

# Package ‘Mfuzz’

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**Title** Soft clustering of omics time series data

**Author** Matthias Futschik <matthias.futschik@sysbiolab.eu>

**Maintainer** Matthias Futschik <matthias.futschik@sysbiolab.eu>

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**Imports** tcltk, tkWidgets

**Suggests** marray

**Description** The Mfuzz package implements noise-robust soft clustering of omics time-series data, including transcriptomic, proteomic or metabolomic data. It is based on the use of c-means clustering. For convenience, it includes a graphical user interface.

**biocViews** Microarray, Clustering, TimeCourse, Preprocessing, Visualization

**License** GPL-2

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acore

*Extraction of alpha cores for soft clusters*

---

### Description

This function extracts genes forming the alpha cores of soft clusters

### Usage

```
acore(eset, cl, min.acore=0.5)
```

### Arguments

eset	object of the class <i>ExpressionSet</i> .
cl	An object of class <i>flcust</i> as produced by <i>mfuzz</i> .
min.acore	minimum membership values of gene belonging to the cluster core.

### Value

The function produces an list of alpha cores including genes and their membership values for the corresponding cluster.

### Author(s)

Matthias E. Futschik (<http://www.sysbiolab.eu/matthias.html>)

**Examples**

```

if (interactive()){
### Data loading and pre-processing
data(yeast) # data set includes 17 measurements
yeastF <- filter.NA(yeast)
yeastF <- fill.NA(yeastF)
yeastF <- standardise(yeastF)

### Soft clustering and visualisation
cl <- mfuzz(yeastF,c=20,m=1.25)
acore.list <- acore(yeastF,cl=cl,min.acore=0.7)
}

```

---

cselection	<i>Repeated soft clustering for detection of empty clusters for estimation of optimised number of clusters</i>
------------	--

---

**Description**

This function performs repeated soft clustering for a range of cluster numbers  $c$  and reports the number of empty clusters detected.

**Usage**

```
cselection(eset,m,crange=seq(4,32,4),repeats=5,visu=TRUE,...)
```

**Arguments**

eset	object of class <i>ExpressionSet</i> .
m	value of fuzzy c-means parameter $m$ .
crange	range of number of clusters $c$ .
repeats	number of repeated clusterings.
visu	If visu=TRUE plot of number of empty clusters is produced.
...	additional arguments for underlying mfuzz.

**Details**

A soft cluster is considered as empty, if none of the genes has a corresponding membership value larger than 0.5

**Value**

A matrix with the number of empty clusters detected is generated.

**Note**

The cselection function may help to determine an accurate cluster number. However, it should be used with care, as the determination remains difficult especially for short time series and overlapping clusters. Alternatively, the [Dmin](#) function can be used to select an optimal number of clusters based on the distances between centroids. Another way to select the cluster number is to use external annotation. For instance, one might perform clustering with a range of cluster numbers and subsequently assess their biological relevance e.g. by GO analyses.

**Author(s)**

Matthias E. Futschik (<http://www.sysbiolab.eu>)

**References**

M.E. Futschik and B. Charlisle, Noise robust clustering of gene expression time-course data, *Journal of Bioinformatics and Computational Biology*, 3 (4), 965-988, 2005

L. Kumar and M. Futschik, Mfuzz: a software package for soft clustering of microarray data, *Bioinformatics*, 2(1) 5-7,2007

**Examples**

```

if (interactive()){
data(yeast)
# Data pre-processing
yeastF <- filter.NA(yeast)
yeastF <- fill.NA(yeastF)
yeastF <- standardise(yeastF)

#### parameter selection
# Empty clusters should not appear
c1 <- mfuzz(yeastF,c=20,m=1.25)
mfuzz.plot(yeastF,c1=c1,mfrow=c(4,5))

# Note: The following calculation might take some time

tmp <- cselection(yeastF,m=1.25,crange=seq(5,40,5),repeats=5,visu=TRUE)
# derivation of number of non-empty clusters (crosses) from diagonal
# line indicate appearance of empty clusters

# Empty clusters might appear
c1 <- mfuzz(yeastF,c=40,m=1.25)
mfuzz.plot(yeastF,c1=c1,mfrow=c(4,5))
}

```

---

Dmin

*Calculation of minimum centroid distance for a range of cluster numbers for estimation of optimised number of clusters*

---

**Description**

This function performs repeated soft clustering for a range of cluster numbers *c* and reports the minimum centroid distance.

**Usage**

```
Dmin(eset,m,crange=seq(4,40,4),repeats=3,visu=TRUE)
```

**Arguments**

eset	object of class <i>ExpressionSet</i> .
m	value of fuzzy c-means parameter m.
crange	range of number of clusters c.
repeats	number of repeated clusterings.
visu	If visu=TRUE plot of average minimum centroid distance is produced

**Details**

The minimum centroid distance is defined as the minimum distance between two cluster centers produced by the c-means clusterings.

**Value**

The average minimum centroid distance for the given range of cluster number is returned.

