Package 'prada'

October 16, 2019

Version 1.60.0

Title Data analysis for cell-based functional assays

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Depends R (>= 2.10), Biobase, RColorBrewer, grid, methods, rrcov

Suggests cellHTS2, tcltk

Imports Biobase, BiocGenerics, graphics, grDevices, grid, MASS, methods, RColorBrewer, rrcov, stats4, utils

Collate AllGenerics.R AllClasses.R methods-cytoFrame.R methods-cytoSet.R methods-gate.R methods-gateSet.R methods-misc.R analysePlate.R as.all.R barploterrbar.R combineFrames.R csApply.R combineGates.R cytoFunctions.R fitNorm2.R gateMatrix.R getPradaPar.R plotNorm2.R plotPlate.R readCytoSet.R readFCS.R readSDM.R removeCensored.R thresholds.R touchFCS.R tcltkProgress.R getAlphanumeric.R

Description Tools for analysing and navigating data from high-throughput phenotyping experiments based on cellular assays and fluorescent detection (flow cytometry (FACS), high-content screening microscopy).

License LGPL

biocViews ImmunoOncology, CellBasedAssays, Visualization

LazyLoad yes

git_url https://git.bioconductor.org/packages/prada

git_branch RELEASE_3_9

git_last_commit 5b534be

git_last_commit_date 2019-05-02

Date/Publication 2019-10-15

R topics documented:

`analysePlate` *Apply a statistic to the data from each well in a plate*

Description

Apply a statistic to the data from each well in a plate

Usage

```
analysePlate(x, wellcol="well", wellrange, statfun, platename, plotdir=".", ...)
```

Arguments

`x`	data frame. It must contain a column whose name is the value of `wellcol`, and further columns that are needed by the function named by `stat`.
`wellcol`	character of length 1. Name of a column in `x` that contains the well ID.
`wellrange`	vector of the same type as `x[,wellcol]`. All values `x[,wellcol]` must be contained in `wellrange`.
`statfun`	character of length 1. Name of a function that can calculate a statistic from selected rows of `x`.
`platename`	character of length 1. The name or ID of this plate, which will be used for graphics output filenames and as the value of the column `platename` of the return value.

plotdir	character of length 1. The name of directory where diagnostic plots will be saved.
...	further arguments that are passed on to statfun.

Details

The semantics of this function are similar to tapply, but some additional checking and reporting is performed, and the return value is a data frame.

Value

A data frame with number of rows equal to length(wellrange). Rows (wells) for which there is no data contains NAs. The columns comprise platename, well-ID (from x[,wellcol]), and the results from statfun.

Author(s)

Wolfgang Huber

Examples

```
##see vignette
```

---

as.all *Coercion without introduction of NAs*

Description

Coercion without introduction of NAs

Usage

```
as.all(x, what)
```

Arguments

x	an object.
what	character of length 1.

Details

The function calls do.call(paste("as.",what,sep=""),list(x)), and checks whether any NAs were introduced.

Value

A vector of type what

Author(s)

Wolfgang Huber

See Also

as

Examples

```
as.all(runif(5)*10, "integer")
```

---

barploterrbar *Barplot with error bars.*

Description

Barplot with error bars.

Usage

```
barploterrbar(y, yl, yh, barcol="orange", errcol="black", horiz=FALSE,
w=0.2, ylim=c(0, max(yh)*1.05), ...)
```

Arguments

y	Numeric vector.
yl	Numeric vector of same length as y.
yh	Numeric vector of same length as y.
barcol	Color of the bars.
errcol	Color of the error bars.
horiz	Logical. As in barplot.
w	The plot limits. The default value wil cause the error bars to fit nicely on the plotting device.
ylim	Size of the error bar ticks.
...	Further arguments that get passed on to barplot.

Details

The function calls barplot with y and decorates it with error bars according to yl and yh.

Value

The function is called for its side effect, producing a plot.

Author(s)

Wolfgang Huber http://www.dkfz.de/abt0840/whuber

See Also

barplot

Examples

```
y <- matrix(runif(80), ncol=5)
ym <- apply(y, 2, mean)
dy <- apply(y, 2, sd)*2/sqrt(nrow(y))
barploterrbar(ym, ym-dy, ym+dy, barcol="#0000c0", errcol="orange",
  ylim=c(0, max(ym+dy)))
```

`cframe` *A sample cytoFrame object - German Cancer Research Center Heidelberg -*

Description

Archived `cytoFrame` object from a MAP kinase screen conducted at the German Cancer Research Center Heidelberg. In the fluorescence channel 3 the expression of a YFP tag and in channel 7 the activation state of ERK2 was measured.

Usage

```
##cytoFrame object, see examples for details
```

Format

cytoFrame object

Source

German Cancer Research Center Heidelberg, Germany

Examples

```
data(cytoFrame)
```

`combineFrames` *Combine the cytoFrames within a cytoSet according to some grouping factor*

Description

Combine the cytoFrames within a cytoSet according to some grouping factor.

