Package 'flowCHIC'

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Description A package to analyze flow cytometric data of complex microbial communities based on histogram images

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`flowCHIC-package` *Analyze flow cytometric data using histogram information*

Description

A package to analyze flow cytometric data of complex microbial communities based on histogram images.

Details

Package:	flowCHIC
Type:	Package
Version:	1.0.1
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abiotic_incol fcs_to_img flowCHIC img_sub calculate_overlaps_xor plot_nmds Results_xor_mix Results_xor_incol Results_overlaps_mix Results_overlaps_incol

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References

Christin Koch, Ingo Fetzer, Hauke Harms, and Susann Müller. CHIC - An Automated Approach for the Detection of Dynamic Variations in Complex Microbial Communities. Cytometry Part A, 2013.

See Also

flowCore,flowViz,flowCyBar

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abiotic_incol *Dataset with abiotic data*

Description

Example dataset containing abiotic parameters used for the NMDS plot of the second downloadable dataset (see manual).

Usage

```
data(abiotic_incol)
```

Format

Data frame with 17 observations of 6 variables.

Value

Abiotic data of the second downloadable dataset.

---

calculate_overlaps_xor
Calculate overlap and XOR images

Description

Calculate overlap and XOR images for each combination of every subset histogram image.

Usage

```
## S4 method for signature 'character'
calculate_overlaps_xor(subsets,verbose=FALSE)
```

Arguments

subsets	List of the subset image files. All files have to be in one folder. See the manual for more information about creating the list.
verbose	logical (default=FALSE). Change to TRUE to print the calculated values to two new files called "Results_overlaps.txt" and "Results_xor.txt" to the working directory.

Details

After saving a list containing the filenames of the subset histogram images this method calculates the XOR and overlap images/values for each combination of every image, returns the values and is able to write the values to two new files called "Results_overlaps.txt" and "Results_xor.txt" (see example section). See reference Koch et al. 2013 for more information about the calculation.

Value

The *calculate_overlaps_xor()* method calculates the overlap and XOR images and returns a list with two data frames containing the calculated data.

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References

Christin Koch, Ingo Fetzer, Hauke Harms, and Susann Müller. CHIC - An Automated Approach for the Detection of Dynamic Variations in Complex Microbial Communities. Cytometry Part A, 2013.

Examples

```
require(EBImage)

## Calculate the overlap and XOR images
## Save the returned values as a list

# Get a list of the filenames of the FCS files
files <- list.files(system.file("extdata",package="flowCHIC"),
full=TRUE,pattern="*.fcs")
# Create histogram images and save them
fcs_to_img(files)
# Create subsets
img_sub(files,x_start=200,x_end=3500,y_start=1000,y_end=3000,maxv=160)
# Get a list of the filenames of the PNG files
subsets <- list.files(path=paste(getwd(),"chic_subset",sep="/"),full=TRUE,pattern="*.png")
# Calculate and save values as a list
results<-calculate_overlaps_xor(subsets)

## Calculate the overlap and XOR images
## Two new files called "Results_overlaps.txt" and
## "Results_xor.txt" are written to the working directory

# Get a list of the filenames of the FCS files
files <- list.files(system.file("extdata",package="flowCHIC"),
full=TRUE,pattern="*.fcs")
# Create histogram images and save them
fcs_to_img(files)
# Create subsets
img_sub(files,x_start=200,x_end=3500,y_start=1000,y_end=3000,maxv=160)
```

```
# Get a list of the filenames of the PNG files
subsets <- list.files(path=paste(getwd(),"chic_subset",sep="/"),full=TRUE,pattern="*.png")
# Calculate
calculate_overlaps_xor(subsets,verbose=TRUE)
```

`fcs_to_img` *FCS files to images*

Description

Create histogram images of FCS files.