**Note**

The minimum centroid distance can be used as cluster validity index. For an optimal cluster number, we may see a 'drop' of minimum centroid distance wh plotted versus a range of cluster number and a slower decrease of the minimum centroid distance for higher cluster number. More information and some examples can be found in the study of Schwaemmle and Jensen (2010). However, it should be used with care, as the determination remains difficult especially for short time series and overlapping clusters. Alternatively, the function *cselection* can be used or functional enrichment analysis (e.g. using Gene Ontology) can help to adjust the cluster number.

**Author(s)**

Matthias E. Futschik (<http://www.sysbiolab.eu/matthias.html>)

**References**

- M.E. Futschik and B. Charlisle, Noise robust clustering of gene expression time-course data, *Journal of Bioinformatics and Computational Biology*, 3 (4), 965-988, 2005
- L. Kumar and M. Futschik, Mfuzz: a software package for soft clustering of microarray data, *Bioinformatics*, 2(1) 5-7,2007
- Schwaemmle and Jensen, *Bioinformatics*, Vol. 26 (22), 2841-2848, 2010

**Examples**

```
if (interactive()){
  data(yeast)
  # Data pre-processing
  yeastF <- filter.NA(yeast)
  yeastF <- fill.NA(yeastF)
  yeastF <- standardise(yeastF)

  ##### parameter selection
  # For fuzzifier m, we could use mestimate
  m1 <- mestimate(yeastF)
  m1 # 1.15
```

```

# or the function partcoef (see example there)

# For selection of c, either cselection (see example there)
# or

tmp <- Dmin(yeastF,m=m1,crange=seq(4,40,4),repeats=3,visu=TRUE)# Note: This calculation might take some time

# It seems that the decrease for c ~ 20 - 25 24 and thus 20 might be
# a suitable number of clusters

}

```

---

fill.NA

*Replacement of missing values*


---

### Description

Methods for replacement of missing values. Missing values should be indicated by NA in the expression matrix.

### Usage

```
fill.NA(eset,mode="mean",k=10)
```

### Arguments

eset	object of the class <i>ExpressionSet</i> .
mode	method for replacement of missing values: <ul style="list-style-type: none"> <li>• <i>mean</i>- missing values will be replaced by the mean expression value of the gene,</li> <li>• <i>median</i>- missing values will be replaced by the median expression value of the gene,</li> <li>• <i>knn</i>- missing values will be replaced by the averging over the corresponding expression values of the k-nearest neighbours,</li> <li>• <i>knnw</i>-same replacement method as <i>knn</i>, but the expression values averaged are weighted by the distance to the corresponding neighbour</li> </ul>
k	Number of neighbours, if one of the knn method for replacement is chosen (knn, knnw).

### Value

The function produces an object of the *ExpressionSet* class with missing values replaced.

### Note

The replacement methods *knn* and *knnw* can computationally intensive for large gene expression data sets. It may be a good idea to run these methods as a 'lunchtime' or 'overnight' job.

### Author(s)

Matthias E. Futschik (<http://itb.biologie.hu-berlin.de/~futschik>) and Lokesh Kumar

**Examples**

```
if (interactive()){  
  data(yeast) # data set includes 17 measurements  
  yeastF <- filter.NA(yeast)  
  yeastF <- fill.NA(yeastF)  
}
```

---

filter.NA	<i>Filtering of genes based on number of non-available expression values.</i>
-----------	---

---

**Description**

This function can be used to exclude genes with a large number of expression values not available.

**Usage**

```
filter.NA(eset, thres=0.25)
```

**Arguments**

eset	object of the class “ExpressionSet”.
thres	threshold for excluding genes. If the percentage of missing values (indicated by NA in the expression matrix) is larger than thres, the corresponding gene will be excluded.

**Value**

The function produces an object of the ExpressionSet class. It is the same as the input eset object, except for the genes excluded.

**Author(s)**

Matthias E. Futschik (<http://www.sysbiolab.eu>)

**Examples**

```
if (interactive()){  
  data(yeast) # data set includes 17 measurements  
  yeastF <- filter.NA(yeast) # genes are excluded if more than 4 measurements are missing  
}
```

---

filter.std	<i>Filtering of genes based on their standard deviation.</i>
------------	--

---

### Description

This function can be used to exclude genes with low standard deviation.

### Usage

```
filter.std(eset,min.std,visu=TRUE)
```

### Arguments

eset	object of the class <i>ExpressionSet</i> .
min.std	threshold for minimum standard deviation. If the standard deviation of a gene's expression is smaller than min.std the corresponding gene will be excluded.
visu	If visu is set to TRUE, the ordered standard deviations of genes' expression values will be plotted.

### Value

The function produces an object of the *ExpressionSet* class. It is the same as the input eset object, except for the genes excluded.

### Note

As soft clustering is noise robust, pre-filtering can usually be avoided. However, if the number of genes with small expression changes is large, such pre-filtering may be necessary to reduce noise.

