable properties and preferences
`vizmap.props`	User-configurable visual mapping preferences
`cytoscape.sh`	Shell script used to run Cytoscape (Linux, Mac OS X)
`cytoscape.bat`	Shell script used to run Cytoscape (Windows)
`LICENSE.txt`	Cytoscape GNU License
`Cytoscape2_0Manual.pdf`	Cytoscape 2.0 Manual (the document you are reading now)
`sampleData/`	
`galFiltered.gml`	Sample molecular interaction network file *
`galFiltered.sif`	Identical network in Simple Interaction Format *
`galExpData.pvals`	Sample gene expression matrix file *
`BINDyeast.sif`	Network of all yeast protein-protein interactions in the BIND database as of February, 2002 **
`GO/`	Directory containing Gene Ontology database entries (currently for yeast only). Info in this directory is used to associate gene names with synonyms as well as process, function, and cellular location data.
`plugins/`	Directory containing cytoscape plug-ins, in .jar format.

* Sample data sets taken from Ideker et al, Science 292:929 (2001)
** Obtained from data hosted at http://www.bind.ca/

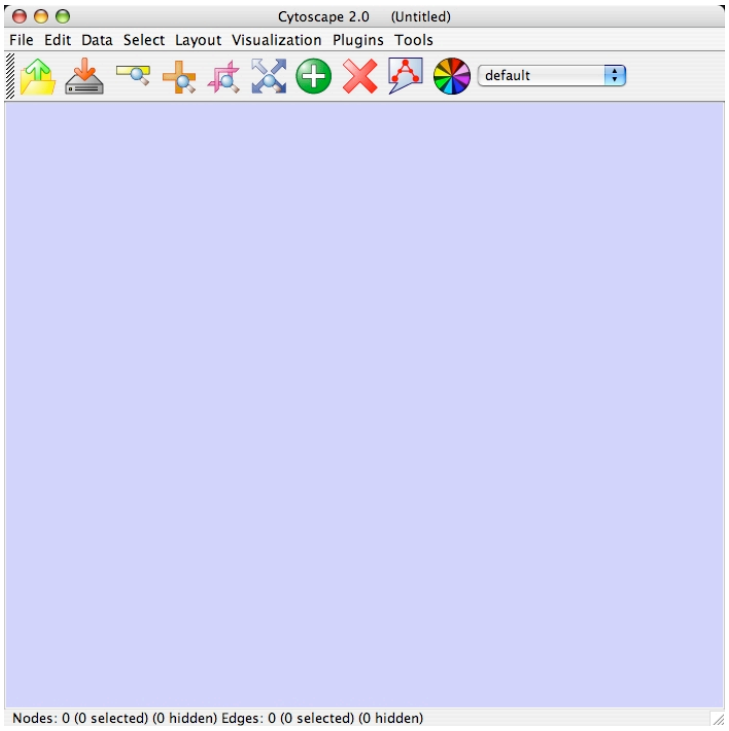
(2) If necessary, install Java. If not already installed on your computer, download and install the Java 2 Runtime Environment, version 1.4.1 or higher. It can be found at:

http://java.sun.com/j2se/index.jsp

(3) Launch the application by running "cytoscape.sh" from the command line (Linux or Mac OS X) or double-clicking "cytoscape.bat" (Windows). Alternatively, you can pass the .jar file to Java directly using the command "java -jar cytoscape.jar". In Windows, it is also possible to directly double-click the .jar file to launch it. However, this does not allow specification of command-line arguments (such as the location of the GO data directory, see the *Command Line Arguments* section for details).

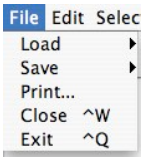
> **! Important Note:**
> For the application to work properly, ALL FILES MUST BE LEFT IN THE DIRECTORY IN WHICH THEY ARE UNPACKED. The core Cytoscape application assumes this directory structure when looking for certain files, such as cytoscape.props, vizmap.props, and the GO/ database].

Cytoscape Window
When you succeed in launching Cytoscape, a window will appear that looks like this:



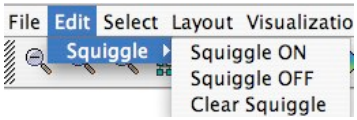
3. Quick Tour of The Menus

File



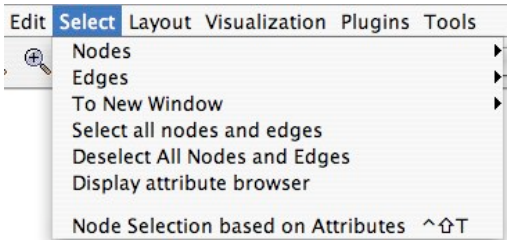
The File menu contains most basic file functionality: File / Load for loading files; File / Save for saving; File / Print for printing to either a printer or a PostScript file. File / Close closes only this window of Cytoscape, leaving other Cytoscape windows open. If this is this is the last open Cytoscape window, File / Close also exits Cytoscape. File / Exit closes all windows of Cytoscape and exits the program. Details of the Load and Save sub-menus can be found in the *Building and Storing Networks* section.

