AutoSOME Version 1.0 Documentation

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Introduction

AutoSOME is a powerful new unsupervised clustering method that identifies clusters of diverse geometries from potentially large, multi-dimensional, datasets without prior knowledge of cluster number or structure. In addition, fuzzy relationships among data points in complex, noisy datasets like microarrays can also be detected by AutoSOME and usefully visualized as two-dimensional fuzzy cluster networks. We have implemented the AutoSOME method as a Graphical User Interface (GUI) and command line tool to facilitate its use by the academic research community. Below, instructions are provided for using both versions of AutoSOME, and a protocol is given for generating fuzzy cluster networks in Cytoscape [Shannon et al., 2005].

System Requirements

AutoSOME is coded in Java to promote platform independence. To launch the GUI from the AutoSOME website [http://jimcooperlab.mcdb.ucsb.edu/autosome], Java Web Start technology needs to be installed on your computer along with Java Standard Edition 1.6, both of which are freely available at http://java.sun.com. The command line version of AutoSOME will run in JAVA 1.5 and perhaps earlier versions. In general, the more memory and processors available, the better the performance of AutoSOME. Without user intervention, both the GUI and command line versions will greedily use all available CPUs. For 32-bit Windows systems, the maximum amount of memory that can be allocated to AutoSOME is about 1.6 GB, while 64-bit operating systems with 64-bit Java can allocate up to ~30 GB of memory. Microarray datasets like the HG U133 Plus 2.0 Affymetrix chipset (>54k probes) with dozens of samples can be run with 1.6 GB RAM.

The 'Java Web Start' launch button on the 'AutoSOME Web Portal' homepage will automatically allocate up to 1 GB of RAM. To run AutoSOME with more or less memory, download the .jar file from: http://jimcooperlab.mcdb.ucsb.edu/autosome/downloads.

Launching AutoSOME from the Command Line

To invoke the AutoSOME GUI from the command line, type:

java –Xmx1600m –Xms1600m –jar autosome.jar

The -Xmx,-Xms arguments allocate additional memory to the Java Virtual Machine necessary for running large datasets. Allocate more or less memory as needed for your input dataset, parameters, and machine architecture.

To run AutoSOME without the GUI, enter an input file (and possible parameter arguments):

java –Xmx1600m –Xms1600m –jar autosome.jar myInput.txt

See *AutoSOME Command Line Version* below for all parameters and definitions.

Using AutoSOME

Input Format
The input file is a table of numerical values with optional column labels (row 1) and mandatory row labels (column 1). If column labels are specified, column 1 also needs a label in row 1. All entries must be tab, comma, or space delimited (if your input is space-delimited, make sure there are no spaces in your labels). Input data can be easily formatted using Microsoft Excel. See Table 1 below for an example.

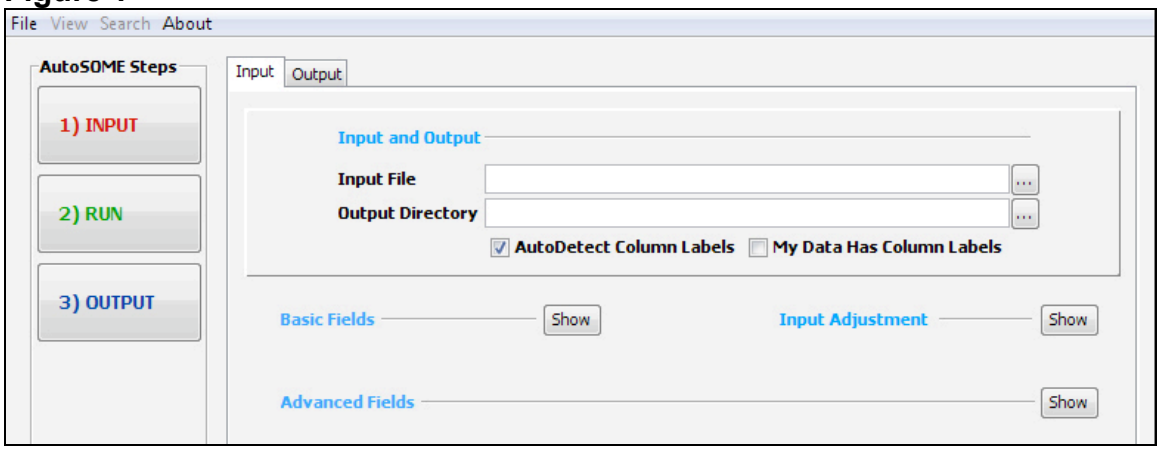
Table 1

Probe	hESC-1	hESC-2	hESC-3	hESC-4	iPSC-1	iPSC-2	iPSC-3
212853_at	8.221449	8.297634	7.694108	8.215596	10.1284	10.25488	10.21546
212854_x_at	8.523748	8.706556	8.044123	8.992252	8.87927	8.974617	9.083473
212855_at	10.64296	10.4093	10.60148	11.03563	12.08599	11.91321	12.04592
212856_at	8.936721	9.218977	9.109346	8.425392	8.431269	8.418733	8.450901
212857_x_at	6.195244	5.929077	4.432911	6.764105	4.344787	4.786857	4.953329
212858_at	7.795604	7.565154	7.744513	7.58388	8.657005	8.613815	8.781187
212859_x_at	10.26586	10.44094	9.274517	9.467813	9.218634	9.221389	9.472309
212860_at	6.178299	6.260259	6.218979	6.416278	6.758869	6.275902	6.270324
212861_at	14.36244	14.37399	14.52238	14.38661	14.50383	14.52275	14.45975

AutoSOME GUI

The AutoSOME GUI is launched using Java Web Start from your browser or via the command line, and is used to run AutoSOME as well as to browse and generate publication quality visualizations of the cluster output (see Figure 1).

