prada
November 11, 2009

R topics documented:

analysePlate ................................................... 1
as.all ............................................................ 2
barploterrbar ..................................................... 3
combineFrames .................................................. 4
csApply .......................................................... 5
cytoFrame-class .................................................. 6
cytoSet-class .................................................... 7
cframe ........................................................... 9
cset ............................................................... 10
devDims .......................................................... 10
devRes ........................................................... 11
fitNorm2 .......................................................... 12
gate-class ........................................................ 13
gateSet-class ..................................................... 14
getPradaPar ...................................................... 16
Internal functions .............................................. 16
plotNorm2 ........................................................ 17
plotPlate ......................................................... 18
readCytoSet .................................................... 20
readFCSaux ...................................................... 21
readFCS .......................................................... 21
removeCensored .................................................. 23
progress .......................................................... 24
threePanelPlot ................................................... 25
thresholds ......................................................... 26
touchFCS ......................................................... 27
vpLocation ....................................................... 27

Index ............................................................... 29
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, platenam, 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.
platenam 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 platenam 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 platenam, well-ID (from x[, wellcol]), and the results from statfun.

Author(s)
Wolfgang Huber http://www.dkfz.de/abt0840/whuber

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

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 will 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](http://www.dkfz.de/abt0840/whuber)

See Also

`barplot`

Examples

```r
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)))
```

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

Description

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

Usage

```r
combineFrames(x, by)
```
csApply

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)
sml <- 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(sml, fac, sum)))

Description
Apply a function over the intensities in a 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](http://www.ebi.ac.uk/huber)

See Also

`sapply`

Examples

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

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

```r
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. See `gateMatrix` for further details.

`hist` Draw a histogram of the data

Author(s)

Florian Hahne, Wolfgang Huber

See Also

`readFCS`, `cytoSet`, `gate`, `gateSet`, `gateMatrix`

Examples

```r
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]
```
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

```r
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

```r
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

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

Author(s)

Florian Hahne, Wolfgang Huber [http://www.ebi.ac.uk/huber](http://www.ebi.ac.uk/huber)

See Also

`readCytoSet, cytoFrame, gate, gateSet`

Examples

```r
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)
```
cframe

**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**

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

**Format**

`cytoFrame` object

**Source**

German Cancer Research Center Heidelberg, Germany

**Examples**

```r
data(cytoFrame)
```

cset

**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**

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

**Format**

`cytoSet` object

**Source**

German Cancer Research Center Heidelberg, Germany
Examples

data(cytoSet)

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

```r
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)
```

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[prada]{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, gateMatrix, gateSet`

Examples

```r
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")
```
gateSet-class

```r
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-dimensional 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`
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

Examples

```r
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]]
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)
```
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](http://www.ebi.ac.uk/huber)

Examples

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

---

Internal functions called by other gating functions

Description

These functions are for internal use by other gating functions and not to be called by the user.

Author(s)

Joern Toedling ⟨toedling@ebi.ac.uk⟩

plotNorm2

Plot fitted bivariate normal distribution.

Description

Plots objects derived from function `fitNorm2` in false color representation.

Usage

```r
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`.
plotPlate

Details

Produces a scatterplot of paired data showing the densities of the fitted bivariate distribution from function *fitNorm* 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, addEllipse

Examples

```r
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)
```

Description

Plot a well statistic for microtiter plates.

Usage

```r
plotPlate(x, nrow = 8, ncol = 12, col = c("red", "blue"),
ind = 1:(ncol*nrow), xrange = range(x, 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*.
plotPlate

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 address the problem of missing values. Each well that is not allocated a value of \( x \) by \( \text{ind} \) will not be plotted.

xrange
Range of \( x \) that is mapped into the color scale.