Edit



The Edit menu contains a Squiggle feature that enables you to mark up your network. This can be particularly useful during live presentations.

Select

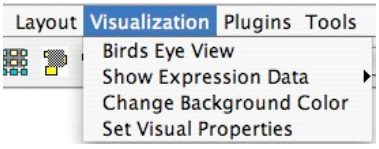


The Select menu contains methods and operations for selecting nodes and edges, operating on existing selections, and displaying the attribute browser. More details about the Nodes, Edges, and To New Window sub-menus can be found in the *Selection and Filtering* section.

Layout

The Layout menu has an array of features for organizing the graph visually; these features are explored in-depth in the *Visualization* section. The main features include arranging the entire graph according to one of several algorithms; aligning and rotating groups of nodes; and adjusting the size of the graph.

Visualization



The Visualization menu provides options for changing the mapping from biological data to a visual representation: colors of nodes, thickness of edges, etc. These features are explored in-depth in the *Visual Styles* section. This menu also provides a Bird's Eye view of your entire graph, and multiple options for viewing expression data.

Plugins

The Plugins menu will contain all plug-ins that you have chosen to load in your cytoscape.props file.

Note: A complete list of Cytoscape PlugIns is available online at: http://cytoscape.org/plugins.html

4. Command Line Arguments

Cytoscape recognizes a number of optional command line arguments, including run-time specification of network files and expression data:

-g <GML network filename>	(xxx.gml) Loads a network file in GML format (see *5. Building and Storing Interaction* Networks)
-i <SIF interactions filename>	(yyy.sif) Loads a network file in SIF format (see *5. Building and Storing Interaction* Networks)
-b <bioData directory>	(e.g. GO/annotationsAndSynonyms) Specifies which directory to use for the BioDataServer annotations.
-e <expression filename>	(zzz.pvals) Loads an expression data file (see *6. Loading Gene Expression* Data)
-x	Prevents expression file from also loading into Cytoscape's *7. Node* and Edge Attributes.
-n <nodeAttributes filename>	(zero or more) Loads a node attributes file (see *7. Node and Edge* Attributes)
-j <edgeAttributes filename>	(zero or more) Loads an edge attributes file (see *7. Node and Edge* Attributes)
-h	help: display these command line arguments
-v	display version
--JLD	specifies a directory in which plug-in .jar's reside.

Most data sets may also be loaded after Cytoscape is running. See the sections on *6. Loading Gene Expression* Data and *7. Node and Edge* Attributes for details.

Additional command line arguments that are not recognized by the Cytoscape core are passed to the plug-in modules. Please refer to the documentation for each specific plug-in for more details.

5. Building and Storing Interaction Networks

Cytoscape reads an interaction network in two ways: from a simple interaction file (SIF or .sif format) or from a universal format known as Graph Markup Language (GML or .gml format). SIF specifies nodes and interactions only, while GML stores additional information about network layout and allows network data exchange with a variety of other network display programs. Typically, SIF is used to import interactions when building a network for the first time. Once the interactions have been loaded and layout has been performed, the network may be saved to and subsequently reloaded from GML format in future Cytoscape sessions. Both SIF and GML are implemented as ASCII text files.

SIF FORMAT:
Lines in the SIF file specify a source node, an interaction type, and one or more target nodes:

```
geneA <interaction type> geneB
geneC <interaction type> geneA
geneD <interaction type> geneE geneF geneB
geneG
...
geneY <interaction type> geneZ
```

In the network specified by this file, genes are represented by nodes, and interactions are represented by edges between nodes. For compactness, a gene also represents its corresponding protein. Nodes may also be used to represent compounds and reactions instead of genes, but this is non-standard, as yet. Note that it is possible to specify an isolated node with no interactions, as in the line "geneG" above.

Gene names must be unique. If the network is to be integrated with Gene Ontology (GO) or gene expression data, the gene names must exactly match the systematic ORF names specified in the other data files. We strongly encourage naming genes and proteins by their systematic ORF name; common names may be displayed on the screen for ease of interpretation, so long as these are available to the program in the bioData directory (Cytoscape ships with all yeast ORF-to-common name mappings in a synonym table within the GO/ directory).

The tag `<interaction type>` must be one of:
- `pp` ..... protein -- protein
- `pd` ..... protein -> DNA

Additional interaction types are also possible, but as yet, nonstandard:
- `pr` ..... protein -> reaction
- `rc` ..... reaction -> compound
- `cr` ..... compound -> reaction
- `gl` ..... genetic lethal relationship

Any text string will work, but these are the conventions that have been followed thus far.