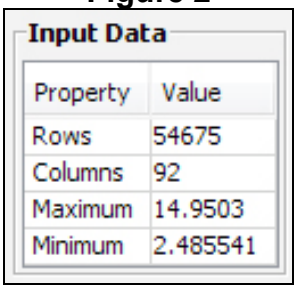
Figure 1



Load Input

To begin, load your properly formatted input file (see Input Format above) by either pressing the large '**INPUT**' button in the '**AutoSOME Steps**' panel on the top left, or by clicking the browse button adjacent to the input file text box in the '**Input and Output**' section (see Figure 1). The GUI will attempt to automatically detect the presence of column labels. If the input has column labels containing numerical data, select the '**My Data Has Column Labels**' checkbox. Once loaded, the output directory is automatically shown, and can be changed by clicking the browse button next to the output directory text box. The GUI will deploy an error message if the input file is incorrectly formatted.

Figure 2



After loading, the number of rows and columns will be displayed in the '**Input Data**' panel on the left side of the GUI (see Figure 2). The maximum and minimum values over the entire dataset are also provided. When finished clustering, this table will dynamically update based on the contents of selected clusters in the cluster tree (see Output below).

Input Adjustment

AutoSOME implements several input normalization methods in addition to log2 scaling. For an excellent overview of these techniques, see the manual to the Cluster software available at http://rana.lbl.gov/manuals/ClusterTreeView.pdf [Eisen et al., 1998]. Press the '**Show**' button to the right of the '**Input Adjustment**' label to access input scaling and normalization options (see Figure 3). All input adjustment operations are performed in the order listed in the GUI, from top to bottom. In brief:

i) Log2 Scaling: This is routinely used for microarray datasets to amplify small fold changes in gene expression, and is completely reversible.

All other implemented input adjustment methods irreversibly change the input to make it more suitable for analysis.

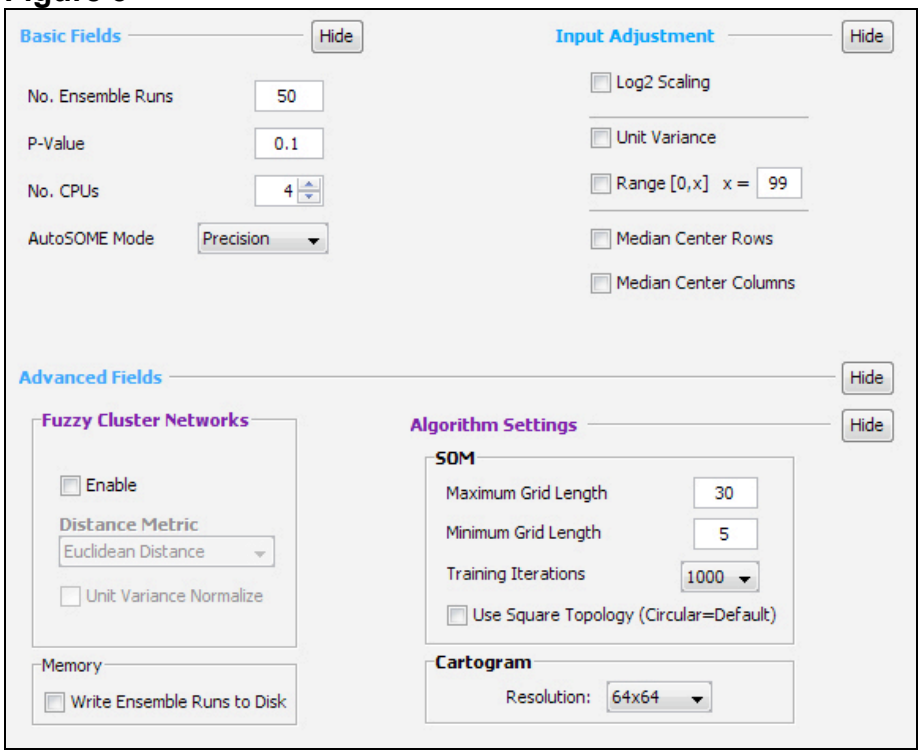
ii) Unit variance: forces all columns to have zero mean and a standard deviation of one, and is commonly used when there is no *a priori* reason to treat any column differently from any other.

iii) Range [0,x]: Alternatively, data in all columns can be normalized to share lowest and highest values (0,*x*) by specifying an upper bound *x*.

iv) Median Center Rows/Arrays: For microarray analysis, median centering genes (rows) and/or arrays (columns) eliminates amplitude shifts to highlight the most prominent patterns in the expression dataset.