### Author(s)

Matthias E. Futschik (<http://itb.biologie.hu-berlin.de/~futschik>)

### Examples

```
data(yeast) # data set includes 17 measurements
yeastF <- filter.NA(yeast) # filtering of genes based on missing values
yeastF <- filter.std(yeastF,min.std=0.3) # filtering of genes based on standard deviation
```

---

kmeans2	<i>K-means clustering for gene expression data</i>
---------	--

---

### Description

This function is a wrapper function for `kmeans` of the `e1071` package. It performs hard clustering of genes based on their expression values using the k-means algorithm.

### Usage

```
kmeans2(eset,k,iter.max=100)
```

**Arguments**

eset            object of the class *ExpressionSet*.  
k                number of clusters.  
iter.max        maximal number of iterations.

**Value**

An list of clustering components (see [kmeans](#)).

**Author(s)**

Matthias E. Futschik (<http://itb.biologie.hu-berlin.de/~futschik>)

**See Also**

[kmeans](#)

**Examples**

```
if (interactive()){  
  data(yeast)  
  # Data pre-processing  
  yeastF <- filter.NA(yeast)  
  yeastF <- fill.NA(yeastF)  
  yeastF <- standardise(yeastF)  
  
  # K-means clustering and visualisation  
  k1 <- kmeans2(yeastF,k=20)  
  kmeans2.plot(yeastF,k1=k1,mfrow=c(2,2))  
}
```

---

kmeans2.plot

*Plotting results for k-means clustering*

---

**Description**

This function visualises the clusters produced by `kmeans2`.

**Usage**

```
kmeans2.plot(eset,k1,mfrow=c(1,1))
```

**Arguments**

eset            object of the class "ExpressionSet".  
k1              list produced by `kmeans2`.  
mfrow          determines splitting of graphic window.

**Value**

The function displays the temporal profiles of clusters detected by k-means.

**Author(s)**

Matthias E. Futschik (<http://itb.biologie.hu-berlin.de/~futschik>)

**Examples**

```
if (interactive()){
  data(yeast)
  # Data pre-processing
  yeastF <- filter.NA(yeast)
  yeastF <- fill.NA(yeastF)
  yeastF <- standardise(yeastF)

  # K-means clustering and visualisation
  k1 <- kmeans2(yeastF,k=20)
  kmeans2.plot(yeastF,k1=k1,mfrow=c(2,2))
}
```

---

membership

*Calculating of membership values for new data based on existing clustering*

---

**Description**

Function that calculates the membership values of genes based on provided data and existing clustering

**Usage**

```
membership(x,clusters,m)
```

**Arguments**

x	expression vector or expression matrix
clusters	cluster centroids from existing clustering
m	fuzzification parameter

**Value**

Matrix of membership values for new genes

**Note**

This function calculates membership values for new data based on existing cluster centroids and fuzzification parameter. It can be useful, for instance, when comparing two time series, to assess whether the same gene in the different time series changes its cluster association.

**Author(s)**

Matthias E. Futschik (<http://www.sysbiolab.eu>)

**Examples**

```
if (interactive()){
  data(yeast)
  yeastF <- filter.NA(yeast)
  yeastF <- fill.NA(yeastF) # for illustration only; rather use knn method
  yeastF <- standardise(yeastF)

  cl <- mfuzz(yeastF,c=20,m=1.25)

  m <- 1.25
  clusters <- cl[[1]]
  x <- matrix(rnorm(2*17),nrow=2) # new expression matrix with two genes
  mem.tmp <- membership(x,clusters=clusters,m=m) #membership values
}
```

---

mestimate

*Estimate for optimal fuzzifier m*

---

**Description**

This function estimates an optimal setting of fuzzifier  $m$

**Usage**

```
mestimate(eset)
```

**Arguments**

eset                    object of class "ExpressionSet"

**Details**

Schwaemmle and Jensen proposed an method to estimate of  $m$ , which was motivated by the evaluation of fuzzy clustering applied to randomized datasets. The estimated  $m$  should give the minimum fuzzifier value which prevents clustering of randomized data.

**Value**

Estimate for optimal fuzzifier.

**Author(s)**

Matthias E. Futschik (<http://itb.biologie.hu-berlin.de/~futschik>)

**References**

Schwaemmle and Jensen, Bioinformatics, Vol. 26 (22), 2841-2848, 2010

## Examples

```
if (interactive()){
  data(yeast)
  # Data pre-processing
  yeastF <- filter.NA(yeast)
  yeastF <- fill.NA(yeastF)
  yeastF <- standardise(yeastF)

  ##### parameter selection

  ##### parameter selection
  # For fuzzifier m, we could use mestimate
  m1 <- mestimate(yeastF)
  m1 # 1.15

  cl <- mfuzz(yeastF,c=20,m=m1)
  mfuzz.plot(yeastF,cl=cl,mfrow=c(4,5))
}
```

---

mfuzz

*Function for soft clustering based on fuzzy c-means.*

---

## Description

This function is a wrapper function for `cmeans` of the `e1071` package. It performs soft clustering of genes based on their expression values using the fuzzy c-means algorithm.

