# Package 'flowCore'

## September 24, 2012

Title flowCore: Basic structures for flow cytometry data
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Author B. Ellis, P. Haaland, F. Hahne, N. Le Meur, N. Gopalakrishnan
<b>Description</b> Provides S4 data structures and basic functions to deal with flow cytometry data.
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flowCore-package Provides S4 data structures and basic infrastructure and functions to deal with flow cytometry data.

#### **Description**

Define important flow cytometry data classes: flowFrame, flowSet and their accessors.

Provide important transformation, filter, gating, workflow, and summary functions for flow cytometry data analysis.

Most of flow cytometry related Bioconductor packages (such as flowStats, flowFP, flowQ, flowViz, flowMerge, flowClust) are heavily depended on this package.

#### **Details**

Package: flowCore
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### Author(s)

Maintainer: Florian Hahne <fhahne@fhcrc.org>

Authors: B. Ellis, P. Haaland, F. Hahne, N. Le Meur, N. Gopalakrishnan

actionItem-class Class "actionItem"

### Description

Class and method to capture standard operations in a flow cytometry workflow.

### **Details**

actionItems provide a means to bind standard operations on flow cytometry data in a workflow. Usually, the user doesn't have to create these objects, instead they will be automatically created when applying one of the standard operations (gating, transformation, compensation) to a workFlow object. Each actionItem creates one or several new views, which again can be the basis to apply further operations. One can conceptualize actionItems being the edges in the workflow tree connecting views, which are the nodes of the tree. There are more specific subclasses for the three possible types of operation: gateActionItem for gating operations, transformActionItem for transformations, and compensateActionItem for compensation operations. See their documentation for details.

arcsinhTransform 5

#### **Objects from the Class**

A virtual Class: No objects may be created from it.

#### **Slots**

```
    ID: Object of class "character". A unique identifier for the actionItem.
    name: Object of class "character". A more human-readable name
    parentView: Object of class "fcViewReference". A reference to the parent view the actionItem is applied on.
    alias: Object of class "fcAliasReference". A reference to the alias table.
    env: Object of class "environment". The evaluation environment in the workFlow.
```

#### Methods

```
identifier signature(object = "actionItem"): Accessor for the ID slot.
names signature(x = "actionItem"): Accessor for the name slot.
parent signature(object = "actionItem"): Accessor for the parentView slot. Note that the reference is resolved, i.e., the view object is returned.
alias signature(object = "actionItem"): Get the alias table from a actionItem.
Rm signature(symbol = "actionItem", envir = "workFlow", subSymbol = "character"):
    Remove a actionItem from a workFlow. This method is recursive and will also remove all dependent views and actionItems.
```

#### Author(s)

Florian Hahne

### See Also

workFlow, gateActionItem, transformActionItem, compensateActionItem, view

### **Examples**

```
showClass("view")
```

arcsinhTransform Create the definition of an arcsinh transformation function (base specified by user) to be applied on a data set

### **Description**

Create the definition of the arcsinh Transformation that will be applied on some parameter via the transform method. The definition of this function is currently x<-asinh(a+b\*x)+c). The transformation would normally be used to convert to a linear valued parameter to the natural logarithm scale. By default a and b are both equal to 1 and c to 0.

### Usage

```
arcsinhTransform(transformationId="defaultArcsinhTransform", a=1, b=1, c=0)
```

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### **Arguments**

transformationId

character string to identify the transformation

a positive double that corresponds to the base of the logarithm.

b positive double that corresponds to a scale factor.

c positive double that corresponds to a scale factor

#### Value

Returns an object of class transform.

#### Author(s)

B. Ellis

#### See Also

```
transform-class, transform, asinh
```

### **Examples**

```
samp <- read.FCS(system.file("extdata",
   "0877408774.B08", package="flowCore"))
asinhTrans <- arcsinhTransform(transformationId="ln-transformation", a=1, b=1, c=1)
dataTransform <- transform(samp, 'FSC-H'=asinhTrans('FSC-H'))</pre>
```

asinht-class

Class "asinht"

### **Description**

Inverse hyperbolic sin transformation is defined by the function

```
f(parameter, a, b) = sinh^{-1}(a * parameter) * b
```

### **Objects from the Class**

Objects can be created by calls to the constructor asinht(parameter,a,b,transformationId)

### **Slots**

```
.Data: Object of class "function" ~~
a: Object of class "numeric" -non zero constant
b: Object of class "numeric" -non zero constant
parameters: Object of class "transformation" -flow parameter to be transformed
transformationId: Object of class "character" -unique ID to reference the transformation
```

#### **Extends**

```
Class "singleParameterTransform", directly. Class "transform", by class "singleParameterTransform", distance 2. Class "transformation", by class "singleParameterTransform", distance 3. Class "characterOrTransformation", by class "singleParameterTransform", distance 4.
```

biexponentialTransform

#### Methods

No methods defined with class "asinht" in the signature.

#### Note

The inverse hyperbolic sin transformation object can be evaluated using the eval method by passing the data frame as an argument. The transformed parameters are returned as a matrix with a single column. (See example below)

### Author(s)

Gopalakrishnan N, F.Hahne

### References

Gating-ML Candidate Recommendation for Gating Description in Flow Cytometry V 1.5

#### See Also

sinht

### **Examples**

```
dat <- read.FCS(system.file("extdata","0877408774.B08", package="flowCore"))
  asinh1<-asinht(parameters="FSC-H",a=2,b=1,transformationId="asinH1")
  transOut<-eval(asinh1)(exprs(dat))</pre>
```

biexponentialTransform

Compute a transform using the 'biexponential' function

### **Description**

The 'biexponential' is an over-parameterized inverse of the hyperbolic sine. The function to be inverted takes the form biexp(x) = a\*exp(b\*(x-w))-c\*exp(-d\*(x-w))+f with default parameters selected to correspond to the hyperbolic sine.

### Usage

biexponential Transform (transformation Id="defaultBiexponential Transform", a = 0.5, b = 1, c = 0.5

### Arguments

a b

С

d

transformationId

A name to assign to the transformation. Used by the transform/filter integration routines.

See the function description above. Defaults to 0.5

See the function description above. Defaults to 1.0

See the function description above. Defaults to 0.5 (the same as a)

See the function description above. Defaults to 1 (the same as b)

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f	A constant bias for the intercept. Defaults to 0.
W	A constant bias for the 0 point of the data. Defaults to 0.
tol	A tolerance to pass to the inversion routine (uniroot usually)
maxit	A maximum number of iterations to use, also passed to uniroot

#### Value

Returns values giving the inverse of the biexponential within a certain tolerance. This function should be used with care as numerical inversion routines often have problems with the inversion process due to the large range of values that are essentially 0. Do not be surprised if you end up with population splitting about w and other odd artifacts.

### Author(s)

B. Ellis, N Gopalakrishnan

### See Also

transform

### **Examples**

```
# Construct some "flow-like" data which tends to be hetereoscedastic.
data(GvHD)
biexp <- biexponentialTransform("myTransform")
after.1 <- transform(GvHD, 'FSC-H'= biexp('FSC-H'))

biexp <- biexponentialTransform("myTransform", w=10)
after.2 <- transform(GvHD, 'FSC-H'= biexp('FSC-H'))

opar = par(mfcol=c(3, 1))
plot(density(exprs(GvHD[[1]])[, 1]), main="Original")
plot(density(exprs(after.1[[1]])[, 1]), main="Standard Transform")
plot(density(exprs(after.2[[1]])[, 1]), main="Shifted Zero Point")</pre>
```

boundaryFilter-class Class "boundaryFilter"

### **Description**

Class and constructor for data-driven filter objects that discard margin events.

### Usage

```
boundaryFilter(x, tolerance=.Machine$double.eps, side=c("both", "lower",
"upper"), filterId="defaultBoundaryFilter")
```

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#### **Arguments**

X	Character giving the name(s) of the measurement parameter(s) on which the filter is supposed to work. Note that all events on the margins of ay of the channels provided by x will be discarded, which is often not desired. Such events may not convey much information in the particular channel on which their value falls on the margin, however they may well be informative in other channels.
tolerance	Numeric vector, used to set the tolerance slot of the object. Can be set separately for each element in x. R's recycling rules apply.
side	Character vector, used to set the side slot of the object. Can be set separately for each element in x. R's recycling rules apply.
filterId	An optional parameter that sets the filterId slot of this filter. The object can later be identified by this name.

#### **Details**

Flow cytomtery instruments usually operate on a given data range, and the limits of this range are stored as keywords in the FSC files. Depending on the amplification settings and the dynamic range of the measured signal, values can occur that are outside of the measurement range, and most instruments will simply pile those values at the minimum or maximum range limit. The boundaryFilter removes these values, either for a single parameter, or for a combination of parameters. Note that it is often desirable to treat boundary events on a per-parameter basis, since their values might be uninformative for one particular channel, but still be useful in all of the other channels.

The constructor boundaryFilter is a convenience function for object instantiation. Evaluating a boundaryFilter results in a single sub-populations, an hence in an object of class filterResult.

### Value

Returns a boundaryFilter object for use in filtering flowFrames or other flow cytometry objects.

#### **Extends**

```
Class "parameterFilter", directly.

Class "concreteFilter", by class parameterFilter, distance 2.

Class "filter", by class parameterFilter, distance 3.
```

### **Slots**

```
tolerance: Object of class "numeric". The machine tolerance used to decide whether an event is on the measurement boundary. Essentially, this is done by evaluating x>minRange+tolerance & x<maxRange-tolside: Object of class "character". The margin on which to evaluate the filter. Either upper for
```

the upper margin or lower for the lower margin or both for both margins.

### **Objects from the Class**

Objects can be created by calls of the form new("boundaryFilter", ...) or using the constructor boundaryFilter. Using the constructor is the recommended way of object instantiation:

#### Methods

```
%in% signature(x = "flowFrame", table = "boundaryFilter"): The workhorse used to evaluate the filter on data. This is usually not called directly by the user, but internally by calls to the filter methods.
```

show signature(object = "boundaryFilter"): Print information about the filter.

### Author(s)

Florian Hahne

#### See Also

flowFrame, flowSet, filter for evaluation of boundaryFilters and Subset for subsetting of flow cytometry data sets based on that.

### **Examples**

```
## Loading example data
dat <- read.FCS(system.file("extdata","0877408774.B08",
    package="flowCore"))

## Create directly. Most likely from a command line
boundaryFilter("FSC-H", filterId="myBoundaryFilter")

## To facilitate programmatic construction we also have the following
bf <- boundaryFilter(filterId="myBoundaryFilter", x=c("FSC-H"))

## Filtering using boundaryFilter
fres <- filter(dat, bf)
fres
summary(fres)

## We can subset the data with the result from the filtering operation.
Subset(dat, fres)

## A boundaryFilter on the lower margins of several channels
bf2 <- boundaryFilter(x=c("FSC-H", "SSC-H"), side="lower")</pre>
```

characterOrTransformation-class

Class "characterOrTransformation"

### **Description**

~~ A concise (1-5 lines) description of what the class is. ~~

### **Objects from the Class**

A virtual Class: No objects may be created from it.

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#### Methods

No methods defined with class "characterOrTransformation" in the signature.

#### References

~put references to the literature/web site here ~

### **Examples**

```
showClass("characterOrTransformation")
```

coerce

Convert an object to another class

### **Description**

These functions manage the relations that allow coercing an object to a given class.

### Arguments

from, to

The classes between which def performs coercion. (In the case of the coerce function, these are objects from the classes, not the names of the classes, but you're not expected to call coerce directly.)

### **Details**

The function supplied as the third argument is to be called to implement as(x, to) when x has class from. Need we add that the function should return a suitable object with class to.

### Author(s)

F. Hahne, B. Ellis

### **Examples**

```
samp1 <- read.FCS(system.file("extdata","0877408774.E07", package="flowCore"))
samp2 <- read.FCS(system.file("extdata","0877408774.B08",package="flowCore"))
samples <-list("sample1"=samp1,"sample2"=samp2)
experiment <- as(samples,"flowSet")</pre>
```

```
compensateActionItem-class
```

Class "compensateActionItem"

### **Description**

Class and method to capture compensation operations in a flow cytometry workflow.

### Usage

```
compensateActionItem(ID = paste("compActionRef", guid(), sep = "_"),
name = paste("action", identifier(get(compensate)), sep = "_"),
parentView, compensate, workflow)
```

### **Arguments**

workflow An object of class workflow for which a view is to be created.

ID A unique identifier of the view, most likely created by using the internal guid

function.

name A more human-readable name of the view.

parentView, compensate

References to the parent view and compensation objects, respectively.

### **Details**

compensateActionItems provide a means to bind compensation operations in a workflow. Each compensateActionItem represents a single compensation.

### Value

A reference to the compensateActionItem that is created inside the workFlow environment as a side effect of calling the add method.

A compensateActionItem object for the constructor.

### **Objects from the Class**

Objects should be created using the add method, which creates a compensateActionItem from a compensation object and directly assigns it to a workFlow. Alternatively, one can use the compensateActionItem constructor function for more programmatic access.

#### **Slots**

compensate: Object of class "fcCompensateReference". A reference to the compensation object that is used for the compensation operation.

ID: Object of class "character". A unique identifier for the actionItem.

name: Object of class "character". A more human-readable name

parentView: Object of class "fcViewReference". A reference to the parent view the compensateActionItem is applied on.

env: Object of class "environment". The evaluation environment in the workFlow.

#### **Extends**

```
Class "actionItem", directly.
```

#### Methods

```
print signature(x = "compensateActionItem"): Print details about the object.

Rm signature(symbol = "compensateActionItem", envir = "workFlow", subSymbol = "character"):
    Remove a compensateActionItem from a workFlow. This method is recursive and will also
    remove all dependent views and actionItems.
```

#### Author(s)

Florian Hahne

#### See Also

```
workFlow, actionItem, gateActionItem, transformActionItem, view
```

### **Examples**

```
showClass("view")
```

```
compensatedParameter-class
```

Class "compensatedParameter"

**show** signature(object = "compensateActionItem"): Print details about the object.

### **Description**

Emission spectral overlap can be corrected by subtracting the the amount of spectral overlap from the total detected signals. This compensation process can be described by using spillover matrices. compensatedParameter objects allow for compensation of specific parameters the user is interested in by creating compensatedParameter objects and evaluating them. This allows for use of compensatedParameter in gate definitions.

### **Objects from the Class**

Objects can be created by calls of the form compensatedParameter(parameters, spillRefId, transformationId, se

### **Slots**

```
.Data: Object of class "function" ~~

parameters: Object of class "character" -flow parameters to be compensated

spillRefId: Object of class "character" -name of the compensation object (The compensation object contains the spillover Matrix)

searchEnv: Object of class "environment" -environment in which the compensation object is defined

transformationId: Object of class "character" -unique Id to reference the compensatedParameter object
```

#### **Extends**

Class "transform", directly. Class "transformation", by class "transform", distance 2. Class "characterOrTransformation", by class "transform", distance 3.

#### Methods

No methods defined with class "compensatedParameter" in the signature.

#### Note

The transformation object can be evaluated using the eval method by passing the data frame as an argument. The transformed parameters are returned as a matrix with a single column. (See example below)

### Author(s)

Gopalakrishnan N,F.Hahne

### References

~~

#### See Also

compensation

### **Examples**

```
samp <- read.flowSet(path=system.file("extdata", "compdata", "data", package="flowCore"))
cfile <- system.file("extdata","compdata","compmatrix", package="flowCore")
comp.mat <- read.table(cfile, header=TRUE, skip=2, check.names = FALSE)
comp.mat

## create a compensation object
comp <- compensation(comp.mat,compensationId="comp1")
## create a compensated parameter object
cPar1<-compensatedParameter(c("FL1-H","FL3-H"),"comp",searchEnv=.GlobalEnv)
compOut<-eval(cPar1)(exprs(samp[[1]]))</pre>
```

```
compensateView-class Class "compensateView"
```

### Description

Class and method to capture the result of compensation operations in a flow cytometry workflow.

### Usage

```
\label{lem:compensateView} compensateView(workflow, ID=paste("compViewRef", guid(), sep="\_"), \\ name="default", action, data)
```

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### **Arguments**

workflow An object of class workflow for which a view is to be created.

ID A unique identifier of the view, most likely created by using the internal guid

function.

name A more human-readable name of the view.

data, action References to the data and actionItem objects, respectively.

### Value

A reference to the compensateView that is created inside the workFlow environment as a side effect of calling the add method.

A compensateView object for the constructor.

### **Objects from the Class**

Objects should be created using the add method, which creates a compensateView from a compensation object and directly assigns it to a workFlow. Alternatively, one can use the compensateView constructor function for more programmatic access.

#### Slots

```
ID: Object of class "character". A unique identifier for the view.
```

name: Object of class "character". A more human-readable name

action: Object of class "fcActionReference". A reference to the actionItem that generated the view.

env: Object of class "environment". The evaluation environment in the workFlow.

data: Object of class "fcDataReference" A reference to the data that is associated to the view.

#### **Extends**

```
Class "view", directly.
```

#### Methods

Rm signature(symbol = "compensateView", envir = "workFlow", subSymbol = "character"):
 Remove a compensateView from a workFlow. This method is recursive and will also remove
 all dependent views and actionItems.

### Author(s)

Florian Hahne

### See Also

```
workFlow, view, gateView, transformView, normalizeView, actionItem
```

### **Examples**

```
showClass("view")
```

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compensation-class

Class "compensation"

### **Description**

Class and methods to compensate for spillover between channels by applying a spillover matrix to a flowSet or a flowFrame assuming a simple linear combination of values.

### Usage

```
compensation(..., spillover,
compensationId="defaultCompensation")
compensate(x, spillover, ...)
```

### Arguments

spillover The spillover or compensation matrix.

compensationId The identifier for the compensation object.

x An object of class flowFrame or flowSet.

... Further arguments.

The constructor is designed to be useful in both programmatic and interactive settings, and ...serves as a container for possible arguments. The following combinations of values are allowed:

Elements in ... are character scalars of parameter names or transform objects and the colnames in spillover match to these parameter names.

The first element in ... is a character vector of parameter names or a list of character scalars or transform objects and the colnames in spillover match to these parameter names.

Argument spillover is missing and the first element in . . . is a matrix, in which case it is assumed to be the spillover matrix.

 $\ldots$  is missing, in which case all parameter names are taken from the colnames of spillover.

### Details

The essential premise of compensation is that some fluorochromes may register signals in detectors that do not correspond to their primary detector (usually a photomultiplier tube). To compensate for this fact, some sort of standard is used to obtain the background signal (no dye) and the amount of signal on secondary channels for each fluorochrome relative to the signal on their primary channel.

To calculate the spillover percentage we use either the mean or the median (more often the latter) of the secondary signal minus the background signal for each dye to obtain n by n matrix, S, of so-called spillover values, expressed as a percentage of the primary channel. The observed values are then considered to be a linear combination of the true fluorescence and the spillover from each other channel so we can obtain the true values by simply multiplying by the inverse of the spillover matrix.

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The spillover matrix can be obtained through several means. Some flow cytometers provide a spillover matrix calculated during acquisition, possibly by the operator, that is made available in the metadata of the flowFrame. While there is a theoretical standard keyword \$SPILL it can also be found in the SPILLOVER or SPILL keyword depending on the cytometry. More commonly the spillover matrix is calculated using a series of compensation cells or beads collected before the experiment. If you have set of FCS files with one file per fluorochrome as well as an unstained FCS file you can use the spillover method for flowSets to automatically calculate a spillover matrix.

The compensation class is essentially a wrapper around a matrix that allows for transformed parameters and method dispatch.

#### Value

A compensation object for the constructor.

A flowFrame or flowSet for the compensate methods.

### **Objects from the Class**

Objects should be created using the constructor compensation(). See the Usage and Arguments sections for details.

#### **Slots**

```
spillover: Object of class matrix; the spillover matrix.

compensationId: Object of class character. An identifier for the object.

parameters: Object of class parameters. The flow parameters for which the compensation is defined. This can also be objects of class transform, in which case the compensation is performed on the compensated parameters.
```

### Methods

```
compensate signature(x = "flowFrame", spillover = "compensation"): Apply the com-
    pensation defined in a compensation object on a flowFrame. This returns a compensated
    flowFrame.
     Usage:
        compensate(flowFrame, compensation)
compensate signature(x = "flowFrame", spillover = "matrix"): Apply a compensation
    matrix to a flowFrame. This returns a compensated flowFrame.
     Usage:
        compensate(flowFrame, matrix)
compensate signature(x = "flowFrame", spillover = "data.frame"):Try to coerce the data.frame
    to a matrix and apply that to a flowFrame. This returns a compensated flowFrame.
        compensate(flowFrame, data.frame)
identifier, identifier<- signature(object = "compensation"): Accessor and replacement meth-
    ods for the compensationId slot.
     Usage:
        identifier(compensation)
        identifier(compensation) <- value</pre>
```

18 concreteFilter-class

```
parameters signature(object = "compensation"): Get the parameter names of the compensation
    object. This method also tries to resolve all transforms and transformReferences before
    returning the parameters as character vectors. Unresolvable references return NA.
    Usage:
        parameters(compensation)
show signature(object = "compensation"): Print details about the object.
        Usage:
        This method is automatically called when the object is printed on the screen.
```

#### Author(s)

F.Hahne, B. Ellis, N. Le Meur

#### See Also

spillover

### **Examples**

concreteFilter-class Class "concreteFilter"

### **Description**

The concreteFilter serves as a base class for all filters that actually implement a filtering process. At the moment this includes all filters except filterReference, the only non-concrete filter at present.

### **Objects from the Class**

Objects of this class should never be created directly. It serves only as a point of inheritance.

### **Slots**

filterId: The identifier associated with this class.

curv1Filter-class 19

#### **Extends**

```
Class "filter", directly.
```

#### Author(s)

B. Ellis

### See Also

```
parameterFilter
```

curv1Filter-class

Class "curv1Filter"

### **Description**

Class and constructor for data-driven filter objects that selects high-density regions in one dimension.

### Usage

```
curv1Filter(x, bwFac=1.2, gridsize=rep(401, 2),
filterId="defaultCurv1Filter")
```

### **Arguments**

Х

Character giving the name of the measurement parameter on which the filter is supposed to work on. This can also be a list containing a single character scalar for programmatic access.

filterId

An optional parameter that sets the filterId slot of this filter. The object can later be identified by this name.

bwFac, gridsize

Numerics of length 1 and 2, respectively, used to set the bwFac and gridsize slots of the object.

### **Details**

Areas of high local density in one dimensions are identified by detecting significant curvature regions. See *Duong, T. and Cowling, A. and Koch, I. and Wand, M.P., Computational Statistics and Data Analysis 52/9, 2008* for details. The constructor curv1Filter is a convenience function for object instantiation. Evaluating a curv1Filter results in potentially multiple sub-populations, an hence in an object of class multipleFilterResult. Accordingly, curv1Filters can be used to split flow cytometry data sets.

### Value

Returns a curv1Filter object for use in filtering flowFrames or other flow cytometry objects.

20 curv1Filter-class

#### **Extends**

```
Class "parameterFilter", directly.

Class "concreteFilter", by class parameterFilter, distance 2.

Class "filter", by class parameterFilter, distance 3.
```

#### Slots

```
bwFac: Object of class "numeric". The bandwidth factor used for smoothing of the density estimate.gridsize: Object of class "numeric". The size of the bins used for density estimation.parameters: Object of class "character", describing the parameter used to filter the flowFrame.filterId: Object of class "character", referencing the filter.
```

### **Objects from the Class**

Objects can be created by calls of the form new("curvFilter", ...) or using the constructor curv1Filter. Using the constructor is the recommended way of object instantiation:

### Methods

```
%in% signature(x = "flowFrame", table = "curv1Filter"): The workhorse used to eval-
uate the filter on data. This is usually not called directly by the user, but internally by calls to
the filter methods.
show signature(object = "curv1Filter"): Print information about the filter.
```

### Note

See the documentation in the flowViz package for plotting of curv1Filters.

#### Author(s)

Florian Hahne

### See Also

curv2Filter, flowFrame, flowSet, filter for evaluation of curv1Filters and split for splitting of flow cytometry data sets based on that.