As an example, to run AutoSOME co-expression analysis, one may apply log2 scaling, unit variance normalization, and median-centering of genes (and arrays). To run AutoSOME transcriptome clustering (fuzzy cluster networks, see tutorial below), it is generally recommended to apply unit variance normalization to your dataset. Other normalization settings may also be desirable. Input can also be adjusted using Microsoft Excel or the Cluster software [Eisen et al., 1998] and then imported into AutoSOME.

Figure 3



Basic Fields

Press the '**Show**' button to expand the '**Basic Fields**' section (see Figure 3).

i) Ensemble Runs: The default of 50 ensemble iterations should be sufficient to begin investigating the cluster structure of most datasets. Although in practice, AutoSOME clustering results can be quite stable with ~50

ensemble iterations (and even as little as 20), for final clustering results, it is recommended to increase this number to at least 100.

ii) P-Value: AutoSOME has been extensively benchmarked on a highly diverse array of clustering problems using a P-value cutoff of 0.1. Reduce the p-value threshold to identify tighter clusters.

iii) No. CPUs: To liberate processor resources, decrease 'No. CPUs'. AutoSOME running time will decrease approximately linearly with respect to the number of dedicated CPUs.

iv) AutoSOME Mode: This parameter alters advanced AutoSOME algorithm settings to switch between 'Precision' and 'Speed' modes of operation. 'Precision' takes longer, but provides greater training of the SOM node lattice (2X1000 iterations) and greater resolution for density-equalization of the SOM error surface (64X64). For enhanced performance, and especially for first-pass exploratory cluster analysis, choose 'Speed' for less SOM iterations (2X500) and less resolution for density-equalization (32X32). In our experiments, 'Speed' works quite well, and in fact, often yields comparable results to 'Precision'. In addition, 'Speed' is roughly 4 times faster.

Advanced Fields

Fuzzy Cluster Networks. To cluster columns (e.g. individual microarrays), select the 'Enable' checkbox (see Figure 3). Then, pick the distance metric for calculating the input distance matrix. Euclidean distance is selected by default. See the review by D'haeseleer (2005) for descriptions of Euclidean, Pearson's and Uncentered correlation distance metrics. In brief, Euclidean distance is sensitive to amplitude shifts while correlation is not. Experiment with these metrics to get a feel for how they work. If smoothing out the distance matrix is desired, 'Unit Variance Normalize' can be selected to normalize the distance matrix columns to unit variance. When finished clustering, AutoSOME will automatically generate additional output files for building fuzzy cluster networks in Cytoscape (see Fuzzy Cluster Networks below).

Memory. Write Intermediate Runs to Disk: To decrease RAM consumption, the results from individual ensemble iterations can be written to disk instead of memory. This may be necessary on systems with insufficient RAM to process large datasets over many ensemble iterations (e.g. 45k probes, 100 samples, and 100 ensemble iterations), however, overall running time will be slower due to reading and writing to disk. If AutoSOME crashes (or seems to hang for a long period of time), then 'Write Intermediate Runs to Disk' should be selected (see Figure 3) as an out of memory error is likely. All intermediate files will be written to a temporary folder in the output directory.

Algorithm Settings. Press the '**Show**' button to expand the '**Algorithm Settings**' section (see Figure 3). The GUI permits modification of the most critical algorithm

parameters. For access to all algorithm parameters, use the command line version of AutoSOME.

SOM.

i) Maximum Grid Length: The maximum number of nodes for the x/y length of the SOM node lattice.

ii) Minimum Grid Length: The minimum number of nodes for the x/y length of the SOM node grid.

In general increase 'Maximum Grid Length' to provide greater resolution for cluster separation in the SOM, and to increase the number of possible clusters. Increase 'Minimum Grid Length' to force AutoSOME to use an SOM lattice with specific minimum dimensions. Set both parameters to the same value to disable automatic adjustment of grid length by the algorithm. The SOM grid length is set, by default, between 5 and 30 for a balance between practical running time, memory consumption, and accurate clustering. *If a dataset with at least 10,000 rows is imported (e.g. whole-genome microarray), the maximum grid length will be automatically set to 20 for more efficient running time.*

iii) Training Iterations: The No. iterations for training the SOM node lattice. The value of this parameter dictates the number of iterations for each of two phases, coarse-grained followed by fine-grained training. This value can be toggled between '1000' iterations for accuracy and '500' iterations for speed.

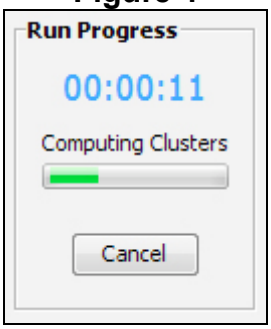
iv) Use Square Topology: Select this checkbox to use a square-shaped node lattice rather than a circular node lattice (default). Benchmarking results indicate both topologies yield comparable results. See the Manuscript for more details.