Usage

```
combineFrames(x, by)
```

Arguments

`x`	cytoSet.
`by`	factor. Length must be same as that of `x`.

Value

cytoSet.

Author(s)

Wolfgang Huber <huber@ebi.ac.uk>

Examples

```
cset <- readCytoSet(path=system.file("extdata", package="prada"),
                pattern="[A-Z][0-9][0-9]$")
nr1 <- csApply(cset, nrow)
sm1 <- csApply(cset, sum)

fac <- factor(c(1,1,2,2,2,2))
cc  <- combineFrames(cset, fac)
nr2 <- csApply(cc, nrow)
sm2 <- csApply(cc, sum)

stopifnot(all(nr2==tapply(nr1, fac, sum)))
stopifnot(all(sm2==tapply(sm1, fac, sum)))
```

---

csApply *Apply a function over the intensities in a cytoSet*

Description

This a wrapper for sapply for objects of class cytoSet.

Usage

```
csApply(X, FUN, ..., simplify = TRUE)
```

Arguments

X	cytoSet.
FUN	the function to be applied.
...	optional arguments to FUN.
simplify	logical; should the result be simplified to a vector or matrix if possible? Gets passed on the sapply.

Details

A wrapper for sapply.

Value

Like sapply: If FUN always returns a scalar, then the value of this function is a named vector. If FUN always returns a vector of length n, then the value of this function is an n x length(X) matrix with dimnames. Else, the value of this function is a named list whose values are the return values of the individual calls to FUN.

Author(s)

Wolfgang Huber http://www.ebi.ac.uk/huber

See Also

sapply

Examples

```
cset=readCytoSet(path=system.file("extdata", package="prada"),
  pattern="[A-Z][0-9][0-9]$")
csApply(cset, nrow)
csApply(cset, colMeans)
```

---

cset *A sample cytoSet object - German Cancer Research Center Heidelberg -*

Description

Archived cytoSet object from a MAP kinase screen conducted at the German Cancer Research Center Heidelberg. In the fluorescence channel 3 the expression of a YFP tag and in channel 7 the activation state of ERK2 was measured. The set contains measurements from 5 wells of a 96 well plate

Usage

```
##cytoSet object, see examples for details
```

Format

cytoSet object

Source

German Cancer Research Center Heidelberg, Germany

Examples

```
data(cytoSet)
```

`cytoFrame-class` *'cytoFrame': a class for storing observed quantitative properties from a population of cells, most likely from a FACS run or, alternatively, from automated microscopy*

Description

This class represents the data contained in a FCS 3.0 file or similar data structures.

Details

Although objects of class `cytoFrame` can be used to hold arbitrary data of cell populations, the main focus lies on flow-cytometry data.

FCS 3.0 is the Data File Standard for Flow Cytometry, Version FCS3.0. See the vignette of this package for additional information on using the object system for handling of flow-cytometry data.

Creating Objects

Objects can be created using

```
new('cytoFrame',
exprs = ....,# Object of class matrix
description = .... # Object of class character
)
```

or the function `readFCS`.

Slots

`exprs`**:** Object of class `matrix` containing the measured intensities. Rows correspond to cells, columns to the different channels. The `colnames` attribute of the matrix is supposed to hold the names or identifiers for the channels. The `rownames` attribute would usually not be set.

`description`**:** A named character vector containing the experiment description as key-value pairs.

`well`**:** A single integer vector giving the position of the well on a microtitre plate. This only applies when using the object within a `cytoSet` collection and will usually be filled in by the function `readCytoSet`.

`gate`**:** An object of class `gateSet`. This object can be used to select defined subsets of the data, a process referred to as `gating` in the analysis of flow-cytometry data.

Methods

[subsetting. Returns an object of class `cytoFrame`. The subsetting is applied to the `exprs` slot, while the `description` slot is unchanged.

exprs, exprs<- extract or replace the intensities.

description, description<- extract or replace the description.

show display summary.

plot scatterplot for `cytoFrame` objects. The additional argument `gate` can be used to plot subsets of the data defined by either an object of class `gate` or by a character vector giving the name of one of the gates in the list.

gate,gate<- extract or replace the list of gates.

ncol,nrow extract the dimensions of the data matrix.

appendGate Append a gate or gateSet to the gate slot.

drawGate Create an object of class gate or gateSet based on a selection made from the data.

hist Draw a histogram of the data

Author(s)

Florian Hahne, Wolfgang Huber

See Also

readFCS, cytoSet, gate, gateSet

Examples

```
intens <- matrix(runif(100), ncol=4)
colnames(intens) <- c("FL1-H", "FL2-H", "FL3-H", "FL4-H")

a <- new("cytoFrame",
          exprs=intens,
          description=c(name="example data", date=date()))

description(a)
dim(exprs(a))

a[1:3, -4]

plot(a)
## Not run:
g1 <- drawGate(a, name="Gate1")

## End(Not run)
```

---

cytoSet-class *'cytoSet': a class for storing raw data from a quantitative cell-based assay*

Description

This class is a container for a set of cytoFrame objects

Creating Objects

Objects can be created using the function readCytoSet or via

```
new('cytoSet',
frames = ....,# environment with cytoFrames
phenoData = .... # object of class phenoData
colnames = .... # object of class character
)
```

Slots

`frames`: An `environment` containing one or more `cytoFrame` objects.