### **Examples**

```
## Loading example data
dat <- read.FCS(system.file("extdata","0877408774.B08",
package="flowCore"))

## Create directly. Most likely from a command line
curv1Filter("FSC-H", filterId="myCurv1Filter", bwFac=2)

## To facilitate programmatic construction we also have the following
c1f <- curv1Filter(filterId="myCurv1Filter", x=list("FSC-H"), bwFac=2)

## Filtering using curv1Filter</pre>
```

curv2Filter-class 21

```
fres <- filter(dat, c1f)
fres
summary(fres)
names(fres)

## The result of curv1 filtering are multiple sub-populations
## and we can split our data set accordingly
split(dat, fres)

## We can limit the splitting to one or several sub-populations
split(dat, fres, population="rest")
split(dat, fres, population=list(keep=c("peak 2", "peak 3")))</pre>
```

curv2Filter-class

Class "curv2Filter"

### **Description**

Class and constructor for data-driven filter objects that selects high-density regions in two dimensions.

### Usage

```
curv2Filter(x, y, filterId="defaultCurv2Filter", bwFac=1.2,
gridsize=rep(151, 2))
```

### **Arguments**

х,у

Characters giving the names of the measurement parameter on which the filter is supposed to work on. y can be missing in which case x is expected to be a character vector of length 2 or a list of characters.

filterId

An optional parameter that sets the filterId slot of this filter. The object can later be identified by this name.

bwFac, gridsize

Numerics of length 1 and 2, respectively, used to set the bwFac and gridsize slots of the object.

#### **Details**

Areas of high local density in two dimensions are identified by detecting significant curvature regions. See *Duong, T. and Cowling, A. and Koch, I. and Wand, M.P., Computational Statistics and Data Analysis 52/9, 2008* for details. The constructor curv2Filter is a convenience function for object instantiation. Evaluating a curv2Filter results in potentially multiple sub-populations, an hence in an object of class multipleFilterResult. Accordingly, curv2Filters can be used to split flow cytometry data sets.

### Value

Returns a curv2Filter object for use in filtering flowFrames or other flow cytometry objects.

22 curv2Filter-class

#### **Extends**

```
Class "parameterFilter", directly.

Class "concreteFilter", by class parameterFilter, distance 2.

Class "filter", by class parameterFilter, distance 3.
```

#### Slots

```
bwFac: Object of class "numeric". The bandwidth factor used for smoothing of the density estimate.gridsize: Object of class "numeric". The size of the bins used for density estimation.parameters: Object of class "character", describing the parameters used to filter the flowFrame.filterId: Object of class "character", referencing the filter.
```

### **Objects from the Class**

Objects can be created by calls of the form new("curv2Filter", ...) or using the constructor curv2Filter. The constructor is the recommended way of object instantiation:

### Methods

```
%in% signature(x = "flowFrame", table = "curv2Filter"): The workhorse used to eval-
uate the filter on data. This is usually not called directly by the user, but internally by calls to
the filter methods.
```

**show** signature(object = "curv2Filter"): Print information about the filter.

### Note

See the documentation in the flowViz package for plotting of curv2Filters.

#### Author(s)

Florian Hahne

### See Also

curv1Filter, flowFrame, flowSet, filter for evaluation of curv2Filters and split for splitting of flow cytometry data sets based on that.

### **Examples**

```
## Loading example data
dat <- read.FCS(system.file("extdata","0877408774.B08",
package="flowCore"))

## Create directly. Most likely from a command line
curv2Filter("FSC-H", "SSC-H", filterId="myCurv2Filter")

## To facilitate programmatic construction we also have the following
c2f <- curv2Filter(filterId="myCurv2Filter", x=list("FSC-H", "SSC-H"),
bwFac=2)
c2f <- curv2Filter(filterId="myCurv2Filter", x=c("FSC-H", "SSC-H"),
bwFac=2)</pre>
```

dg1polynomial-class 23

```
## Filtering using curv2Filter
fres <- filter(dat, c2f)
fres
summary(fres)
names(fres)

## The result of curv2 filtering are multiple sub-populations
## and we can split our data set accordingly
split(dat, fres)

## We can limit the splitting to one or several sub-populations
split(dat, fres, population="rest")
split(dat, fres, population=list(keep=c("area 2", "area 3")))

curv2Filter("FSC-H", "SSC-H", filterId="test filter")</pre>
```

dg1polynomial-class

Class "dg1polynomial"

### **Description**

dg1polynomial allows for scaling, linear combination and translation within a single transformation defined by the function

```
f(parameter_1, ..., parameter_n, a_1, ..., a_n, b) = b + \sum_{i=1}^n a_i * parameter_i
```

### **Objects from the Class**

Objects can be created by using the constructor dg1polynomial(parameter,a,b,transformationId).

### **Slots**

```
.Data: Object of class "function" ~~

parameters: Object of class "parameters" -the flow parameters that are to be transformed

a: Object of class "numeric" - coefficients of length equal to the number of flow parameters

b: Object of class "numeric" - coefficient of length 1 that performs the translation

transformationId: Object of class "character" unique ID to reference the transformation
```

### Extends

```
Class "transform", directly. Class "transformation", by class "transform", distance 2. Class "characterOrTransformation", by class "transform", distance 3.
```

### Methods

No methods defined with class "dg1polynomial" in the signature.

24 each\_col

#### Note

The transformation object can be evaluated using the eval method by passing the data frame as an argument. The transformed parameters are returned as a matrix with a single column. (See example below)

### Author(s)

Gopalakrishnan N, F.Hahne

#### References

Gating-ML Candidate Recommendation for Gating Description in Flow Cytometry V 1.5

### See Also

ratio,quadratic,squareroot

### **Examples**

```
dat <- read.FCS(system.file("extdata","0877408774.B08",
package="flowCore"))
dg1<-dg1polynomial(c("FSC-H","SSC-H"),a=c(1,2),b=1,transformationId="dg1")
transOut<-eval(dg1)(exprs(dat))</pre>
```

each\_col

Method to apply functions over flowFrame margins

### Description

Returns a vector or array of values obtained by applying a function to the margins of a flowFrame. This is equivalent of running apply on the output of exprs(flowFrame).

### Usage

```
each_col(x, FUN, ...)
each_row(x, FUN, ...)
```

### **Arguments**

```
X Object of class flowFrame.
FUN the function to be applied. In the case of functions like '+', '%*%', etc., the function name must be backquoted or quoted.
... optional arguments to 'FUN'.
```

### Author(s)

```
B. Ellis, N. LeMeur, F. Hahne
```

### See Also

```
apply
```

EHtrans-class 25

#### **Examples**

```
samp <- read.FCS(system.file("extdata", "0877408774.B08", package="flowCore"),
transformation="linearize")
each_col(samp, summary)</pre>
```

EHtrans-class

Class "EHtrans"

### **Description**

EH transformation of a parameter is defined by the function

$$EH(parameter, a, b) = 10^{(\frac{parameter}{a})} + \frac{b * parameter}{a} - 1 \quad parameter >= 0$$

$$-10^{(\frac{-parameter}{a})} + \frac{b*parameter}{a} + 1 \quad parameter < 0$$

### **Objects from the Class**

Objects can be created by calls to the constructor EHtrans(parameters, a, b, transformationId)

### **Slots**

```
.Data: Object of class "function" ~~

a: Object of class "numeric" -numeric constant greater than zero
b: Object of class "numeric" -numeric constant greater than zero
parameters: Object of class "transformation" - flow parameter to be transformed
transformationId: Object of class "character"- unique ID to reference the transformation
```

### Extends

Class "singleParameterTransform", directly. Class "transform", by class "singleParameterTransform", distance 2. Class "transformation", by class "singleParameterTransform", distance 3. Class "characterOrTransformation", by class "singleParameterTransform", distance 4.

### Methods

No methods defined with class "EHtrans" in the signature.

### Note

The transformation object can be evaluated using the eval method by passing the data frame as an argument. The transformed parameters are returned as a matrix with a single column. (See example below)

### Author(s)

Gopalakrishnan N, F.Hahne

26 ellipsoidGate-class

#### References

Gating-ML Candidate Recommendation for Gating Description in Flow Cytometry V 1.5

#### See Also

hyperlog

### **Examples**

ellipsoidGate-class

Class "ellipsoidGate"

### **Description**

Class and constructor for n-dimensional ellipsoidal filter objects.

### Usage

```
ellipsoidGate(..., .gate, mean, distance=1, filterId="defaultEllipsoidGate")
```

### **Arguments**

filterId An optional parameter that sets the filterId of this gate.

. gate A definition of the gate via a covariance matrix.

mean Numeric vector of equal length as dimensions in .gate.

distance Numeric scalar giving the Mahalanobis distance defining the size of the ellipse.

This mostly exists for compliance reasons to the gatingML standard as mean and gate should already uniquely define the ellipse. Essentially, distance is merely

a factor that gets applied to the values in the covariance matrix.

... You can also directly describe the covariance matrix through named arguments,

as described below.

### **Details**

A convenience method to facilitate the construction of a ellipsoid filter objects. Ellipsoid gates in n dimensions ( $n \ge 2$ ) are specified by a covarinace matrix and a vector of mean values giving the center of the ellipse.

This function is designed to be useful in both direct and programmatic usage. In the first case, simply describe the covariance matrix through named arguments. To use this function programmatically, you may pass a covarince matrix and a mean vector directly, in which case the parameter names are the colnames of the matrix.

ellipsoidGate-class 27

#### Value

Returns a ellipsoidGate object for use in filtering flowFrames or other flow cytometry objects.

#### **Extends**

```
Class "parameterFilter", directly.

Class "concreteFilter", by class parameterFilter, distance 2.

Class "filter", by class parameterFilter, distance 3.
```

#### **Slots**

mean: Objects of class "numeric". Vector giving the location of the center of the ellipse in n dimensions.

cov: Objects of class "matrix". The covariance matrix defining the shape of the ellipse.

distance: Objects of class "numeric". The Mahalanobis distance defining the size of the ellipse.

parameters: Object of class "character", describing the parameter used to filter the flowFrame.

filterId: Object of class "character", referencing the filter.

### **Objects from the Class**

Objects can be created by calls of the form new("ellipsoidGate", ...) or by using the constructor ellipsoidGate. Using the constructor is the recommended way of object instantiation:

#### Methods

```
%in% signature(x = "flowFrame", table = "ellipsoidGate"): The workhorse used to eval-
uate the filter on data. This is usually not called directly by the user, but internally by calls to
the filter methods.
```

show signature(object = "ellipsoidGate"): Print information about the filter.

#### Note

See the documentation in the flowViz package for plotting of ellipsoidGates.

#### Author(s)

```
F.Hahne, B. Ellis, N. LeMeur
```

### See Also

flowFrame, polygonGate, rectangleGate, polytopeGate, filter for evaluation of rectangleGates and split and Subsetfor splitting and subsetting of flow cytometry data sets based on that.

#### **Examples**

```
## Loading example data
dat <- read.FCS(system.file("extdata","0877408774.B08",
package="flowCore"))
## Defining the gate
cov <- matrix(c(6879, 3612, 3612, 5215), ncol=2,</pre>
```

28 exponential-class

```
dimnames=list(c("FSC-H", "SSC-H"), c("FSC-H", "SSC-H")))
mean <- c("FSC-H"=430, "SSC-H"=175)
eg <- ellipsoidGate(filterId= "myEllipsoidGate", .gate=cov, mean=mean)
## Filtering using ellipsoidGates
fres <- filter(dat, eg)
fres
summary(fres)

## The result of ellipsoid filtering is a logical subset
Subset(dat, fres)

## We can also split, in which case we get those events in and those
## not in the gate as separate populations
split(dat, fres)</pre>
```

exponential-class

Class "exponential"

### **Description**

Exponential transform class defines a transformation given by the function

$$f(parameter, a, b) = e^{parameter/b} * \frac{1}{a}$$

### **Objects from the Class**

Objects can be created by calls to the constructorexponential(parameters,a,b).

#### Slots

```
.Data: Object of class "function" ~~

a: Object of class "numeric"- non zero constant
b: Object of class "numeric"- non zero constant
parameters: Object of class "transformation"- flow parameter to be transformed
transformationId: Object of class "character" -unique ID to reference the transformation
```

### **Extends**

Class "singleParameterTransform", directly. Class "transform", by class "singleParameterTransform", distance 2. Class "transformation", by class "singleParameterTransform", distance 3. Class "characterOrTransformation", by class "singleParameterTransform", distance 4.

#### Methods

No methods defined with class "exponential" in the signature.

### Note

The exponential transformation object can be evaluated using the eval method by passing the data frame as an argument. The transformed parameters are returned as a matrix with a single column

expressionFilter-class 29

### Author(s)

Gopalakrishnan N, F.Hahne

#### References

Gating-ML Candidate Recommendation for Gating Description in Flow Cytometry V 1.5

### See Also

logarithm

### **Examples**

```
dat <- read.FCS(system.file("extdata","0877408774.B08",
  package="flowCore"))
  exp1<-exponential(parameters="FSC-H",a=1,b=37,transformationId="exp1")
  transOut<-eval(exp1)(exprs(dat))</pre>
```

```
expressionFilter-class
```

Class "expressionFilter"

### **Description**

A filter holding an expression that can be evaluated to a logical vector or a vector of factors.

### Usage

```
expressionFilter(expr, ..., filterId="defaultExpressionFilter")
char2ExpressionFilter(expr, ..., filterId="defaultExpressionFilter")
```

### **Arguments**

filterId	An optional parameter that sets the filterId of this filter. The object can later be identified by this name.
expr	A valid R expression or a character vector that can be parsed into an expression.
•••	Additional arguments that are passed to the evaluation environment of the expression.

### **Details**

The expression is evaluated in the environment of the flow cytometry values, hence the parameters of a flowFrame can be accessed through regular R symbols. The convenience function char2ExpressionFilter exists to programmatically construct expressions.

### Value

Returns a expressionFilter object for use in filtering flowFrames or other flow cytometry objects.

30 expressionFilter-class

#### **Extends**

```
Class "concreteFilter", directly.
Class "filter", by class concreteFilter, distance 2.
```

### **Slots**

```
expr: The expression that will be evaluated in the context of the flow cytometry values.

args: An environment providing additional parameters.

deparse: A character scalar of the deparsed expression.

filterId: The identifier of the filter
```

### **Objects from the Class**

Objects can be created by calls of the form new("expressionFilter", ...), using the expressionFilter constructor or, programmatically, from a character string using the char2ExpressionFilter function.

#### Methods

```
%in% signature(x = "flowFrame", table = "expressionFilter"): The workhorse used
to evaluate the gate on data. This is usually not called directly by the user, but internally by
calls to the filter methods.
```

**show** signature(object = "expressionFilter"): Print information about the gate.

#### Author(s)

```
F. Hahne, B. Ellis
```

### See Also

flowFrame, filter for evaluation of sampleFilters and split and Subsetfor splitting and subsetting of flow cytometry data sets based on that.

### **Examples**

```
## Loading example data
dat <- read.FCS(system.file("extdata","0877408774.B08",
package="flowCore"))

#Create the filter
ef <- expressionFilter('FSC-H' > 200, filterId="myExpressionFilter")
ef

## Filtering using sampeFilters
fres <- filter(dat, ef)
fres
summary(fres)

## The result of sample filtering is a logical subset
newDat <- Subset(dat, fres)
all(exprs(newDat)[,"FSC-H"] > 200)
```

```
## We can also split, in which case we get those events in and those
## not in the gate as separate populations
split(dat, fres)

## Programmatically construct an expression
dat <- dat[,-8]
r <- range(dat)
cn <- paste("'", colnames(dat), "'", sep="")
exp <- paste(cn, ">", r[1,], "&", cn, "<", r[2,], collapse=" & ")
ef2 <- char2ExpressionFilter(exp, filterId="myExpressionFilter")
ef2
fres2 <- filter(dat, ef2)
fres2
summary(fres2)</pre>
```

fcReference-class

Class "fcReference" and its subclasses

### **Description**

Classes and methods to provide reference-based semantics for flow cytometry workflows.

#### Usage

```
fcReference(ID=paste("genericRef", guid(), sep="_"), env=new.env(parent=emptyenv()))
fcTreeReference(ID=paste("treeRef", guid(), sep="_"), env=new.env(parent=emptyenv()))
fcAliasReference(ID=paste("aliasRef", guid(), sep="_"), env=new.env(parent=emptyenv()))
fcDataReference(ID=paste("dataRef", guid(), sep="_"), env=new.env(parent=emptyenv()))
fcActionReference(ID=paste("actionRef", guid(), sep="_"), env=new.env(parent=emptyenv()))
fcViewReference(ID=paste("viewRef", guid(), sep="_"), env=new.env(parent=emptyenv()))
fcFilterResultReference(ID=paste("fresRef", guid(), sep="_"), env=new.env(parent=emptyenv()))
fcFilterReference(ID=paste("filterRef", guid(), sep="_"), env=new.env(parent=emptyenv()))
fcCompensateReference(ID=paste("compRef",
                                           guid(), sep="_"),
                                  env=new.env(parent=emptyenv()))
fcNormalizationReference(ID=paste("normRef",
                                           guid(), sep="_"),
                                  env=new.env(parent=emptyenv()))
fcSubsettingReference(ID=paste("subRef",
                                           guid(), sep="_"),
```

### **Arguments**

x, f, symbol An object of class or inheriting from class fcReference.

ID The reference identifier.

value An arbitrary R object which is supposed to be assigned to the environment in

the workFlow object and to which a reference is returned.

env An environment, usually within a workFlow object.

pos, envir Objects of class workFlow. inherits, immediate, mode, subSymbol, ...

Further arguments from the generics that are not used in this context.

### **Details**

These classes provide references to objects within an R environment and allow for method dispatch based on the nature of the referenced object. The parent fcReference class is used for references to all R objects, unless there exists a more specific subclass. fcTreeReference, fcViewReference, and fcActionReference are used to reference to graphNEL, view, and actionItem objects, respectively. fcDataReference should be used for flowFrame or flowSet objects, whereas fcFilterResultReference, fcFilterReference, fcTransformReference, fcCompensateReference, and fcNormalizationReference link to filterResult, filter, transform and compensation objects. fsStructureReference only exists to jointly dispatch on certain subgroups of references.

#### Value

An object of class fcReference or one of its subclasses for the assign constructor.

The object referenced to for the get method.

A character string of the object symbol for the identifier method.

A logical scalar for the isNull method.

#### **Extends**

```
fcStructureReference:
Class "fcReference", directly.
fcTreeReference:
Class "fcStructureReference", directly. Class "fcReference", by class "fcStructureReference",
distance 2.
fcAliasReference:
Class "fcStructureReference", directly. Class "fcReference", by class "fcStructureReference",
distance 2.
fcDataReference:
Class "fcReference", directly.
fcActionReference:
Class "fcStructureReference", directly. Class "fcReference", by class "fcStructureReference",
distance 2.
fcViewReference:
Class "fcStructureReference", directly. Class "fcReference", by class "fcStructureReference",
distance 2
fcFilterResultReference:
Class "fcReference", directly.
fcFilterReference:
Class "fcReference", directly.
fcCompensateReference:
Class "fcReference", directly.
fcTransformReference:
Class "fcReference", directly.
fcNormalizationReference:
Class "fcReference", directly.
fcNullReference:
Class "fcDataReference", directly. Class "fcActionReference", directly. Class "fcViewReference",
directly. Class "fcFilterResultReference", directly. Class "fcFilterReference", directly.
Class "fcCompensateReference", directly. Class "fcTransformReference", directly. Class
"fcNormalizationReference", directly. Class "fcTreeReference", directly. Class "fcAliasReference",
directly. Class "fcReference", by class "fcDataReference", distance2. Class "fcStructureReference",
by class "fcActionReference", distance 2. Class "fcReference", by class "fcActionReference",
distance 3. Class "fcStructureReference", by class "fcViewReference", distance 2. Class "fcReference",
by class "fcViewReference", distance3. Class "fcReference", by class "fcFilterResultReference",
distance 2. Class "fcReference", by class "fcFilterReference", distance 2. Class "fcReference",
by class "fcCompensateReference", distance 2. Class "fcReference", by class "fcTransformRefer-
ence", distance 2. Class "fcStructureReference", by class "fcTreeReference", distance 2. Class
"fcReference", by class "fcTreeReference", distance 3.
```

#### **Objects from the Class**

Objects should be created via the assign constructor. Whenever an object is assigned to a workFlow using the assign method, an appropriate instance of class fcReference or one of its subclasses is returned. In addition, there are the usual constructor functions of same names as the classes that can be used for object instantiation without assignment. Note that this might lead to unresolvable references unless the object referenced to is available in the environment.

#### Slots

```
ID: Object of class "character" The name of the object in env referenced to.
```

env: Object of class "environment" An environment that contains the referenced objects. Usually, this will be the environment that's part of a workFlow object.

#### Methods

```
get signature(x = "fcReference", pos = "missing", envir = "missing", mode = "missing", inherits
    Resolve the reference, i.e., get the object from the environment.
```

get signature(x = "fcNullReference", pos = "missing", envir = "missing", mode = "missing", inheri
 Resolve the reference. This always returns NULL.

identifier signature(object = "fcReference"): Return a character string of the object name.

**isNull** signature(f = "fcReference"): Check whether a fcReference is a NULL reference. Note that this is different from a unresolvable reference.

Rm signature(symbol = "fcReference", envir = "missing", subSymbol = "character"):

Remove the object referenced to by a fcReference from its environment. The argument subSymbol will be automatically set by the generic and should never be provided by the user.

Rm signature(symbol = "fcReference", envir = "workFlow", subSymbol = "character"):

Remove the object referenced to by a fcReference from a workFlow. The argument subSymbol will be automatically set by the generic and should never be provided by the user.

Rm signature(symbol = "fcNullReference", envir = "missing", subSymbol = "character"): Essentially, this doesn't do anything since there is no object referenced to.

```
show signature(object = "fcReference"): Print details about the object.
show signature(object = "fcNullReference"): Print details about the object.
```

#### Author(s)

Florian Hahne

### See Also

workFlow

### **Examples**

```
showClass("fcReference")
```

filter 35

filter FCS files

### **Description**

These methods link filter descriptions to a particular set of flow cytometry data allowing for the lightweight calculation of summary statistics common to flow cytometry analysis.

### Usage

```
filter(x, filter, ...)
```

### **Arguments**

```
x Object of class flowFrame or flowSet.filter An object of class filter or a named list filters.... Optional arguments
```

### **Details**

The filter method conceptually links a filter description, represented by a filter object, to a particular flowFrame. This is accomplished via the filterResult object, which tracks the linked frame as well as caching the results of the filtering operation itself, allowing for fast calculation of certain summary statistics such as the percentage of events accepted by the filter. This method exists chiefly to allow the calculation of these statistics without the need to first Subset a flowFrame, which can be quite large.

When applying on a flowSet, the filter argument can either be a single filter object, in which case it is recycled for all frames in the set, or a named list of filter objects. The names are supposed to match the frame identifiers (i.e., the output of sampleNames(x) of the flowSet. If some frames identifiers are missing, the particular frames are skipped during filtering. Accordingly, all filters in the filter list that can't be mapped to the flowSet are ignored. Note that all filter objects in the list must be of the same type, e.g. rectangleGates.

### Value

A filterResult object or a filterResultList object if x is a flowSet. Note that filterResult objects are themselves filters, allowing them to be used in filter expressions or Subset operations.

### Author(s)

```
F Hahne, B. Ellis, N. Le Meur
```

#### See Also

```
Subset, filterResult
```

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#### **Examples**

```
## Filtering a flowFrame
samp <- read.FCS(system.file("extdata","0877408774.B08", package="flowCore"))</pre>
rectGate <- rectangleGate(filterId="nonDebris", "FSC-H"=c(200,Inf))</pre>
fr <- filter(samp,rectGate)</pre>
class(fr)
summary(fr)
## filtering a flowSet
data(GvHD)
foo <- GvHD[1:3]</pre>
fr2 <- filter(foo, rectGate)</pre>
class(fr2)
summary(fr2)
## filtering a flowSet using different filters for each frame
rg2 <- rectangleGate(filterId="nonDebris","FSC-H"=c(300,Inf))</pre>
rg3 <- rectangleGate(filterId="nonDebris","FSC-H"=c(400,Inf))</pre>
flist <- list(rectGate, rg2, rg3)</pre>
names(flist) <- sampleNames(foo)</pre>
fr3 <- filter(foo, flist)</pre>
```

filter-and-methods

Take the intersection of two filters

### Description

There are two notions of intersection in flowCore. First, there is the usual intersection boolean operator & that has been overridden to allow the intersection of two filters or of a filter and a list for convenience. There is also the %&% or %subset% operator that takes an intersection, but with subset semantics rather than simple intersection semantics. In other words, when taking a subset, calculations from summary and other methods are taken with respect to the right hand filter. This primarily affects calculations, which are ordinarily calculated with respect to the entire population as well as data-driven gating procedures which will operate only on elements contained by the right hand filter. This becomes especially important when using filters such as norm2Filter

### Author(s)

B. Ellis

filter-class

A class for representing filtering operations to be applied to flow data.