Cartogram. Resolution: The density-equalizing cartogram algorithm requires an input array with dimensions that are a power of two. Although all benchmarking tests were conducted using an array size of 64X64, greater resolutions may yield more accurate density-equalization (especially when the SOM dimensions are large). On the other hand, going from 64X64 to 32X32 will increase running time dramatically (~4X), and is sufficient for accurate clustering results in many cases (especially when the SOM dimensions are less than 30X30).

Running AutoSOME

After the input file and parameters are specified, execute AutoSOME clustering by pressing the green '**RUN**' button in the '**AutoSOME Steps**' panel (see Figure 1). Progress is shown in the lower left corner of the GUI and elapsed time is displayed (see Figure 4). When finished, the GUI will automatically be redirected to the output panel for browsing the cluster output. The input and output panels can be toggled back and forth using the tabs or the '**INPUT**' and '**OUTPUT**' buttons in the control panel.

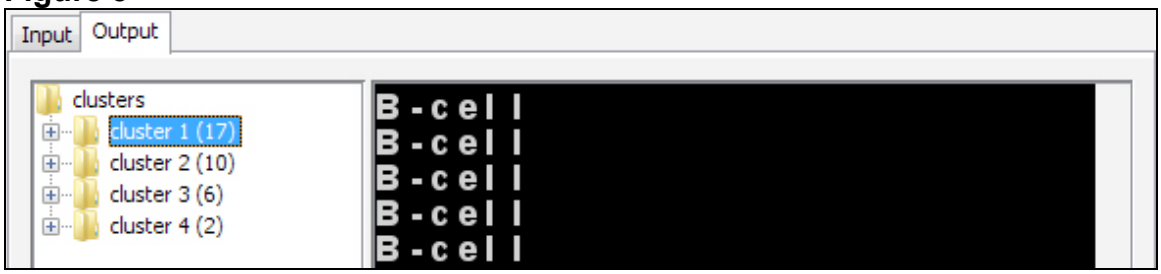
Figure 4



Output

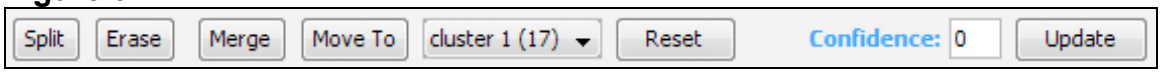
Cluster tree. As shown in Figure 5, all clusters are listed using a dynamic tree structure and are ordered from top to bottom by decreasing size.

Figure 5



Once a cluster node is selected using your mouse, the cluster tree can be rapidly traversed by pressing the '↑' and '↓' keys. To select more than one cluster, hold down the 'Shift' or 'Control' key and select using your mouse. Clusters can be manually modified using the control panel below the cluster tree (see Figure 6). Use 'Split' to make a new cluster from a selected group of data points (first expand a cluster node by double-clicking, then select data points with mouse). Use 'Erase' (or press Delete key) to erase entire clusters or specific data points. Press 'Merge' to combine the contents of all selected clusters into a single cluster. Use 'Move To' for moving selected data points from one cluster to another cluster. Press 'Reset' to return to the original clustering output. To filter clustering results by cluster confidence (metric ranging from 1-100, where 100=data point always in cluster x) type in confidence threshold ≤100 in the '**Confidence**' text box and press 'Update'.

Figure 6



By default, the contents of the selected cluster(s) will be displayed as a list of cluster labels. For additional viewing options, see below.

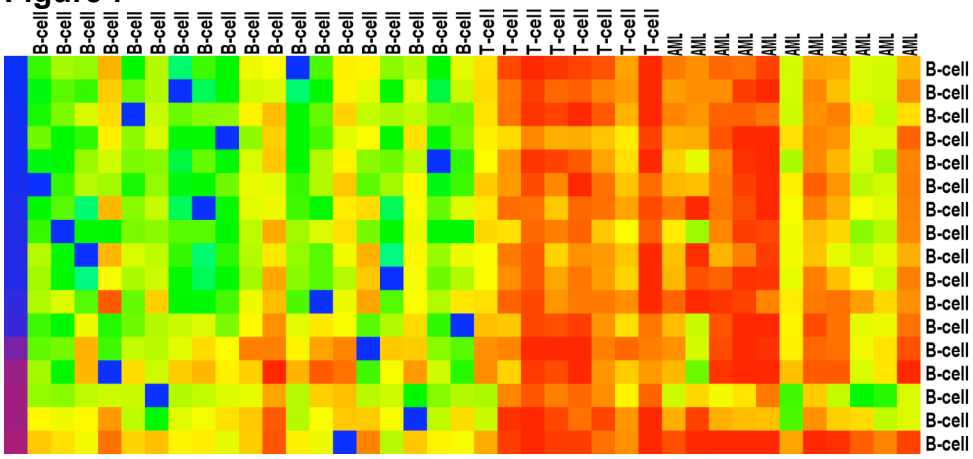
Heatmaps. Several heatmap visualizations are available. Go to 'View' in the menu bar, and select 'green red', 'rainbow', 'gray scale', or 'blue white' from the 'heatmap' submenu. Table 2 summarizes the key attributes of the available heatmap color modes.