## Usage

```
mfuzz(eset,centers,m,...)
```

## Arguments

<code>eset</code>	object of the class “ExpressionSet”.
<code>centers</code>	number of clusters.
<code>m</code>	fuzzification parameter.
<code>...</code>	additional parameters for <code>cmeans</code> .

## Details

This function is the core function for soft clustering. It groups genes based on the Euclidean distance and the c-means objective function which is a weighted square error function. Each gene is assigned a membership value between 0 and 1 for each cluster. Hence, genes can be assigned to different clusters in a gradual manner. This contrasts hard clustering where each gene can belong to a single cluster.

**Value**

An object of class `flclust` (see [cmeans](#)) which is a list with components:

<code>centers</code>	the final cluster centers.
<code>size</code>	the number of data points in each cluster of the closest hard clustering.
<code>cluster</code>	a vector of integers containing the indices of the clusters where the data points are assigned to for the closest hard clustering, as obtained by assigning points to the (first) class with maximal membership.
<code>iter</code>	the number of iterations performed.
<code>membership</code>	a matrix with the membership values of the data points to the clusters.
<code>withinerror</code>	the value of the objective function.
<code>call</code>	the call used to create the object.

**Note**

Note that the clustering is based solely on the `exprs` matrix and no information is used from the `phenoData`. In particular, the ordering of samples (arrays) is the same as the ordering of the columns in the `exprs` matrix. Also, replicated arrays in the `exprs` matrix are treated as independent by the `mfuzz` function i.e. they should be averaged prior to clustering or placed into different distinct “ExpressionSet” objects.

**Author(s)**

Matthias E. Futschik (<http://www.sysbiolab.eu>)

**References**

M.E. Futschik and B. Charlisle, Noise robust clustering of gene expression time-course data, *Journal of Bioinformatics and Computational Biology*, 3 (4), 965-988, 2005

L. Kumar and M. Futschik, `Mfuzz`: a software package for soft clustering of microarray data, *Bioinformatics*, 2(1) 5-7,2007

**See Also**

[cmeans](#)

**Examples**

```
if (interactive()){
  data(yeast)
  # Data pre-processing
  yeastF <- filter.NA(yeast)
  yeastF <- fill.NA(yeastF) # for illustration only; rather use knn method
  yeastF <- standardise(yeastF)

  # Soft clustering and visualisation
  cl <- mfuzz(yeastF,c=20,m=1.25)
  mfuzz.plot(yeastF,cl=cl,mfrow=c(2,2))

  # Plotting center of cluster 1
  X11(); plot(cl[[1]][1,],type="l",ylab="Expression")
}
```

```
# Getting the membership values for the first 10 genes in cluster 1
cl[[4]][1:10,1]
}
```

mfuzz.plot

*Plotting results for soft clustering***Description**

This function visualises the clusters produced by mfuzz.

**Usage**

```
mfuzz.plot(eset,cl,mfrow=c(1,1),colo,min.mem=0,time.labels,new.window=TRUE)
```

**Arguments**

eset	object of the class <i>ExpressionSet</i> .
cl	object of class <i>flclust</i> .
mfrow	determines splitting of graphic window.
colo	color palette to be used for plotting. If the color argument remains empty, the default palette is used.
min.mem	Genes with membership values below min.mem will not be displayed.
time.labels	labels can be given for the time axis.
new.window	should a new window be opened for graphics.

**Value**

The function generates plots where the membership of genes is color-encoded.

**Author(s)**

Matthias E. Futschik (<http://www.sysbiolab.eu/matthias>)

**Examples**

```
if (interactive()){
data(yeast)
# Data pre-processing
yeastF <- filter.NA(yeast)
yeastF <- fill.NA(yeastF)
yeastF <- standardise(yeastF)

# Soft clustering and visualisation
cl <- mfuzz(yeastF,c=20,m=1.25)
mfuzz.plot(yeastF,cl=cl,mfrow=c(2,2))

# display of cluster cores with alpha = 0.5
mfuzz.plot(yeastF,cl=cl,mfrow=c(2,2),min.mem=0.5)

# display of cluster cores with alpha = 0.7
mfuzz.plot(yeastF,cl=cl,mfrow=c(2,2),min.mem=0.7)
}
```

mfuzz.plot2

*Plotting results for soft clustering with additional options***Description**

This function visualises the clusters produced by mfuzz. it is similar to mfuzz.plot, but offers more options for adjusting the plots.