### Description

The filter class is the virtual base class for all filter/gating objects in flowCore. In general you will want to subclass or create a more specific filter.

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#### **Slots**

filterId: A character vector that identifies this filter. This is typically user specified but can be automatically deduced by certain filter operations, particularly boolean and set operations.

### **Objects from the Class**

All filter objects in flowCore should be instantiated through their constructors. These are functions that share the same name with the respective filter classes. E.g., rectangleGate() is the constructor function for rectangular gates, and kmeansFilter() creates objects of class kmeansFilter. Usually these constructors can deal with various different inputs, allowing to utilize the same function in different programmatic or interactive settings. For all filters that operate on specific flow parameters (i.e., those inheriting from parameterFilter), the parameters need to be passed to the constructor, either as names or colnames of additional input arguments or explicitly as separate arguments. See the documentation of the respective filter classes for details. If parameters are explicitly defined as separate arguments, they may be of class character, in which case they will be evaluated literally as colnames in a flowFrame, or of class transform, in which case the filtering is performed on a temporarily transformed copy of the input data. See here for details.

#### Methods

- %in% Used in the usual way this returns a vector of values that identify which events were accepted by the filter. A single filter may encode several populations so this can return either a logical vector, a factor vector or a numeric vector of probabilities that the event is accepted by the filter. Minimally, you must implement this method when creating a new type of filter
- &, |,! Two filters can be composed using the usual boolean operations returning a filter class of a type appropriate for handling the operation. These methods attempt to guess an appropriate filterId for the new filter
- %subset%, %&% Defines a filter as being a subset of another filter. For deterministic filters the results will typically be equivalent to using an & operation to compose the two filters, though summary methods will use subset semantics when calculating proportions. Additionally, when the filter is data driven, such as norm2Filter, the subset semantics are applied to the data used to fit the filter possibly resulting in quite different, and usually more desirable, results.
- %on% Used in conjunction with a transformList to create a transformFilter. This filter is similar to the subset filter in that the filtering operation takes place on transformed values rather than the original values.
- filter A more formal version of %in%, this method returns a filterResult object that can be used in subsequent filter operations as well as providing more metadata about the results of the filtering operation
- summarizeFilter When implementing a new filter this method is used to update the filterDetails slot of a filterResult. It is optional and typically only needs to be implemented for data-driven filters.

## Author(s)

B. Ellis, P.D. Haaland and N. LeMeur

### See Also

transform, filter

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filter-in-methods

Filter-specific membership methods

## **Description**

Membership methods must be defined for every object of type filter with respect to a flowFrame object. The operation is considered to be general and may return a logical, numeric or factor vector that will be handled appropriately. The ability to handle logical matrices as well as vectors is also planned but not yet implemented.

## Author(s)

F.Hahne, B. Ellis

filterDetails-methods Obtain details about a filter operation

## **Description**

A filtering operation captures details about its metadata and stores it in a filterDetails slot that is accessed using the filterDetails method. Each set of metadata is indexed by the filterId of the filter allowing for all the metadata in a complex filtering operation to be recovered after the final filtering.

## Methods

result = "filterResult", filterId = "missing" When no particular filterId is specified all the details are returned

result = "filterResult", filterId = "ANY" You can also obtain a particular subset of details

# Author(s)

B. Ellis, P.D. Haaland and N. LeMeur

filterList-class

Class "filterList"

# Description

Container for a list of filter objects. The class mainly exists for method dispatch.

```
filterList(x, filterId=identifier(x[[1]]))
```

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### **Arguments**

A list of filter objects.
 The global identifier of the filter list. As default, we take the filterId of the first filter object in x.

### Value

A filterList object for the constructor

## **Objects from the Class**

Objects are created from regular lists using the constructor filterList.

## **Slots**

```
.Data: Object of class "list". The class directly extends list, and this slot holds the list data. filterId: Object of class "character". The identifier for the object.
```

#### **Extends**

```
Class "list", from data part.
```

#### Methods

```
show signature(object = "filterList"): Print details about the object.
identifier, identifier<- signature(object = "filteList"): Accessor and replacement method
for the object's filterId slot.</pre>
```

## Author(s)

Florian Hahne

## See Also

```
filter,
```

```
f1 <- rectangleGate(FSC=c(100,200), filterId="testFilter")
f2 <- rectangleGate(FSC=c(200,400))
f1 <- filterList(list(a=f1, b=f2))
fl
identifier(f1)</pre>
```

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```
filterReference-class Class filterReference
```

## **Description**

A reference to another filter inside a reference. Users should generally not be aware that they are using this class, but it is used heavily by "filterSet" classes.

## **Objects from the Class**

Objects are generally not created by users so there is no constructor function.

### **Slots**

```
name: The R name of the referenced filter
env: The environment where the filter must live
filterId: The filterId, not really used since you always resolve
```

### **Extends**

```
Class "filter", directly.
```

### Author(s)

B. Ellis

## See Also

```
"filterSet"
```

filterResult-class

Class "filterResult"

## **Description**

Container to store the result of applying a filter on a flowFrame object

## **Slots**

### **Extends**

```
Class "filter", directly.
```

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#### Methods

```
== test equality
```

### Author(s)

B. Ellis, N. LeMeur

#### See Also

```
filter, "logicalFilterResult", "multipleFilterResult", "randomFilterResult"
```

### **Examples**

```
showClass("filterResult")
```

```
filterResultList-class
```

Class "filterResultList"

## **Description**

Container to store the result of applying a filter on a flowSet object

## **Objects from the Class**

Objects are created by applying a filter on a flowSet. The user doesn't have to deal with manual object instantiation.

### **Slots**

```
.Data: Object of class "list". The class directly extends list, and this slot holds the list data.

frameId: Object of class "character" The IDs of the flowFrames in the filtered flowSet.

filterDetails: Object of class "list". Since filterResultList inherits from filterResult, this slot has to be set. It contains only the input filter.

filterId: Object of class "character". The identifier for the object.
```

### **Extends**

```
Class "list", from data part. Class "filterResult", directly. Class "concreteFilter", by class "filterResult", distance 2. Class "filter", by class "filterResult", distance 3.
```

# Methods

```
[ signature(x = "filterResultList", i = "ANY"): Subset to filterResultList.
[[ signature(x = "filterResultList", i = "ANY"): Subset to individual filterResult.
names signature(x = "filterResultList"): Accessor to the frameId slot.
parameters signature(object = "filterResultList"): Return parameters on which data has been filtered.
show signature(object = "filterResultList"): Print details about the object.
```

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```
split signature(x = "flowSet", f = "filterResultList"): Split a flowSet based on the
    results in the filterResultlIst. See split for details.
```

summary signature(object = "filterResultList"): Summarize the filtering operation. This
creates a filterSummaryList object.

## Author(s)

Florian Hahne

### See Also

filter, filterResult, logicalFilterResult, multipleFilterResult, randomFilterResult

## **Examples**

```
## Loading example data and creating a curv1Filter
data(GvHD)
dat <- GvHD[1:3]</pre>
c1f <- curv1Filter(filterId="myCurv1Filter", x=list("FSC-H"), bwFac=2)</pre>
## applying the filter
fres <- filter(dat, c1f)</pre>
fres
## subsetting the list
fres[[1]]
fres[1:2]
## details about the object
parameters(fres)
names(fres)
summary(fres)
## splitting based on the filterResults
split(dat, fres)
```

 $\verb|filterSet-class||$ 

Class filterSet

# Description

A container for a collection of related filters.

## **Objects from the Class**

There are several ways to create a filterSet object. There is the filterSet constructor, which creates an empty filterSet object (see the details section for more information). filterSet objects can also be coerced to and from list objects using the as function.

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#### **Slots**

env: The environment that actually holds the filters name: A more descriptive name of the set.

### Methods

**names** An unsorted list of the names of the filters contained within the set.

**sort** Returns a topological sort of the names of the filters contained within the set. Primarily used by internal functions (such as filter), this method is also useful for planning gating strategy layouts and the like.

filterReference Retrieves references to a filter inside a filterSet

- [ Returns the filter reference used inside the filter. See Details.
- [[ Retrieves the actual filters from a filterSet. Note that composed filters can still contain references.
- [[<- Put a filter into a filterSet. As a convenience, assigning to the """" or NULL name will use the filter's name for assignment. Composed filters can be added easily using formulas rather than attempting to construct filters the long way. The formula interface is also lazy, allowing you to add filters in any order.

### **Details**

filterSet objects are intended to provide a convenient grouping mechanism for a particular gating strategy. To accomplish this, much like the flowSet object, the filterSet object introduces reference semantics through the use of an environment, allowing users to change an upstream filter via the usual assignment mechanism and have that change reflected in all dependent filters. We do this by actually creating two filters for each filter in the filterSet. The first is the actual concrete filter, which is assigned to a variable of the form .name where name is the original filter name. A second filterReference filter is the created with the original name to point to the internal name. The allows us to evaluate a formula in the environment without creating a copy of the original filter.

## Author(s)

B. Ellis

### See Also

filterSet

```
fs = new("filterSet")

## Simple assignment. Note that the filterId slot for the rectangle gate
## is changed.
fs[["filter1"]] = rectangleGate("FSC-H"=c(.2,.8),"SSC-H"=c(0,.8))

## Convenience assignment using the filterId slot.
fs[[""]] = norm2Filter("FSC-H","SSC-H",scale.factor=2,filterId="Live Cells")

## We also support formula interfaces. These two statements are equivalent.
fs[["Combined"]] = ~ filter1 %subset% 'Live Cells'
fs[[""]] = Combined ~ filter1 %subset% 'Live Cells'
```

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```
fs
as(fs,"list")
as(as(fs,"list"),"filterSet")
```

```
filterSummary-class Class "filterSummary"
```

## **Description**

Class and methods to handle the summary information of a gating operation.

## Usage

```
## S4 method for signature 'filterResult'
summary(object, ...)
```

## **Arguments**

object An object inheriting from class filterResult which is to be summarized.

... Further arguments that are passed to the generic.

## **Details**

Calling summary on a filterResult object prints summary information on the screen, but also creates objects of class filterSummary for computational access.

## Value

An object of class filterSummary for the summary constructor, a named list for the subsetting operators. The \$ operator returns a named vector of the respective value, where each named element corresponds to one sub-population.

## **Objects from the Class**

Objects are created by calling summary on a link{filterResult} object. The user doesn't have to deal with manual object instantiation.

# Slots

```
name: Object of class "character" The name(s) of the populations created in the filtering operation. For a logicalFilterResult this is just a single value; the name of the link{filter}.
true: Object of class "numeric". The number of events within the population(s).
count: Object of class "numeric". The total number of events in the gated flowFrame.
p: Object of class "numeric" The percentage of cells in the population(s).
```

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### Methods

```
[[ signature(x = "filterSummary", i = "numeric"): Subset the filterSummary to a single
    population. This only makes sense for multipleFilterResults. The output is a list of
    summary statistics.

[[ signature(x = "filterSummary", i = "character"): see above

\$ signature(x = "filterSummary", name = "ANY"): A list-like accessor to the slots and more.
    Valid values are n and count (those are identical), true and in (identical), false and out
        (identical), name, p and q (1-p).

coerce signature(from = "filterSummary", to = "data.frame"): Coerce object to data.frame.

length signature(x = "filterSummary"): The number of populations in the filterSummary.

names signature(x = "filterSummary"): Print details about the object.

show signature(object = "filterSummary"): Print details about the object.

toTable signature(x = "filterSummary"): Coerce object to data.frame.
```

## Author(s)

Florian Hahne, Byron Ellis

### See Also

 $filter Result, logical Filter Result, multiple Filter Result, flow Frame\ filter Summary List Filter Result, flow Frame\ filter Filter Result, flow Frame\ filter Filter Filter Filter Result, flow Frame\ filter Filter$ 

```
## Loading example data, creating and applying a curv1Filter
dat <- read.FCS(system.file("extdata","0877408774.B08",
    package="flowCore"))
c1f <- curv1Filter(filterId="myCurv1Filter", x=list("FSC-H"), bwFac=2)
fres <- filter(dat, c1f)

## creating and showing the summary
summary(fres)
s <- summary(fres)

## subsetting
s[[1]]
s[["peak 2"]]

##accessing details
s$true
s$n
toTable(s)</pre>
```

```
filterSummaryList-class
```

Class "filterSummaryList"

# Description

Class and methods to handle summary statistics for from filtering operations on whole flowSets.

### **Arguments**

object An object of class filterResultList which is to be summarized.
... Further arguments that are passed to the generic.

### **Details**

Calling summary on a filterResult object prints summary information on the screen, but also creates objects of class filterSummary for computational access.

#### Value

An object of class filterSummaryList.

## **Objects from the Class**

Objects are created by calling summary on a link{filterResultList} object. The user doesn't have to deal with manual object instantiation.

## **Slots**

.Data: Object of class "list". The class directly extends list, and this slot holds the list data.

## **Extends**

```
Class "list", from data part.
```

# Usage

```
summary(object,\dots)
```

### Methods

**toTable** signature(x = "filterSummaryList"): Coerce object to data.frame. Additional factors are added to indicate list items in the original object.

#### Author(s)

Florian Hahne

# See Also

```
filter Result, filter Result List, logical Filter Result, multiple Filter Result, flow Frame filter Summary\\
```

### **Examples**

```
## Loading example data, creating and applying a curv1Filter
data(GvHD)
dat <- GvHD[1:3]
c1f <- curv1Filter(filterId="myCurv1Filter", x=list("FSC-H"), bwFac=2)
fres <- filter(dat, c1f)

## creating and showing the summary
summary(fres)
s <- summary(fres)

## subsetting
s[[1]]

##accessing details
toTable(s)</pre>
```

flowFrame-class

'flowFrame': a class for storing observed quantitative properties for a population of cells from a FACS run

## **Description**

This class represents the data contained in a FCS file or similar data structure. There are three parts of the data:

- 1. a numeric matrix of the raw measurement values with rows=events and columns=parameters
- 2. annotation for the parameters (e.g., the measurement channels, stains, dynamic range)
- 3. additional annotation provided through keywords in the FCS file

#### **Details**

Objects of class flowFrame can be used to hold arbitrary data of cell populations, acquired in flow-cytometry.

FCS is the Data File Standard for Flow Cytometry, the current version is FCS 3.0. 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("flowFrame",

exprs = ..., Object of class matrix

parameters = ..., Object of class AnnotatedDataFrame

description = ..., Object of class list
)
```

or the constructor flow Frame, with mandatory arguments exprs and optional arguments parameters and description.

```
flowFrame(exprs, parameters, description=list())
```

To create a flowFrame directly from an FCS file, use function read.FCS. This is the recommended and safest way of object creation, since read.FCS will perform basic data quality checks upon import. Unless you know exactly what you are doing, creating objects using new or the constructor is discouraged.

#### **Slots**

exprs: Object of class matrix containing the measured intensities. Rows correspond to cells, columns to the different measurement 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.

parameters: An AnnotatedDataFrame containing information about each column of the flowFrame. This will generally be filled in by read. FCS or similar functions using data from the FCS keywords describing the parameters.

description: A list containing the meta data included in the FCS file.

#### Methods

There are separate documentation pages for most of the methods listed here which should be consulted for more details.

Subsetting. Returns an object of class flowFrame. The subsetting is applied to the exprs slot, while the description slot is unchanged. The syntax for subsetting is similar to that of data. frames. In addition to the usual index vectors (integer and logical by position, character by parameter names), flowFrames can be subset via filterResult and filter objects.

Usage:

```
flowFrame[i,j]
flowFrame[filter,]
flowFrame[filterResult,]
```

Note that the value of argument drop is ignored when subsetting flowFrames.

- \\$ \\$ubsetting by channel name. This is similar to subsetting of columns of data.frames, i.e., frame\$FSC.H is equivalent to frame[, "FSC.H"]. Note that column names may have to be quoted if they are no valid R symbols (e.g. frame\$"FSC-H").
  - **exprs, exprs<-** Extract or replace the raw data intensities. The replacement value must be a numeric matrix with colnames matching the parameter definitions. Implicit subsetting is allowed (i.e. less columns in the replacement value compared to the original flowFrame, but all have to be defined there).

```
Usage:
```

```
exprs(flowFrame)
exprs(flowFrame) <- value</pre>
```

head, tail Show first/last elements of the raw data matrix

Usage:

```
head(flowFrame)
tail(flowFrame)
```

**description, description<-** Extract or replace the whole list of annotation keywords. Usually one would only be interested in a subset of keywords, in which case the keyword method is more appropriate. The optional hideInternal parameter can be used to exclude internal FCS parameters starting with\$.

```
description(flowFrame)
description(flowFrame) <- value</pre>
```

**keyword, keyword<-** Extract ore replace one or more entries from the description slot by keyword. Methods are defined for character vectors (select a keyword by name), functions (select a keyword by evaluating a function on their content) and for lists (a combination of the above). See keyword for details.

Usage:

```
keyword(flowFrame)
keyword(flowFrame, character)
keyword(flowFrame, list)
keyword(flowFrame) <- list(value)</pre>
```

parameters, parameters<- Extract parameters and return an object of class AnnotatedDataFrame, or replace such an object. To access the actual parameter annotation, use pData(parameters(frame)). Replacement is only valid with AnnotatedDataFrames containing all varLabels name, desc, range, minRange and maxRange, and matching entries in the name column to the colnames of the exprs matrix. See parameters for more details.</p>

Usage:

```
parameters(flowFrame)
parameters(flowFrame) <- value</pre>
```

**show** Display details about the flowFrame object.

**summary** Return descriptive statistical summary (min, max, mean and quantile) for each channel *Usage:* 

```
summary(flowFrame)
```

plot Basic plots for flowFrame objects. If the object has only a single parameter this produces a histogram. For exactly two parameters we plot a bivariate density map (see smoothScatter and for more than two parameters we produce a simple splom plot. To select specific parameters from a flowFrame for plotting, either subset the object or specify the parameters as a character vector in the second argument to plot. The smooth parameters lets you toggle between density-type smoothScatter plots and regular scatterplots. For far more sophisticated plotting of flow cytometry data, see the flowViz package.

Usage:

```
plot(flowFrame, ...)
plot(flowFrame, character, ...)
plot(flowFrame, smooth=FALSE, ...)
```

**ncol, nrow, dim** Extract the dimensions of the data matrix.

Usage:

```
ncol(flowFrame)
nrow(flowFrame)
dim(flowFrame)
```

**featureNames, colnames, colnames<-** Extract parameter names (i.e., the colnames of the data matrix). colnames and featureNames are synonyms. For colnames there is also a replacement method. This will update the name column in the parameters slot as well.

```
featureNames(flowFrame)
colnames(flowFrame) <- value</pre>
```

names Extract pretty formated names of the parameters including parameter descriptions.

```
Usage:
```

```
names(flowFrame)
```

**identifier** Extract GUID of a flowFrame. Returns the file name if no GUID is available. See identifier for details.

Usage:

```
identifier(flowFrame)
```

range Get dynamic range of the flowFame. Note that this is not necessarily the range of the actual data values, but the theoretical range of values the measurement instrument was able to capture. The values of the dynamic range will be transformed when using the transformation methods for flowFrames. Additional character arguments are evaluated as parameter names for which to return the dynamic range.

Usage:

```
range(flowFrame, ...)
```

each\\_row, each\\_col Apply functions over rows or columns of the data matrix. These are convenience methods. See each\_col for details.

Usage:

```
each_row(flowFrame, function, ...)
each_col(flowFrame, function, ...)
```

**transform** Apply a transformation function on a flowFrame object. This uses R's transform function by treating the flowFrame like a regular data.frame. flowCore provides an additional inline mechanism for transformations (see %on%) which is strictly more limited than the out-of-line transformation described here.

Usage:

```
transform(flowFrame, ...)
```

**filter** Apply a filter object on a flowFrame object. This returns an object of class **filterResult**, which could then be used for subsetting of the data or to calculate summary statistics. See **filter** for details.

Usage:

```
filter(flowFrame, filter)
```

**split** Split flowFrame object according to a filter, a filterResult or a factor. For most types of filters, an optional flowSet=TRUE parameter will create a flowSet rather than a simple list. See split for details.

Usage:

```
split(flowFrame, filter, flowSet=FALSE, ...)
split(flowFrame, filterResult, flowSet=FALSE, ...)
split(flowFrame, factor, flowSet=FALSE, ...)
```

**Subset** Subset a flowFrame according to a filter or a logical vector. The same can be done using the standard subsetting operator with a filter, filterResult, or a logical vector as first argument.

Usage:

```
Subset(flowFrame, filter)
Subset(flowFrame, logical)
```

**cbind2** Expand a flowFrame by the data in a numeric matrix of the same length. The matrix must have column names different from those of the flowFrame. The additional method for numerics only raises a useful error message.

### Author(s)

F. Hahne, B. Ellis, P. Haaland and N. Le Meur

### See Also

flowSet, read.FCS

```
## load example data
data(GvHD)
frame <- GvHD[[1]]</pre>
## subsetting
frame[1:4,]
frame[,3]
frame[,"FSC-H"]
frame$"SSC-H"
## accessing and replacing raw values
head(exprs(frame))
exprs(frame) <- exprs(frame)[1:3000,]</pre>
frame
exprs(frame) <- exprs(frame)[,1:6]</pre>
frame
## access FCS keywords
head(description(frame))
keyword(frame, c("FILENAME", "$FIL"))
## parameter annotation
parameters(frame)
pData(parameters(frame))
## summarize frame data
summary(frame)
## plotting
```

```
plot(frame)
if(require(flowViz)){
plot(frame)
plot(frame, c("FSC-H", "SSC-H"))
plot(frame[,1])
plot(frame, c("FSC-H", "SSC-H"), smooth=FALSE)
## frame dimensions
ncol(frame)
nrow(frame)
dim(frame)
## accessing and replacing parameter names
featureNames(frame)
all(featureNames(frame) == colnames(frame))
colnames(frame) <- make.names(colnames(frame))</pre>
colnames(frame)
parameters(frame)$name
names(frame)
## accessing a GUID
identifier(frame)
identifier(frame) <- "test"</pre>
## dynamic range of a frame
range(frame)
range(frame, "FSC.H", "FL1.H")
range(frame)$FSC.H
## iterators
head(each_row(frame, mean))
head(each_col(frame, mean))
## transformation
opar <- par(mfcol=c(1:2))</pre>
if(require(flowViz))
plot(frame, c("FL1.H", "FL2.H"))
frame <- transform(frame, FL1.H=log('FL1.H'), FL2.H=log('FL2.H'))</pre>
if(require(flowViz))
plot(frame, c("FL1.H", "FL2.H"))
par(opar)
range(frame)
## filtering of flowFrames
rectGate <- rectangleGate(filterId="nonDebris", "FSC.H"=c(200,Inf))</pre>
fres <- filter(frame, rectGate)</pre>
summary(fres)
## splitting of flowFrames
split(frame, rectGate)
split(frame, rectGate, flowSet=TRUE)
split(frame, fres)
f <- cut(exprs(frame$FSC.H), 3)</pre>
split(frame, f)
## subsetting according to filters and filter results
```

```
Subset(frame, rectGate)
Subset(frame, fres)
Subset(frame, as.logical(exprs(frame$FSC.H) < 300))
frame[rectGate,]
frame[fres,]

## accessing the spillover matrix
try(spillover(frame))

## check equality
frame2 <- frame
frame == frame2
exprs(frame2) <- exprs(frame)*2
frame == frame2</pre>
```

flowSet-class

'flowSet': a class for storing flow cytometry raw data from quantitative cell-based assays

### **Description**

This class is a container for a set of flowFrame objects

## **Creating Objects**

```
Objects can be created using
new('flowSet',
frames = ..., # environment with flowFrames
phenoData = .... # object of class AnnotatedDataFrame
colnames = .... # object of class character
)
```

or via the constructor flowSet, which takes arbitrary numbers of flowFrames, either as a list or directly as arguments, along with an optional AnnotatedDataFrame for the phenoData slot and a character scalar for the name by which the object can be referenced.

```
flowSet(..., phenoData)
```

Alternatively, flowSets can be coerced from list and environment objects.

```
as(list("A"=frameA, "B"=frameB), "flowSet")
```

The safest and easiest way to create flowSets directly from FCS files is via the read.flowSet function, and there are alternative ways to specify the files to read. See the separate documentation for details.