Usage

```
## S4 method for signature 'character'
fcs_to_img(files,transformation=FALSE,ch1="FS.Log",ch2="FL.4.Log",width=300,height=300,...)
```

Arguments

`files`	List of all .fcs files. All files have to be in one folder. See the manual for more information about creating the list.
`transformation`	Character string to define the type of data transformation (default=*FALSE*). Fore more details type "?read.FCS" into R command line.
`ch1`	Character string indicating the first channel of the histogram (x-axis) (default=*"FS.Log"*). See the manual for more details.
`ch2`	Character string indicating the second channel of the histogram (y-axis) (default=*"FL.4.Log"*). See the manual for more details.
`width`	Width (pixel) of the resulting histogram image (default=*300*).
`height`	Height (pixel) of the resulting histogram image (default=*300*).
`...`	Additional parameters used for reading the FCS files, for creating the PNG images and for creating the plots. Fore more details type "?read.FCS", "?png" or "??ggplot2" into R command line.

Details

This method creates histogram images of FCS files using the *ggplot* method of the package "ggplot2" (see reference Wickham 2009). After creating a list containing the names of the FCS files a new folder called "chic_images" is created in the working directory that contains the histogram images. Choose the two channels that are used for plotting on the x/y-axis.

Value

The *fcs_to_img()* method creates histogram images of FCS files.

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References

H. Wickham. ggplot2: elegant graphics for data analysis. Springer New York,2009.

Examples

```
require(flowCore)
require(ggplot2)

## Write the histogram images of the FCS files that are included
## to the package in a new subfolder of the working directory
## called "chic_images"

# Get a list of the filenames of the FCS files
files <- list.files(system.file("extdata",package="flowCHIC"),
full=TRUE,pattern="*.fcs")
# Create histogram images and save them
fcs_to_img(files)
```

---

img_sub *Create image subsets*

Description

Create subsets of FCS files and the resulting histogram images.

Usage

```
## S4 method for signature 'character'
img_sub(files,transformation=FALSE,ch1="FS.Log",ch2="FL.4.Log",x_start=0,x_end=4095,y_start=0,y_end
```

Arguments

files	Character list of .fcs files. All files have to be in one folder. See the manual for more information about creating the list.
transformation	Character string to define the type of data transformation (default=*FALSE*). Fore more details type "?read.FCS" into R command line.
ch1	Character string indicating the first channel of the histogram (x-axis) (default=*"FS.Log"*). See the manual for more details.
ch2	Character string indicating the second channel of the histogram (y-axis) (default=*"FL.4.Log"*). See the manual for more details.
x_start	Start of the rectangle gate on the x-axis (default=*0*). See the manual for more details.

x_end	End of the rectangle gate on the x-axis (default=*4095*). See the manual for more details.
y_start	Start of the rectangle gate on the y-axis (default=*0*). See the manual for more details.
y_end	End of the rectangle gate on the y-axis (default=*4095*). See the manual for more details.
xbin	Number of bins within the histogram (default=*128*).
maxv	Maximal value of the expressions within the histogram that is set to the highest color value (black) (default=*200*). See the manual for more details.
width	Width (pixel) of the resulting histogram image (default=*300*).
height	Height(pixel) of the resulting histogram image (default=*300*).
...	Additional parameters used for reading the FCS files, creating the PNG images and for plotting. For more details type "?read.FCS", "?png" or "??ggplot2" into R command line.

Details

This method creates subsets of FCS files and the resulting histogram images using the *ggplot* method of the package "ggplot2" (see reference Wickham 2009). After creating a list containing the names of the FCS files a new folder called "chic_subset" is created in the working directory that contains the subset histogram images. Choose the two channels that are used for plotting on the x/y-axis. Define the start and the end of the rectangle gate of both axes. See the manual for more details.

Value

The *img_sub()* method creates subsets of the histogram images.