`phenoData`: A `phenoData`. Each row corresponds to one of the cytoFrames in the `frames` slot. It is mandatory that the pData has column named `name`

`colnames`: A `character` object with the (common) column names of all the data matrices in the cytoFrames.

Methods

[, [[subsetting. If `x` is `cytoSet`, then `x[i]` returns a `cytoSet` object, and `x[[i]]` a `cytoFrame` object. The semantics is similar to the behavior of the subsetting operators for lists.

colnames, colnames<- extract or replace the `colnames` slot.

phenoData, phenoData<- extract or replace the `phenoData` slot.

show display summary.

plot Scatterplot of one or all (consecutively) `cytoFrame` objects. The additional argument `gate` can be used to plot subsets of the data defined by an object of class `gate` or `gateSet`.

hist Draw histogram of the data. The additional argument `variable` can be used to subset to one variable prior to plotting.

Important note on storage and performance

The bulk of the data in a `cytoSet` object is stored in an `environment`, and is therefore not automatically copied when the `cytoSet` object is copied. If `x` is an object of class `cytoSet`, then the code

```
y <- x
```

will create a an object `y` that contains copies of the `phenoData` and administrative data in `x`, but refers to the *same* environment with the actual fluorescence data. See below for how to create proper copies.

The reason for this is performance. The pass-by-value semantics of function calls in `R` can result in numerous copies of the same data object being made in the course of a series of nested function calls. If the data object is large, this can result in a considerable cost of memory and performance. `cytoSet` objects are intended to contain experimental data in the order of hundreds of Megabytes, which can effectively be treated as read-only: typical tasks are the extraction of subsets and the calculation of summary statistics. This is afforded by the design of the `cytoSet` class: an object of that class contains a `phenoData` slot, some administrative information, and a *reference* to an environment with the fluorescence data; when it is copied, only the reference is copied, but not the potentially large set of fluorescence data themselves.

However, note that subsetting operations, such as

```
y <- x[i]
```

do create proper copies, including a copy of the appropriate part of the fluorescence data, as it should be expected. Thus, to make a proper copy of a `cytoSet` `x`, use

```
y <- x[seq(along=x)]
```

Author(s)

Florian Hahne, Wolfgang Huber http://www.ebi.ac.uk/huber

See Also

readCytoSet, cytoFrame, gate, gateSet

Examples

```
cset<-readCytoSet(path=system.file("extdata", package="prada"),
  pattern="[A-Z][0-9][0-9]$")
cset
pData(cset)
cset[[1]]
cset[["fas-Bcl2-plate323-04-04.A02"]]
cset["fas-Bcl2-plate323-04-04.A02"]
cset[1:3]

cset[[1]] <- exprs(cset[[1]])[1:100, ]

## Not run:
plot(cset[2])

## End(Not run)
```

---

devDims *Device Dimensions for plate plots*

Description

Calculate device dimensions for plate plots

Usage

```
devDims(width, height, ncol=12, nrow=8, res=72)
```

Arguments

width	Device width in inches.
height	Device width in inches.
ncol	Number of columns for plate plot.
nrow	Number of rows for plate plot.
res	The resolution of the graphic device used for plotting.

Details

The function computes the device dimensions needed to create plate plots that fit perfectly in the device. This is necessary to retain the aspect ratio of the plots.

One of width or height need to be specified, the missing value will be computed.

Value

A list with items width,height,pwidth and pheight. These are the width and height values in inches and pixels respectively.

Author(s)

Florian Hahne

See Also

plotPlate

Examples

```
devDims(width=10)
```

---

devRes *Resolution of current plotting device*

Description

Calculates what R thinks to be the resolution of the current graphic device.

Usage

```
devRes()
```

Details

This function may be used to get the resolution of the current graphics device. This can be important when calculating pixel coordinates for the output graphic.

Value

A vector with items `xres` and `yres`, the resolution in `x` and `y` direction respectively.

Author(s)

Florian Hahne

See Also

plotPlate

Examples

```
devRes()
```

`fitNorm2` *Fit bivariate normal distribution.*

Description

Fits a bivariate normal distribution into a data set of paired values and selects data points according to their standard deviation from the fitted distribution.

Usage

```
fitNorm2(x, y=NA, scalefac=1, method="covMcd", noise, gateName = "fitNorm")
```

Arguments

`x`	Numeric vector containing x-value or n by 2 matrix containing x and y values or object of class `cytoFrame`.
`y`	Numeric vector containing y-value (optional). The length of `x` must be the same as that of `y`.
`scalefac`	Numeric vector giving factor of standard deviations used for data selection (all points within `scalefac` standard deviations are selected).
`method`	One of `covMcd` or `cov.rob` defining method used for computation of covariance matrix.
`noise`	Numeric or logical index vector defining value pairs in x that are not used for fitting of distributions. Can be used to deal with noisy data.
`gateName`	Character giving the name of the gate object.