# Slots

frames: An environment containing one or more flowFrame objects.

phenoData: A AnnotatedDataFrame containing the phenotyoic data for the whole data set. Each row corresponds to one of the flowFrames in the frames slot. The sampleNames of phenoData (see below) must match the names of the flowFrame in the frames environment.

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

#### Methods

[, [[ Subsetting. x[i] where i is a scalar, returns a flowSet object, and x[[i]] a flowFrame object. In this respect the semantics are similar to the behavior of the subsetting operators for lists. x[i, j] returns a flowSet for which the parameters of each flowFrame have been subset according to j, x[[i,j]] returns the subset of a single flowFrame for all parameters in j. Similar to data frames, valid values for i and j are logicals, integers and characters.

Usage:

```
flowSet[i]
flowSet[i,j]
flowSet[[i]]
```

\\$ Subsetting by frame name. This will return a single flowFrame object. Note that names may have to be quoted if they are no valid R symbols (e.g. flowSet\$"sample 1"

colnames, colnames<- Extract or replace the colnames slot.

```
Usage:
   colnames(flowSet)
   colnames(flowSet) <- value</pre>
```

**identifier**, **identifier**<- Extract or replace the name item from the environment.

```
Usage:
  identifier(flowSet)
  identifier(flowSet) <- value</pre>
```

phenoData, phenoData<- Extract or replace the AnnotatedDataFrame from the phenoData slot.

Usage:

```
phenoData(flowSet)
phenoData(flowSet) <- value</pre>
```

**pData, pData<-** Extract or replace the data frame (or columns thereof) containing actual phenotypic information from the phenoData slot.

```
Usage:
```

```
pData(flowSet)
pData(flowSet)$someColumn <- value</pre>
```

varLabels, varLabels - Extract and set varLabels in the AnnotatedDataFrame of the phenoData slot.

```
Usage:
```

```
varLabels(flowSet)
varLabels(flowSet) <- value</pre>
```

**sampleNames** Extract and replace sample names from the phenoData object. Sample names correspond to frame identifiers, and replacing them will also replace the GUID slot for each frame. Note that sampleName need to be unique.

Usage:

```
sampleNames(flowSet)
sampleNames(flowSet) <- value</pre>
```

**keyword** Extract or replace keywords specified in a character vector or a list from the description slot of each frame. See keyword for details.

```
keyword(flowSet, list(keywords))
keyword(flowSet, keywords)
keyword(flowSet) <- list(foo="bar")</pre>
```

```
length number of flowFrame objects in the set.
     Usage:
        length(flowSet)
show display object summary.
summary Return descriptive statistical summary (min, max, mean and quantile) for each channel
     of each flowFrame
     Usage:
        summary(flowSet)
fsApply Apply a function on all frames in a flowSet object. Similar to sapply, but with additional
     parameters. See separate documentation for details.
     Usage:
        fsApply(flowSet, function, ...)
        fsApply(flowSet, function, use.exprs=TRUE, ...)
compensate Apply a compensation matrix on all frames in a flowSet object. See separate docu-
     mentation for details.
     Usage:
        compensate(flowSet, matrix)
transform Apply a transformation function on all frames of a flowSet object. See separate docu-
     mentation for details.
     Usage:
        transform(flowSet, ...)
filter Apply a filter object on a flowSet object. There are methods for filters, filterSets and
     lists of filters. The latter has to be a named list, where names of the list items are matching
     sampleNames of the flowSet. See filter for details.
     Usage:
        filter(flowSet, filter)
        filter(flowSet, list(filters))
split Split all flowSet objects according to a filter, filterResult or a list of such objects,
     where the length of the list has to be the same as the length of the flowSet. This returns
     a list of flowFrames or an object of class flowSet if the flowSet argument is set to TRUE.
     Alternatively, a flowSet can be split into separate subsets according to a factor (or any vector
     that can be coerced into factors), similar to the behaviour of split for lists. This will return a
     list of flowSets. See split for details.
     Usage:
        split(flowSet, filter)
        split(flowSet, filterResult)
        split(flowSet, list(filters))
        split(flowSet, factor)
Subset Returns a flowSet of flowFrames that have been subset according to a filter or filterResult,
     or according to a list of such items of equal length as the flowSet.
     Usage:
        Subset(flowSet, filter)
        Subset(flowSet, filterResult)
        Subset(flowSet, list(filters))
rbind2 Combine two flowSet objects, or one flowSet and one flowFrame object.
     Usage:
        rbind2(flowSet, flowSet)
        rbind2(flowSet, flowFrame)
```

**spillover** Compute spillover matrix from a compensation set. See separate documentation for details.

### Important note on storage and performance

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

```
y <- x
```

will create 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 considerable cost of memory and performance. flowSet 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 flowSet 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 flowSet x, use y <-x[seq(along=x)]

### Author(s)

F. Hahne, B. Ellis, P. Haaland and N. Le Meur

## See Also

```
flowFrame, read.flowSet
```

```
## load example data and object creation
data(GvHD)

## subsetting to flowSet
set <- GvHD[1:4]
GvHD[1:4,1:2]
sel <- sampleNames(GvHD)[1:2]
GvHD[sel, "FSC-H"]
GvHD[sampleNames(GvHD) == sel[1], colnames(GvHD[1]) == "SSC-H"]

## subsetting to flowFrame
GvHD[[1]]
GvHD[[1, 1:3]]
GvHD[[1, "FSC-H"]]
GvHD[[1, colnames(GvHD[1]) == "SSC-H"]]
GvHD[1, colnames(GvHD[1]) == "SSC-H"]]</pre>
```

```
## constructor
flowSet(GvHD[[1]], GvHD[[2]])
pd <- phenoData(GvHD)[1:2,]</pre>
flowSet(s5a01=GvHD[[1]], s5a02=GvHD[[2]],phenoData=pd)
## colnames
colnames(set)
colnames(set) <- make.names(colnames(set))</pre>
## object name
identifier(set)
identifier(set) <- "test"</pre>
## phenoData
pd <- phenoData(set)</pre>
pd
pd$test <- "test"
phenoData(set) <- pd</pre>
pData(set)
varLabels(set)
varLabels(set)[6] <- "Foo"</pre>
varLabels(set)
## sampleNames
sampleNames(set)
sampleNames(set) <- LETTERS[1:length(set)]</pre>
sampleNames(set)
## keywords
keyword(set, list("transformation"))
## length
length(set)
## compensation
samp <- read.flowSet(path=system.file("extdata","compdata","data",</pre>
package="flowCore"))
cfile <- system.file("extdata","compdata","compmatrix", package="flowCore")</pre>
comp.mat <- read.table(cfile, header=TRUE, skip=2, check.names = FALSE)</pre>
comp.mat
summary(samp[[1]])
samp <- compensate(samp, as.matrix(comp.mat))</pre>
summary(samp[[1]])
## transformation
opar <- par(mfcol=c(1:2))</pre>
plot(set[[1]], c("FL1.H", "FL2.H"))
set <- transform(set, FL1.H=log(FL1.H), FL2.H=log(FL2.H))</pre>
plot(set[[1]], c("FL1.H", "FL2.H"))
par(opar)
## filtering of flowSets
rectGate <- rectangleGate(filterId="nonDebris", FSC.H=c(200,Inf))</pre>
fres <- filter(set, rectGate)</pre>
class(fres)
summary(fres[[1]])
```

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```
rectGate2 <- rectangleGate(filterId="nonDebris2", SSC.H=c(300,Inf))
fres2 <- filter(set, list(A=rectGate, B=rectGate2, C=rectGate, D=rectGate2))
## Splitting frames of a flowSet
split(set, rectGate)
split(set[1:2], rectGate, populatiuon="nonDebris2+")
split(set, c(1,1,2,2))
## subsetting according to filters and filter results
Subset(set, rectGate)
Subset(set, filter(set, rectGate))
Subset(set, list(A=rectGate, B=rectGate2, C=rectGate, D=rectGate2))
## combining flowSets
rbind2(set[1:2], set[3:4])
rbind2(set[1:3], set[[4]])
rbind2(set[[4]], set[1:2])</pre>
```

fsApply

Apply a Function over values in a flowSet

## **Description**

fsApply like many of the apply-style functions in R acts as an iterator for flowSet objects, allowing the application of a function to either the flowFrame or the data matrix itself. The output can the be reconstructed as either a flowSet, a list or a matrix depending on options and the type of objects returned.

## Usage

```
fsApply(x, FUN, ..., simplify=TRUE, use.exprs=FALSE)
```

# Arguments

x flowSet to be used

FUN the function to be applied to each element of x

simplify logical (default: TRUE); if all true and all objects are flowFrame objects, a flowSet object will be constructed. If all of the values are of the same type there will be an attempt to construct a vector or matrix of the appropriate type (e.g. all numeric results will return a matrix).

use.exprs logical (default: FALSE); should the FUN be applied on the flowFrame object or the expression values.

... optional arguments to FUN.

## Author(s)

B. Ellis

## See Also

```
apply, sapply
```

gateActionItem-class 59

### **Examples**

```
fcs.loc <- system.file("extdata",package="flowCore")
file.location <- paste(fcs.loc, dir(fcs.loc), sep="/")
samp <- read.flowSet(file.location[1:3])

#Get summary information about each sample.
fsApply(samp,summary)

#Obtain the median of each parameter in each frame.
fsApply(samp,each_col,median)</pre>
```

```
gateActionItem-class Class "gateActionItem"
```

## **Description**

Class and method to capture gating operations in a flow cytometry workflow.

## Usage

```
gateActionItem(ID = paste("gateActionRef", guid(), sep = "_"), name =
paste("action", identifier(get(gate)), sep = "_"), parentView, gate,
filterResult, workflow)
```

## **Arguments**

workflow An object of class workflow for which a view is to be created.

ID A unique identifier of the view, most likely created by using the internal guid

function.

name A more human-readable name of the view.

parentView, gate, filterResult

References to the parent view, filter, and filterResult objects, respectively.

# **Details**

gateActionItems provide a means to bind gating operations in a workflow. Each gateActionItem represents a single filter.

### Value

A reference to the gateActionItem that is created inside the workFlow environment as a side effect of calling the add method.

A gateActionItem object for the constructor.

### **Objects from the Class**

Objects should be created using the add method, which creates a gateActionItem from a filter object and directly assigns it to a workFlow. Alternatively, one can use the gateActionItem constructor function for more programmatic access.

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#### **Slots**

gate: Object of class "fcFilterReference". A reference to the filter that is used for the gating operation.

filterResult: Object of class "fcFilterResultReference". A reference to the filterResult produced by the gating operation.

ID: Object of class "character". A unique identifier for the actionItem.

name: Object of class "character". A more human-readable name

parentView: Object of class "fcViewReference". A reference to the parent view the gateActionItem is applied on.

env: Object of class "environment". The evaluation environment in the workFlow.

### **Extends**

```
Class "actionItem", directly.
```

#### Methods

```
gate signature(object = "gateActionItem"): Accessor to the gate slot. Note that this re-
solved the reference, i.e., the filter object is returned.
```

```
print signature(x = "gateActionItem"): Print details about the object.
```

Rm signature(symbol = "gateActionItem", envir = "workFlow", subSymbol = "character"):
 Remove a gateActionItem from a workFlow. This method is recursive and will also remove
 all dependent views and actionItems.

show signature(object = "gateActionItem"): Print details about the object.

summary signature(object = "gateActionItem"): Summarize the gating operation and return the appropriate filterSummary object.

## Author(s)

Florian Hahne

# See Also

workFlow, actionItem, transformActionItem, compensateActionItem, view

```
showClass("view")
```

gateView-class 61

# Description

Class and method to capture the result of gating operations in a flow cytometry workflow.

# Usage

## **Arguments**

workflow	An object of class workFlow for which a view is to be created.
ID	A unique identifier of the view, most likely created by using the internal guid function.
name data, action,	A more human-readable name of the view.  filterResult References to the data, filterResult, and actionItem objects, respectively.
indices	A logical vector of indices in the parent data.
frEntry	A character vector indicating the name of the population in the filterResult.

## Details

gateViews provide a means to bind the results of gating operations in a workflow. Each gateView represents one of the populations that arise from the gating. logicalFilterResults create two gateViews (events in the gate and events not in the gate), multipleFilterResults one view for each population. See the documentation of the parent class view for more details.

# Value

A reference to the gateView that is created inside the workFlow environment as a side effect of calling the add method.

A gateView object for the constructor.

# **Objects from the Class**

Objects should be created using the add method, which creates a gateView from a filter object and directly assigns it to a workFlow. Alternatively, one can use the gateView constructor function for more programmatic access.

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#### **Slots**

indices: Object of class "logical". The indices in the parent data for events that are within the filter.

filterResult: Object of class "fcFilterResultReference". A reference to the outcome of the filtering operation.

frEntry: Object of class "character" The population in the filterResult that corresponds to the current view. See details for further explanation.

ID: Object of class "character". A unique identifier for the view.

name: Object of class "character". A more human-readable name

action: Object of class "fcActionReference". A reference to the actionItem that generated the view.

env: Object of class "environment". The evaluation environment in the workFlow.

data: Object of class "fcDataReference" A reference to the data that is associated to the view. Subsets of the data are only generated when a a further action is invoked on a particular gateView. Summary statistics about the view can be acquired through the usual process of summarizing filterResults.

### **Extends**

Class "view", directly.

### Methods

Rm signature(symbol = "gateView", envir = "workFlow", subSymbol = "character"): Remove a gateView from a workFlow. This method is recursive and will also remove all dependent views and actionItems.

**summary** signature(x = "formula", data = "gateView"): Summarize the gating operation.

xyplot signature(x = "formula", data = "gateView"): Plot the data of the gateView along
 with the gate.

## Author(s)

Florian Hahne

### See Also

workFlow, view, transformView, compensateView, actionItem

```
showClass("view")
```

GvHD 63

GvHD	Extract of a (Rizzieri et al., 2	•	Host Disease	monitoring	experiment

## **Description**

A flow cytometry high throughput screening was used to identify biomarkers that would predict the development of GvHD. The GvHD dataset is an extract of a collection of weekly peripheral blood samples obtained from patients following allogenic blood and marrow transplant. Samples were taken at various time points before and after graft.

## Usage

data(GvHD)

#### **Format**

The format is an object of class flowSet composed of 35 flowFrames. Each flowFrame corresponds to one sample at one time point. The phenodata lists:

Patient The patient Id code

Visit The number of visits to the hospital

Days The number of days since the graft. Negative values correpond to days before the graft.

Grade Grade of the cancer

## **Details**

This GvHD dataset represents the measurements of one biomarker (leukocyte) for 5 patients over 7 visits (7 time points). The blood samples were labeled with four different fluorescent probes to identify the biomarker and the fluorescent intensity was determined for at least ten thousand cells per sample.

## **Source**

Complete dataset available at <a href="http://www.ficcs.org/software.html#Data\_Files">http://www.ficcs.org/software.html#Data\_Files</a>, the Flow Informatics and Computational Cytometry Society website (FICCS)

# References

Rizzieri DA et al. J Clin Oncol. 2007 Jan 16; [Epub ahead of print] PMID: 17228020

64 hyperlog-class

hyperlog-class

Class "hyperlog"

### **Description**

Hyperlog transformation of a parameter is defined by the function

$$f(parameter, a, b) = rootEH(y, a, b) - parameter$$

where EH is a function defined by

$$EH(y, a, b) = 10^{(\frac{y}{a})} + \frac{b * y}{a} - 1 \quad y >= 0$$

$$-10^{\left(\frac{-y}{a}\right)} + \frac{b * y}{a} + 1 \quad y < 0$$

## **Objects from the Class**

Objects can be created by calls to the constructor hyperlog(parameter,a,b,transformationId)

### **Slots**

.Data: Object of class "function" ~~

a: Object of class "numeric" - numeric constant treater than zero

b: Object of class "numeric" numeric constant greater than zero

parameters: Object of class "transformation" -flow parameter to be transformed

transformationId: Object of class "character" - unique ID to reference the transformation

### **Extends**

Class "singleParameterTransform", directly. Class "transform", by class "singleParameterTransform", distance 2. Class "transformation", by class "singleParameterTransform", distance 3. Class "characterOrTransformation", by class "singleParameterTransform", distance 4.

# Methods

No methods defined with class "hyperlog" in the signature.

# Note

The transformation object can be evaluated using the eval method by passing the data frame as an argument. The transformed parameters are returned as a matrix with a single column. (See example below)

### Author(s)

Gopalakrishnan N, F.Hahne

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#### References

Gating-ML Candidate Recommendation for Gating Description in Flow Cytometry V 1.5

#### See Also

**EHtrans** 

### **Examples**

```
dat <- read.FCS(system.file("extdata","0877408774.B08",
package="flowCore"))
hlog1<-hyperlog("FSC-H",a=1,b=1,transformationId="hlog1")
transOut<-eval(hlog1)(exprs(dat))</pre>
```

identifier

Retrieve the GUID of flowCore objects

### **Description**

Retrieve the GUID (globally unique identifier) of a flowFrame that was generated by the cytometer or the identifier of a filter or filterResult given by the analyst.

## Usage

```
identifier(object)
```

#### **Arguments**

object

Object of class flowFrame, filter or filterResult.

## Details

GUID or Globally Unique Identifier is a pseudo-random number used in software applications. While each generated GUID is not guaranteed to be unique, the total number of unique keys (2\^128) is so large that the probability of the same number being generated twice is very small.

Note that if no GUID has been recorded along with the FCS file, the name of the file is returned.

## Value

Character vector representing the GUID or the name of the file.

### Methods

```
object = "filter" Return identifier of a filter object.
object = "filterReference" Return identifier of a filterReference object.
object = "filterResult" Return identifier of a filterResult object.
object = "tansform" Return identifier of a transform object.
object = "flowFrame" Return GUID from the description slot of a flowFrame object or, alternatively, the name of the input FCS file in case none can be found. For flowFrame objects there also exists a replacement method.
```

### Author(s)

N. LeMeur

### **Examples**

```
samp <- read.FCS(system.file("extdata","0877408774.B08", package="flowCore"))
identifier(samp)</pre>
```

inverseLogicleTransform

Computes the inverse of the transform defined by the 'logicleTransform' function

## **Description**

inverseLogicleTransform can be use to compute the inverse of the Logicle transformation. The parameters w, t, m, a for calculating the inverse are obtained from the 'trans' input passed to the 'inverseLogicleTransform' function. (The inverseLogicleTransform method makes use of the C++ implementation of the inverse logicle transform contributed by Wayne Moore et al.)

## Usage

inverseLogicleTransform(transformationId, trans)

## **Arguments**

transformationId

A name to assigned to the inverse transformation. Used by the transform routines.

trans

An object of class 'transform' created using the 'logicleTransform' function. The parameters w, t, m, a for calculating the inverse are obtained from the 'trans' input passed to the 'inverseLogicleTransform' function.

## Author(s)

Wayne Moore, N. Gopalakrishnan

### References

Parks D.R., Roederer M., Moore W.A.(2006) A new "logicle" display method avoids deceptive effects of logarithmic scaling for low signals and compensated data. CytometryA, 96(6):541-51.

### See Also

logicleTransform

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## **Examples**

```
data(GvHD)
samp <- GvHD[[1]]
logicle <- logicleTransform(t = 10000, w = 0.5, m = 4.5 , a =0 ,"logicle")
## transform FL1-H parameter using logicle transformation
after <- transform(samp, 'FL1-H' = logicle('FL1-H'))
## Inverse transform the logicle transformed data to retrieve the original data
invLogicle <- inverseLogicleTransform(trans = logicle)
before <- transform (after, 'FL1-H'= invLogicle('FL1-H'))</pre>
```

invsplitscale-class Class "invsplitscale"

## **Description**

The inverse split scale transformation is defined by the function

 $f(parameter, r, maxValue, transitionChannel) = \frac{(parameter-b)}{a} \quad parameter <= t*a+b$ 

$$\frac{10^{parameter*\frac{d}{r}}}{c} \quad parameter > t*a+b$$

where,

$$b = \frac{transitionChannel}{2}$$
 
$$d = \frac{2*log_{10}(e)*r}{transitionChannel} + log_{10}(maxValue)$$
 
$$t = 10^{log_{10}t}$$
 
$$a = \frac{transitionChannel}{2*t}$$
 
$$log_{10}ct = \frac{(a*t+b)*d}{r}$$
 
$$c = 10^{log_{10}ct}$$

## **Objects from the Class**

 $Objects\ can\ be\ created\ by\ calls\ to\ the\ constructor\ invsplits cale (parameters, \texttt{r}, \texttt{maxValue}, \texttt{transitionChannel}, \texttt$ 

### **Slots**

.Data: Object of class "function" ~~

r: Object of class "numeric" -a positive value indicating the range of the logarithmic part of the display

maxValue: Object of class "numeric" -a positive value indicating the maximum value the transformation is applied to

transitionChannel: Object of class "numeric" -non negative value that indicates where to split the linear vs. logarithmic transformation

parameters: Object of class "transformation" - flow parameter to be transformed transformationId: Object of class "character" -unique ID to reference the transformation

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#### **Extends**

Class "singleParameterTransform", directly. Class "transform", by class "singleParameterTransform", distance 2. Class "transformation", by class "singleParameterTransform", distance 3. Class "characterOrTransformation", by class "singleParameterTransform", distance 4.

#### Methods

No methods defined with class "invsplitscale" in the signature.

#### Note

The transformation object can be evaluated using the eval method by passing the data frame as an argument. The transformed parameters are returned as a matrix with a single column. (See example below)

### Author(s)

Gopalakrishnan N,F.Hahne

#### References

Gating-ML Candidate Recommendation for Gating Description in Flow Cytometry

#### See Also

splitscale

## **Examples**

```
dat <- read.FCS(system.file("extdata","0877408774.B08",package="flowCore"))
sp1<-invsplitscale("FSC-H",r=512,maxValue=2000,transitionChannel=512)
transOut<-eval(sp1)(exprs(dat))</pre>
```

keyword-methods

Methods to retrieve keywords of a flowFrame

# Description

Accessor and replacement methods for items in the description slot (usually read in from a FCS file header). It lists the keywords and its values for a flowFrame specified by a character vector. Additional methods for function and lists exists for more programmatic access to the keywords.

## Usage

```
keyword(object, keyword)
```

# Arguments

object Object of class flowFrame.

keyword Character vector or list of potential keywords or function. If missing all key-

words are returned.

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#### **Details**

The keyword methods allow access to the keywords stored in the FCS files, either for a flowFrame or for a list of frames in a flowSet. The most simple use case is to provide a character vector or a list of character strings of keyword names. A more sophisticated version is to provide a function which has to take one mandatory argument, the value of this is the flowFrame. This can be used to query arbitrary information from the flowFrames description slot or even the raw data. The function has to return a single character string. The list methods allow to combine functional and direct keyword access. The replacement method takes a named character vector or a named list as input. R's usual recycling rules apply when replacing keywords for a whole flowSet

## Methods

- **object = "flowFrame", keyword = "character"** Return values for all keywords from the description slot in object that match the character vector keyword.
- **object = "flowFrame", keyword = "function"** Apply the function in keyword on the flowFrame object. The function needs to be able to cope with a single argument and it needs to return a single character string. A typical use case is for instance to paste together values from several different keywords or to compute some statistic on the flowFrame and combine it with one or several other keywords.
- **object = "flowFrame", keyword = "list"** Combine characters and functions in a list to select keyword values.
- **object = "flowFrame", keyword = "missing"** This is essentially an alias for description and returns all keyword-value pairs.
- object = "flowSet", keyword = "list" This is a wrapper around fsApply(object, keyword, keyword)
   which essentially iterates over the frames in the flowSet.
- **object = "flowSet", keyword = "ANY"** This first coerces the keyword (mostly a character vector) to a list and then calls the next applicable method.

## Author(s)

N LeMeur, F Hahne, B Ellis

# See Also

description

```
samp <- read.FCS(system.file("extdata","0877408774.B08", package="flowCore"))
keyword(samp)
keyword(samp, "FCSversion")
keyword(samp, function(x,...) paste(keyword(x, "SAMPLE ID"), keyword(x,
"GUID"), sep="_"))
keyword(samp) <- list(foo="bar")
data(GvHD)
keyword(GvHD, list("GUID", cellnumber=function(x) nrow(x)))
keyword(GvHD) <- list(sample=sampleNames(GvHD))</pre>
```

70 kmeansFilter-class

kmeansFilter-class

Class "kmeansFilter"

### **Description**

A filter that performs one-dimensional k-means (Lloyd-Max) clustering on a single flow parameter.

## Usage

```
kmeansFilter(..., filterId="defaultKmeansFilter")
```

### **Arguments**

. . .

kmeansFilter are defined by a single flow parameter and an associated list of k population names. They can be given as a character vector via a named argument, or as a list with a single named argument. In both cases the name will be used as the flow parameter and the content of the list or of the argument will be used as population names, after coercing to character. For example

```
kmeansFilter(FSC=c("a", "b", "c"))
```

or

kmeansFilter(list(SSC=1:3))

If the parameter is not fully realized, but instead is the result of a transformation operation, two arguments need to be passed to the constructor: the first one being the transform object and the second being a vector of population names which can be coerced to a character. For example

kmeansFilter(tf, c("D", "E"))

filterId

An optional parameter that sets the filterId of the object. The filter can later be identified by this name.

## **Details**

The one-dimensional k-means filter is a multiple population filter capable of operating on a single flow parameter. It takes a parameter argument associated with two or more populations and results in the generation of an object of class multipleFilterResult. Populations are considered to be ordered such that the population with the smallest mean intensity will be the first population in the list and the population with the highest mean intensity will be the last population listed.

# Value

Returns a kmeansFilter object for use in filtering flowFrames or other flow cytometry objects.

# Extends

```
Class parameterFilter, directly.
```

Class concreteFilter, by class parameterFilter, distance 2.

Class filter, by class parameterFilter, distance3.

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#### **Slots**

populations: Object of class character. The names of the k populations (or clusters) that will be created by the kmeansFilter. These names will later be used for the respective subpopulations in split operations and for the summary of the filterResult.

parameters: Object of class parameters, defining a single parameter for which the data in the flowFrame is to be clustered. This may also be a transformation object.

filterId: Object of class character, an identifier or name to reference the kmeansFilter object later on.

# **Objects from the Class**

Like all other filter objects in flowCore, kmeansFilter objects should be instantiated through their constructor kmeansFilter(). See the Usage section for details.

## Methods

```
%in% signature(x = "flowFrame", table = "kmeansFilter"): The workhorse used to eval-
uate the filter on data.
    Usage:
    This is usually not called directly by the user, but internally by the filter methods.
show signature(object = "kmeansFilter"): Print information about the filter.
```

The method is called automatically whenever the object is printed on the screen.

### Note

See the documentation in the flowViz package for plotting of kmeansFilters.

## Author(s)

```
F. Hahne, B. Ellis, N. LeMeur
```

## See Also

flowFrame, flowSet, filter for evaluation of kmeansFilters and split for splitting of flow cytometry data sets based on the result of the filtering operation.