**Usage**

```
mfuzz.plot2(eset, cl, mfrow=c(1,1), colo, min.mem=0, time.labels, time.points,
  ylim.set=c(0,0), xlab="Time", ylab="Expression changes", x11=TRUE,
  ax.col="black", bg = "white", col.axis="black", col.lab="black",
  col.main="black", col.sub="black", col="black", centre=FALSE,
  centre.col="black", centre.lwd=2,
  Xwidth=5, Xheight=5, single=FALSE, ...)
```

**Arguments**

eset	object of the class <i>ExpressionSet</i> .
cl	object of class <i>flclust</i> .
mfrow	determines splitting of graphic window. Use mfrow=NA if layout is used (see example).
colo	color palette to be used for plotting. If the color argument remains empty, the default palette is used. If the colo = "fancy", an alternative (fancier) palette will be used.
min.mem	Genes with membership values below min.mem will not be displayed.
time.labels	labels for ticks on x axis.
time.points	numerical values for the ticks on x axis. These can be used if the measured time points are not equidistant.
ylim.set	Vector of min. and max. y-value set for plotting. If ylim.set=c(0,0), min. and max. value will be determined automatically.
xlab	label for x axis
ylab	label for y axis
x11	If TRUE, a new window will be open for plotting.
ax.col	Color of axis line.
bg	Background color.
col.axis	Color for axis annotation.
col.lab	Color for axis labels.
col.main	Color for main titles.
col.sub	Color for sub-titles.
col	Default plotting color.
centre	If TRUE, a line for the cluster centre will be drawn.
centre.col	Color of the line for the cluster centre
centre.lwd	Width of the line for the cluster centre

Xwidth	Width of window.
Xheight	Height of window.
single	Integer if a specific cluster is to be plotted, otherwise it should be set to FALSE.
...	Additional, optional plotting arguments passed to plot.default and axes functions such as cex.lab,cex.main,cex.axis

### Value

The function generates plots where the membership of genes is color-encoded.

### Author(s)

Matthias E. Futschik (<http://www.sysbiolab.eu/matthias>)

### Examples

```

if (interactive()){
  data(yeast)
  # Data pre-processing
  yeastF <- filter.NA(yeast)
  yeastF <- fill.NA(yeastF)
  yeastF <- standardise(yeastF)

  # Soft clustering and visualisation
  cl <- mfuzz(yeastF,c=20,m=1.25)
  mfuzz.plot2(yeastF,cl=cl,mfrow=c(2,2)) # same output as mfuzz.plot
  mfuzz.plot2(yeastF, cl=cl,mfrow=c(2,2),centre=TRUE) # lines for cluster centres will be included

  # More fancy choice of colors
  mfuzz.plot2(yeastF,cl=cl,mfrow=c(2,2),colo="fancy",
  ax.col="red",bg = "black",col.axis="red",col.lab="white",
  col.main="green",col.sub="blue",col="blue",cex.main=1.3,cex.lab=1.1)

  ### Single cluster with colorbar (cluster # 3)
  X11(width=12)
  mat <- matrix(1:2,ncol=2,nrow=1,byrow=TRUE)
  l <- layout(mat,width=c(5,1))
  mfuzz.plot2(yeastF,cl=cl,mfrow=NA,colo="fancy", ax.col="red",bg = "black",col.axis="red",col.lab="white",
  col.main="green",col.sub="blue",col="blue",cex.main=2, single=3,x11=FALSE)

  mfuzzColorBar(col="fancy",main="Membership",cex.main=1)

  ### Single cluster with colorbar (cluster # 3)
  X11(width=14)
  mat <- matrix(1:2,ncol=2,nrow=1,byrow=TRUE)
  l <- layout(mat,width=c(5,1))
  mfuzz.plot2(yeastF,cl=cl,mfrow=NA,colo="fancy", ax.col="red",bg =
  "black",col.axis="red",col.lab="white",time.labels = c(paste(seq(0,160,10),"min")),
  col.main="green",col.sub="blue",col="blue",cex.main=2, single=3,x11=FALSE)

  mfuzzColorBar(col="fancy",main="Membership",cex.main=1)

```

```
}
```

---

mfuzzColorBar                    *Plots a colour bar*

---

## Description

This function produces a (separate) colour bar for graphs produced by mfuzz.plot

## Usage

```
mfuzzColorBar(col, horizontal=FALSE,...)
```

## Arguments

col	vector of colours used. If missing, the same vector as the default vector for mfuzz.plot is used. If col="fancy", an alternative color palette is used (see mfuzz.plot2).
horizontal	If TRUE, a horizontal colour bar is generated, otherwise a vertical one will be produced.
...	additional parameter passed to maColorBar (see also example in mfuzz.plot2)

## Author(s)

Matthias E. Futschik (<http://www.sysbiolab.eu/matthias.html>)

## References

M.E. Futschik and B. Charlisle, Noise robust clustering of gene expression time-course data, Journal of Bioinformatics and Computational Biology, 3 (4), 965-988, 2005

L. Kumar and M. Futschik, Mfuzz: a software package for soft clustering of microarray data, Bioinformatics, 2(1) 5-7,2007

## See Also

[maColorBar](#)

## Examples

```
if (interactive()){
  X11(w=1.5,h=5);
  par(mar=c(1,1,1,5))
  mfuzzColorBar()
  mfuzzColorBar(col="fancy",main="Membership value")
  mfuzzColorBar(rev(heat.colors(100))) # example of using heat colors with red indicating high membership values
}
```

**Description**

The function `Mfuzzgui` provides a graphical user interface for clustering of microarray data and visualisation of results. It is based on the functions of the `Mfuzz` package.