Table 2

Heatmap Mode	High Value (R,G,B)	Low Value (R,G,B)	Middle Value(s)
green red	Green (0,255,0)	Red (255,0,0)	Black
rainbow	Red (255,0,0)	Blue (0,0,255)	Blue, Light Blue, Green, Yellow, Red (evenly spaced)
gray scale	Black (0,0,0)	White (255,255,255)	shades of Gray
blue white	Blue (0,0,255)	White (255,255,255)	shades of Blue

The mouse scrollbar is used to zoom in or out. By default, cluster confidence is shown as a vertical bar to the left of the heatmap with blue=high and red=low confidence (see rainbow-colored heatmap in Figure 7 below). For fine-grained control over heatmap visualization parameters, select '**pixel settings**'.

Figure 7



Heatmap>pixel settings. The pixel settings window provides several options for customizing the heatmap display (see Figure 8).

i) Row height: Determines the vertical resolution of each heatmap pixel and can be used to vertically compress the heatmap image.

ii) Contrast: Adjusts the maximum and minimum values used for heatmap display. Contrast *C* increases the original maximum value *M* and decreases the original minimum value *m* by (*C*-1)*(1+*M*-*m*)/2.

   a. Manually adjust range for contrast: Select this option to manually input maximum and minimum values for contrast adjustment. By default, the maximum and minimum values of the entire input dataset are used. To see the maximum and minimum values for a

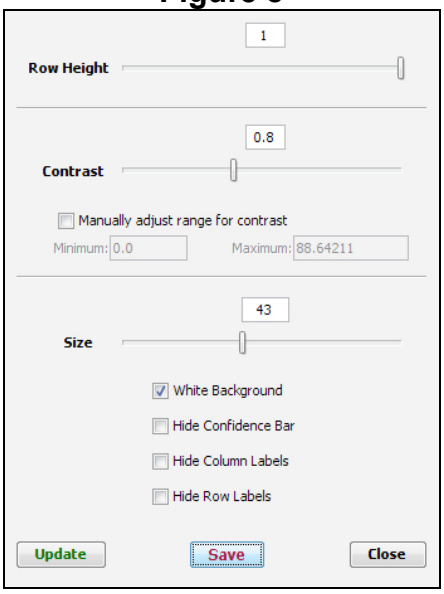
particular cluster (or set of clusters), check the 'Input Data' table on the left panel of the GUI.

iii) Size: This slider controls the size scale of each heatmap pixel and can be used for saving high-resolution images.

iv) Checkboxes: Self-explanatory.

To update the heatmap image after changing parameters, press 'Update'. Click 'Save' to launch a file browser for saving your heatmap output. All images are saved in the Portable Network Graphics (PNG) format. For Windows machines, we recommend appending the .png extension to your file name for easier future access.

Figure 8

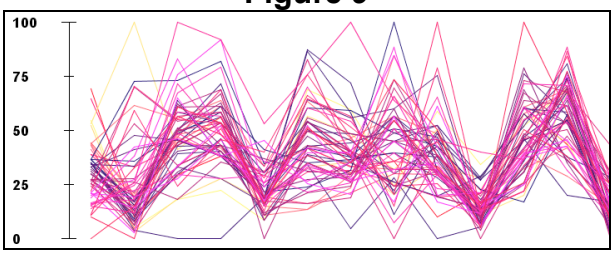


Signal plots. As indicated in Figure 9, signal plots display all numerical vectors in the selected cluster(s) as a line graph across all column categories (x-axis). Go to 'View' in the menu bar, and select 'rainbow' or 'red' from the 'signal plot' submenu.

i) Scale bar: By default, a scale bar is shown on the y-axis. Deselect the 'scale bar' checkbox in View>signal plot to remove. To increase or decrease the scale-bar resolution, use the up and down arrow keys in the number pad (8 and 2), respectively. To change the decimal precision of each number, use the left and right arrow keys in the number pad (4 and 6).

ii) Mean signal: Select the 'mean signal' checkbox in View>signal plot. Collapses signal plot into one line representing the mean of all vectors in the selected cluster(s).

Figure 9



View>Settings. scale using entire dataset: When selected (go to View>settings), the contrast setting in the heatmap, and range of the y-axis for signal plots, are scaled using the maximum and minimum values of the dataset. When deselected, maximum and minimum values are set based on the selected cluster(s). Deselecting is useful for amplifying cluster-specific signals that are otherwise washed out in the context of the entire input dataset.

Search. To find a particular data item in the cluster output, go to 'Search>Find'. This will invoke a search window. Either find your data point from the 'Choose Identifier' list of manually enter your data item identifier, then click 'Submit'. If it is found, your data item will be highlighted in yellow using the heatmap display (you might need to scroll down to find it). Please make sure your data items are uniquely labeled for this feature to work properly.

Saving data.

i) Images: All images can be saved by selecting 'File>Export>save image' from the menu. Make sure to append ".png" to the end of your file name.

ii) Tabular Data: To export tabular data from the selected cluster(s), go to 'File>Export>save tabular data'. This option will save all cluster contents, along with cluster labels and cluster confidence for each data point. The output format is identical to output file 3 (see *Files written to disk* below).