```
## Loading example data
dat <- read.FCS(system.file("extdata","0877408774.B08",
package="flowCore"))

## Create the filter
kf <- kmeansFilter("FSC-H"=c("Pop1","Pop2","Pop3"), filterId="myKmFilter")

## Filtering using kmeansFilters
fres <- filter(dat, kf)
fres
summary(fres)
names(fres)

## The result of quadGate filtering are multiple sub-populations</pre>
```

72 linearTransform

```
## and we can split our data set accordingly
split(dat, fres)

## We can limit the splitting to one or several sub-populations
split(dat, fres, population="Pop1")
split(dat, fres, population=list(keep=c("Pop1", "Pop2")))
```

linearTransform

Create the definition of a linear transformation function to be applied on a data set

## **Description**

Create the definition of the linear Transformation that will be applied on some parameter via the transform method. The definition of this function is currently  $x <- a^*x + b$ 

### Usage

linearTransform(transformationId="defaultLinearTransform", a = 1, b = 0)

### **Arguments**

transformationId

character string to identify the transformation

- a double that corresponds to the multiplicative factor in the equation
- b double that corresponds to the additive factor in the equation

## Value

Returns an object of class transform.

# Author(s)

N. LeMeur

# See Also

```
transform-class, transform
```

```
samp <- read.FCS(system.file("extdata",
   "0877408774.B08", package="flowCore"))
linearTrans <- linearTransform(transformationId="Linear-transformation", a=2, b=0)
dataTransform <- transform(samp, 'FSC-H'=linearTrans('FSC-H'))</pre>
```

InTransform 73

lnTransform	Create the definition of a ln transformation function (natural logarthim) to be applied on a data set
	rthim) to be applied on a data set

# Description

Create the definition of the ln Transformation that will be applied on some parameter via the transform method. The definition of this function is currently  $x < -\log(x) * (r/d)$ . The transformation would normally be used to convert to a linear valued parameter to the natural logarithm scale. Typically r and d are both equal to 1.0. Both must be positive.

# Usage

```
lnTransform(transformationId="defaultLnTransform", r=1, d=1)
```

# Arguments

#### Value

Returns an object of class transform.

### Author(s)

```
B. Ellis and N. LeMeur
```

#### See Also

```
transform-class, transform
```

```
data(GvHD)
  lnTrans <- lnTransform(transformationId="ln-transformation", r=1, d=1)
  ln1 <- transform(GvHD, 'FSC-H'=lnTrans('FSC-H'))

opar = par(mfcol=c(2, 1))
  plot(density(exprs(GvHD[[1]])[ ,1]), main="Original")
  plot(density(exprs(ln1[[1]])[ ,1]), main="Ln Transform")</pre>
```

74 logarithm-class

logarithm-class

Class "logarithm"

## **Description**

Logartithmic transformation of an argument is a transformation defined by the function

```
f(parameter, a, b) = ln(a * prarameter) * b \quad a * parameter > 0 0 \quad a * parameter <= 0
```

# **Objects from the Class**

Objects can be created by calls to the constructor logarithm(parameters, a, b, transformationId)

#### **Slots**

```
.Data: Object of class "function" ~~
a: Object of class "numeric" -non zero multiplicative constant
b: Object of class "numeric" -non zero multiplicative constant
parameters: Object of class "transformation"-flow parameters to be transformed
transformationId: Object of class "character"-unique ID to reference the transformation
```

### **Extends**

```
Class "singleParameterTransform", directly. Class "transform", by class "singleParameterTransform", distance 2. Class "transformation", by class "singleParameterTransform", distance 3. Class "characterOrTransformation", by class "singleParameterTransform", distance 4.
```

## Methods

No methods defined with class "logarithm" in the signature.

#### Note

The logarithm transformation object can be evaluated using the eval method by passing the data frame as an argument. The transformed parameters are returned as a matrix with a single column. (See example below)

## Author(s)

Gopalakrishnan N, F.Hahne

#### References

Gating-ML Candidate Recommendation for Gating Description in Flow Cytometry V 1.5

### See Also

exponential, quadratic

logicalFilterResult-class 75

# **Examples**

```
dat <- read.FCS(system.file("extdata","0877408774.B08",
   package="flowCore"))
   lg1<-logarithm(parameters="FSC-H",a=2,b=1,transformationId="lg1")
   transOut<-eval(lg1)(exprs(dat))</pre>
```

```
logicalFilterResult-class
```

Class "logicalFilterResult"

# **Description**

Container to store the result of applying a filter on a flowFrame object

# **Slots**

```
subSet: Object of class "numeric"
frameId: Object of class "character" referencing the flowFrame object filtered. Used for sanity checking.
filterDetails: Object of class "list" describing the filter applied
filterId: Object of class "character" referencing the filter applied
```

## Extends

```
Class "filterResult", directly. Class "filter", by class "filterResult", distance 2.
```

## Author(s)

B. Ellis

# See Also

filter

```
showClass("logicalFilterResult")
```

76 logicleTransform

logicleTransform	Computes a transform using the 'logicle_transform' function

# Description

Logicle transformation creates a subset of biexponentialTransform hyperbolic sine transformation functions that provides several advantages over linear/log transformations for display of flow cytometry data. (The logicleTransform method makes use of the C++ implementation of the logicle transform contributed by Wayne Moore et al.)

# Usage

```
logicleTransform(transformationId="defaultLogicleTransform", w = 0.5, t = 262144, m = 4.5, a = 0) estimateLogicle(x, channels)
```

# **Arguments**

transformationId

transformationid		
	A name to assign to the transformation. Used by the transform/filter routines.	
W	w is the linearization width in asymptotic decades. w should be $> 0$ and determines the slope of transformation at zero. w can be estimated using the equation $w=(m-\log 10(t/abs(r)))/2$ , where r is the most negative value to be included in the display	
t	Top of the scale data value, e.g, $10000$ for common 4 decade data or $262144$ for a 18 bit data range. t should be greater than zero	
m	$\boldsymbol{m}$ is the full width of the transformed display in asymptotic decades. $\boldsymbol{m}$ should be greater than zero	
a	Additional negative range to be included in the display in asymptotic decades. Positive values of the argument brings additional negative input values into the transformed display viewing area. Default value is zero corresponding to a Standard logicle function.	
X	Input flow frame for which the logicle transformations are to be estimated.	
channels	Flouresence channels for which the logicle transformation is to be estimated.	

# Author(s)

Wayne Moore, N Gopalakrishnan

## References

Parks D.R., Roederer M., Moore W.A.(2006) A new "logicle" display method avoids deceptive effects of logarithmic scaling for low signals and compensated data. CytometryA, 96(6):541-51.

### See Also

inverse Logicle Transform, estimate Logicle

logTransform 77

#### **Examples**

```
data(GvHD)
samp <- GvHD[[1]]
## User defined logicle function
lgcl <- logicleTransform( w = 0.5, t= 10000, m =4.5)
after <- transform(samp, 'FL1-H' = lgcl('FL1-H'), 'FL2-H' = lgcl('FL2-H'))
invLgcl <- inverseLogicleTransform(trans = lgcl)
before <- transform (after, 'FL1-H'= invLgcl('FL1-H'), 'FL2-H' = invLgcl('FL2-H'))
## Automatically estimate the logicle transformation based on the data
lgcl <- estimateLogicle(samp, channels = c("FL1-H", "FL2-H", "FL3-H", "FL2-A", "FL4-H"))
## transform parameters using the estimated logicle transformation
after <- transform(samp, lgcl)</pre>
```

logTransform

Create the definition of a log transformation function (base specified by user) to be applied on a data set

# **Description**

Create the definition of the log Transformation that will be applied on some parameter via the transform method. The definition of this function is currently  $x < -\log(x, \log base) * (r/d)$ . The transformation would normally be used to convert to a linear valued parameter to the natural logarithm scale. Typically r and d are both equal to 1.0. Both must be positive. logbase = 10 corresponds to base 10 logarithm.

# Usage

```
logTransform(transformationId="defaultLogTransform", logbase=10, r=1, d=1)
```

# **Arguments**

transformationId

character string to identify the transformation

logbase positive double that corresponds to the base of the logarithm.

positive double that corresponds to a scale factor.positive double that corresponds to a scale factor

# Value

Returns an object of class transform.

### Author(s)

B. Ellis, N. LeMeur

#### See Also

```
transform-class, transform
```

#### **Examples**

```
samp <- read.FCS(system.file("extdata",
    "0877408774.B08", package="flowCore"))
logTrans <- logTransform(transformationId="log10-transformation", logbase=10, r=1, d=1)
dataTransform <- transform(samp, 'FSC-H'=logTrans('FSC-H'))</pre>
```

```
manyFilterResult-class
```

Class "manyFilterResult"

# **Description**

The result of a several related, but possibly overlapping filter results. The usual creator of this object will usually be a filter operation of filterSet object on a flowFrame object.

#### **Slots**

```
subSet: Object of class "matrix"
frameId: Object of class "character" referencing the flowFrame object filtered. Used for sanity checking.
filterDetails: Object of class "list" describing the filter applied
filterId: Object of class "character" referencing the filter applied
dependency: Any dependencies between the filters. Currently not used.
```

# Extends

```
Class "filterResult", directly. Class "filter", by class "filterResult", distance 2.
```

#### Methods

[, [[ subsetting. If x is manyFilterResult, then x[[i]] a filterResult object. The semantics is similar to the behavior of the subsetting operators for lists.

```
length number of filterResult objects in the set.
```

names names of the filterResult objects in the set.

summary summary filterResult objects in the set.

# Author(s)

B. Ellis

#### See Also

filterResult

```
showClass("manyFilterResult")
```

```
multipleFilterResult-class
```

Class "multipleFilterResult"

# Description

Container to store the result of applying filter on set of flowFrame objects

# **Slots**

```
subSet: Object of class "factor"
frameId: Object of class "character" referencing the flowFrame object filtered. Used for sanity checking.
filterDetails: Object of class "list" describing the filter applied
filterId: Object of class "character" referencing the filter applied
```

#### **Extends**

```
Class "filterResult", directly. Class "filter", by class "filterResult", distance 2.
```

# Methods

[, [[ subsetting. If x is multipleFilterResult, then x[[i]] a FilterResult object. The semantics is similar to the behavior of the subsetting operators for lists.

**length** number of FilterResult objects in the set.

names names of the FilterResult objects in the set.

summary summary FilterResult objects in the set.

# Author(s)

B. Ellis

### See Also

filterResult

```
showClass("multipleFilterResult")
```

80 norm2Filter-class

norm2Filter-class Class "norm2Filter"

## **Description**

Class and constructors for a filter that fits a bivariate normal distribution to a data set of paired values and selects data points according to their standard deviation from the fitted distribution.

# Usage

```
norm2Filter(x, y, method="covMcd", scale.factor=1, n=50000,
filterId="defaultNorm2Filter")
```

### **Arguments**

х,у	Characters giving the names of the measurement parameter on which the filter is supposed to work on. y can be missing in which case x is expected to be a character vector of length 2 or a list of characters.
filterId	An optional parameter that sets the filterId slot of this filter. The object can later be identified by this name.
scale.factor, n	
	Numerics of length 1, used to set the scale.factor and n slots of the object.
method	Character in covMcd or cov.rob, used to set the method slot of the object.

#### **Details**

The filter fits a bivariate normal distribution to the data and selects all events within the Mahalanobis distance multiplied by the scale.factor argument. The constructor norm2Filter is a convenience function for object instantiation. Evaluating a curv2Filter results in an object of class logicalFilterResult. Accordingly, norm2Filters can be used to subset and to split flow cytometry data sets.

### Value

Returns a norm2Filter object for use in filtering flowFrames or other flow cytometry objects.

## **Extends**

```
Class "parameterFilter", directly.

Class "concreteFilter", by class parameterFilter, distance 2.

Class "filter", by class parameterFilter, distance 3.
```

#### **Slots**

method: One of covMcd or cov.rob defining method used for computation of covariance matrix. scale.factor: Numeric vector giving factor of standard deviations used for data selection (all points within scalefac standard deviations are selected).

norm2Filter-class 81

n: Object of class "numeric", the number of events used to compute the covariance matrix of the bivariate distribution.

filterId: Object of class "character" referencing the filter.

parameters: Object of class "ANY" describing the parameters used to filter the flowFrame or flowSet.

### **Objects from the Class**

Objects can be created by calls of the form new("norm2Filter", ...) or using the constructor norm2Filter. The constructor is the recommended way of object instantiation:

### Methods

```
%in% signature(x = "flowFrame", table = "norm2Filter"): The workhorse used to eval-
uate the filter on data. This is usually not called directly by the user, but internally by calls to
the filter methods.
```

show signature(object = "norm2Filter"): Print information about the filter.

#### Note

See the documentation in the flowViz package for plotting of norm2Filters.

### Author(s)

F. Hahne

## See Also

cov.rob, CovMcd, filter for evaluation of norm2Filters and split and Subsetfor splitting and subsetting of flow cytometry data sets based on that.

```
## Loading example data
dat <- read.FCS(system.file("extdata","0877408774.B08",</pre>
package="flowCore"))
## Create directly. Most likely from a command line
norm2Filter("FSC-H", "SSC-H", filterId="myCurv2Filter")
## To facilitate programmatic construction we also have the following
n2f <- norm2Filter(filterId="myNorm2Filter", x=list("FSC-H", "SSC-H"),</pre>
scale.factor=2)
n2f <- norm2Filter(filterId="myNorm2Filter", x=c("FSC-H", "SSC-H"),</pre>
scale.factor=2)
## Filtering using norm2Filter
fres <- filter(dat, n2f)</pre>
fres
summary(fres)
## The result of norm2 filtering is a logical subset
Subset(dat, fres)
```

82 normalization-class

```
## We can also split, in which case we get those events in and those
## not in the gate as separate populations
split(dat, fres)
```

normalization-class C

Class "normalization"

### **Description**

Class and methods to normalize a a flowSet using a potentially complex normalization function.

### Usage

```
\label{lem:normalization} normalization (parameters, normalization Id="defaultNormalization", \\ normFunction, arguments=list()) \\ normalize(data, x)
```

## **Arguments**

parameters Character vector of parameter names.

 ${\tt normalizationId}$ 

The identifier for the normalization object.

x An object of class flowSet.

normFunction The normalization function

arguments The list of additional arguments to normFunction

data The flowSet to normalize.

#### **Details**

Data normalization of a flowSet is a rather fuzzy concept and the class mainly exists for method dispatch in the workflow tools. The idea is to have a rather general function that takes a flowSet and a list of parameter names as input and applies any kind of normalization to the respective data columns. The output of the function has to be a flowSet again. Although we don't formally check for it, the dimensions of the input and of the output set should remain the same. Additional arguments may be passed to the normalization function via the arguments list. Internally we evaluate the function using do.call and one should check its documentation for details.

Currently, the most prominent example for a normalization function is warping, as provided by the flowStats package.

# Value

A normalization object for the constructor.

A flowSet for the normalize methods.

normalizeActionItem-class 83

#### **Objects from the Class**

Objects should be created using the constructor normalization(). See the Usage and Arguments sections for details.

### **Slots**

parameters: Object of class "character". The flow parameters that are supposed to be normalized by the normalization function.

normalizationId: Object of class "character". An identifier for the object.

normFunction: Object of class "function" The normalization function. It has to take two mandatory arguments: x, the flowSet, and parameters, a character of parameter names that are to be normalized by the function. Additional arguments have to be passed in via arguments.

arguments: Object of class "list" A names list of additional arguments. Can be NULL.

#### Methods

```
add signature(wf = "workFlow", action = "normalization"): The constructor for the work-
Flow.
```

identifier<- signature(object = "normalization", value = "character"): Set method for
 the identifier slot.</pre>

identifier signature(object = "normalization"): Get method for the identifier slot.

normalize signature(data = "flowSet", x = "normalization"): Apply a normalization to a flowSet.

parameters signature(object = "normalization"): The more generic constructor.

# Author(s)

F. Hahne

```
normalizeActionItem-class
```

Class "normalizeActionItem"

# Description

Class and method to capture normalization operations in a flow cytometry workflow.

### Usage

```
normalizeActionItem(ID = paste("normActionRef", guid(), sep = "_"),
name = paste("action", identifier(get(normalization)), sep = "_"),
parentView, normalization, workflow)
```

#### **Arguments**

workflow An object of class workflow for which a view is to be created.

ID A unique identifier of the view, most likely created by using the internal guid

function.

name A more human-readable name of the view.

parentView, normalization

References to the parent view and normalization objects, respectively.

#### **Details**

normalizeActionItems provide a means to bind normalization operations like warping in a work-flow. Each normalizeActionItem represents a single normalization.

# Value

A reference to the normalizeActionItem that is created inside the workFlow environment as a side effect of calling the add method.

A normalizeActionItem object for the constructor.

### **Objects from the Class**

Objects should be created using the add method, which creates a normalizeActionItem from a normalization object and directly assigns it to a workFlow. Alternatively, one can use the normalizeActionItem constructor function for more programmatic access.

## Slots

```
normalization: Object of class "fcNormalizationReference". A reference to the normalization object that is used for the compensation operation.
```

ID: Object of class "character". A unique identifier for the actionItem.

name: Object of class "character". A more human-readable name

parentView: Object of class "fcViewReference". A reference to the parent view the normalizeActionItem is applied on.

env: Object of class "environment". The evaluation environment in the workFlow.

## Extends

```
Class "actionItem", directly.
```

# Methods

```
print signature(x = "normalizeActionItem"): Print details about the object.
```

```
Rm signature(symbol = "normalizeActionItem", envir = "workFlow", subSymbol = "character"):
    Remove a normalizeActionItem from a workFlow. This method is recursive and will also
    remove all dependent views and actionItems.
```

**show** signature(object = "normalizeActionItem"): Print details about the object.

# Author(s)

Florian Hahne

normalizeView-class 85

#### See Also

workFlow, actionItem, gateActionItem, transformActionItem, compensateActionItem, view

#### **Examples**

```
showClass("view")
```

normalizeView-class

Class "normalizeView"

# **Description**

Class and method to capture the result of normalization operations in a flow cytometry workflow.

# Usage

# **Arguments**

workflow An object of class workflow for which a view is to be created.

ID A unique identifier of the view, most likely created by using the internal guid

function.

name A more human-readable name of the view.

data, action References to the data and actionItem objects, respectively.

### Value

A reference to the normalizeView that is created inside the workFlow environment as a side effect of calling the add method.

A normalizeView object for the constructor.

# **Objects from the Class**

Objects should be created using the add method, which creates a normalizeView from a normalization object and directly assigns it to a workFlow. Alternatively, one can use the normalizeView constructor function for more programmatic access.

#### **Slots**

```
ID: Object of class "character". A unique identifier for the view.
```

name: Object of class "character". A more human-readable name

action: Object of class "fcActionReference". A reference to the actionItem that generated the view.

env: Object of class "environment". The evaluation environment in the workFlow.

data: Object of class "fcDataReference" A reference to the data that is associated to the view.

86 parameterFilter-class

#### **Extends**

```
Class "view", directly.
```

### Methods

```
Rm signature(symbol = "normalizeView", envir = "workFlow", subSymbol = "character"):

Remove a normalizeView from a workFlow. This method is recursive and will also remove all dependent views and actionItems.
```

# Author(s)

Florian Hahne

### See Also

```
workFlow, view, gateView, transformView, compensateView, actionItem
```

# **Examples**

```
showClass("view")
```

```
parameterFilter-class Class "parameterFilter"
```

# **Description**

A concrete filter that acts on a set of parameters.

# **Objects from the Class**

parameterFilter objects are never created directly. This class serves as an inheritance point for filters that depends on particular parameters.

#### **Slots**

```
parameters: The names of the parameters employed by this filter filterId: The filter identifier
```

# **Extends**

```
Class\ "concrete Filter", directly.\ Class\ "filter", by\ class\ "concrete Filter", distance\ 2.
```

#### Methods

No methods defined with class "parameterFilter" in the signature.

# Author(s)

B. Ellis

parameters 87

parameters

Obtain information about parameters for flow cytometry objects.

# **Description**

Many different objects in flowCore are associated with one or more parameters. This includes filter, flowFrame and parameterFilter objects that all either describe or use parameters.

# Usage

```
parameters(object, ...)
```

# **Arguments**

```
objectObject of class filter, flowFrame or parameterFilter....Further arguments that get passed on to the methods.
```

### Value

When applied to a flowFrame object, the result is an AnnotatedDataFrame describing the parameters recorded by the cytometer. For other objects it will usually return a vector of names used by the object for its calculations.

### Methods

```
object = "filter" Returns for all objects that inherit from filter a vector of parameters on which
    a gate is defined.

object = "parameterFilter" see above

object = "setOperationFilter" see above

object = "filterReference" see above

object = "flowFrame" Returns an AnnotatedDataFrame containing detailed descriptions about
    the measurement parameters of the flowFrame. For flowFrame objects there also exists a
    replacement method.
```

# Author(s)

```
B. Ellis, N. Le Meur, F. Hahne
```

```
samp <- read.FCS(system.file("extdata","0877408774.B08", package="flowCore"))
parameters(samp)
print(samp@parameters@data)</pre>
```

parameters-class

Class "parameters"

## **Description**

A representation of flow parameters that allows for referencing.

# **Objects from the Class**

Objects will be created internally whenever necessary and this should not be of any concern to the user.

# **Slots**

.Data: A list of the individual parameters

# **Extends**

```
Class "list", from data part. Class "vector", by class "list", distance 2.
```

### Methods

No methods defined with class "parameters" in the signature.

# Author(s)

Nishant Gopalakrishnan

```
parameterTransform-class
```

Class "parameterTransform"

### **Description**

Link a transformation to particular flow parameters

# **Objects from the Class**

Objects are created by using the %on% operator and are usually not directly instantiated by the user.

# Slots

```
.Data: Object of class "function", the transformation function.

parameters: Object of class "character" The parameters the transformation is applied to.

transformationId: Object of class "character". The identifier for the object.
```

### **Extends**

```
Class "transform", directly. Class "function", by class "transform", distance 2.
```

polygonGate-class 89

#### Methods

```
%on% signature(e1 = "filter", e2 = "parameterTransform"): Apply the transforma-
tion.
%on% signature(e1 = "parameterTransform", e2 = "flowFrame"): see above
parameters signature(object = "parameterTransform"): Accessor to the parameters slot
```

### Author(s)

Byron Ellis

polygonGate-class

Class "polygonGate"

### **Description**

Class and constructor for 2-dimensional polygonal filter objects.

## Usage

```
polygonGate(..., .gate, boundaries, filterId="defaultPolygonGate")
```

## **Arguments**

filterId An optional parameter that sets the filterId of this gate.

.gate, boundaries

A definition of the gate. This can be either a list or a named matrix as described below. Note the argument boundaries is deprecated and will go away in the next release.

You can also directly describe a gate without wrapping it in a list or matrix, as described below.

# **Details**

Polygons are specified by the coordinates of their vertices in two dimensions. The constructor is designed to be useful in both direct and programmatic usage. It takes either a list or a named matrix with 2 columns and at least 3 rows containing these coordinates. Alternatively, vertices can be given as named arguments, in which case the function tries to convert the values into a matrix.

## Value

Returns a polygonGate object for use in filtering flowFrames or other flow cytometry objects.

# Extends

```
Class "parameterFilter", directly.