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References

H. Wickham. ggplot2: elegant graphics for data analysis. Springer New York,2009.

Examples

```
require(flowCore)
require(ggplot2)

## Write the subset histogram images of the FCS files that are included
## to the package in a new subfolder of the working directory
## called "chic_subset"

# Get a list of the filenames of the FCS files
files <- list.files(system.file("extdata",package="flowCHIC"),
```

```
full=TRUE,pattern="*.fcs")
# Create subsets
img_sub(files,x_start=200,x_end=3500,y_start=1000,y_end=3000,maxv=160)
```

`plot_nmds` *NMDS plot of samples*

Description

NMDS plot of samples based on the calculated XOR and overlap values

Usage

```
## S4 method for signature 'data.frame,data.frame'
plot_nmds(x,y,show_cluster=FALSE,type="p",main="",col_nmds="black",cex=0.6,pos=1,
group,legend_pos="topleft",abiotic,p.max=0.05,col_abiotic="magenta",verbose=FALSE,...)
```

Arguments

`x`	Table with calculated overlap data.
`y`	Table with calculated XOR data.
`show_cluster`	logical (default=*FALSE*). Change to TRUE if cluster dendrogram shall be plotted.
`type`	Type of the plot (default=*"p"*). The *"p"* indicates points without connecting lines within the plot. Only used if **group**=FALSE. For more details type "?points" into R command line.
`main`	Character string used as title of the NMDS plot (default="").
`col_nmds`	Color used for the plotted data points if **group**=FALSE (default=*"black"*).
`cex`	numeric (default=0.6). Character expansion factor. Used for the final size of the characters.
`pos`	Position of the text (default=2). Values of 1, 2, 3 and 4, respectively indicate positions below, to the left of, above and to the right of the specified coordinates.
`group`	Data frame containing group assignments. The order and the number of these groups has to be identical to the order and the number of the samples printed in R. Use only integer values in the range from 1 to 25. See the manual for more details.
`legend_pos`	Position of the legend (default=*"topleft"*) if **group**=TRUE. For more details type "?legend" into R command line.
`abiotic`	Table with abiotic data. Should be a tab-delimited text file using '.' as decimal delimiter. Use one row for one sample and one column for one abiotic or experimental parameter. Use the first column for the first parameter and the first line as header. The order and the number of the lines has to be identical to the order and the number of the samples printed in R.

p.max	Decimal number defining the significance level of the abiotic parameters (default=0.05) if **abiotic**=TRUE. Only parameters less/equal this value are plotted.
col_abiotic	Color used for the plotted abiotic parameters (default=*"magenta"*).
verbose	logical (default=FALSE). Do not print additional information. Change to TRUE to print results of the metaMDS method and the p-values of the abiotic parameters.
...	Additional parameters used for plotting the data points if **group**=FALSE. For more details type "?points" into R command line.

Details

This method is used for calculating the similarities found in the histogram images of cytometric data. A dissimilarity matrix is generated from the pairwise comparison of histogram images based on the values returned by the method *calculate_overlaps_xor* or saved in the files "Results_overlaps.txt" and "Results_xor.txt". See reference Koch et al. 2013 for more details. Ensuing from this matrix nonmetric multidimensional scaling (NMDS) is performed to show the results. The NMDS plot is calculated using the *metaMDS* method of the package "vegan" (see reference Warnes et al. 2013). In addition, a cluster analysis can be performed to reveal samples with high similarities.

Value

The *plot_nmds()* method calculates a NMDS plot of the samples and an additional cluster dendrogram.

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References

Christin Koch, Ingo Fetzer, Hauke Harms, and Susann Müller. CHIC - An Automated Approach for the Detection of Dynamic Variations in Complex Microbial Communities. Cytometry Part A, 2013.