GML FORMAT:

In contrast to SIF, GML is a rich graph format language supported by many other graph visualization packages. Its file format specification is available at:

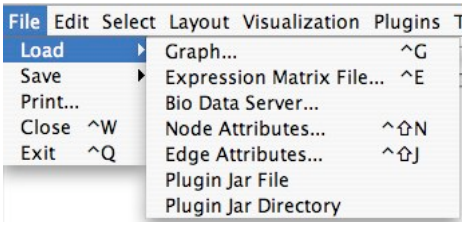
http://www.infosun.fmi.uni-passau.de/Graphlet/GML/

It is generally not necessary to modify the content of a GML file directly. Once a network is built in SIF format and then laid out, the layout is preserved by saving to and loading from GML.

COMMANDS:

Load and save network files using the File menu of Cytoscape. Network files may also be loaded directly from the command line using the –i (SIF format) or -g (GML format) options.

FOR EXAMPLE:



To load a sample molecular interaction network in SIF format, use the menu File / Load / Graph. In the resulting file dialog box, select the file "sampleData/galFiltered.sif". After a few seconds, a small network of 329 nodes should appear in the main window. To load the same interaction network as a GML, use the menu: File / Load / Graph again. In the resulting file dialog box, select the file "sampleData/galFiltered.gml". As of Cytoscape version 1.1, Plug-ins can also be loaded from the File / Load menu, as can node and edge attribute files.

6. Loading Gene Expression Data

Interaction networks are certainly useful as stand-alone models. However, they are most powerful when integrated with information about the biological states that are induced by the network, such as gene or protein expression levels. Once loaded, expression ratios/levels may be visually superimposed on the network, used in a filter to select a subset of nodes, or used to identify active modules and subsystems (see sections below). Expression data are only relevant once a network has been loaded.

FORMAT:

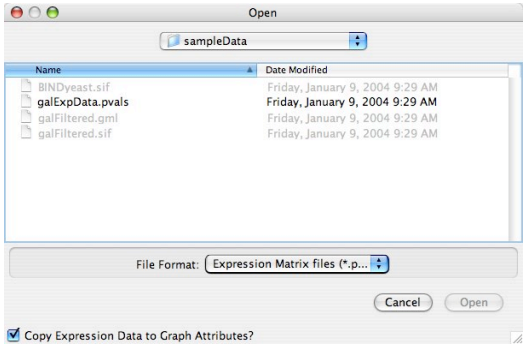
Gene expression ratios are specified over one or more experiments using an ASCII text file. The file consists of a number of space- or tab-delimited fields, one line per gene, with the following format:

```
GeneName [CommonName] ratio1 ratio2 ... ratioN [pval1 pval2 ... pvalN]
```

Brackets [] indicate fields that are optional. The first two fields are the systematic gene name followed by an optional common name. Expression ratios are provided for each experiment, optionally followed by a p-value per experiment or other measure of the significance of each ratio, i.e. whether the ratio represents a true change in expression. Significance values are generated by a variety of software packages for analyzing expression data generated by DNA microarrays, for instance our program VERA (http://www.systemsbiology.org/VERAandSAM). A list of other microarray analysis packages is available at: http://linkage.rockefeller.edu/wli/microarray/soft.html

COMMANDS:
Load an expression data file using the File menu of Cytoscape, or by specifying the filename using the -e option at the command line. The –x command line option indicates that the expression data should not be loaded into node attributes. This is an advanced option, and is typically only used when the number of expression conditions is sufficiently large that it becomes unwieldy in the normal user interface.

FOR EXAMPLE:
Load a sample gene expression data set using the menu: File / Load / Expression Matrix File. In the resulting file dialog box (shown at right), select the file "sampleData/galExpData.pvals". As described in the following sections, Cytoscape is now ready to integrate these data with the underlying molecular interaction network. **Note:** the checkbox in the lower left corner of the file dialog asks whether to "Copy Expression Data to Graph Attributes" – un-checking this box has the same effect is as the command line option -x, and it is left checked by default.



7. Node and Edge Attributes

Node and edge attribute files are very simply formatted: A node attribute file begins with the name of the attribute on the first line, and on each following line, has the name of the node, followed by an equals sign, followed by the value of that attribute. For example:

```
FunctionalCategory
YAL001C = metabolism
YAR002W = apoptosis
YBL007C = ribosome
```

An edge attribute file has much the same structure, except that the name of the edge is the source node name, followed by the interaction type in parentheses, followed by the target node name. Directionality counts, so switching the source and target will refer to a different (or perhaps non-existent) edge. Following is an example edge attributes file:

```
InteractionStrength
```

```
YAL001C (pp) YBR043W = 0.82
YMR022W (pd) YDL112C = 0.441
YDL112C (pd) YMR022W = 0.9013
```

Note that the second and third edge attribute values refer to two different edges (source and target are reversed, though the nodes involved are the same).