**Usage**

```
Mfuzzgui()
```

**Details**

The function `Mfuzzgui` launches a graphical user interface for the `Mfuzz` package. It is based on Tk widgets using the R TclTk interface by Peter Dalgaard. It also employs some pre-made widgets from the `tkWidgets` Bioconductor-package by Jianhua Zhang for the selection of objects/files to be loaded.

`Mfuzzgui` provides a convenient interface to most functions of the `Mfuzz` package without restriction of flexibility. An exception is the batch processes such as `partcoeff` and `cselection` routines which are used for parameter selection in fuzzy c-means clustering of microarray data. These routines are not included in `Mfuzzgui`. To select various parameters, the underlying `Mfuzz` routines may be applied.

Usage of `Mfuzzgui` does not require assumes an pre-built `exprSet` object but can be used with tab-delimited text files containing the gene expression data. Note, however, that the clustering is based on the the ordering of samples (arrays) as of the columns in the expression matrix of the `exprSet` object or in the uploaded table, respectively. Also, replicated arrays in the expression matrix (or table) are treated as independent by the `mfuzz` function and, thus, should be averaged prior to clustering.

For a overview of the functionality of `Mfuzzgui`, please refer to the package vignette. For a description of the underlying functions, please refer to the `Mfuzz` package.

**Value**

`Mfuzzgui` returns a `tclObj` object.

**Note**

The newest versions of `Mfuzzgui` can be found at the `Mfuzz` webpage (<http://itb.biologie.hu-berlin.de/~futschik/software/R/Mfuzz>).

**Author(s)**

Matthias E. Futschik (<http://itb.biologie.hu-berlin.de/~futschik>) and Lokesh Kumar

**References**

1. M.E. Futschik and B. Charlisle, Noise robust clustering of gene expression time-course data, **Journal of Bioinformatics and Computational Biology**, Vol. 3, No. 4, 965-988, 2005.

2. Cho RJ, Campbell MJ, Winzeler EA, Steinmetz L, Conway A, Wodicka L, Wolfsberg TG, Gabrielian AE, Landsman D, Lockhart DJ, Davis RW, A genome-wide transcriptional analysis of the mitotic cell cycle, *Mol Cell*,(2):65-73, 1998.
3. Mfuzz web-page: <http://itb.biologie.hu-berlin.de/~futschik/software/R/Mfuzz>

### See Also

[mfuzz](#)

---

overlap

*Calculation of the overlap of soft clusters*

---

### Description

This function calculates the overlap of clusters produced by mfuzz.

### Usage

```
overlap(cl)
```

### Arguments

cl                    object of class *fclust*

### Value

The function generates a matrix of the normalised overlap of soft clusters. The overlap indicates the extent of “shared” genes between clusters. For a mathematical definition of the overlap, see the vignette of the package or the reference below.

### Author(s)

Matthias E. Futschik (<http://itb.biologie.hu-berlin.de/~futschik>)

### References

M.E. Futschik and B. Charlisle, Noise robust clustering of gene expression time-course data, *Journal of Bioinformatics and Computational Biology*, 3 (4), 965-988, 2005

### Examples

```
if (interactive()){
  data(yeast)
  # Data pre-processing
  yeastF <- filter.NA(yeast)
  yeastF <- fill.NA(yeastF)
  yeastF <- standardise(yeastF)

  # Soft clustering and visualisation
  cl <- mfuzz(yeastF, c=20, m=1.25)
  mfuzz.plot(yeastF, cl=cl, mfrow=c(4,5))

  # Calculation of cluster overlap and visualisation
```

```
O <- overlap(c1)
X11()
Ptmp <- overlap.plot(c1,over=0,thres=0.05)
}
```

---

overlap.plot

*Visualisation of cluster overlap and global clustering structure*

---

## Description

This function visualises the cluster overlap produced by `overlap`.

## Usage

```
overlap.plot(c1,overlap,thres=0.1,scale=TRUE,magni=30,P=NULL)
```

## Arguments

<code>c1</code>	object of class “fclust”
<code>overlap</code>	matrix of cluster overlap produced by <code>overlap</code>
<code>thres</code>	threshold for visualisation. Cluster overlaps below the threshold will not be visualised.
<code>scale</code>	Scale parameter for principal component analysis by <a href="#">prcomp</a>
<code>magni</code>	Factor for increase the line width for cluster overlap.
<code>P</code>	Projection matrix produced by principal component analysis.

## Value

A plot is generated based on a principal component analysis of the cluster centers. The overlap is visualised by lines with variable width indicating the strength of the overlap. Additionally, the matrix of principal components is returned. This matrix can be re-used for other projections to compare the overlap and global cluster structure of different clusterings.