Files written to disk. AutoSOME writes the following files to your hard drive:

1) AutoSOME_inputName_E**x**_Pval**#**_Summary.html = summary table of file name, parameters used, and cluster output table with size of each cluster, mean cluster confidence, and hyperlinks to data content of each cluster.
2) AutoSOME_inputName_E**x**_Pval**#**.html = all clusters and data contents, including individual cluster confidence for each data point.
3) AutoSOME_inputName_E**x**_Pval**#**.txt = text file with same data as file (2). First row = column labels (if provided as input), first column 'CLUST'=cluster label, second column 'CONF'=cluster confidence, third column 'NAME' =data point label, all other columns=data vectors

E**x** = **x** ensemble iterations (e.g. E100), Pval**#** = p-value cutoff (e.g. Pval0.1)

If '**Fuzzy Cluster Networks**' is enabled (see Figure 3), three additional files are written to disk:

4) AutoSOME_inputName_E**x**_Pval**#**_Nodes.txt = All clustered data points: first column= unique data point label, second column=cluster label, third column=original data point label
5) AutoSOME_inputName_E**x**_Pval**#**_Edges.txt = fuzzy edges among data points: first two columns denote connected nodes, third column = pairwise affinity or the fraction of times the two data points were co-clustered (pairwise affinity ranges from -.5, never co-clustered, to 0.5, always co-clustered).
6) AutoSOME_inputName_E**x**_Pval**#**_Matrix.txt = pairwise affinity matrix of all data points compared to all data points; essentially the same information content of output file (5) presented in a form suitable for a heatmap display. This file can be immediately read as input into the Cluster 3.0 software [Eisen et al., 1998] to hierarchically reorder the matrix. Results can be visualized with Java TreeView [Saldanha, 2004].

Since Fuzzy Cluster Networks are computed from a distance matrix of all vertical data vectors (e.g. cell samples), output files (1)-(3) and (6) contain data points represented as a distance matrix (not the original input data).

Opening old clustering results. To browse previous clustering results, adjust the input using 'Input Adjustment' first (e.g. select Unit Variance and Median Centering if these settings were used for clustering), then select 'Open AutoSOME Results' from the 'File' menu. Use the file browser to find output file (3), the text file, and press 'Open'. The GUI will immediately redirect to the 'output' window and display the cluster tree.

AutoSOME Command Line Version

>Usage:
java -jar autosome.jar [Input] [Options]

>maximum JVM memory recommended, e.g.
java -jar -Xmx1600m -Xms1600m -jar autosome.jar input.txt -p.01 -e30

To display all input parameters, run AutoSOME with the parameter –o (letter 'o' for options).

***Make sure to precede all parameters with '-' dash symbol.**

As indicated below, the command line version of AutoSOME has many options not available in the GUI, including the option to use alternative clustering algorithms (i.e K-Means, Hierachical Clustering with four linkage types) and alternative dimensional reduction techniques (i.e. density-equalized SOM, normal SOM, and Sammon's Mapping). For alternative clustering methods, the number of input clusters needs to be specified using the –k parameter (see below). In addition, all of these clustering methods, including AutoSOME, can be benchmarked with the –b option as long as the data item labels in the input file correspond to numerical cluster labels starting with 1 (1,2,…,No. clusters *n*).

parameter || description (default value):

t[integer] || set number of threads (available CPUs)

e[integer] || set number of runs to merge into ensemble (10)

p[0-1] || set p-value threshold for minimum spanning tree clustering (0.1)

D[directory] || set output directory (C:\)

C || read in column headers from first row of input file (false)

v || launch cluster viewer (false)

v2 || display previous clustering results: input=clustering output text file (false)

n[integer] || normalize input by unit variance '-n1', to log2 '-n0' or into range [0,X] '-nX' (false)

j1,j2,j3 || j1=perform median center normalization on all rows;j2=columns;j3=rows and columns

N || normalize input to log2 with unit variance (false)

|| apply unit variance normalization to distance matrix (false)

l[integer] || start reading numerical data from this column, lowest column = 0 (1)

Q || transform columns from input into Euclidean distance matrix (false)

Q2,Q3 || distance matrix metric; Q2 = Pearson's, Q3 = Uncentered Correlation (Euclidean)

b || do benchmarking: 'F-measure, Precision, Recall, NMI, corrected Rand Index', [data items must be labeled: 1,2,3,...,total number of clusters] (false)

c[integer] || set number of Monte Carlo simulations for MST clustering (10)

g[integer] || set x of SOM grid xy, where y=x (square root of input size*2)

M[integer] || set maximum x/y grid of SOM (30)

m[integer] || set minimum x/y grid of SOM (5)

P || set SOM distance metric to Pearson Correlation (Euclidean)

P2 || set SOM distance metric to Uncentered Correlation (Euclidean)

s || set SOM topology to square (circle)

i[integer] || set number of SOM iterations (1000)

x[integer] || set SOM error surface exponent (3)

r[power of 2] || set density-equalizing cartogram resolution (64)

E || disable Density-Equalizing Cartogram (false)

S || invoke Sammon Mapping instead of SOM (false)