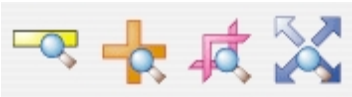
Node and edge attributes may be loaded at the command line using the –n and –j options, via the File / Load menu, or using Ctrl-Shift-N and Ctrl-Shift-J.

When expression data is loaded using an expression matrix file (*6. Loading* Gene Expression Data), it is automatically copied into the Node Attributes data structure unless explicitly specified not to.

Edge and Node attributes can be mapped to visual properties (colors, shapes, etc.) using Visual Styles (see Section 9).

8. Navigation and Layout

BASIC FEATURES:
Use the zooming buttons located on the toolbar to zoom in / out of the interaction network shown in the current network display. Zoom icons are detailed below:



From Left to Right:

- Zoom In
- Zoom Out
- Zoom Selected Region
- Zoom out to Display all of Current Graph

You can also zoom in/out by right clicking and moving the mouse to the right (zoom in) or left (zoom out).

Use the left mouse button to select a node (hold down the Shift key to select more than one node). Use the right mouse button to launch a context sensitive menu with additional information about the node.

NETWORK LAYOUT:
To lay out your network using a Spring Embedded Layout, select Layout → Apply Spring Embedded Layout from the main menu. Sample screenshot is provided below:

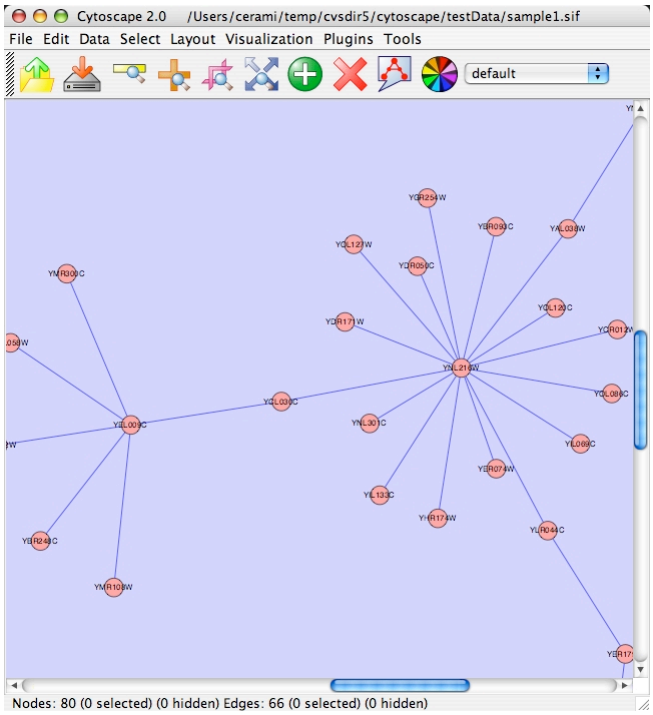


Figure: Applying the Spring Embedded Layout to a sample graph.

9. Visual Styles

With the Cytoscape Visual Style feature, you can easily customize the visual appearance of your graph. For example, you can specify a default color and shape for all nodes, use specific line types to indicate different types of interactions, or visualize gene expression data using a color gradient. All these features are available by selecting Visualization → Set Visual Properties from the main menu or clicking on the color wheel in the main button bar menu.

9.1 Introduction to Visual Styles

The Cytoscape distribution you have downloaded includes three predefined visual styles to get you started. To demonstrate these styles, try out the following example:

- Load a sample graph: From the main menu, select File → Load → Graph, and select sampleData/galFiltered.sif.

- Load a sample set of expression data: From the main menu, select File → Load → Expression Matrix File, and select sampleData/galExpData.pvals.

By default, the Visual Style labeled "default" will be automatically applied to your graph. This default style has a blue background, circular pink nodes, and blue edges (see sample screenshot below).

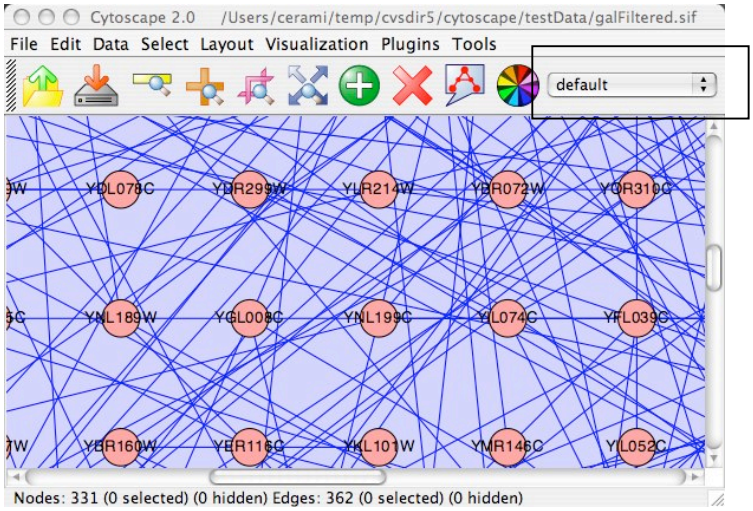


Figure: Using the default Visual Style.