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, \( \text{na.action} \) is only applied to rows containing nothing but NAs. Further special treatment of NA values in matrices need to be implemented in \( \text{gridFun} \).

main
Character of length 1. Plot title.

char
An optional character vector of equal length as \( x \) (except if argument \( \text{ind} \) 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 \( \text{drawCircle} \) and \( \text{drawPie} \)

funArgs
Dataframe with argument values to be passed to \( \text{gridCall} \). For each argument specified in \( \text{gridCall} \) there must be one column with the argument name as col-name 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. \( \text{plotPlate} \) passes all data values for the respective well to the plotting function. Any further arguments may be passed on using argument \( \text{funArgs} \). See \( \text{drawCircle} \) and \( \text{drawPie} \) for examples of valid plotting functions and the vignette for detailed information. Note that using \( \text{funCall} \) overrides some of the default functionalities, e.g. plotting of legends and alters the treatment of NA values.

Argument \( \text{ind} \) 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 \( \text{char} \). Each element of \( \text{char} \neq \text{NA} \) will be superimposed on the respective well (see examples).
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.phenoData.
...  Further arguments that get passed on to read.phenoData, see details.
Details

There are three different ways to specify the file names:

First, if the argument `phenoData` is present and is of class `phenoData`, 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 `phenoData` object from the file with that name by calling `read.phenoData(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](http://www.ebi.ac.uk/huber)

See Also

`readFCSdata`

Examples

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

---

**readFCSaux**  
*Auxiliary functions for readFCS*

**Description**

Auxiliary functions for readFCS - not normally called by the user

**Usage**

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

**Arguments**

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><code>x</code></td>
<td>Named character vector.</td>
</tr>
<tr>
<td><code>pnam</code></td>
<td>Character vector, its elements must be contained in <code>names(x)</code>.</td>
</tr>
<tr>
<td><code>con</code></td>
<td>Connection.</td>
</tr>
<tr>
<td><code>offsets</code></td>
<td>Integer vector of length 6 with byte offsets of the header, text, and data blocks.</td>
</tr>
</tbody>
</table>
Details

These functions are not normally called by the user. See \texttt{readFCS} instead.

Value

Various.

Author(s)

Wolfgang Huber \url{http://www.ebi.ac.uk/huber}

See Also

\texttt{readFCS}

\begin{verbatim}
readFCS
\end{verbatim}

\begin{verbatim}
Read an FCS file
\end{verbatim}

Description

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

Usage

\begin{verbatim}
read.fcs(filename=NULL, objectModel="prada", ...)
readFCS(filename)
\end{verbatim}

Arguments

\begin{verbatim}
filename    Character of length 1: filename
objectModel Character of length 1: the object model to use for the output. Either 'prada' for
cytoFrame objects or 'FCS' for rflowcyt's FCS objects.
...         Arguments that get passed on to higher-level import functions.
\end{verbatim}

Details

The function \texttt{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 function is an object of class \texttt{cytoFrame}.

\texttt{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 \texttt{R} using the \texttt{prada} or \texttt{rflowcyt} packages. If the \texttt{filename} argument to \texttt{read.fcs} is a character vector of length \texttt{> 1}, multiple FCS files can be imported. Please see the documentation for \texttt{readCytoSet} and \texttt{read.series.FCS} for alternatives ways to import multiple FCS files and for more details on the higher-level import function.

Be aware that \texttt{rflowcyt} needs to be installed when the function is run with argument \texttt{objectModel="FCS"}. For specifications of FCS 3.0 see \url{http://www.isac-net.org} and the file \\../doc/fcs3.html in the doc directory of the package.
Value

An object of class `cytoFrame` or `FCS`.