S[integer] || set number of Sammon Mapping iterations (100)

k[integer] || specify number of clusters in dataset (false)

K || invoke K-Means Clustering; requires option -k (false)

A || invoke Agglomerative Clustering; requires option -k (false)

A1,A3,A3,A4 || A1=Single, A2=Complete, A3=Average, A4=Ward's (Ward’s)

V || print verbose output (false)

Creating Fuzzy Cluster Networks

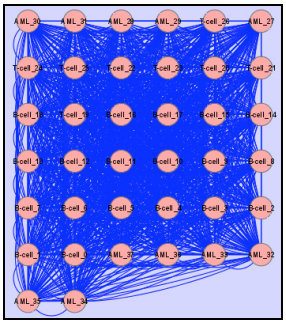
Fuzzy cluster networks highlight the fuzzy relationships among clustered data points using an intuitive two-dimensional network display. A powerful application of this approach is the visualization of differences between cell lines on the basis of differential gene expression. To display fuzzy cluster networks, the network visualization tool Cytoscape needs to be installed on your computer [Shannon et al., 2005]. Cytoscape is freely available from http://www.cytoscape.org/.

1) Import your input file into AutoSOME, set fields and adjust input.
2) Select 'Fuzzy Cluster Networks' and choose distance metric. (Please remember that only vertical data vectors are clustered (e.g. cell samples or time series of a microarray dataset)
3) Run AutoSOME
4) Launch Cytoscape
5) In Cytoscape (All screenshots below taken from Cytoscape 2.6.0):
    a. Go to File>Import>Network from Table (Text/MS Excel)…
    b. Go to 'Select File(s)' and locate AutoSOME output file (5) containing all edges (see '**Files written to disk'** in Output above).
    c. Set 'Source Interaction' to 'Column 1' and 'Target Interaction' to 'Column 2'. Finally, click on Column 3 in the data Preview window to activate it (it will turn blue).

AutoSOME_golub_autFormat_E250_Pval0.1_Edges.txt

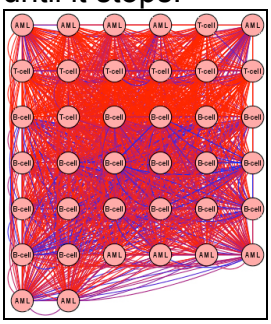
Column 1	Column 2	Column 3
B-cell 0	B-cell 1	0.454545455
B-cell 0	B-cell 2	0.363636364
B-cell 0	B-cell 3	0.05785124
B-cell 0	B-cell 4	0.421487603
B-cell 0	B-cell 5	-0.132231405
B-cell 0	B-cell 6	0.466942149
B-cell 0	B-cell 7	0.409090909
B-cell 0	B-cell 8	0.462809917
B-cell 0	B-cell 9	-0.136363636

    d. Select 'Import' and then 'Close'. A raw network will appear as a grid.



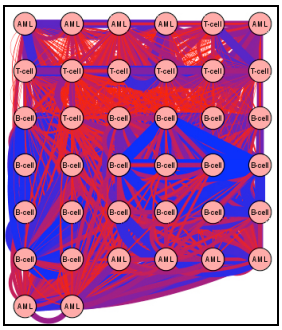
    e. Go to File>Import>Attribute from Table (Text/MS Excel)…

f. Go to 'Select File(s)' and locate AutoSOME output file (4) containing all nodes and attributes (see '**Files written to disk'** in Output above).
g. Select 'Import'.
h. Change global properties of the network:
    i. Click in the 'Defaults' window (shows a source and target pair with a blue background). A new window will appear.
    ii. Select the 'Global' tab in the bottom right.
    iii. Change the background color to white.
    iv. Go back to the 'Node' tab and change the NODE_BORDER_COLOR property to black and increase the 'NODE_LINE_WIDTH' to 2.
    v. Select 'Apply'
i. In the Control Panel, select the VizMapper™ tab.
j. Next to 'Node Label', click 'ID' and select 'Column 3'. All nodes should now be relabeled according to the original data labels. Minimize the 'Node Label' property by selecting the minus icon.
k. Under 'Unused Properties' find 'Edge Color' and double-click it.
    i. Select 'Column 3' as a value.
    ii. Then, select 'Continuous Mapper' for 'Mapping Type'.
    iii. Click on the black-to-white gradient next to 'Graphical View' to launch a Gradient Editor.
    iv. There are two fixed triangles, one on each end, and two adjustable triangles. Double-click the two leftmost triangles and set their colors to pure red (255,0,0). Double-click the two rightmost triangles and set their colors to pure blue (0,0,255). Drag the leftmost adjustable triangle all the way to the left and likewise drag the rightmost triangle to the right until it stops.