The VizMap.props File: All Cytoscape Visual Style settings are automatically stored in a file called vizmap.props. Upon startup, Cytoscape will first try to locate the vizmap.props file in the "user home" directory. For example, on Windows XP, this corresponds to the user "Documents and Settings" directory, e.g. c:\Documents and Settings\cerami. On Linux or Mac OS X, this corresponds to the user home directory, e.g. /Users/cerami. If no vizmap.props file is found in the user's home directory, Cytoscape will next search the current local directory.

[!] If you are upgrading from Cytoscape 1.1: If you are upgrading from Cytoscape 1.1, you may have an existing vizmap.props file in your home directory. If this is the case, you will not have the sample1 and sample2 visual styles described below. To get around this issue, backup your current vizmap.props file to safe place, and copy the new Cytoscape 2.0 vizmap.props file to your home directory.

You can flip through different visual styles by making a selection from the Visual Style pull down menu. For example, if you select "Sample1", a new visual style will be applied to your graph, and you will see a green background and round blue nodes. Additionally, protein-DNA interactions (specified with the label: pd) are drawn with dashed edges, whereas protein-protein interactions (specified with the label: pp) are drawn with solid edges (see sample screenshot below).

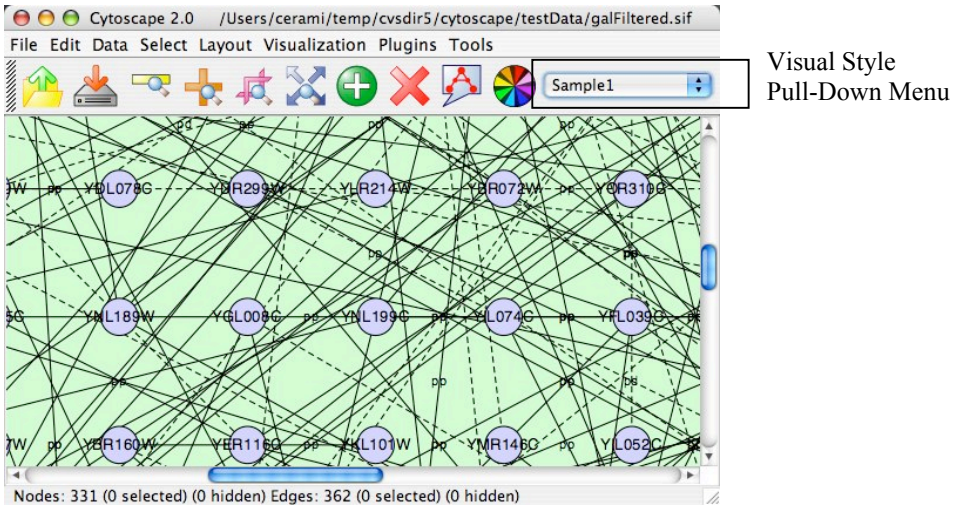


Figure: Using the Sample1 Visual Style. Protein-Protein interactions (solid lines) are now distinguishable from Protein-DNA interactions (dashed lines).

Finally, if you select "Sample2", gene expression values for each node will be colored along a color gradient between red and green (see sample screenshot below).