## Author(s)

Matthias E. Futschik (<http://itb.biologie.hu-berlin.de/~futschik>)

## See Also

[prcomp](#)

## Examples

```
if (interactive()){
  data(yeast)
  # Data pre-processing
  yeastF <- filter.NA(yeast)
  yeastF <- fill.NA(yeastF)
  yeastF <- standardise(yeastF)

  # Soft clustering
```

```

c1 <- mfuzz(yeastF,c=20,m=1.25)
X11();mfuzz.plot(yeastF,c1=c1,mfrow=c(4,5))
0 <- overlap(c1)
X11();Ptmp <- overlap.plot(c1,over=0,thres=0.05)

# Alternative clustering
c1 <- mfuzz(yeastF,c=10,m=1.25)
X11();mfuzz.plot(yeastF,c1=c1,mfrow=c(3,4))
0 <- overlap(c1)

X11();overlap.plot(c1,over=0,P=Ptmp,thres=0.05)
# visualisation based on principal compents from previous projection
}

```

---

partcoef

---

*Calculation of the partition coefficient matrix for soft clustering*


---

### Description

This function calculates partition coefficient for clusters within a range of cluster parameters. It can be used to determine the parameters which lead to uniform clustering.

### Usage

```
partcoef(eset, crange=seq(4, 32, 4), mrange=seq(1.05, 2, 0.1), ...)
```

### Arguments

eset	object of class "ExpressionSet".
crange	range of number of clusters c.
mrange	range of clustering paramter m.
...	additional arguments for underlying mfuzz.

### Details

Introduced by Bezdek (1981), the partition coefficient F is defined as the sum of squares of values of the partition matrix divided by the number of values. It is maximal if the partition is hard and reaches a minimum for  $U=1/c$  when every gene is equally assigned to every cluster.

It is well-known that the partition coefficient tends to decrease monotonically with increasing n. To reduce this tendency we defined a normalized partition coefficient where the partition for uniform partitions are subtracted from the actual partition coefficients (Futschik and Kasabov,2002).

### Value

The function generates the matrix of partition coefficients for a range of c and m values. It also produces a matrix of normalised partition coefficients as well as a matrix with partition coefficient for uniform partitions.

### Author(s)

Matthias E. Futschik (<http://itb.biologie.hu-berlin.de/~futschik>)

## References

1. J.C.Bezdek, Pattern recognition with fuzzy objective function algorithms, Plenum, 1981
2. M.E. Futschik and N.K. Kasabov. Fuzzy clustering of gene expression data, Proceedings of World Congress of Computational Intelligence WCCI 2002, Hawaii, IEEE Press, 2002

## Examples

```

if (interactive()){
data(yeast)
# Data pre-processing
yeastF <- filter.NA(yeast)
yeastF <- fill.NA(yeastF)
yeastF <- standardise(yeastF)

#### parameter selection
yeastFR <- randomise(yeastF)
cl <- mfuzz(yeastFR,c=20,m=1.1)
mfuzz.plot(yeastFR,cl=cl,mfrow=c(4,5)) # shows cluster structures (non-uniform partition)

tmp <- partcoef(yeastFR) # This might take some time.
F <- tmp[[1]];F.n <- tmp[[2]];F.min <- tmp[[3]]

# Which clustering parameters result in a uniform partition?
F > 1.01 * F.min

cl <- mfuzz(yeastFR,c=20,m=1.25) # produces uniform partition

mfuzz.plot(yeastFR,cl=cl,mfrow=c(4,5))
# uniform coloring of temporal profiles indicates uniform partition
}

```

---

randomise

*Randomisation of data*

---

## Description

This function randomise the time order for each gene separately.

## Usage

```
randomise(eset)
```

## Arguments

eset                    object of the class *ExpressionSet*.

## Value

The function produces an object of the *ExpressionSet* class with randomised expression data.

## Author(s)

Matthias E. Futschik (<http://itb.biologie.hu-berlin.de/~futschik>)

**Examples**

```
data(yeast) # data set includes 17 measurements
yeastR <- randomise(yeast)
```

---

standardise	<i>Standardization of expression data for clustering.</i>
-------------	---

---

**Description**

Standardisation of the expression values of every gene/transcript/protein is carried out, so that the average expression value for each gene/transcript/protein is zero and the standard deviation of its expression profile is one.

**Usage**

```
standardise(eset)
```

**Arguments**

eset                    object of the classe *ExpressionSet*.

**Value**

The function produces an object of the *ExpressionSet* class with standardised expression values.

**Note**

Mfuzz assumes that the given expression data are preprocessed (including the normalisation). The function *standardise* does not replace the normalisation step. Note the difference: Normalisation is carried out to make different samples comparable, while standardisation (in Mfuzz) is carried out to make transcripts (genes) comparable.

**Author(s)**

Matthias E. Futschik (<http://www.sysbiolab.eu>)

**Examples**

```
if (interactive()){
  data(yeast)
  # Data pre-processing
  yeastF <- filter.NA(yeast)
  yeastF <- fill.NA(yeastF)
  yeastF <- standardise(yeastF)

  # Soft clustering and visualisation
  cl <- mfuzz(yeastF,c=20,m=1.25)
  mfuzz.plot(yeastF,cl=cl,mfrow=c(4,5))
}
```

---

`standardise2`*Standardization in regards to selected time-point*

---

### Description

Standardisation of the expression values of every gene is performed, so that the expression values at a chosen time point are zero and the standard deviation of expression profiles of individual genes/transcripts/proteins is one.