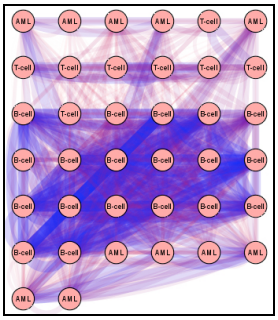


l. Find 'Edge Line Width' under 'Unused Properties' and double-click it.
    i. Select 'Column 3' as a value.
    ii. Then, select 'Continuous Mapper' for 'Mapping Type'.
    iii. Click on the graph next to the 'Graphical View' property to launch the 'Continuous Editor'.
    iv. Adjust the minimum and maximum values denoted by red squares (double-click on squares for precision, otherwise

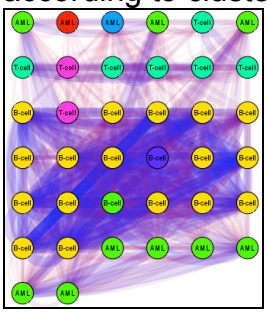
slide squares up or down). For example, set minimum to 0.5 and maximum to 20. Then, exit ‘Continuous Editor’.



m. Find ‘Edge Opacity’ under ‘Unused Properties’ and double-click it.
    i. Select ‘Column 3’ as a value.
    ii. Then, select ‘Continuous Mapper’ for ‘Mapping Type’.
    iii. Click on the graph next to the ‘Graphical View’ property to launch the ‘Continuous Editor’.
    iv. Adjust the minimum and maximum values denoted by red squares (double-click on squares for precision, otherwise slide squares up or down). For example, set minimum to 0.5 and maximum to 60. Then, exit ‘Continuous Editor’.

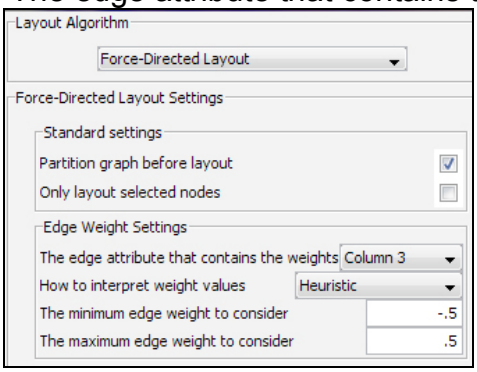


n. Finally, find ‘Node Color’ under ‘Unused Properties’ and double-click it.
    i. Select ‘Column 2’ as a value.
    ii. Then, select ‘Discrete Mapping’ for ‘Mapping Type’.
    iii. Right click on ‘Discrete Mapping’ and go to ‘Generate Discrete Values’>’Rainbow 1’. All nodes are now colored according to cluster labels. Adjust colors as necessary.

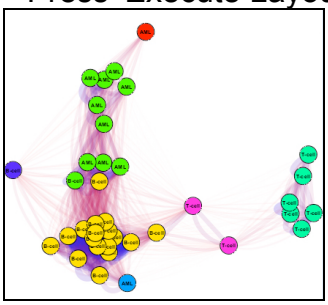


o. Go to ‘Layout’ in the main menu and select ‘Settings’
    i. Choose ‘Force-Directed Layout’ for ‘Layout Algorithm’

ii. Under ‘Edge Weight Settings’, set ‘The minimum edge weight to consider’ to -0.5, and set ‘The maximum edge weight to consider’ to 0.5. Further, select Column 3 from ‘The edge attribute that contains the weights’.



iii. Press ‘Execute Layout’ to run the layout algorithm.



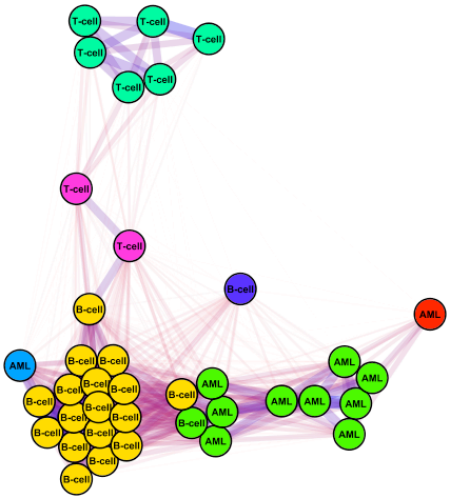
iv. Although the network topology is generally preserved, different runs of the layout algorithm can yield slightly different results in terms of network rotation and local node placement. To increase the repulsion between neighboring nodes (for evenly spaced nodes within a cluster), increase ‘Default Node Mass’ under ‘Algorithm settings’. Another layout algorithm that can yield comparable results is the ‘Edge-weighted Spring Embedded’ algorithm. Before executing the layout, make sure the ‘Edge Weight Settings’ are adjusted as in (ii) above. This layout algorithm can yield more evenly spaced nodes, but is less stable than ‘Force-Directed Layout’. Run a few times.

v. Notice that all edges are slightly curved. To straighten edges, save and reopen the Cytoscape file.

vi. At this point, make any desirable fine-grained changes to the ‘Edge Color’, ‘Edge Line Width’ and ‘Edge Opacity’ parameters to emphasize the fuzziness in the network.

p. To export the final network, go to ‘File’>’Export’>’Network View as Graphics…’

q. Then, select file format and save image!



r. To expedite this process for next time, start with your saved network. All visualization parameters will still be specified. Then, simply input your edge and node files and perform the layout. Note that for the Node Color property, it is important to assign colors again using the process given in step (n).

References

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4) Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T: **Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks.** *Genome Research* 2003, **13:**2498-2504.