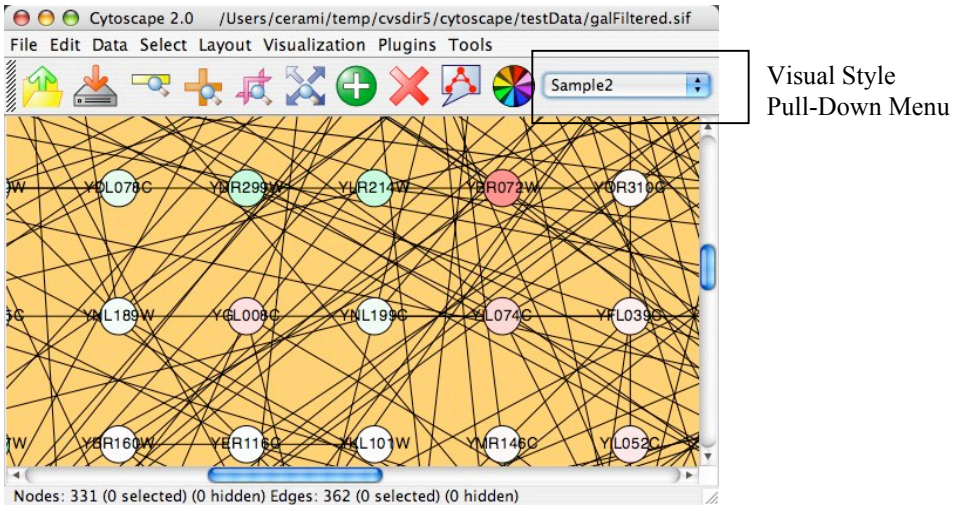


Figure: Using the Sample2 Visual Style. Gene expression values are now displayed along a red/green color gradient.

9.2 Visual Attributes, Graph Attributes and Visual Mappers

The Cytoscape Visual Mapper has three core components: *visual attributes, graph attributes* and *visual mappers*:

- A *visual attribute* is any visual setting that can be applied to your graph. For example, you can change all nodes to squares by setting the node shape visual property.

- A *graph attribute* is any attribute associated with a node or an edge. For example, each edge in a graph may be associated with a label, such as "pd" (protein-DNA interactions), or "pp" (protein-protein interactions).

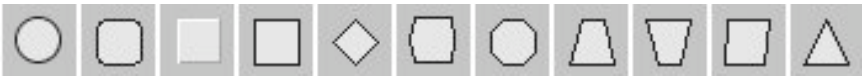
- A *visual mapper* maps graph attributes to visual attributes. For example, a visual mapper can map all protein-DNA interactions to the color blue, and all protein-protein interactions to the color red.

Cytoscape includes a large number of visual attributes. These are summarized in the tables below.

Visual Attributes Associated with Nodes:

- Node Color
- Node Border Color
- Node Border Type. The following options are available:

- Node Shape. The following options are available:



- Node Size: width and height of each node.
- Node Label: the text label for each node.
- Node Font: node font and size.

Visual Attributes Associated with Edges:

- Edge Color
- Edge Line Type. The following options are available:

- Edge Source Arrow. The following options are available:

- Edge Target Arrow. The following options are available:

- Edge Label: the text label for each edge.
- Edge Font: edge font and size.

Global Visual Properties:

- Background Color

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

- **Passthrough Mapper:** graph attributes are passed directly through to visual attributes. A passthrough mapper only works for node / edge labels. For example, a passthrough mapper can draw the gene name on all nodes.
- **Discrete Mapper:** discrete graph attributes are mapped to discrete visual attributes. For example, a discrete mapper can map all protein-protein interactions to the color blue.
- **Continuous Mapper**: continuous attributes are mapped to visual attributes. Depending on the visual attribute, there are two types of continuous mappers:
    - **continuous to continuous mapper**: for example, you can map a continuous value (0..1) to a color gradient (red..green) or node/font size (10..100).
    - **continuous to discrete mapper**: for example, all values below 0 are mapped to square nodes, and all values above 0 are mapped to circular nodes. However, there is no way to smoothly morph between circular nodes and square nodes.

The matrix below shows visual mapper support for each visual property.

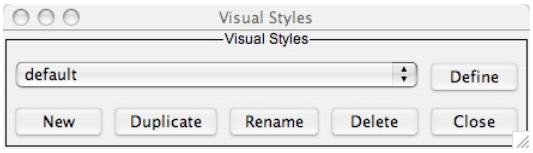
	Passthrough Mapper	Discrete Mapper	Continuous Mapper

Node Properties			
Node Color	⊘	●	●
Node Border Color	⊘	●	●
Node Border Type	⊘	●	◗
Node Shape	⊘	●	◗
Node Size	⊘	●	●
Node Label	●	●	◗
Node Font Family	⊘	●	◗
Node Font Size	⊘	●	●
Edge Properties			
Edge Color	⊘	●	●
Edge Line Type	⊘	●	◗
Edge Source Arrow	⊘	●	◗
Edge Target Arrow	⊘	●	◗
Edge Label	●	●	◗
Edge Font Family	⊘	●	◗
Edge Font Size	⊘	●	●

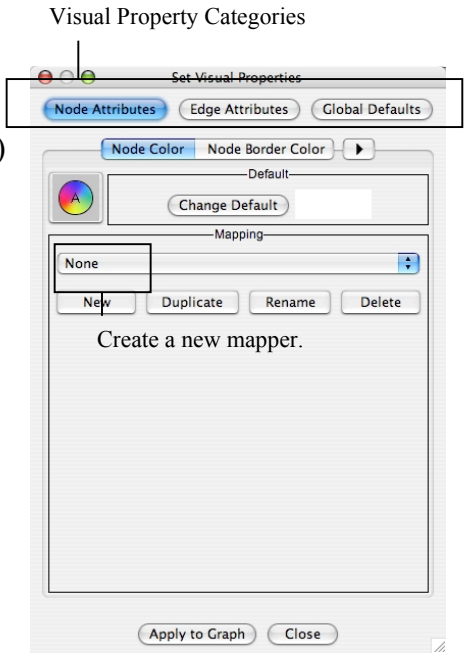
Legend	
⊘	Mapping is not supported for specified visual property.
●	Mapping is fully supported for specified visual property.
◗	Mapping is partially supported for specified visual property. Support for "continuous to continuous" mapping is not supported.