Class "concreteFilter", by class parameterFilter, distance 2.

Class "filter", by class parameterFilter, distance 3.
```

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#### **Slots**

boundaries: Object of class "matrix". The vertices of the polygon in two dimensions. There need to be at least 3 vertices specified for a valid polygon.

parameters: Object of class "character", describing the parameter used to filter the flowFrame. filterId: Object of class "character", referencing the filter.

### **Objects from the Class**

Objects can be created by calls of the form new("polygonGate", ...) or by using the constructor polygonGate. Using the constructor is the recommended way of object instantiation:

#### Methods

```
%in% signature(x = "flowFrame", table = "polygonGate"): The workhorse used to eval-
uate the filter on data. This is usually not called directly by the user, but internally by calls to
the filter methods.
```

**show** signature(object = "polygonGate"): Print information about the filter.

#### Note

See the documentation in the flowViz package for plotting of polygonGates.

#### Author(s)

```
F.Hahne, B. Ellis N. Le Meur
```

# See Also

flowFrame, rectangleGate, ellipsoidGate, polytopeGate, filter for evaluation of rectangleGates and split and Subsetfor splitting and subsetting of flow cytometry data sets based on that.

```
## Loading example data
dat <- read.FCS(system.file("extdata","0877408774.B08",</pre>
package="flowCore"))
## Defining the gate
sqrcut <- matrix(c(300,300,600,600,50,300,300,50),ncol=2,nrow=4)</pre>
colnames(sqrcut) <- c("FSC-H","SSC-H")</pre>
pg <- polygonGate(filterId="nonDebris", boundaries= sqrcut)</pre>
pg
## Filtering using polygonGates
fres <- filter(dat, pg)</pre>
fres
summary(fres)
## The result of polygon filtering is a logical subset
Subset(dat, fres)
## We can also split, in which case we get those events in and those
## not in the gate as separate populations
```

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```
split(dat, fres)
```

polytopeGate-class

Define filter boundaries

#### **Description**

Convenience methods to facilitate the construction of filter objects

### Usage

```
polytopeGate(..., .gate, b, filterId="defaultPolytopeGate")
```

### **Arguments**

filterId An optional parameter that sets the filterId of this gate.

.gate A definition of the gate. This can be either a list, vector or matrix, described

below.

b Need documentation

... You can also directly describe a gate without wrapping it in a list or matrix, as

described below.

#### **Details**

These functions are designed to be useful in both direct and programmatic usage.

For rectangle gate in n dimensions, if n=1 the gate correspond to a range gate. If n=2, the gate is a rectangle gate. To use this function programmatically, you may either construct a list or you may construct a matrix with n columns and 2 rows. The first row corresponds to the minimal value for each parameter while the second row corresponds to the maximal value for each parameter. The names of the parameters are taken from the column names as in the third example.

Rectangle gate objects can also be multiplied together using the \* operator, provided that both gate have orthogonal axes.

For polygon gate, the boundaries are specified as vertices in 2 dimensions, for polytope gate objects as vertices in n dimensions.

For quadrant gates, the boundaries are specified as a named list or vector of length two.

# Value

Returns a rectangleGate or polygonGate object for use in filtering flowFrames or other flow cytometry objects.

# Author(s)

```
F.Hahne, B. Ellis N. Le Meur
```

### See Also

```
flowFrame, filter
```

92 quadGate-class

# **Description**

Class and constructors for quadrant-type filter objects.

### Usage

```
quadGate(..., .gate, filterId="defaultQuadGate")
```

### **Arguments**

filterId	An optional parameter that sets the filterId of this filter. The object can later be identified by this name.
.gate	A definition of the gate for programmatic access. This can be either a named list or a named numeric vector, as described below.
•••	The parameters of quadGates can also be directly described using named function arguments, as described below.

#### **Details**

quadGates are defined by two parameters, which specify a separation of a two-dimensional parameter space into four quadrants. The quadGate function is designed to be useful in both direct and programmatic usage:

For the interactive use, these parameters can be given as additional named function arguments, where the names correspond to valid parameter names in a flowFrame or flowSet. For a more programmatic approach, a named list or numeric vector of the gate boundaries can be passed on to the function as argument .gate.

Evaluating a quadGate results in four sub-populations, and hence in an object of class multipleFilterResult. Accordingly, quadGates can be used to split flow cytometry data sets.

#### Value

Returns a quadGate object for use in filtering flowFrames or other flow cytometry objects.

### **Extends**

```
Class "parameterFilter", directly.

Class "concreteFilter", by class parameterFilter, distance 2.

Class "filter", by class parameterFilter, distance 3.
```

# Slots

```
boundary: Object of class "numeric", length 2. The boundaries of the quadrant regions. parameters: Object of class "character", describing the parameter used to filter the flowFrame. filterId: Object of class "character", referencing the gate.
```

quadGate-class 93

#### **Objects from the Class**

Objects can be created by calls of the form new("quadGate", ...) or using the constructor quadGate. The latter is the recommended way of object instantiation:

#### Methods

```
%in% signature(x = "flowFrame", table = "quadGate"): The workhorse used to evaluate
the gate on data. This is usually not called directly by the user, but internally by calls to the
filter methods.
```

```
show signature(object = "quadGate"): Print information about the gate.
```

#### Note

See the documentation in the flowViz package for plotting of quadGates.

# Author(s)

```
F.Hahne, B. Ellis N. Le Meur
```

#### See Also

flowFrame, flowSet, filter for evaluation of quadGates and split for splitting of flow cytometry data sets based on that.

```
## Loading example data
dat <- read.FCS(system.file("extdata","0877408774.B08",</pre>
package="flowCore"))
## Create directly. Most likely from a command line
quadGate(filterId="myQuadGate1", "FSC-H"=100, "SSC-H"=400)
## To facilitate programmatic construction we also have the following
quadGate(filterId="myQuadGate2", list("FSC-H"=100, "SSC-H"=400))
## FIXME: Do we want this?
##quadGate(filterId="myQuadGate3", .gate=c("FSC-H"=100, "SSC-H"=400))
## Filtering using quadGates
qg <- quadGate(filterId="quad", "FSC-H"=600, "SSC-H"=400)</pre>
fres <- filter(dat, qg)</pre>
fres
summary(fres)
names(fres)
## The result of quadGate filtering are multiple sub-populations
## and we can split our data set accordingly
split(dat, fres)
## We can limit the splitting to one or several sub-populations
split(dat, fres, population="FSC-H-SSC-H-")
split(dat, fres, population=list(keep=c("FSC-H-SSC-H-",
"FSC-H-SSC-H+")))
```

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quadratic-class

Class "quadratic"

# **Description**

Quadratic transform class defines a transformation defined by the function

```
f(parameter, a) = a * parameter^2
```

# **Objects from the Class**

Objects can be created by calls to the constructor quadratic (parameters, a, transformationId)

#### Slots

```
.Data: Object of class "function" ~~
a: Object of class "numeric"-non zero multiplicative constant
parameters: Object of class "transformation"-flow parameter to be transformed
transformationId: Object of class "character"-unique ID to reference the transformation
```

### **Extends**

```
Class "singleParameterTransform", directly. Class "transform", by class "singleParameterTransform", distance 2. Class "transformation", by class "singleParameterTransform", distance 3. Class "characterOrTransformation", by class "singleParameterTransform", distance 4.
```

## Methods

No methods defined with class "quadratic" in the signature.

#### Note

The quadratic transformation object can be evaluated using the eval method by passing the data frame as an argument. The transformed parameters are returned as a column vector. (See example below)

# Author(s)

Gopalakrishnan N, F.Hahne

# References

Gating-ML Candidate Recommendation for Gating Description in Flow Cytometry V 1.5

#### See Also

dg1polynomial,ratio,squareroot

quadraticTransform 95

# **Examples**

```
dat <- read.FCS(system.file("extdata","0877408774.B08",
package="flowCore"))
quad1<-quadratic(parameters="FSC-H",a=2,transformationId="quad1")
transOut<-eval(quad1)(exprs(dat))</pre>
```

quadratic Transform

Create the definition of a quadratic transformation function to be applied on a data set

# **Description**

Create the definition of the quadratic Transformation that will be applied on some parameter via the transform method. The definition of this function is currently  $x <- a*x^2 + b*x + c$ 

### Usage

```
quadraticTransform(transformationId="defaultQuadraticTransform", a = 1, b = 1, c = 0)
```

# **Arguments**

transformationId

character string to identify the transformation

- a double that corresponds to the quadratic coefficient in the equation
- b double that corresponds to the linear coefficient in the equation
- c double that corresponds to the intercept in the equation

#### Value

Returns an object of class transform.

### Author(s)

N. Le Meur

#### See Also

```
transform-class, transform
```

```
samp <- read.FCS(system.file("extdata",
   "0877408774.B08", package="flowCore"))
quadTrans <- quadraticTransform(transformationId="Quadratic-transformation", a=1, b=1, c=0)
dataTransform <- transform(samp, 'FSC-H'=quadTrans('FSC-H'))</pre>
```

96 ratio-class

```
randomFilterResult-class
```

Class "randomFilterResult"

# **Description**

Container to store the result of applying a filter on a flowFrame object

#### Slots

```
subSet: Object of class "numeric"
frameId: Object of class "character" referencing the flowFrame object filtered. Used for sanity checking.
filterDetails: Object of class "list" describing the filter applied
filterId: Object of class "character" referencing the filter applied
```

### **Extends**

Class "filterResult", directly. Class "filter", by class "filterResult", distance 2.

# Author(s)

B. Ellis

### See Also

filter

ratio-class

Class "ratio"

# **Description**

ratio transform calculates the ratio of two parameters defined by the function

$$f(parameter_1, parameter_2) = \frac{parameter_1}{parameter_2}$$

# **Objects from the Class**

Objects can be created by calls to the constructor ratio(parameter1, parameter2, transformationId).

## **Slots**

```
.Data: Object of class "function" ~~
numerator: Object of class "transformation" -flow parameter to be transformed
denominator: Object of class "transformation" -flow parameter to be transformed
transformationId: Object of class "character" unique ID to reference the transformation
```

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#### **Extends**

Class "transform", directly. Class "transformation", by class "transform", distance 2. Class "characterOrTransformation", by class "transform", distance 3.

### Methods

No methods defined with class "ratio" in the signature.

#### Note

The ratio transformation object can be evaluated using the eval method by passing the data frame as an argument. The transformed parameters are returned as matrix with one column. (See example below)

# Author(s)

Gopalakrishnan N, F.Hahne

# References

Gating-ML Candidate Recommendation for Gating Description in Flow Cytometry V 1.5

### See Also

dg1polynomial,quadratic,squareroot

## **Examples**

```
dat <- read.FCS(system.file("extdata","0877408774.B08",
package="flowCore"))
rat1<-ratio("FSC-H","SSC-H",transformationId="rat1")
transOut<-eval(rat1)(exprs(dat))</pre>
```

read.FCS

Read an FCS file

# Description

Check validity and Read Data File Standard for Flow Cytometry

# Usage

98 read.FCS

### **Arguments**

files A vector of filenames

filename Character of length 1: filename

transformation An character string that defines the type of transformation. Valid values are

linearize (default) or scale. The linearize transformation applies the appropriate power transform to the data while the scale transformation scales all columns to  $[0,10^{decades}]$ . defaulting to decades=0 as in the FCS4 specification. A logical can also be used: TRUE is equal to linearize and FALSE

corresponds to no transformation.

which.lines Numeric vector to specify the indices of the lines to be read. If NULL all

the records are read, if of length 1, a random sample of the size indicated by

which.lines is read in.

alter.names boolean indicating whether or not we should rename the columns to valid R

names using make.names. The default is FALSE.

column.pattern An optional regular expression defining parameters we should keep when load-

ing the file. The default is NULL.

decades When scaling is activated, the number of decades to use for the output.

ncdf Instead of reading all data into memory, this switches to file-based data storage.

A netCDF file is creates for each flowFrame in the .flowCoreNcdf subdirectory. For large data sets this significantly reduces the memory profile of the R session, to the cost of speed and disk space. The exprs and exprs<- methods make sure that the user always gets a matrix of data values. Please note that currently all operations that call exprs<-, either explicitly or implicitely, will result in the creation of a new netCDF file. This behaviour may change in the future. Currently the software does not remove any of the netCDF files and it is up to the user to do clean up. The easiest way to do that is to delete the whole netCDF

directory. To this end, one can invoke the cleanup function.

min.limit The minimum value in the data range that is allowed. Some instruments produce

extreme artifactual values. The positive data range for each parameter is completely defined by the measurement range of the instrument and all larger values are set to this threshold. The lower data boundary is not that well defined, since compensation might shift some values below the original measurement range of the instrument. The default value of -111 copies the behavior of flowJo. It can be set to an arbitrary number or to NULL, in which case the original values are

kept.

dataset The FCS file specification allows for multiple data segments in a single file.

Since the output of read. FCS is a single flowFrame we can't automatically read in all available sets. This parameter allows to chose one of the subsets for import. Its value is supposed to be an integer in the range of available data sets. This argument is imported if there is only a single data segment in the ECS file.

argument is ignored if there is only a single data segment in the FCS file.

emptyValue boolean indicating whether or not we allow empty value for keyword values in

TEXT segment. It affects how the double delimiters are treated. IF TRUE, The double delimiters are parsed as a pair of start and end single delimiter for an empty value. Otherwise, double delimiters are parsed one part of string as the

keyword value. default is TRUE.

# Details

The function is FCS file determines whether its arguments are valid FCS files.

read.FCSheader 99

The function read.FCS works with the output of the FACS machine software from a number of vendors (FCS 2.0, FCS 3.0 and List Mode Data LMD). 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 flowFrame.

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.

The nlines and sampling arguments allow you to read a subset of the record as you might not want to read the thousands of events recorded in the FCS file.

The which.lines argument allows you to read a specific number of records.

#### Value

isFCSfile returns a logical vector.

read.FCS returns an object of class flowFrame that contains the data in the exprs slot, the parameters monitored in the parameters slot and the keywords and value saved in the header of the FCS file.

### Author(s)

F. Hahne, N.Le Meur

#### See Also

```
read.flowSet
```

#### **Examples**

```
## a sample file
fcsFile <- system.file("extdata", "0877408774.B08", package="flowCore")

## read file and linearize values
samp <- read.FCS(fcsFile, transformation="linearize")
exprs(samp[1:3,])
description(samp)[3:6]
class(samp)

## Only read in lines 2 to 5
subset <- read.FCS(fcsFile, which.lines=2:5, transformation="linearize")
exprs(subset)

## Read in a random sample of 100 lines
subset <- read.FCS(fcsFile, which.lines=100, transformation="linearize")
nrow(subset)</pre>
```

read.FCSheader

Read the TEXT section of a FCS file

# **Description**

Read (part of) the TEXT section of a Data File Standard for Flow Cytometry that contains FACS keywords.

100 read.flowSet

#### Usage

```
read.FCSheader(files, path=".", keyword=NULL)
```

#### **Arguments**

files Character vector of filenames.

path Directory where to look for the files.

keyword An optional character vector that specifies the FCS keyword to read.

### **Details**

The function read. FCSheader works with the output of the FACS machine software from a number of vendors (FCS 2.0, FCS 3.0 and List Mode Data LMD). The output of the function is the TEXT section of the FCS files. The user can specify some keywords to limit the output to the information of interest.

#### Value

A list of character vector. Each element of the list correspond to one FCS file.

#### Author(s)

N.Le Meur

#### See Also

```
link[flowCore]{read.flowSet}, link[flowCore]{read.FCS}
```

# **Examples**

```
samp <- read.FCSheader(system.file("extdata",
    "0877408774.B08", package="flowCore"))
samp

samp <- read.FCSheader(system.file("extdata",
    "0877408774.B08", package="flowCore"), keyword=c("$DATE", "$FIL"))
samp</pre>
```

read.flowSet

Read a set of FCS files

# **Description**

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

# Usage

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#### **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 An object of class AnnotatedDataFrame, character or a list of values to be

extracted from the flowFrame object, see details.

descriptions Character vector to annotate the object of class flowSet.

name.keyword An optional character vector that specifies which FCS keyword to use as the

sample names. If this is not set, the GUID of the FCS file is used for sample-Names, and if that is not present (or not unique), then the file names are used.

alter.names see read.FCS for details.
transformation see read.FCS for details.
which.lines see read.FCS for details.
column.pattern see read.FCS for details.
decades see read.FCS for details.

sep Separator character that gets passed on to read. AnnotatedDataFrame.

as.is Logical that gets passed on to read. AnnotatedDataFrame. This controls the

automatic coercion of characters to factors in the phenoDataslot.

name An optional character scalar used as name of the object.

ncdf Instead of reading all data into memory, this switches to file-based data storage.

See read. FCS for details.

dataset see read. FCS for details.

... Further arguments that get passed on to read.AnnotatedDataFrame, see de-

tails.

#### **Details**

There are four different ways to specify the file from which data is to be imported:

First, if the argument phenoData is present and is of class AnnotatedDataFrame, then the file names are obtained from its sample names (i.e. 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(file.path(path,phenoData),...).

In some cases the file names are not a reasonable selection criterion and the user might want to import files based on some keywords within the file. One or several keyword value pairs can be given as the phenoData argument in form of a named list.

Third, 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.

Fourth, 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 flowSet.

102 rectangleGate-class

#### Author(s)

```
F. Hahne, N.Le Meur, B. Ellis
```

### **Examples**

```
fcs.loc <- system.file("extdata",package="flowCore")
file.location <- paste(fcs.loc, dir(fcs.loc), sep="/")
samp <- read.flowSet(file.location[1:3])</pre>
```

rectangleGate-class

Class "rectangleGate"

### **Description**

Class and constructor for n-dimensional rectangular filter objects.

# Usage

```
rectangleGate(..., .gate, filterId="defaultRectangleGate")
```

## Arguments

filterId	An optional parameter that sets the filterId of this gate. The object can later be identified by this name.
.gate	A definition of the gate. This can be either a list, or a matrix, as described below.
•••	You can also directly provide the boundaries of a rectangleGate as additional named arguments, as described below.

## **Details**

This class describes a rectangular region in n dimensions, which is a Cartesian product of n orthogonal intervals in these dimensions. n=1 corresponds to a range gate, n=2 to a rectangle gate, n=3 corresponds to a box region and n>3 to a hyper-rectangular regions. Intervals may be open on one side, in which case the value for the boundary is supposed to be Inf or -Inf, respectively. rectangleGates are inclusive, that means that events on the boundaries are considered to be in the gate.

The constructor is designed to be useful in both direct and programmatic usage. To use it programmatically, you may either construct a named list or you may construct a matrix with n columns and 2 rows. The first row corresponds to the minimal value for each parameter while the second row corresponds to the maximal value for each parameter. The names of the parameters are taken from the column names or from the list names, respectively. Alternatively, the boundaries of the rectangleGate can be given as additional named arguments, where each of these arguments should be a numeric vector of length 2; the function tries to collapse these boundary values into a matrix.

Note that boundaries of rectangleGates where min > max are syntactically valid, however when evaluated they will always be empty.

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rectangleGate objects can also be multiplied using the \* operator, provided that both gates have orthogonal axes. This results in higher-dimensional rectangleGates. The inverse operation of subsetting by parameter name(s) is also available.

Evaluating a rectangleGate generates an object of class logicalFilterResult. Accordingly, rectangleGates can be used to subset and to split flow cytometry data sets.

#### Value

Returns a rectangleGate object for use in filtering flowFrames or other flow cytometry objects.

#### **Extends**

```
Class "parameterFilter", directly.

Class "concreteFilter", by class parameterFilter, distance 2.

Class "filter", by class parameterFilter, distance 3.
```

### **Slots**

min, max: Objects of class "numeric". The minimum and maximum values of the n-dimensional rectangular region.

parameters: Object of class "character", indicating the parameters for which the rectangleGate is defined.

filterId: Object of class "character", referencing the filter.

# **Objects from the Class**

Objects can be created by calls of the form new("rectangleGate", ...), by using the constructor rectangleGate or by combining existing rectangleGates using the \* method. Using the constructor is the recommended way of object instantiation:

# Methods

```
%in% signature(x = "flowFrame", table = "rectangleGate"): The workhorse used to eval-
uate the filter on data. This is usually not called directly by the user, but internally by calls to
the filter methods.
```

```
show signature(object = "rectangleGate"): Print information about the filter.
```

\* signature(e1 = "rectangleGate", e2 = "rectangleGate"): combining two rectangleGates into one higher dimensional representation.

```
\[ \signature(x = "rectangleGate", i = "character"): Subsetting of a rectangleGate by parameter name(s). This is essentially the inverse to *.
```

### Note

See the documentation in the flowViz package for details on plotting of rectangleGates.

### Author(s)

```
F.Hahne, B. Ellis N. Le Meur
```

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#### See Also

flowFrame, polygonGate, ellipsoidGate, polytopeGate, filter for evaluation of rectangleGates and split and Subsetfor splitting and subsetting of flow cytometry data sets based on that.

### **Examples**

```
## Loading example data
dat <- read.FCS(system.file("extdata","0877408774.B08",</pre>
package="flowCore"))
#Create directly. Most likely from a command line
rectangleGate(filterId="myRectGate", "FSC-H"=c(200, 600), "SSC-H"=c(0, 400))
#To facilitate programmatic construction we also have the following
rg <- rectangleGate(filterId="myRectGate", list("FSC-H"=c(200, 600),</pre>
"SSC-H"=c(0, 400)))
mat <- matrix(c(200, 600, 0, 400), ncol=2, dimnames=list(c("min", "max"),</pre>
c("FSC-H", "SSC-H")))
rg <- rectangleGate(filterId="myRectGate", .gate=mat)</pre>
## Filtering using rectangleGates
fres <- filter(dat, rg)</pre>
fres
summary(fres)
## The result of rectangle filtering is a logical subset
Subset(dat, fres)
## We can also split, in which case we get those events in and those
## not in the gate as separate populations
split(dat, fres)
## Multiply rectangle gates
rg1 <- rectangleGate(filterId="FSC-", "FSC-H"=c(-Inf, 50))
rg2 <- rectangleGate(filterId="SSC+", "SSC-H"=c(50, Inf))
rg1 * rg2
## Subset rectangle gates
rg["FSC-H"]
```

sampleFilter-class

Class "sampleFilter"

# **Description**

This non-parameter filter selects a number of events from the primary flowFrame.

# Usage

```
sampleFilter(size, filterId="defaultSampleFilter")
```

sampleFilter-class 105

## **Arguments**

filterId An optional parameter that sets the filterId of this filter. The object can later be identified by this name.

Size The number of events to select.

### **Details**

Selects a number of events without replacement from a flowFrame.

### Value

Returns a sampleFilter object for use in filtering flowFrames or other flow cytometry objects.