Details

Computes the densities of a bivariate normal distribution from the covariance matrix of the paired data. Covariance matrices are acquired either by function `covMcd` (considerably faster) or by function `cov.rob`.

Value

A list containing items `mu` (midpoint of distribution), `S` (covariance matrix), `p` (density values for each data pair), `sel` (selection of data points), `scalefac` (factor of standard deviations used for data selection), `data` (x and y values of data points) and `gate`, an object of class `gate` containing the selection.

Author(s)

Florian Hahne

See Also

`cov.rob`, `covMcd`, `plotNorm2`

Examples

```
sampdat <- readFCS(system.file("extdata",
  "fas-Bcl2-plate323-04-04.A01", package="prada"))
nfit    <- fitNorm2(exprs(sampdat[,1:2]), scalefac=2)
plotNorm2(nfit, selection=TRUE, ellipse=TRUE)
```

---

gate-class *'gate': a class for subsetting flow-cytometry data by defining regions in two-dimesional projections of the data*

Description

In flow-cytometry analysis, regions in two-dimensional projections of the data space often have to be selected. Objects of this class can store the properties of these selections.

Creating Objects

Objects can be created using methods of the generic function drawGate or via

```
new("gate",
gateFun = ....,# function returning logical vector
colnames = .... # object of class character and length 2
logic = .... # object of class character
)
```

Slots

name: A character vector for the name of the gate object. You can reference the object by its name for subsequent operations (e.g. plotting).

gateFun: A function call together with necessary arguments to produce a logical vector when applied on the data.

colnames: The colnames of the data matrix to which the gating function is to be applied.

logic: A character object, either & or |. This specifies the logical operation that will be applied when combining the selection from the gate with other object of that class. See link{gateSet} for additional information on combining gates.

type: A character giving the type of the object. This is currently not used but might become important in the future.

boundaries: A matrix with two columns giving the boundaries of the gate in two dimensional space. Can be used to superimpose the gate boundaries on a plot using lines().

Methods

applyGate: applyGate(x,data) applies the gating of object x on data objects of class cytoFrame or matrix. In the former case x may be of class gate, gateSet, character, numeric or logical. See vignette for details.

show display summary.

names, names<- access and replace slot name.

as.gateSet Convert gate object to gateSet object

combineGates Combine multiple gate objects to one gateSet object

lines Draw the boundaries of the gate.

Author(s)

Florian Hahne

See Also

cytoFrame, gateSet

Examples

```
sampdat <- readFCS(system.file("extdata", "fas-Bcl2-plate323-04-04.A01",
                               package="prada"))
g1 <- new("gate", name="test1", gateFun=function(x)x[,"FSC-H"]<500, logic="&",
          colnames="FSC-H", type="misc")
g1
g2 <- new("gate", name="test2", gateFun=function(x)x[,"SSC-H"]>800, logic="&",
          colnames="SSC-H", type="misc")
gs1 <- combineGates(g1,g2)
gs2 <- as.gateSet(g2)
names(g1)
names(g1) <- "testName"
applyGate(sampdat, g1)
applyGate(exprs(sampdat), g2)
gate(sampdat) <- g1
applyGate(sampdat, 1)
applyGate(sampdat, "testName")
applyGate(sampdat, TRUE)
```

---

gateSet-class — *'gateSet': a class for subsetting flow-cytometry data by defining multiple regions in two-dimesional projections of the data*

Description

In flow-cytometry analysis, regions in two-dimensional projections of the data space often have to be selected. Objects of this class can store the properties for several of these selections

Creating Objects

Objects can be created using methods of the generic function drawGate or via
`new("gateSet",`
`glist = ....,# object of class list`
`)`

Slots

name: Object of class `character` giving the name of the object. You can reference the object by its name for subsequent operations (e.g. plotting).

glist: Object of class `"list"` with items of class gate. The individual gate objects will be combined according to the value of their slot `logic`.

Methods

applyGate: `applyGate(x,data)` applies the gating of object `x` on data objects of class `cytoFrame` or `matrix`

length length of slot `glist`

show `display summary`

names, names<- extract or replace the names of the individual `gate` objects.

[subset to `gateSet` objects.