### Usage

```
standardise2(eset, timepoint=1)
```

### Arguments

<code>eset</code>	object of the class <i>ExpressionSet</i> .
<code>timepoint</code>	integer: which time point should have expression values of zero.

### Value

The function produces an object of the *ExpressionSet* class with standardised expression values.

### Note

Mfuzz assumes that the given expression data are preprocessed (including the normalisation). The function `standardise2` does not replace the normalisation step. Note the difference: Normalisation is carried out to make different samples comparable, while standardisation (in Mfuzz) is carried out to make transcripts (genes) comparable.

### Author(s)

Matthias E. Futschik (<http://www.sysbiolab.eu>)

### Examples

```
if (interactive()){
  data(yeast)
  # Data pre-processing
  yeastF <- filter.NA(yeast)
  yeastF <- fill.NA(yeastF)
  yeastF <- standardise2(yeastF, timepoint=1)

  # Soft clustering and visualisation
  cl <- mfuzz(yeastF, c=20, m=1.25)
  mfuzz.plot(yeastF, cl=cl, mfrow=c(4, 5))
}
```

---

table2eset	<i>Conversion of table to Expression set object.</i>
------------	--

---

**Description**

A expression matrix stored as a table (in a defined format) is read and converted to Expression Set object.

**Usage**

```
table2eset(filename)
```

**Arguments**

filename            name of file to be scanned in

**Details**

The expression matrix stored as table in the file has to follow some conventions in order to be able to be converted to an Expression Set object: The first row of the file contains sample labels and optionally, the second column can contains the time points. If the second row is used for the input the time, the first field in the second row must contain "Time". Similarly, the first column contains unique gene IDs and optionally second row can contain gene names. If the second row contains gene names, the second field in the first row must contain "Gene.Name". The rest of the file contains expression data. As example, two tables with expression data are provided. These examples can be viewed by inputing `data(yeast.table)` and `data(yeast.table2)` in the R console.

**Value**

An Expression Set object is generated.

**Author(s)**

Matthias E. Futschik (<http://www.sysbiolab.eu>)

---

top.count	<i>Determines the number for which each gene has highest membership value in all cluster</i>
-----------	--

---

**Description**

This function calculates the number,for which each gene appears to have the top membership score in the partition matrix of clusters produced by `mfuzz`.

**Usage**

```
top.count(c1)
```

**Arguments**

c1                    object of class "fclust"

**Value**

The function generates a vector containing a count for each gene, which is just the number of times that particular gene has acquired the top membership score.

**Author(s)**

Lokesh Kumar and Matthias E. Futschik (<http://itb.biologie.hu-berlin.de/~futschik>)

**Examples**

```
if (interactive()){
  data(yeast)
  # Data pre-processing
  yeastF <- filter.NA(yeast)
  yeastF <- fill.NA(yeastF)
  yeastF <- standardise(yeastF)

  # Soft clustering and visualisation
  cl <- mfuzz(yeastF,c=20,m=1.25)
  top.count(cl)
}
```

---

yeast

*Gene expression data of the yeast cell cycle*

---

**Description**

The data contains gene expression measurements for 3000 randomly chosen genes of the yeast mutant *cdc28* as performed and described by Cho *et al.* For details, see the reference.

**Usage**

```
data(yeast)
```

**Format**

An object of class “ExpressionSet”.

**Source**

The data was downloaded from *Yeast Cell Cycle Analysis Project* website and converted to an *ExpressionSet* object.

**References**

Cho et al., A genome-wide transcriptional analysis of the mitotic cell cycle, *Mol Cell.* 1998 Jul;2(1):65-73.

---

`yeast.table`*Gene expression data of the yeast cell cycle as table*

---

**Description**

The data serves as an example for the format required for uploading tables with expression data into *Mfuzzgui*. The first row contains the names of the samples, the second row contains the measured time points. Note that “TIME” has to be placed in the first field of the second row.

The first column contains unique identifiers for genes; optionally the second row can contain gene names if “GENE.NAMES” is in the second field in the first row.

An example for a table without optional fields is the dataset [yeast.table2](#).

The exemplary tables can be found in the data sub-folder of the *Mfuzzgui* package.

**References**

Cho et al., A genome-wide transcriptional analysis of the mitotic cell cycle, *Mol Cell*. 1998 Jul;2(1):65-73.

**See Also**

[yeast.table2](#)

---

`yeast.table2`*Gene expression data of the yeast cell cycle as table*

---

**Description**

The data serves as an example for the format required to upload tables with expression data into *Mfuzzgui*. The first row contains the names of the samples and the first column contains unique identifiers for genes. To input measurement time and gene names, refer to [yeast.table](#).

The exemplary tables can be found in the data sub-folder of the *Mfuzzgui* package.

**References**

Cho et al., A genome-wide transcriptional analysis of the mitotic cell cycle, *Mol Cell*. 1998 Jul;2(1):65-73.

**See Also**

[yeast.table](#)

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