Author(s)

Wolfgang Huber [http://www.ebi.ac.uk/huber](http://www.ebi.ac.uk/huber), Florian Hahne

See Also

`read.FCS`, `read.series.FCS`, `readCytoSet`

Examples

```r
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)
```

```r
removeCensored

Remove rows that contain censored data

Description

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

Usage

```r
## 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, `lrcol(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 <f.hahne@dkfz.de>, Wolfgang Huber <huber@ebi.ac.uk>

Examples

```r
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])
```

---

progress $\quad$ A simple tcltk progress window

Description

Show progress of a task in a tcltk window as percentage

Usage

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

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 via `updateProgress`
- **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 explicitly 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**

```r
if(interactive()){
  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")
  }
}
```

---

**threePanelPlot**  
*Visualize cytometry data*

**Description**

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

**Usage**

```r
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 \((0, 1023)\)

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

```r
# 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)
```
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 separating 'left' and 'right'.
ythr  y value separating '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)

Check for FCS files

The function reads the header of a file or of a range of files and checks whether 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 icles respectively.

Author(s)

Florian Hahne

See Also

plotPlate, devRes

Examples

vpLocation()
Index

*Topic IO
readCytoSet, 19
readFCS, 21
readFCSaux, 20
touchFCS, 26

*Topic classes
  cytoFrame-class, 5
cytoSet-class, 7
gate-class, 12
gateSet-class, 14

*Topic datasets
cframe, 9
cset, 9

*Topic hplot
  barploterrbar, 3
  plotPlate, 17
  threePanelPlot, 24

*Topic internal
  Internal functions, 16

*Topic manip
  analysePlate, 1
  as.all, 2
  csApply, 4
  getPradaPar, 15

*Topic misc
  progress, 23
  [,cytoFrame,ANY,ANY,ANY-method
   (cytoFrame-class), 5
  [,cytoSet,ANY,missing,missing-method
   (cytoSet-class), 7
  [,gateSet,ANY,missing,missing-method
   (gateSet-class), 14
  ][,cytoSet,ANY,missing-method
   (cytoSet-class), 7
  ][,gateSet,ANY,missing-method
   (gateSet-class), 14
  ][<-,cytoSet-method
   (cytoSet-class), 7
  $ . cytoFrame (cytoFrame-class), 5

  addEllipse, 17
  analysePlate, 1
  appendGates (gateSet-class), 14

  appendGates, gateSet_method
   (gateSet-class), 14
  appendGates, cytoFrame-method
   (cytoFrame-class), 5
  appendGates, gateSet-method
   (gate-class), 12
  applyGate (gateSet-class), 14
  applyGate, cytoFrame, character-method
   (cytoFrame-class), 5
  applyGate, cytoFrame, gate-method
   (cytoFrame-class), 5
  applyGate, cytoFrame, gateSet-method
   (cytoFrame-class), 5
  applyGate, cytoFrame, logical-method
   (cytoFrame-class), 5
  applyGate, cytoFrame, numeric-method
   (cytoFrame-class), 5
  applyGate, matrix, gate-method
   (gate-class), 12
  applyGate, matrix, gateSet-method
   (gateSet-class), 14
  as, 2
  as.all, 2
  as.gateSet (gate-class), 12
  as.gateSet, gate-method
   (gate-class), 12

  barplot, 3
  barploterrbar, 3
cframe, 9
colnames, cytoFrame-method
   (cytoFrame-class), 5
colnames, cytoSet-method
   (cytoSet-class), 7
colnames<-, cytoFrame-method
   (cytoFrame-class), 5
colnames<-, cytoSet-method
   (cytoSet-class), 7
colorRampPalette, 17
combineFrames, 4
combineGates (gate-class), 12
cov.robd, 12
covMcd, 12
removeCensored, 22
removeCensored, cytoFrame-method (removeCensored), 22
removeCensored, data.frame-method (removeCensored), 22
removeCensored, matrix-method (removeCensored), 22

sapply, 4, 5
setPradaPars (getPradaPar), 15
show, cytoFrame-method (cytoFrame-class), 5
show, cytoSet-method (cytoSet-class), 7
show, gate-method (gate-class), 12
show, gateSet-method (gateSet-class), 14
smoothScatter, 25
split, cytoSet, ANY, ANY-method (cytoSet-class), 7
split, cytoSet, ANY-method (cytoSet-class), 7
split, cytoSet-method (cytoSet-class), 7

tapply, 1
threePanelPlot, 24
thresholds, 26
touchFCS, 26

updateProgress (progress), 23
vpLocation, 27