[[subset to individual `gate` objects.

appendGates append a `gate` or `gateSet` to a `cytoFrame`

Author(s)

Florian Hahne

See Also

`cytoFrame`, `gate`

Examples

```
sampdat <- readFCS(system.file("extdata", "fas-Bcl2-plate323-04-04.A01",
                               package="prada"))
g1 <- new("gate", name="G1", gateFun=function(x)x[,"FSC-H"]<500, logic="&",
          colnames="FSC-H")
g2 <- new("gate", name="G2", gateFun=function(x)x[,"SSC-H"]>800, logic="&",
          colnames="SSC-H")
g3 <- new("gate", name="G3", gateFun=function(x)x[,"FL1-H"]>800, logic="&",
          colnames="FL1-H")
gs <- new("gateSet", name="Set1", glist=list(G1=g1, G2=g2))
length(gs)
gs[[1]]
gs[1]
gsnames <- names(gs)
names(gs) <- gsnames
applyGate(sampdat, gs)
applyGate(exprs(sampdat), gs)
gate(sampdat) <- gs
applyGate(sampdat, 1)
applyGate(sampdat, "G1")
applyGate(sampdat, TRUE)
appendGates(sampdat, g3)
```

---

getAlphaNumeric *Convert from plate coordinates to alphanumeric notation.*

Description

Given an array of (x,y) well coordinates, this function returns the corresponding alphanumeric notation.

Usage

```
getAlphaNumeric(horizontal, vertical)
```

Arguments

horizontal	Integer coordinate of horizontal well location.
vertical	Integer coordinate of vertical well location.

Value

`getAlphaNumeric` returns a list containing `id`, the full alphanumeric id of the well(s), `id.alpha`, the alpha part of the id, and `id.num`, the numeric part of the id.

Author(s)

Joseph Barry <joseph.barry@embl.de>

See Also

convertWellCoordinates

Examples

```
## To obtain the alpha, numeric and alphanumeric information for a single well
getAlphaNumeric(horizontal=1,vertical=1)

## To obtain only the alphanumeric ids of a tetrad in the corner of a 1536 well plate
getAlphaNumeric(horizontal=c(31,31,32,32),vertical=c(47,48,47,48))$id
```

---

getPradaPar *Set and query global parameters for functions in the prada package*

Description

Set and query global parameters for functions in the prada package

Usage

```
setPradaPars(pars)
getPradaPar(parname)
```

Arguments

pars	Named list
parname	Character string of length 1

Details

TBA

Value

For getPradaPar, the value of the list element with name parname of the global parameters list. The function setPradaPars is invoked for its side effect, which is assigning a value to the global parameters list. It returns the value invisible(NULL).

Author(s)

Wolfgang Huber http://www.ebi.ac.uk/huber

Examples

```
setPradaPars(list(tomato=1, apple="two", pear=as.integer(3)))
getPradaPar("pear")
```

---

plotNorm2 *Plot fitted bivariate normal distribution.*

Description

Plots objects derived from function fitNorm2 in false color represenation.

Usage

```
plotNorm2(fn, colrange=c("gray82", "blue"), center=TRUE, selection=FALSE,
                      ellipse=FALSE, pch=20, cex=1, col="dens", ...)
```

Arguments

fn	List. Object derived from function fitNorm2
colrange	Character vector with valid color identifiers (eg name or RGB values) from which a smooth color palette is derived.
center	Logical. Assign center of distribution.
selection	Logical. Mark all points beyond selection.
ellipse	Logical. Plot area and borders of selection as ellipse.
pch	see par
cex	see par
col	see par or special cases dens for coloring according to density and prob for coloring according to probability.
...	further arguments that are passed on to plot.

Details

Produces a scatterplot of paired data showing the densities of the fitted bivariate distribution from function fitNorm2 in false color representation. Additionally a selection of data points can be highlighted either by marking outliers or by showing its area.

Value

A list containing items p,cov,mu,S (density values for each data pair, resulting object from call to cov.rob, midpoint of distribution, covariance matrix).

Author(s)

Florian Hahne

See Also

fitNorm2

Examples

```
sampdat <- readFCS(system.file("extdata",
  "fas-Bcl2-plate323-04-04.A01", package="prada"))
nfit   <- fitNorm2(exprs(sampdat[,1:2]), scalefac=2)
plotNorm2(nfit, selection=TRUE, ellipse=TRUE)
```

---

plotPlate *Plot a well statistic for microtiter plates.*

Description

Plot a well statistic in false color representation or using a self-defined grid plotting function. The plot is supposed to resemble the physical geometry of a microtitre plate.

Usage

```
plotPlate(x,nrow = 8, ncol = 12, col=c("red", "blue"),
ind = 1:(ncol*nrow), xrange=function(y) range(y, na.rm=TRUE), na.action = "zero",
main, char, desc = character(2), add=FALSE, gridFun="default",
funArgs=NULL,...)
```

Arguments

x	Numeric vector of length ncol*nrow or matrix with ncol*nrow rows (except if argument ind is specified). If of class matrix, the use of argument gridFun is expected.
nrow	Numeric of length 1. The number of rows of the plate.
ncol	Numeric of length 1. The number of columns of the plate.
col	Character vector. Usually the names of two or three colors between which the color map is interpolated, using the function colorRampPalette.
ind	Optional integer vector of equal length as x. It indicates the position of the respective value of x on the plate. Can be used to adress the problem of missing values. Each well that is not allocated a value of x by ind will not be plotted.
xrange	Numeric vector of length two giving thwe range of x that is mapped into the color scale. Alternatively, this can be a function which takes the values of x as input and creates such a vector.
na.action	Character. One of "zero" "omit" or "xout". How should the wells for which x is NA be treated? For "zero", they are plotted as if the value were 0. For "omit", they are omitted. For "xout", they are crossed out. When x is a matrix, na.action is only applied to rows containing nothing but NAs. Further special treatment of NA values in matrices need to be implemented in gridFun.