#### **Extends**

```
Class "concreteFilter", directly.
Class "filter", by class concreteFilter, distance 2.
```

### **Slots**

```
size: Object of class "numeric". Then number of events that are to be selected. filterId: A character vector that identifies this filter.
```

### **Objects from the Class**

Objects can be created by calls of the form new("sampleFilter", ...) or using the constructor sampleFilter. The latter is the recommended way of object instantiation:

# Methods

```
%in% signature(x = "flowFrame", table = "sampleFilter"): The workhorse used to evaluate the gate on data. This is usually not called directly by the user, but internally by calls to the filter methods.
```

```
show signature(object = "sampleFilter"): Print information about the gate.
```

## Author(s)

```
B. Ellis, F.Hahne
```

## See Also

flowFrame, filter for evaluation of sampleFilters and split and Subsetfor splitting and subsetting of flow cytometry data sets based on that.

```
## Loading example data
dat <- read.FCS(system.file("extdata","0877408774.B08",
package="flowCore"))

#Create the filter
sf <- sampleFilter(filterId="mySampleFilter", size=500)</pre>
```

106 scaleTransform

```
## Filtering using sampeFilters
fres <- filter(dat, sf)
fres
summary(fres)

## The result of sample filtering is a logical subset
Subset(dat, fres)

## We can also split, in which case we get those events in and those
## not in the gate as separate populations
split(dat, fres)</pre>
```

scaleTransform

Create the definition of a scale transformation function to be applied on a data set

# **Description**

Create the definition of the scale Transformation that will be applied on some parameter via the transform method. The definition of this function is currently x = (x-a)/(b-a). The transformation would normally be used to convert to a 0-1 scale. In this case, b would be the maximum possible value and a would be the minimum possible value.

# Usage

```
scaleTransform(transformationId="defaultScaleTransform", a, b)
```

# Arguments

transformationId

character string to identify the transformation

- a double that corresponds to the value that will be transformed to 0
- b double that corresponds to the value that will be transformed to 1

# Value

Returns an object of class transform.

# Author(s)

P. Haaland

### See Also

transform-class, transform

setOperationFilter-class 107

## **Examples**

```
samp <- read.FCS(system.file("extdata",
    "0877408774.B08", package="flowCore"))
scaleTrans <- scaleTransform(transformationId="Truncate-transformation", a=1, b=10^4)
dataTransform <- transform(samp, 'FSC-H'=scaleTrans('FSC-H'))</pre>
```

```
setOperationFilter-class
```

Class "setOperationFilter"

# Description

Description goes here

### **Slots**

```
filters: Object of class "list" filterId: Object of class "character" referencing the filter applied
```

#### **Extends**

```
Class "filter", directly.
```

## Author(s)

B. Ellis

# See Also

filter

```
\verb|singleParameterTransform-class|\\
```

Class "singleParameterTransform"

# **Description**

A transformation that operates on a single parameter

# **Objects from the Class**

Objects can be created by calls of the form new("singleParameterTransform", ...).

### **Slots**

```
.Data: Object of class "function". The transformation.
```

parameters: Object of class "transformation". The parameter to transform. Can be a derived parameter from another transformation.

transformationId: Object of class "character". An identifier for the object.

108 sinht-class

#### **Extends**

Class "transform", directly. Class "transformation", by class "transform", distance 2. Class "characterOrTransformation", by class "transform", distance 3.

#### Methods

No methods defined with class "singleParameterTransform" in the signature.

# Author(s)

F Hahne

### **Examples**

```
showClass("singleParameterTransform")
```

sinht-class

Class "sinht"

### **Description**

~~ A concise (1-5 lines) description of what the class is. ~~

### **Objects from the Class**

Objects can be created by calls of the form new("sinht", parameters, ...).  $\sim$  describe objects here  $\sim$ 

### **Slots**

```
.Data: Object of class "function" ~~
a: Object of class "numeric"- non zero constant
b: Object of class "numeric"- non zero constant
parameters: Object of class "transformation" -flow parameters to be transformed
transformationId: Object of class "character" -unique ID to reference the transformation
```

#### Extends

```
Class "singleParameterTransform", directly. Class "transform", by class "singleParameterTransform", distance 2. Class "transformation", by class "singleParameterTransform", distance 3. Class "characterOrTransformation", by class "singleParameterTransform", distance 4.
```

## Methods

No methods defined with class "sinht" in the signature.

### Note

The transformation object can be evaluated using the eval method by passing the data frame as an argument. The transformed parameters are returned as a matrix with a single column. (See example below)

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## Author(s)

Gopalakrishnan N, F.Hahne

## References

Gating-ML Candidate Recommendation for Gating Description in Flow Cytometry V 1.5

## See Also

asinht

#### **Examples**

```
dat <- read.FCS(system.file("extdata","0877408774.B08", package="flowCore"))
sinh1<-sinht(parameters="FSC-H",a=1,b=2000,transformationId="sinH1")
transOut<-eval(sinh1)(exprs(dat))</pre>
```

spillover

Compute a spillover matrix from a flowSet

# Description

Spillover information for a particular experiment is often obtained by running several tubes of beads or cells stained with a single color that can then be used to determine a spillover matrix for use with compensate.

# Usage

# **Arguments**

X	A flowSet of compensation beads or cells
unstained	The name of index of the unstained negative control
patt	An optional regular expression defining which parameters should be considered.
fsc	The name or index of the forward scatter parameter
ssc	The name or index of the side scatter parameter
method	The statistic to use for calculation. Traditionally, this has been the median so it is the default. The mean is sometimes more stable.
useNormFilt	Logical Indicating whether to apply a norm2Filter first before computing the spillover

# Details

The algorithm used is fairly simple. First, using the scatter parameters, we restrict ourselves to the most closely clustered population to reduce the amount of debris. The selected statistic is then calculated on all appropriate parameters and the unstained values swept out of the matrix. Every sample is then normalized to [0,1] with respect to the maximum value of the sample, giving the spillover in terms of a proportion of the primary channel intensity.

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#### Value

A matrix for each of the parameters

#### Author(s)

B. Ellis

#### References

C. B. Bagwell & E. G. Adams (1993). Fluorescence spectral overlap compensation for any number of flow cytometry parameters. in: Annals of the New York Academy of Sciences, 677:167-184.

## See Also

compensate

split-methods

Methods to split flowFrames and flowSets according to filters

## **Description**

Divide a flow cytometry data set into several subset according to the results of a filtering operation. There are also methods available to split according to a factor variable.

# Details

The splitting operation in the context of flowFrames and flowSets is the logical extension of subsetting. While the latter only returns the events contained within a gate, the former splits the data into the groups of events contained within and those not contained within a particular gate. This concept is extremely useful in applications where gates describe the distinction between positivity and negativity for a particular marker.

The flow data structures in flowCore can be split into subsets on various levels:

flowFrame: row-wise splitting of the raw data. In most cases, this will be done according to the outcome of a filtering operation, either using a filter that identifiers more than one sub-population or by a logical filter, in which case the data is split into two populations: "in the filter" and "not in the filter". In addition, the data can be split according to a factor (or a numeric or character vector that can be coerced into a factor).

flowSet: can be either split into subsets of flowFrames according to a factor or a vector that can be coerced into a factor, or each individual flowFrame into subpopulations based on the filters or filterResults provided as a list of equal length.

Splitting has a special meaning for filters that result in multipleFilterResults or manyFilterResults, in which case simple subsetting doesn't make much sense (there are multiple populations that are defined by the gate and it is not clear which of those should be used for the subsetting operation). Accordingly, splitting of multipleFilterResults creates multiple subsets. The argument population can be used to limit the output to only one or some of the resulting subsets. It takes as values a character vector of names of the populations of interest. See the documentation of the different filter classes on how population names can be defined and the respective default values. For splitting of logicalFilterResults, the population argument can be used to set the population names since there is no reasonable default other than the name of the gate. The content of the argument prefix

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will be prepended to the population names and '+' or '-' are finally appended allowing for more flexible naming schemes.

The default return value for any of the split methods is a list, but the optional logical argument filterSet can be used to return a flowSet instead. This only applies when splitting flowFrames, splitting of flowSets always results in lists of flowSet objects.

#### Methods

#### flowFrame methods:

- x = "flowFrame", f = "ANY", drop = "ANY" Catch all input and cast an error if there is no method for f to dispatch to.
- x = "flowFrame", f = "factor", drop = "ANY" Split a flowFrame by a factor variable. Length of f should be the same as nrow(x), otherwise it will be recycled, possibly leading to undesired outcomes. The optional argument drop works in the usual way, in that it removes empty levels from the factor before splitting.
- x = "flowFrame", f = "character", drop = "ANY" Coerce f to a factor and split on that.
- x = "flowFrame", f = "numeric", drop = "ANY" Coerce f to a factor and split on that.
- x = "flowFrame", f = "filter", drop = "ANY" First applies the filter to the flowFrame and then splits on the resulting filterResult object.
- x = "flowFrame", f = "filterSet", drop = "ANY" First applies the filterSet to the flowFrame
  and then splits on the resulting final filterResult object.
- x = "flowFrame", f = "logicalFilterResult", drop = "ANY" Split into the two subpopulations (in and out of the gate). The optional argument population can be used to control the names of the results.
- x = "flowFrame", f = "manyFilterResult", drop = "ANY" Split into the several subpopulations identified by the filtering operation. Instead of returning a list, the additional logical argument codeflowSet makes the method return an object of class flowSet. The optional population argument takes a character vector indicating the subpopulations to use for splitting (as identified by the population name in the filterDetails slot).
- x = "flowFrame", f = "multipleFilterResult", drop = "ANY" Split into the several subpopulations identified by the filtering operation. Instead of returning a list, the additional logical argument codeflowSet makes the method return an object of class flowSet. The optional population argument takes a character vector indicating the subpopulations to use for splitting (as identified by the population name in the filterDetails slot). Alternatively, this can be a list of characters, in which case the populations for each list item are collapsed into one flowFrame.

#### flowSet methods:

- x = "flowSet", f = "ANY", drop = "ANY" Catch all input and cast an error if there is no method for f to dispatch to.
- x ="flowSet", f ="factor", drop = "ANY" Split a flowSet by a factor variable. Length of f needs to be the same as length(x). The optional argument drop works in the usual way, in that it removes empty levels from the factor before splitting.
- x = "flowSet", f = "character", drop = "ANY" Coerce f to a factor and split on that.
- x = "flowSet", f = "numeric", drop = "ANY" Coerce f to a factor and split on that.

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x = "flowSet", f = "list", drop = "ANY" Split a flowSet by a list of filterResults (as typically returned by filtering operations on a flowSet). The length of the list has to be equal to the length of the flowSet and every list item needs to be a filterResult of equal class with the same parameters. Instead of returning a list, the additional logical argument codeflowSet makes the method return an object of class flowSet. The optional population argument takes a character vector indicating the subpopulations to use for splitting (as identified by the population name in the filterDetails slot). Alternatively, this can be a list of characters, in which case the populations for each list item are collapsed into one flowFrame. Note that using the population argument implies common population names for allfilterResults in the list and there will be an error if this is not the case.

#### Author(s)

F Hahne, B. Ellis, N. Le Meur

# **Examples**

```
data(GvHD)
qGate <- quadGate(filterId="qg", "FSC-H"=200, "SSC-H"=400)
## split a flowFrame by a filter that creates
## a multipleFilterResult
samp <- GvHD[[1]]
fres <- filter(samp, qGate)</pre>
split(samp, qGate)
## return a flowSet rather than a list
split(samp, fres, flowSet=TRUE)
## only keep one population
names(fres)
##split(samp, fres, population="FSC-Height+SSC-Height+")
## split the whole set, only keep two populations
##split(GvHD, qGate, population=c("FSC-Height+SSC-Height+",
##"FSC-Height-SSC-Height+"))
## now split the flowSet according to a factor
split(GvHD, pData(GvHD)$Patient)
```

splitscale-class

Class "splitscale"

# Description

The split scale transformation class defines a transformation that has a logarithmic scale at high values and a linear scale at low values. The transition points are chosen so that the slope of the transformation is continuous at the transition points.

The split scale transformation is defined by the function

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 $f(parameter, r, maxValue, transitionChannel) = a * parameter + b \quad parameter <= t$ 

$$log_{10}(c*parameter)*\frac{r}{d}$$
 parameter > t

where,

$$b = \frac{transitionChannel}{2}$$

$$d = \frac{2 * log_{10}(e) * r}{transitionChannel} + log_{10}(maxValue)$$

$$t = 10^{log_{10}t}$$

$$a = \frac{transitionChannel}{2 * t}$$

$$log_{10}ct = \frac{(a * t + b) * d}{r}$$

$$c = 10^{log_{10}ct}$$

#### **Objects from the Class**

Objects can be created by calls to the constructor splitscale(parameters, r, maxValue, transitionChannel, transf

#### **Slots**

.Data: Object of class "function" ~~

r: Object of class "numeric"-a positive value indicating the range of the logarithmic part of the display

maxValue: Object of class "numeric" -a positive value indicating the maximum value the transformation is applied to

transitionChannel: Object of class "numeric" -non negative value that indicates where to split the linear vs. logarithmic transformation

 $\verb"parameters:" Object of class" \verb"transformation" - flow parameter to be transformed$ 

transformationId: Object of class "character"-unique ID to reference the transformation

#### **Extends**

Class "singleParameterTransform", directly. Class "transform", by class "singleParameterTransform", distance 2. Class "transformation", by class "singleParameterTransform", distance 3. Class "characterOrTransformation", by class "singleParameterTransform", distance 4.

## Methods

No methods defined with class "splitscale" in the signature.

## Note

The transformation object can be evaluated using the eval method by passing the data frame as an argument. The transformed parameters are returned as a matrix with a single column. (See example below)

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#### Author(s)

Gopalakrishnan N, F.Hahne

#### References

Gating-ML Candidate Recommendation for Gating Description in Flow Cytometry

#### See Also

invsplitscale

# **Examples**

```
dat <- read.FCS(system.file("extdata","0877408774.B08",package="flowCore"))
sp1<-splitscale("FSC-H",r=768,maxValue=10000,transitionChannel=256)
transOut<-eval(sp1)(exprs(dat))</pre>
```

splitScaleTransform

Compute the split-scale transformation describe by FL. Battye

# **Description**

The split scale transformation described by Francis L. Battye [B15] (Figure 13) consists of a logarithmic scale at high values and a linear scale at low values with a fixed transition point chosen so that the slope (first derivative) of the transform is continuous at that point. The scale extends to the negative of the transition value that is reached at the bottom of the display.

## Usage

splitScaleTransform(transformationId="defaultSplitscaleTransform", maxValue=1023, transitionChar

# Arguments

transformationId

A name to assign to the transformation. Used by the transform/filter integration routines.

maxValue

Maximum value the transformation is applied to, e.g., 1023

transitionChannel

Where to split the linear versus the logarithmic transformation, e.g., 64

r

Range of the logarithm part of the display, ie. it may be expressed as the max-Channel - transitionChannel considering the maxChannel as the maximum value to be obtained after the transformation.

#### Value

Returns values giving the inverse of the biexponential within a certain tolerance. This function should be used with care as numerical inversion routines often have problems with the inversion process due to the large range of values that are essentially 0. Do not be surprised if you end up with population splitting about w and other odd artifacts.

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#### Author(s)

N. LeMeur

#### References

Battye F.L. A Mathematically Simple Alternative to the Logarithmic Transform for Flow Cytometric Fluorescence Data Displays. http://www.wehi.edu.au/cytometry/Abstracts/AFCG05B.html.

## See Also

transform

## **Examples**

```
data(GvHD)
ssTransform <- splitScaleTransform("mySplitTransform")
after.1 <- transform(GvHD, 'FSC-H'= ssTransform('FSC-H'))

opar = par(mfcol=c(2, 1))
plot(density(exprs(GvHD[[1]])[, 1]), main="Original")
plot(density(exprs(after.1[[1]])[, 1]), main="Split-scale Transform")</pre>
```

squareroot-class

Class "squareroot"

# **Description**

Square root transform class defines a transformation defined by the function

$$f(parameter, a) = \sqrt{|\frac{parameter}{a}|}$$

# **Objects from the Class**

Objects can be created by calls to the constructor squareroot(parameters, a, transformationId)

#### **Slots**

```
.Data: Object of class "function" ~~
a: Object of class "numeric" -non zero multiplicative constant
parameters: Object of class "transformation"-flow parameter to be transformed
transformationId: Object of class "character" -unique ID to reference the transformation
```

# **Extends**

Class "singleParameterTransform", directly. Class "transform", by class "singleParameterTransform", distance 2. Class "transformation", by class "singleParameterTransform", distance 3. Class "characterOrTransformation", by class "singleParameterTransform", distance 4.

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#### Methods

No methods defined with class "squareroot" in the signature.

#### Note

The squareroot transformation object can be evaluated using the eval method by passing the data frame as an argument. The transformed parameters are returned as a column vector. (See example below)

## Author(s)

Gopalakrishnan N, F.Hahne

#### References

Gating-ML Candidate Recommendation for Gating Description in Flow Cytometry

#### See Also

dg1polynomial, ratio,quadratic

# **Examples**

```
dat <- read.FCS(system.file("extdata","0877408774.B08",
package="flowCore"))
sqrt1<-squareroot(parameters="FSC-H",a=2,transformationId="sqrt1")
transOut<-eval(sqrt1)(exprs(dat))</pre>
```

Subset

Subset a flowFrame or a flowSet

# **Description**

An equivalent of a subset function for flowFrame or a flowSet object. Alternatively, the regular subsetting operators can be used for most of the topics documented here.

# Usage

```
Subset(x, subset, ...)
```

## **Arguments**

x The flow object, frame or set, to subset.
 subset A filter object or, in the case of flowSet subsetting, a named list of filters.
 ... Like the original subset function, you can also select columns.

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#### **Details**

The Subset method is the recommended method for obtaining a flowFrame that only contains events consistent with a particular filter. It is functionally equivalent to frame[as(filter(frame, subset), "logical") when used in the flowFrame context. Used in the flowSet context, it is equivalent to using fsApply to apply the filtering operation to each flowFrame.

Additionally, using Subset on a flowSet can also take a named list as the subset. In this case, the names of the list object should correspond to the sampleNames of the flowSet, allowing a different filter to be applied to each frame. If not all of the names are used or excess names are present, a warning will be generated but the valid filters will be applied for the rare instances where this is the intended operation. Note that a filter operation will generate a list of filterResult objects that can be used directly with Subset in this manner.

## Value

Depending on the original context, either a flowFrame or a flowSet.

## Author(s)

B. Ellis

#### See Also

split, subset

## **Examples**

```
sample <- read.flowSet(path=system.file("extdata", package="flowCore"),
pattern="0877408774")
result <- filter(sample, rectangleGate("FSC-H"=c(-Inf, 1024)))
result
Subset(sample,result)</pre>
```

subsetting-class

Class "subsetting"

## **Description**

Class and methods to subset a a flowSet. This is only needed for method dispatch in the workFlow framework.

# Usage

```
subsetting(indices, subsettingId="defaultSubsetting")
```

## **Arguments**

indices Character or numeric vector of sample names. subsettingId The identifier for the subsetting object.

## **Details**

The class mainly existst for method dispatch in the workflow tools.

## Value

A subsetting object.

## **Objects from the Class**

Objects should be created using the constructor subsetting(). See the Usage and Arguments sections for details.

#### Slots

```
subsettingId: Object of class "character". An identifier for the object. indices: Object of class "numericOrCharacter". Indices into a flowSet.
```

## Methods

```
add signature(wf = "workFlow", action = "subsetting"): The constructor for the work-
Flow.

identifier<- signature(object = "subsetting", value = "character"): Set method for the
    identifier slot.

identifier signature(object = "subsetting"): Get method for the identifier slot.

show signature(object = "subsetting"): Show details about the object.</pre>
```

## Author(s)

F. Hahne

# **Description**

Class and method to capture subsetting operations in a flow cytometry workflow.

# Usage

```
subsettingActionItem(ID = paste("subActionRef", guid(), sep = "_"),
name = paste("action", identifier(get(subsetting)), sep = "_"),
parentView, subsetting, workflow)
```

## **Arguments**

workflow An object of class workflow for which a view is to be created.

ID A unique identifier of the view, most likely created by using the internal guid

function.

name A more human-readable name of the view.

parentView, subsetting

References to the parent view and subsetting objects, respectively.

#### **Details**

subsettingActionItems provide a means to bind subsetting operations in a workflow. Each subsettingActionItem represents a single subsetting.

# Value

A reference to the subsettingActionItem that is created inside the workFlow environment as a side effect of calling the add method.

A subsettingActionItem object for the constructor.

## **Objects from the Class**

Objects should be created using the add method, which creates a subsettingActionItem from a normalization object and directly assigns it to a workFlow. Alternatively, one can use the subsettingActionItem constructor function for more programmatic access.

## Slots

subsetting: Object of class "fcSubsettingReference". A reference to the subsetting object that is used for the operation.

ID: Object of class "character". A unique identifier for the actionItem.

name: Object of class "character". A more human-readable name

parentView: Object of class "fcViewReference". A reference to the parent view the subsettingActionItem is applied on.

env: Object of class "environment". The evaluation environment in the workFlow.

## Extends

```
Class "actionItem", directly.
```

# Methods

```
print signature(x = "subsettingActionItem"): Print details about the object.
```

Rm signature(symbol = "subsettingActionItem", envir = "workFlow", subSymbol = "character"):
 Remove a subsettingActionItem from a workFlow. This method is recursive and will also
 remove all dependent views and actionItems.

show signature(object = "subsettingActionItem"): Print details about the object.

## Author(s)

Florian Hahne

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#### See Also

workFlow, actionItem, gateActionItem, transformActionItem, compensateActionItem, view

#### **Examples**

```
showClass("view")
```

```
subsettingView-class Class "subsettingView"
```

# **Description**

Class and method to capture the result of subsetting operations in a flow cytometry workflow.

# Usage

## **Arguments**

workflow An object of class workflow for which a view is to be created.

ID A unique identifier of the view, most likely created by using the internal guid

function.

name A more human-readable name of the view.

data, action References to the data and actionItem objects, respectively.

#### Value

A reference to the subsettingView that is created inside the workFlow environment as a side effect of calling the add method.

A subsetting View object for the constructor.

# **Objects from the Class**

Objects should be created using the add method, which creates a subsettingView from a subsetting object and directly assigns it to a workFlow. Alternatively, one can use the subsettingView constructor function for more programmatic access.

## **Slots**

```
ID: Object of class "character". A unique identifier for the view.
```

name: Object of class "character". A more human-readable name

action: Object of class "fcActionReference". A reference to the actionItem that generated the view.

env: Object of class "environment". The evaluation environment in the workFlow.

data: Object of class "fcDataReference" A reference to the data that is associated to the view.

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#### **Extends**

```
Class "view", directly.
```

#### Methods

Rm signature(symbol = "subsettingView", envir = "workFlow", subSymbol = "character"):
 Remove a subsettingView from a workFlow. This method is recursive and will also remove
 all dependent views and actionItems.

## Author(s)

Florian Hahne

#### See Also

```
workFlow, view, gateView, transformView, compensateView, actionItem
```

#### **Examples**

```
showClass("view")
```

summarizeFilter-methods

Methods for function summarizeFilter

## **Description**

Internal methods to populate the filterDetails slot of a filterResult object.

# Methods

```
result = "filterResult", filter = "filter" summarizeFilter methods are called during the process of filtering. Their output is a list, and it can be arbitrary data that should be stored along with the results of a filtering operation.
```

```
result = "filterResult", filter = "filterReference" see above
result = "filterResult", filter = "parameterFilter" see above
result = "filterResult", filter = "subsetFilter" see above
result = "logicalFilterResult", filter = "norm2Filter" see above
result = "logicalFilterResult", filter = "parameterFilter" see above
result = "multipleFilterResult", filter = "curv1Filter" see above
result = "multipleFilterResult", filter = "curv2Filter" see above
result = "multipleFilterResult", filter = "parameterFilter" see above
```

122 timeFilter-class

## **Description**

Define a filter that removes stretches of unusual data distribution within a single parameter over time. This can be used to correct for problems during data acquisition like air bubbles or clods.

# Usage

```
timeFilter(..., bandwidth=0.75, binSize, timeParameter,
filterId="defaultTimeFilter")
```

# **Arguments**

... The names of the parameters on which the filter is supposed to work on. Names

can either be given as individual arguments, or as a list or a character vector.

filterId An optional parameter that sets the filterId slot of this gate. The object can

later be identified by this name.

bandwidth, binSize

Numerics used to set the bandwidth and binSize slots of the object.

timeParameter Character used to set the timeParameter slot of the object.