9.3 Tutorial: Creating a New Visual Style

To create a new visual style, select Visualization → Set Visual Properties from the main menu, or select the color wheel icon in the main button bar. You will now see a new Visual Styles dialog box (shown at right.)



Click the New button, and enter a name for your new visual style when prompted. Then click the Define button. You will now see the main Visual Styles Properties dialog box (shown at right.)

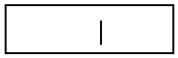


From this dialog box, you can flip between Node Attributes, Edge Attributes, and Global Defaults. You can also specify default values for any visual property, or define a new custom mapping.

For example, to set the default node shape to triangles, select Node Attributes → Node Shape. Then, click the "Change Default" button, and select the Triangle icon from the selection list.

Applying Changes to the Graph

To apply your visual style to your graph, hit the "Apply to Graph" button, available in the bottom of the dialog panel.

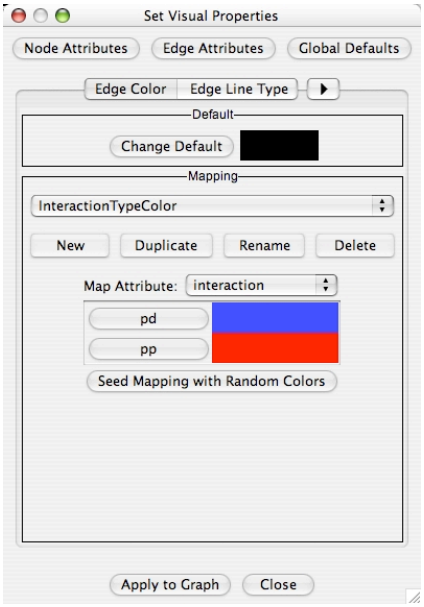


Select the Apply button to apply your newly revised style to the graph.

Saving a Visual Style

When you exit Cytoscape, new visual styles or newly modified visual styles will automatically be saved in the vizmap.props file. You can therefore create a new visual style and apply it to all future graphs.

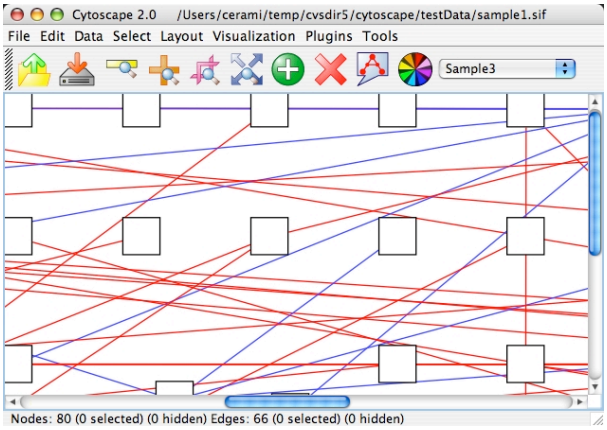
9.4 Tutorial: Creating a New Discrete Mapper



The following tutorial demonstrates how to create a new discrete mapper. The goal is to draw Protein-DNA interactions with blue edges, and Protein-Protein interactions with red edges.

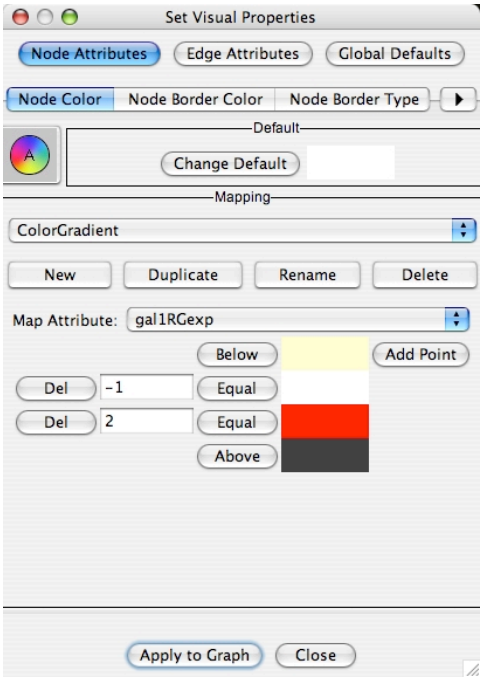
- Load a sample graph: From the main menu, select File → Load → Graph, and select sampleData/galFiltered.sif.
- Select Visualization → Set Visual Properties.
- Select "New" to create a new Visual Style. Name your new style: "Sample3".
- In the "Set Visual Properties" Dialog box, select Edge Attributes → Edge Color.
- Click the New button in the mapping panel.
- You will be prompted to select a mapping type: passthrough mapper, discrete mapper or continuous mapper (for an overview of the differences between these mappers, please refer back to section 8.2.) Select "discrete mapper", and enter a descriptive name. For example, enter: "InteractionTypeColor".
- From the "Map Attribute" pull-down menu, select "interaction." You should now see two buttons, one for pd (Protein-DNA interactions), and one for pp (Protein-Protein interactions).
- Click the "pd" button and select a blue color.
- Click the "pp" button and select a red color.
- Click the "Apply to Graph" button.