main	Character of length 1. Plot title.
char	An optional character vector of equal length as x (except if argument ind is specified) to be used for well annotation. Each element of the vector may contain a string to be superimposed on the respective well or NA for no plotting.
desc	Character of length 2. Legend for the two extremes of the colorbar, e.g. 'act' and 'inh'.
add	Logical. If TRUE add plate plot to current plot. May be used when plotting in grid layout panels.
gridFun	Character. The name of the plotting function to create individual graphs for each well. See functions .drawCircle and .drawPiefor examples.
funArgs	Dataframe with argument values to be passed to gridCall. For each argument specified in gridCall there must be one column with the argument name as colname and the argument values for every well.
...	Further graphical parameters that can be used to control the output of plotPlate. **cex.main:** expansion factor for title. **cex.lab:** expansion factor for label. **cex.char:** expansion factor for well annotation. **cex.legend:** expansion factor for well legend labels. **cex.desc:** expansion factor for well legend description.

Details

You may use this function either to create plots showing a single-value per well statistic for microtiter plates, or you can use a self-made plotting function using a combination of any valid grid commands to produce arbitrary plots in a plate array format. These plots may also show multifactorial data. Self-defined plotting functions need to have data as first argument. plotPlate passes all data values for the respective well to the plotting function. Any further arguments may be passed on using argument funArgs. See .drawCircle and .drawPie for examples of valid plotting functions and the vignette for detailed information. Note that using funCall overrides some of the default functionalities, e.g. plotting of legends and alters the treatment of NA values.

Argument ind allows the user to indicate the position (well number) for each element of vector x on the plate. This can be used either to change the order in which elements of x are to be plotted or to deal with the problem of missing data for some of the wells on a plate.

To further increase the amount of information of the platePlot one may decorate wells with short annotations using argument char. Each element of char != NA will be superimposed on the respective well (see examples).

Value

The function produces a plot in the active graphics device.

It returns a list withfour elements. The element which is a vector with the indices of those elements in x that were plotted (see argument na.action). The element coord is a length(which) by 4 matrix in which each row specifies the corners of a rectangle that contains a well. It is intended to be used as an argument to a subsequent call to imageMap. Elements width and height may be used to open a graphic devices that can hold the plate plot with the correct aspect ratio.

Author(s)

Florian Hahne, Wolfgang Huber http://www.ebi.ac.uk/huber

See Also

imageMap

Examples

```
plotPlate(runif(96), main="example 1", col=c("#0000e0", "#e00000"), desc=c("act", "inh"))
plotPlate(runif(384), nrow=16, ncol=24, main="example 2", col=c("#0000e0", "white", "#e00000"))
plotPlate(runif(48), main="example 3", col=c("#0000e0", "#e00000"), ind=c(1:24, 73:96))
x <- runif(96)
x[sample(96, 10)] <- NA
plotPlate(x, main="example 4", col=c("#0000e0", "#e00000"),
char=c(rep(NA, 72), LETTERS[1:24]), na.action="xout")
plotPlate(runif(96, min=0.1, max=0.5), gridFun=".drawCircle")
plotPlate(matrix(runif(288), ncol=3), gridFun=".drawPie",
funArgs=as.data.frame(matrix(2:4, ncol=3, nrow=96, byrow=TRUE)))
```

---

progress *A simple tcltk progress window*

Description

Show progress of a task in a tcltk window as percentage

Usage

```
progress(title="processing task...", message="", sub="")
updateProgress(percentage, autoKill=FALSE, sub="")
killProgress()
```

Arguments

title	The title of the tcltk window
message	A short test message to add to the window
sub	An additional text field that can be updated viaupdateProgress
percentage	An integer giving the percentage of completion
autoKill	Logical indicating whether to kill the display after 100 is reached

Details

Function progress creates the progress window and sets up the necessary environment. updateProgress takes as argument an integer value indicating the percentage of completion and updates the display. The integer value that gets passed to updateProgress will usually be generated by an iterator (e.g. in a for loop). killProgress may be called explicitely to kill the progress window. Alternatively one can set the argument autoKill of updateProgress to TRUE to automatically kill the window once a value of 100 is reached.

Value

The functions are called for their side effects.