Jari Oksanen, F. Guillaume Blanchet, Roeland Kindt, Pierre Legendre, Peter R. Minchin, R. B. O'Hara, Gavin L. Simpson, Peter Solymos, M. Henry H. Stevens and Helene Wagner (2013). vegan: Community Ecology Package. R package version 2.0-10. http://CRAN.R-project.org/package=vegan

Examples

```
require(vegan)

## Show the NMDS plot of the sample files
## included to the package

# Get a list of the filenames of the FCS files
files <- list.files(system.file("extdata",package="flowCHIC"),
full=TRUE,pattern="*.fcs")
# Create histogram images
```

```
fcs_to_img(files)
# Create subsets
img_sub(files,x_start=200,x_end=3500,y_start=1000,y_end=3000,maxv=160)
# Get a list of the filenames of the subset PNG files
subsets <- list.files(path=paste(getwd(),"chic_subset",sep="/"),full=TRUE,pattern="*.png")
# Calculate
results<-calculate_overlaps_xor(subsets)
# Show NMDS plot ensuing from the returned values
plot_nmds(x=results$overlap,y=results$xor)


## Show the NMDS plot of the dataset "mix""

data(Results_overlaps_mix)
data(Results_xor_mix)
plot_nmds(Results_overlaps_mix,Results_xor_mix)

## Show the NMDS plot of the dataset "incol""

data(Results_overlaps_incol)
data(Results_xor_incol)
plot_nmds(Results_overlaps_incol,Results_xor_incol)

## Show the NMDS plot of the dataset "incol"" with group assignment
## and abiotic data
## Print results of the metaMDS method and the p-values of the abiotic parameters
## Show a cluster dendrogram

data(Results_overlaps_incol)
data(Results_xor_incol)
groups<-data.frame("groups"=c(15,19,19,19,15,22,19,15,22,15,15,22,22,22,22,19,19))
data(abiotic_incol)
plot_nmds(Results_overlaps_incol,Results_xor_incol,show_cluster=TRUE,group=groups,abiotic=abiotic_incol[,-1],v
```

`Results_overlaps` *Dataset with labels and sums of informative pixels within the overlap area of the FCS files included to the package*

Description

Example dataset containing labels and areas of each image pair of the FCS files included to the package.

Usage

```
data(Results_overlaps)
```

Format

Data frame with 3 observations of 2 variables.

Value

Results of the method *calculate_overlaps_xor()* on the FCS files included to the package.

`Results_overlaps_incol` *Dataset with labels and sums of informative pixels within the overlap area of the second downloadable dataset*

Description

Example dataset containing labels and areas of each image pair of the second downloadable dataset (see manual).

Usage

```
data(Results_overlaps_incol)
```

Format

Data frame with 136 observations of 2 variables.

Value

Results of the method *calculate_overlaps_xor()* on the subsets of the second downloadable dataset.

`Results_overlaps_mix` *Dataset with labels and sums of informative pixels within the overlap area of the first downloadable dataset*

Description

Example dataset containing labels and areas of each image pair of the first downloadable dataset (see manual).

Usage

```
data(Results_overlaps_mix)
```

Format

Data frame with 4005 observations of 2 variables.

Value

Results of the method *calculate_overlaps_xor()* on the subsets of the first downloadable dataset.

`Results_xor` *Dataset with labels and intensity values of the FCS files included to the package*

Description

Example dataset containing labels and intensity values of each image pair of the FCS files included to the package.

Usage

```
data(Results_xor)
```

Format

Data frame with 3 observations of 2 variables.

Value

Results of the method *calculate_overlaps_xor()* on the FCS files included to the package.

`Results_xor_incol` *Dataset with labels and intensity values of the second downloadable dataset*

Description

Example dataset containing labels and intensity values of each image pair of the second downloadable dataset (see manual).

Usage

```
data(Results_xor_incol)
```

Format

Data frame with 136 observations of 2 variables.

Value

Results of the method *calculate_overlaps_xor()* on the subsets of the second downloadable dataset.

`Results_xor_mix` *Dataset with labels and intensity values of the first downloadable dataset*

Description

Example dataset containing labels and intensity values of each image pair of the first downloadable dataset (see manual).

Usage

```
data(Results_xor_mix)
```

Format

Data frame with 4005 observations of 2 variables.

Value

Results of the method *calculate_overlaps_xor()* on the subsets of the first downloadable dataset.

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