You graph should now show "pd" interactions in blue, and "pp" interactions in red. Sample screenshot is provided at right



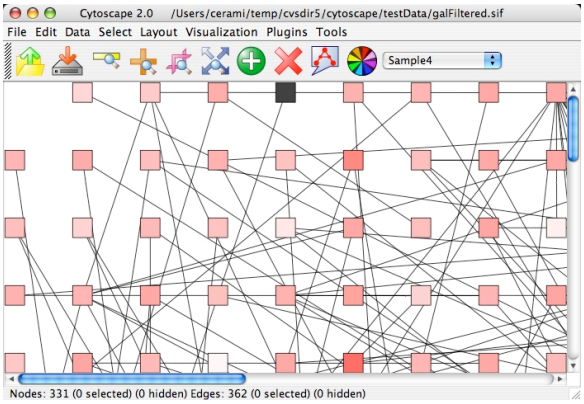
9.5 Tutorial: Visualizing Expression Data on a Network

The following tutorial demonstrates how to create a new continuous mapper. The goal is to superpose gene expression data onto a network, and to display gene expression values along a color gradient.



- Load a sample graph: From the main menu, select File → Load → Graph, and select sampleData/galFiltered.sif.
- Load a sample set of expression data: From the main menu, select File → Load → Expression Matrix File, and select sampleData/galExpData.pvals.
- Select Visualization → Set Visual Properties.
- Select "New" to create a new Visual Style. Name your new style: "Sample4".
- In the "Set Visual Properties" Dialog box, select Node Attributes → Node Color.
- Click the New button in the mapping panel.
- You will be prompted to select a mapping type: passthrough mapper, discrete mapper or continuous mapper (for an overview of the differences between these mappers, please refer back to section 8.2.) Select "continuous mapper", and enter a descriptive name. For example, enter: "ColorGradient".
- From the "Map Attribute" pull-down menu, select "gal1RGexp."
- Click the "Add Point" button twice to add two data points.
- Set the first point to "-1", Below = Yellow, Equal = White.
- Set the second point to "2", Equal = Red, Above = Black.
- Click the "Apply to Graph" button.

This visual mapper will set all nodes with a gal1RGexp value less than –1 to Yellow, and all nodes with a gal1RGExp value greater than 2 to Black. Additionally, all values between –1 and 2 will be painted with a white/red color gradient. Sample screenshot is shown at right.



10. Acknowledgements

Cytoscape is built with a number of open source 3rd party Java libraries. The Cytoscape team gratefully acknowledges the following libraries:

- The Colt Distribution: Open Source Libraries for High Performance Scientific and Technical Computing in Java. Information is available at: http://hoschek.home.cern.ch/hoschek/colt/.

- GNU Getopt in Java. Information is available at: http://www.urbanophile.com/arenn/hacking/download.html.

- Graph INterface librarY a.k.a. GINY. Information is available at: http://csbi.sourceforge.net/.

- JDOM. Information is available at: http://jdom.org.

- JUnit. Information is available at: http://junit.org.

- JGoodies Looks. Information is available at: http://www.jgoodies.com/freeware/looks/index.html.

- Piccolo. Information is available at: http://www.cs.umd.edu/hcil/jazz/.

- Type-Specific Collections Library, from Sosnoski Software Solutions, Inc. Information is available at: http://www.sosnoski.com/opensrc/tclib/.

- Xerces Java XML parser. Information is available at: http://xml.apache.org/xerces-j/.

This product includes software developed by the Apache Software Foundation (http://www.apache.org/).

This product includes software developed by the JDOM Project (http://www.jdom.org/).

Appendix: GNU Lesser General Public License

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Version 2.1, February 1999

[This is the first released version of the Lesser GPL. It also counts as the successor of the GNU Library Public License, version 2, hence the version number 2.1.]

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