#### **Details**

Clods and disturbances in the laminar flow of a FACS instrument can cause temporal aberrations in the data acquisition that lead to artifactual values. timeFilters try to identify such stretches of disturbance by computing local variance and location estimates and to remove them from the data.

#### Value

Returns a timeFilter object for use in filtering flowFrames or other flow cytometry objects.

## **Extends**

```
Class "parameterFilter", directly.

Class "concreteFilter", by class parameterFilter, distance 2.

Class "filter", by class parameterFilter, distance 3.
```

## **Slots**

bandwidth: Object of class "numeric". The sensitivity of the filter, i.e., the amount of local variance of the signal we want to allow.

binSize: Object of class "numeric". The size of the bins used for the local variance and location estimation. If NULL, a reasonable default is used when evaluating the filter.

timeParameter: Object of class "character", used to define the time domain parameter. If NULL, the filter tries to guess the time domain from the flowFrame.

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parameters: Object of class "character", describing the parameters used to filter the flowFrame. filterId: Object of class "character", referencing the filter.

## **Objects from the Class**

Objects can be created by calls of the form new("timeFilter", ...) or using the constructor timeFilter. Using the constructor is the recommended way of object instantiation:

#### Methods

```
%in% signature(x = "flowFrame", table = "timeFilter"): The workhorse used to eval-
uate the filter on data. This is usually not called directly by the user.
show signature(object = "timeFilter"): Print information about the filter.
```

# Note

See the documentation of timeLinePlot in the flowViz package for details on visualizing temporal problems in flow cytometry data.

## Author(s)

Florian Hahne

#### See Also

flowFrame, filter for evaluation of timeFilters and split and Subsetfor splitting and subsetting of flow cytometry data sets based on that.

## **Examples**

```
## Loading example data
data(GvHD)
dat <- GvHD[1:10]
## create the filter
tf <- timeFilter("SSC-H", bandwidth=1, filterId="myTimeFilter")</pre>
## Visualize problems
## Not run:
library(flowViz)
timeLinePlot(dat, "SSC-H")
## End(Not run)
## Filtering using timeFilters
fres <- filter(dat, tf)</pre>
fres[[1]]
summary(fres[[1]])
summary(fres[[7]])
## The result of rectangle filtering is a logical subset
cleanDat <- Subset(dat, fres)</pre>
```

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```
## Visualizing after cleaning up
## Not run:
timeLinePlot(cleanDat, "SSC-H")

## End(Not run)

## We can also split, in which case we get those events in and those
## not in the gate as separate populations
allDat <- split(dat[[7]], fres[[7]])

par(mfcol=c(1,3))
plot(exprs(dat[[7]])[, "SSC-H"], pch=".")
plot(exprs(cleanDat[[7]])[, "SSC-H"], pch=".")
plot(exprs(allDat[[2]])[, "SSC-H"], pch=".")</pre>
```

transform-class

'transform': a class for transforming flow-cytometry data by applying scale factors.

## **Description**

Transform objects are simply functions that have been extended to allow for specialized dispatch. All of the "...Transform" constructors return functions of this type for use in one of the transformation modalities.

## **Slots**

```
.Data: Object of class "function" transformationId: A name for the transformation object
```

## Methods

summary Return the parameters

## Author(s)

N LeMeur

# See Also

linear Transform, ln Transform, logic le Transform, biexponential Transform, arcsinh Transform, quadratic Transform, log Tra

# **Examples**

```
cosTransform <- function(transformId, a=1, b=1){
  t = new("transform", .Data = function(x) cos(a*x+b))
  t@transformationId = transformId
  t
}</pre>
```

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```
cosT <- cosTransform(transformId="CosT",a=2,b=1)
summary(cosT)</pre>
```

transformActionItem-class

Class "transformActionItem"

# **Description**

Class and method to capture transformation operations in a flow cytometry workflow.

# Usage

## Arguments

workflow An object of class workflow for which a view is to be created.

ID A unique identifier of the view, most likely created by using the internal guid

function.

name A more human-readable name of the view.

 ${\tt parentView,\ transform}$ 

References to the parent view and transform objects, respectively.

## **Details**

transformActionItems provide a means to bind transformation operations in a workflow. Each transformActionItem represents a single transform.

# Value

A reference to the transformActionItem that is created inside the workFlow environment as a side effect of calling the add method.

A transformActionItem object for the constructor.

# **Objects from the Class**

Objects should be created using the add method, which creates a transformActionItem from a transform object and directly assigns it to a workFlow. Alternatively, one can use the transformActionItem constructor function for more programmatic access.

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#### **Slots**

transform: Object of class "fcTransformReference". A reference to the transform object that is used for the transformation operation.

ID: Object of class "character". A unique identifier for the actionItem.

name: Object of class "character". A more human-readable name

parentView: Object of class "fcViewReference". A reference to the parent view the transformActionItem is applied on.

env: Object of class "environment". The evaluation environment in the workFlow.

## **Extends**

```
Class "actionItem", directly.
```

#### Methods

```
print signature(x = "transformActionItem"): Print details about the object.
```

Rm signature(symbol = "transformActionItem", envir = "workFlow", subSymbol = "character"):
 Remove a transformActionItem from a workFlow. This method is recursive and will also
 remove all dependent views and actionItems.

**show** signature(object = "transformActionItem"): Print details about the object.

## Author(s)

Florian Hahne

#### See Also

```
workFlow, actionItem, gateActionItem, compensateActionItem, view
```

#### **Examples**

```
showClass("view")
```

 $transformation\hbox{-}class \quad \textit{Class "transformation"}$ 

## **Description**

A virtual class to abstract transformations.

## **Objects from the Class**

A virtual Class: No objects may be created from it.

# Extends

Class "characterOrTransformation", directly.

## Methods

No methods defined with class "transformation" in the signature.

transformFilter-class 127

#### Author(s)

N. Gopalakrishnan

transformFilter-class A class for encapsulating a filter to be performed on transformed parameters

## **Description**

The transformFilter class is a mechanism for including one or more variable transformations into the filtering process. Using a special case of transform we can introduce transformations inline with the filtering process eliminating the need to process flowFrame objects before applying a filter.

# **Objects from the Class**

Objects of this type are not generally created "by hand". They are a side effect of the use of the %on% method with a filter object on the left hand side and a transformList on the right hand side.

## **Slots**

```
transforms: A list of transforms to perform on the target flowFrame filter: The filter to be applied to the transformed frame filterId: The name of the filter (chosen automatically)
```

## Extends

```
Class "filter", directly.
```

## Author(s)

B. Ellis

# See Also

```
"filter", "transform", transform
```

## **Examples**

128 transformList-class

transformList-class Class "transformList"

## **Description**

Class "transformList"

#### Usage

## **Arguments**

from, to Characters giving the names of the measurement parameter on which to trans-

form on and into which the result is supposed to be stored. If both are equal, the

existing parameters will be overwritten.

tfun A list if functions or a character vector of the names of the functions used to

transform the data. R's recycling rules apply, so a single function can be given

to be used on all parameters.

transformationId

The identifier for the object.

# **Objects from the Class**

Objects can be created by calls of the form new("transformList", ...), by calling the transform method with key-value pair arguments of the form key equals character and value equals function, or by using the constructor transformList. See below for details

## **Slots**

```
transforms: Object of class "list", where each list item is of class transformMap. transformationId: Object of class "character", the identifier for the object.
```

## Methods

```
colnames signature(x = "transformList"): This returns the names of the parameters that are
    to be transformed.

c signature(x = "transformList"): Concatenate transformLists or regular lists and transformLists.

% on % signature(e1 = "transformList", e2 = "flowFrame"): Perform a transformation using the transformList on a flowFrame or flowSet.
```

## Author(s)

B. Ellis, F. Hahne

## See Also

transform, transformMap

transformMap-class 129

# **Examples**

```
tl <- transformList(c("FSC-H", "SSC-H"), list(log, asinh))
colnames(tl)
c(tl, transformList("FL1-H", "linearTransform"))
data(GvHD)
transform(GvHD[[1]], tl)</pre>
```

transformMap-class

A class for mapping transforms between parameters

# **Description**

This class provides a mapping between parameters and transformed parameters via a function.

# **Objects from the Class**

Objects of this type are not usually created by the user, except perhaps in special circumstances. They are generally automatically created by the inline transform process during the creation of a transformFilter, or by a call to the transformList constructor.

# **Slots**

```
output: Name of the transformed parameterinput: Name of the parameter to transformf: Function used to accomplish the transform
```

# Methods

```
show signature(object = "transformList"): Print details about the object.
```

## Author(s)

```
B. Ellis, F. Hahne
```

## See Also

```
transform, transformList
```

# **Examples**

```
new("transformMap", input="FSC-H", output="FSC-H", f=log)
```

130 transformView-class

```
transformReference-class
```

Class "transformReference"

# **Description**

Class allowing to reference transforms, for instance as parameters.

# Objects from the Class

Objects will be created internally whenever necessary and this should not be of any concern to the user.

## **Slots**

```
.Data: The list of references searchEnv: The environment into which the reference points. transformationId: The name of the transformation
```

## **Extends**

```
Class "transform", directly. Class "transformation", by class "transform", distance 2. Class "characterOrTransformation", by class "transform", distance 3.
```

## Methods

No methods defined with class "transformReference" in the signature.

# Author(s)

N. Gopalakrishnan

```
transformView-class Class "transformView"
```

# Description

Class and method to capture the result of transformation operations in a flow cytometry workflow.

# Usage

```
\label{lem:constraint}  \mbox{transformView(workflow, ID=paste("transViewRef", guid(), sep="\_"),} \\ \mbox{name="default", action, data)}
```

transformView-class 131

## **Arguments**

workflow An object of class workflow for which a view is to be created.

ID A unique identifier of the view, most likely created by using the internal guid

function.

name A more human-readable name of the view.

data, action References to the data and actionItem objects, respectively.

#### Value

A reference to the transformView that is created inside the workFlow environment as a side effect of calling the add method.

A transformView object for the constructor.

## **Objects from the Class**

Objects should be created using the add method, which creates a transformView from a transform object and directly assigns it to a workFlow. Alternatively, one can use the transformView constructor function for more programmatic access.

#### Slots

```
ID: Object of class "character". A unique identifier for the view.
```

name: Object of class "character". A more human-readable name

action: Object of class "fcActionReference". A reference to the actionItem that generated the view.

env: Object of class "environment". The evaluation environment in the workFlow.

data: Object of class "fcDataReference" A reference to the data that is associated to the view.

#### **Extends**

```
Class "view", directly.
```

#### Methods

Rm signature(symbol = "transformView", envir = "workFlow", subSymbol = "character"): Remove a transformView from a workFlow. This method is recursive and will also remove all dependent views and actionItems.

# Author(s)

Florian Hahne

## See Also

```
workFlow, view, gateView, compensateView, actionItem
```

## **Examples**

```
showClass("view")
```

132 unitytransform-class

truncateTransform	Create the definition of a truncate transformation function to be applied on a data set

# **Description**

Create the definition of the truncate Transformation that will be applied on some parameter via the transform method. The definition of this function is currently x[x<a] <-a. Hence, all values less than a are replaced by a. The typical use would be to replace all values less than 1 by 1.

# Usage

```
truncateTransform(transformationId="defaultTruncateTransform", a=1)
```

## **Arguments**

а

transformationId

character string to identify the transformation

double that corresponds to the value at which to truncate

## Value

Returns an object of class transform.

## Author(s)

P. Haaland

## See Also

```
transform-class, transform
```

# **Examples**

```
samp <- read.FCS(system.file("extdata",
   "0877408774.B08", package="flowCore"))
truncateTrans <- truncateTransform(transformationId="Truncate-transformation", a=5)
dataTransform <- transform(samp, 'FSC-H'=truncateTrans('FSC-H'))</pre>
```

```
unitytransform-class Class "unitytransform"
```

## **Description**

Unity transform class transforms parameters names provided as characters into unity transform objects which can be evaluated to retrieve the corresponding columns from the data frame

# **Objects from the Class**

Objects can be created by calls to the constructor unitytransform(parameters, transformationId).

view-class 133

#### **Slots**

```
.Data: Object of class "function" ~~

parameters: Object of class "character" -flow parameters to be transformed
transformationId: Object of class "character"-unique ID to reference the transformation
```

## **Extends**

```
Class "transform", directly. Class "transformation", by class "transform", distance 2. Class "characterOrTransformation", by class "transform", distance 3.
```

## Methods

No methods defined with class "unitytransform" in the signature.

# Author(s)

Gopalakrishnan N, F.Hahne

# See Also

```
dg1polynomial, ratio
```

# **Examples**

```
dat <- read.FCS(system.file("extdata","0877408774.B08",
package="flowCore"))
un1<-unitytransform(c("FSC-H","SSC-H"),transformationId="un1")
transOut<-eval(un1)(exprs(dat))</pre>
```

view-class

Class "view"

# Description

Class and method to capture the results of standard operations (called "views" here) in a flow cytometry workflow.

## Usage

134 view-class

#### **Arguments**

workflow An object of class workflow for which a view is to be created.

object An object of class view or one of its subclasses.

ID A unique identifier of the view, most likely created by using the internal guid

function.

name A more human-readable name of the view.

data, action References to the data and actionItem objects, respectively.

... Further arguments that get passed to the generic.

#### **Details**

Views provide a means to bind the results of standard operations on flow cytometry data in a work-flow. Each view can be considered the outcome of one operation. There are more specific sub-classes for the three possible types of operation: gateView for gating operations, transformView for transformations, and compensateView for compensation operations. See their documentation for details.

#### Value

A reference to the view that is created inside the workFlow environment as a side effect of calling the constructor.

The parent view (i.e., the view based on which the current view was created) for the parent method.

#### **Objects from the Class**

Objects should be created using the constructor view, which also assigns the view to a workFlow object.

# Slots

ID: Object of class "character". A unique identifier for the view.

name: Object of class "character". A more human-readable name

action: Object of class "fcActionReference". A reference to the actionItem that generated the view

env: Object of class "environment". The evaluation environment in the workFlow.

alias: Object of class "fcAliasReference". A reference to the alias table.

data: Object of class "fcDataReference" A reference to the data that is associated to the view. See gateView for details on copying and subsetting of the raw data in the context of gating.

#### **Methods**

action signature(object = "view"): Accessor for the action slot. Note that this returns the
actual actionItem object, i.e., the reference gets resolved.

**Data** signature(object = "view"): Accessor for the data slot. Note that this returns the actual data object, i.e., the reference gets resolved.

names signature(x = "view"): Accessor to the name slot.

alias signature(object = "view"): Get the alias table from a view.

parent signature(object = "view"): The parent view, i.e., the view based on which the current
view was created.

workFlow-class 135

```
print signature(x = "view"): Print details about the object.

Rm signature(symbol = "view", envir = "workFlow", subSymbol = "character"): Remove
    a view from a workFlow. This method is recursive and will also remove all dependent views
    and actionItems.

show signature(object = "view"): Print details about the object.

xyplot signature(x = "formula", data = "view"): Plot the data underlying the view.

xyplot signature(x = "view", data = "missing"): Plot the data underlying the view.
```

## Author(s)

Florian Hahne

# See Also

```
workFlow, gateView, transformView, compensateView, actionItem
```

# **Examples**

```
showClass("view")
```

workFlow-class

Class "workFlow"

## **Description**

Class and methods to organize standard flow cytometry data analysis operation in a concise work-flow

# Usage

```
workFlow(data, name = "default", env = new.env(parent = emptyenv()))
undo(wf, n=1)
```

# **Arguments**

data	An object of class flowFrame or flowSet for which a basic view is created.
name	A more human-readable name of the view.
env	$Object \ of \ class \ environment. \ The \ evaluation \ environment \ used \ for \ the \ work {\tt Flow}.$
wf	Object of class workFlow.
n	The number of operations to undo.

136 workFlow-class

#### **Details**

workFlow objects organize standard flow data analysis operations like gating, compensation and transformation in one single object. The user can interact with a workFlow object (e.g. adding operations, removing them, summarizing the results) without having to keep track of intermediate objects and names.

The integral part of a workFlow is an evaluation environment which holds all objects that are created during the analysis. The structure of the whole workflow is a tree, where nodes represent link{view}s (or results of an operation) and edges represent actionItems (or the operations themselves).

#### Value

A workFlow object for the constructor

Both applyParentFilter and undo are called for their side-effects.

## **Objects from the Class**

Objects should be created using the constructor workFlow, which takes a flowFrame or flowSet as only mandatory input and creates a basic view for that.

#### **Slots**

```
name: Object of class "character". The name of the workFlow object.
```

tree: Object of class "fcTreeReference". A reference to the graphNEL objects representing the view structure of the workflow.

alias: Object of class "fcAliasReference". A reference to the alias table.

journal: Object of class "fcJournalReference". A reference to the journal.

env: Object of class "environment". The evaluation environment for the workflow in which all objects will be stored.

## Methods

```
add signature(wf = "workFlow", action = "concreteFilter"): Create a new gateActionItem
and gateView from a filter and assign those to the workflow.
```

```
add signature(wf = "workFlow", action = "filterList"): Create a new gateActionItem
and gateView from a filterList and assign those to the workflow.
```

```
add signature(wf = "workFlow", action = "transformList"): Create a new transformActionItem
and transformView from a transform and assign those to the workflow.
```

```
add signature(wf = "workFlow", action = "compensation"): Create a new compensateActionItem
and compensateView from a compensation and assign those to the workflow.
```

```
assign signature(x = "ANY", value = "ANY", pos = "missing", envir = "workFlow", inherits = "miss:
    "missing"): Assign an object to the environment in the workFlow object and return a fcReference
    to it. The symbol for the object is created as a unique identifier.
```

```
assign signature(x = "missing", value = "ANY", pos = "workFlow", envir = "missing", inherits = "r
    "missing"): see above
```

```
assign signature(x = "missing", value = "ANY", pos = "missing", envir = "workFlow", inherits = "r
    "missing"): same as above, but provide custom symbol for the assignment.
```

```
assign signature(x = "character", value = "ANY", pos = "workFlow", envir = "missing", inherit
    "missing"): see above
```

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```
assign signature(x = "fcReference", value = "ANY", pos = "workFlow", envir = "missing", inherits
     = "missing"): same as above, but assign object using an existing fcReference. Note that
     assigning NULL essentially removes the original object.
[ signature(x = "workFlow", i = "ANY"): Cast a useful error message.
[[ signature(x = "workFlow", i = "ANY"): Treat the workFlow object as a regular environ-
     ment. Essentially, this is equivalent to get(x, i).
\$ signature(x = "workFlow", name = "character"): Allow for list-like access. Note that
     completion is only available for views since all other objects in the environment are consid-
     ered to be internal.
get signature(x = "character", pos = "workFlow", envir = "missing", mode = "missing", inherits =
     Get an object identified by symbol x from the environment in the workFlow.
get signature(x = "character", pos = "missing", envir = "workFlow", mode = "missing", inherits =
     see above
ls signature(name = "workFlow", pos = "missing", envir = "missing", all.names = "missing", patter
     List the content of the environment in the workFlow.
ls signature(name = "workFlow", pos = "missing", envir = "missing", all.names = "missing", patter
mget signature(x = "character", envir = "workFlow", mode = "missing", ifnotfound = "missing", inl
     "missing"): Get multiple objects identified by the symbols in x from the environment in the
names signature(x = "workFlow"): List the identifiers for all views and actionItems in the
    workFlow.
plot signature(x = "workFlow", y = "missing"): Plot the structure of the workFlow tree.
Rm signature(symbol = "character", envir = "workFlow",subSymbol = "character"):
     Remove the object identified by the symbol symbol from the workFlow.
undo signature(wf = "workFlow", n = "numeric"): Undo the last n operations on the workFlow.
show signature(object = "workFlow"): Print details about the object.
summary signature(object = "workFlow"): Summarize a view in the workFlow.
nodes signature(object = "workFlow"): Return a named vector of node ids where the names
     are the human readable names stored in the alias table.
actions signature(x = "workFlow"): List the names of the actionItems in the workFlow.
views signature(x = "workFlow"): List the names of only the views in the workFlow.
alias signature(object = "workFlow"): Return the alias table for the workFlow.
alias signature(object = "environment"): Return the alias table from a generic environment.
     The method tries to find 'fcAliasRef' among the object symbols in the environment.
journal signature(object = "workFlow"): Return the journal for the workFlow.
journal signature(object = "environment"): Return the journal from a generic environment.
     The method tries to find 'fcJournalRef' among the object symbols in the environment.
tree signature(object = "workFlow"): Return the tree of the workFlow.
journal signature(object = "environment"): Return the tree from a generic environment.
     The method tries to find 'fcTreeRef' among the object symbols in the environment.
```

## Author(s)

Florian Hahne

write.FCS

#### See Also

```
"view", "actionItem"
```

# **Examples**

```
showClass("view")
```

write.FCS

Write an FCS file

# **Description**

Write FCS file from a flowFrame

## Usage

```
write.FCS(x, filename, what="numeric")
```

# **Arguments**

x A flowFrame.

filename A character scalar giving the output file name. By default, the function will use

the output of identifier(x) as the file name, potentially adding the .fcs suffix

unless a file extension is already present.

what A character scalar defining the output data type. One in integer, numeric,

double. Note that forcing the data type to integer may result in considerable loss of precision if the data has been transformed. We recommend using the

default data type unless disc space is an issue.

## **Details**

The function write.FCS creates FCS 3.0 standard file from an object of class flowFrame.

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

A character of the file name.

# Author(s)

F. Hahne

## See Also

```
link[flowCore]{write.flowSet}
```

write.flowSet 139

## **Examples**

```
## a sample file
inFile <- system.file("extdata", "0877408774.B08", package="flowCore")
foo <- read.FCS(inFile, transform=FALSE)
outFile <- file.path(tempdir(), "foo.fcs")

## now write out into a file
write.FCS(foo, outFile)
bar <- read.FCS(outFile, transform=FALSE)
all(exprs(foo) == exprs(bar))</pre>
```

write.flowSet

Write an FCS file

# Description

Write FCS file for each flowFrame in a flowSet

# Usage

```
write.flowSet(x, outdir=identifier(x), filename, ...)
```

## **Arguments**

x	A flowSet.
filename	A character scalar or vector giving the output file names. By default, the function will use the identifiers of the individual flowFrames as the file name, potentially adding the .fcs suffix unless a file extension is already present. Alternatively, one can supply either a character scalar, in which case the prefix $i_i$ is appended (i being an integer in $seq_len(length(x))$ ), or a character vector of the same length as the flowSet x.
outdir	A character scalar giving the output directory. As the default, the output of identifier(x) is used.
	Further arguments that are passed on to write.FCS.

#### Details

The function write.flowSet creates FCS 3.0 standard file for all flowFrames in an object of class flowSet. In addition, it will write the content of the phenoData slot in the ASCII file "annotation.txt". This file can subsequently be used to reconstruct the whole flowSet using the read.flowSet function, e.g.:

```
read.flowSet(path=outdir, phenoData="annotation.txt"
```

The function uses write. FCS for the actual writing of the FCS files.

## Value

A character of the output directory.

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## Author(s)

F. Hahne

## See Also

```
link[flowCore]{write.FCS}
```

## **Examples**

```
## sample data
data(GvHD)
foo <- GvHD[1:5]
outDir <- file.path(tempdir(), "foo")

## now write out into files
write.flowSet(foo, outDir)
dir(outDir)

## and read back in
bar <- read.flowSet(path=outDir, phenoData="annotation.txt")</pre>
```

%on%

Methods for Function %on% in Package 'flowCore'

# **Description**

This operator is used to construct a transformFilter that first applies a transformList to the data before applying the filter operation. You may also apply the operator to a flowFrame or flowSet to obtain transformed values specified in the list.

# Author(s)

B. Ellis

# **Examples**

```
samp <- read.FCS(system.file("extdata","0877408774.B08", package="flowCore"))
plot(transform("FSC-H"=log, "SSC-H"=log) %on% samp)

rectangle <- rectangleGate(filterId="rectangleGateI","FSC-H"=c(4.5, 5.5))
sampFiltered <- filter(samp, rectangle %on% transform("FSC-H"=log, "SSC-H"=log))
res <- Subset(samp, sampFiltered)

plot(transform("FSC-H"=log, "SSC-H"=log) %on% res)</pre>
```

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