Author(s)

Florian Hahne

Examples

```
if(interactive() && capabilities()["tcltk"]){
  progress(message="This is a progress display...", sub="(step 1 of 50)")
  for(i in 1:50) {
     zz = rnorm(1e5)
     updateProgress(i*2, autoKill=TRUE, sub=paste("(step", i, "of 50)"))
  }
}
```

---

readCytoSet *Create a cytoSet object from one or more FCS 3.0 files*

Description

Create a cytoSet object from one or more FCS 3.0 files

Usage

```
readCytoSet(files=NULL, path=".", pattern=NULL, phenoData, sep="\t", ...)
```

Arguments

files	Optional character vector with filenames
path	Directory where to look for the files
pattern	This argument is passed on to dir (see details).
phenoData	Either an object of class phenoData or character.
sep	Separator character that gets passed on to read.AnnotatedDataFrame.
...	Further arguments that get passed on to read.AnnotatedDataFrame, see details.

Details

There are three different ways to specify the file names:

First, if the argument phenoData is present and is of class AnnotatedDataFrame, then it is obtained from its column name. The column is mandatory, and an error will be generated if it is not there. Alternatively, the argument phenoData can be of class character, in which case this function tries to read a AnnotatedDataFrame object from the file with that name by calling read.AnnotatedDataFrame with arguments file.path(path,phenoData),....

Second, if the argument phenoData is not present and the argument files is not NULL, then files is expected to be a character vector with the file names.

Third, if neither the argument phenoData is present nor files is not NULL, then the file names are obtained by calling dir(path,pattern).

Value

An object of class cytoSet.

Author(s)

Wolfgang Huber http://www.ebi.ac.uk/huber

See Also

readFCSdata

Examples

```
## Please see man page for cytoSet-class
```

---

readFCS *Read an FCS file*

Description

Read one or several FCS files: Data File Standard for Flow Cytometry

Usage

```
read.fcs(filename=NULL, objectModel="prada", ...)
readFCS(filename)
```

Arguments

filename	Character of length 1: filename
objectModel	Character of length 1: the object model to use for the output. Curently only 'prada' for cytoFrame objects is supported.
...	Arguments that get passed on to higher-level import functions.

Details

The function readFCS works with the output of the FACS machine software from a number of vendors. However, the FCS 3.0 standard includes some options that are not yet implemented in this function. If you need extensions, please let me know. The output of the fucntion is an object of class cytoFrame.

read.fcs is a wrapper function that allows the user to specify the class of the output. The purpose of the function is to standardize the way flow cytometry data is imported into R using the prada package. If the filename argument to read.fcs is a character vector of length > 1, multiple FCS files can be imported. Please see the documentation for readCytoSet for alternatives ways to import multiple FCS files and for more details on the higher-level import function.

For specifications of FCS 3.0 see http://www.isac-net.org and the file ../doc/fcs3.html in the doc directory of the package.

Value

An object of class cytoFrame.

Author(s)

Wolfgang Huber http://www.ebi.ac.uk/huber, Florian Hahne

See Also

readCytoSet

Examples

```
sampdat <- readFCS(system.file("extdata", "fas-Bcl2-plate323-04-04.A01",
                               package="prada"))
files <- dir(system.file("extdata", package="prada"),
             pattern="[A-H][0-9][0-9]")
sampdat2 <- read.fcs(system.file("extdata", "fas-Bcl2-plate323-04-04.A01",
                               package="prada"))
sampdat3 <- read.fcs(files, path=system.file("extdata", package="prada"))
sampdat
exprs(sampdat[1:3,])
description(sampdat)[3:6]
class(sampdat3)
```

---

readFCSaux *Auxiliary functions for readFCS*

Description

Auxiliary functions for readFCS - not normally called by the user

Usage

```
readFCSgetPar(x, pnam)
readFCSheader(con)
readFCStext(con, offsets)
readFCSdata(con, offsets, x)
```

Arguments

x	Named character vector.
pnam	Character vector, its elements must be contained in names(x).
con	Connection.
offsets	Integer vector of length 6 with byte offsets of the header, text, and data blocks.

Details

These functions are not normally called by the user. See readFCS instead.

Value

Various.

Author(s)

Wolfgang Huber http://www.ebi.ac.uk/huber

See Also

readFCS

removeCensored *Remove rows that contain censored data*

Description

Remove rows that contain censored data in the colums of x specified by columns.

Usage

```
## S4 method for signature 'matrix'
removeCensored(x, values, columns, na.rm=TRUE)
## S4 method for signature 'data.frame'
removeCensored(x, values, columns, na.rm=TRUE)
## S4 method for signature 'cytoFrame'
removeCensored(x, values, columns, na.rm=TRUE)
```

Arguments

x	Object of class matrix, data.frame, or cytoFrame.
values	Values that correspond to censored data. If missing, range(x) is used.
columns	Numeric or character vector specifying the columns of x that are compared against values. If missing, 1:ncol(x) is used.
na.rm	Logical. If TRUE, rows that contain NA values are also removed.

Details

The function removes all rows that contain, in the columns specified by the columns argument, values that are contained in the values argument. If na.rm is TRUE, then rows that contain NA values are also removed.

An application is with FACS data, where measurements outside of the detector's dynamic range produce minimal or maximal values. For example, if a 16-bit A/D converter was used, top-censored data would have a value of 65535.

Value

Object of the same class as x, with some rows removed.

Author(s)

Florian Hahne, Wolfgang Huber

Examples

```
set.seed(8215)
mat <- matrix(floor(runif(20000)*1024), ncol=4)
range(mat[,1])
mat <- removeCensored(mat, columns=1:2)
range(mat[,1])
range(mat[,3])
```

`threePanelPlot` *Visualize cytometry data*

Description

Function to visualize multivariate (cytometry) data in three two-dimensional plots.

Usage

```
threePanelPlot(data, x.panels = c(1, 4, 5), y.panels = c(2, 3, 6),
               tot.width = 15, tot.height = 5.4, maxcells = 20000,
               limits = c(0, 1023), remove.extremes = TRUE,
               plotTitle = "Three-Panel Plot", use.smoothScatter = TRUE,
               palette = colorRampPalette(brewer.pal(9, "Blues")),
               new.device = TRUE, verbose = TRUE,
               addPoints = NULL, addCol = "red", ...)
```

Arguments

`data`	data matrix to visualize
`x.panels`	which variables (columns) are to be plotted at the x-axis of the three variables
`y.panels`	which variables (columns) are to be plotted at the y-axis of the three variables
`tot.width`	width of a new device to open, see argument `new.device`
`tot.height`	height of a new device to open, see argument `new.device`
`maxcells`	maximum number of observations (cells) for plotting; higher numbers reduce performance
`limits`	minimum and maximum value (theoretically) observed in the data; e.g., with 10-channel digitized data it is c(0,1023)
`remove.extremes`	logical; are extreme values (equal to theoretical `limits`) to be removed before plotting
`plotTitle`	title for the plot
`use.smoothScatter`	logical, should the function `smoothScatter` be employed for plotting the data (plots data densities rather than individual points)
`palette`	if `smoothScatter` is used, which colour palette is it to use
`new.device`	logical; should a new device be opened for the three plots; if `FALSE` the three plots will be plotted to the currently active device
`verbose`	logical; do you want extended output to STDOUT
`addPoints`	should special points be marked after plotting the data; is expected to be a subser of argument `data` with the same number of columns (=variables); if `NULL` no points are marked
`addCol`	in which colour are the points in `addPoints` to be marked
`...`	further arguments passed on to `plot.default`

Value

no value is returned; the function is called to produce three plots

Author(s)

Joern Toedling <toedling@ebi.ac.uk>

See Also

plot.default

Examples

```
# generate some data:
toyData <- cbind(matrix(pmax(0,pmin(runif(3000)+rnorm(3000),4)),ncol=3),
                 matrix(pmax(0,pmin(rnorm(3000,2,1),4)),ncol=3))
colnames(toyData) <- paste("Var",1:6,sep="")
toyQuantiles <- apply(toyData,2,quantile,probs=c(0.25,0.5,0.75))

# plot it and mark the quantiles:
threePanelPlot(toyData,addPoints=toyQuantiles,
               addCol=c("orange","red","purple"),limits=c(0,4),pch=20)
```

---

thresholds — *Discretize a two-dimensional data space into quadrants by applying thresholds*

Description

Discretize a two-dimensional data space into quadrants by applying thresholds.

Usage

```
thresholds(x, y, xthr, ythr)
```

Arguments

x	Vector containing x or matrix containing x and y values of bivariate data.
y	Optional vector containing y values of bivariate data.
xthr	x value seperating 'left' and 'right'.
ythr	y value seperating 'up' and 'down'.

Details

The function returns a 2x2 matrix giving the counts for each quadrant. Events with values equal to the thresholds are counted to the left or down respectively.

Value

2x2 matrix.

Author(s)

Florian Hahne

Examples

```
thresholds(cbind(c(1, 1, 2, 2, 2, 4), c(1, 4, 2, 4, 5, 4)), xthr=3, ythr=3)
```

touchFCS *Check for FCS files*

Description

The function reads the header of a file or of a range of files and checks wether they are valid FCS 2.0 or FCS 3.0 files.

Usage

```
touchFCS(path = ".", file)
```

Arguments

path	character, the path to a folder containing files
file	character, the path to a single file

Details

The user may either specify the path to a directory in which to search for FCS files or the path to a single file.

Value

A character vector with names of the valid FCS files found.

Author(s)

fhahne

vpLocation *Absolute location of current viewport*

Description

Calculates the absolute location and size of the current grid viewport in inches and pixels.

Usage

```
vpLocation()
```

Details

This function may be used to get the absolute location of the current viewport on the current graphics device. It uses function devRes to get the device resolution for calculating pixel values. Locations are given by the two extreme coordinates in x and y direction.

Value

A list with items location,size,ilocation and isize, the location and size of the viewport in pixels and iches respectively.

Author(s)

Florian Hahne

See Also

plotPlate, devRes

Examples

```
vpLocation()
```

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