# Package 'flowCore'

October 16, 2018

Title flowCore: Basic structures for flow cytometry data

**Version** 1.46.2

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**Description** Provides S4 data structures and basic functions to deal with flow cytometry data.

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**Depends** R (>= 2.10.0)

**Imports** Biobase, BiocGenerics (>= 0.1.14), graph, graphics, methods, rrcov, stats, utils, stats4, corpcor, Rcpp, matrixStats

**Suggests** Rgraphviz, flowViz, flowStats, testthat, flowWorkspace, flowWorkspaceData, openCyto, knitr, ggcyto, gridExtra

Collate AllGenerics.R AllClasses.R flowFrame-accessors.R flowSet-accessors.R Rm-methods.R actionItem-accessors.R coerce.R logicalFilterResult-accessors.R summarizeFilter-methods.R filterSummary-accessors.R fcReference-accessors.R manyFilterResult-accessors.R summary-methods.R multipleFilterResult-accessors.R on-methods.R transformList-accessors.R identifier-methods.R parameters-methods.R view-accessors.R initialize-methods.R filterResult-accessors.R in-methods.R rectangleGate-accessors.R workFlow-accessors.R filterResultList-accessors.R IO.R show-methods.R length-methods.R names-methods.R filterSet-accessors.R split-methods.R eval-methods.R gatingML.R FCSTransTransform.R median-logicle-transform.R utils.R RcppExports.R

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biocViews Infrastructure, FlowCytometry, CellBasedAssays

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# R topics documented:

flowCore-package
actionItem-class
arcsinhTransform
asinht-class
asinhtGml2-class
biexponentialTransform
boundaryFilter-class
characterOrTransformation-class
checkOffset
coerce
compensateActionItem-class
compensatedParameter-class
compensateView-class
compensation-class
concreteFilter-class
dg1polynomial-class
each_col
EHtrans-class
ellipsoidGate-class
estimateMedianLogicle
exponential-class
expressionFilter-class
fcReference-class
FCSTransTransform
filter
filter-and-methods
filter-class
filter-in-methods
filterDetails-methods
filterList-class
filterReference-class
filterResult-class
filterResultList-class
filters-class
filterSet-class
filterSummary-class
filterSummaryList-class
flowFrame-class
flowSet-class
fsApply
gateActionItem-class
gateView-class
getChannelMarker
getIndexSort
GvHD
hymanica alaca

hyperlogtGml2-class .			 		 					 	
identifier											
inverseLogicleTransform											
invsplitscale-class											
keyword-methods											
kmeansFilter-class											•
linearTransform											•
											•
lintGml2-class											•
InTransform											•
logarithm-class											•
logicalFilterResult-class											
logicletGml2-class	. <b></b>		 		 					 	
logicleTransform			 		 					 	
logtGml2-class			 		 					 	
logTransform											
manyFilterResult-class											
markernames											
multipleFilterResult-clas											
norm2Filter-class											
normalization-class											
normalizeActionItem-cla											
normalizeView-class .											
parameterFilter-class .	. <b></b>		 		 		 •			 	
parameters	. <b></b>		 		 					 	
parameters-class			 		 					 	
parameterTransform-class	s		 		 					 	
polygonGate-class			 		 					 	
polytopeGate-class											
quadGate-class											
quadratic-class											
quadraticTransform											
*											
randomFilterResult-class											
ratio-class											
ratiotGml2-class											
read.FCS											
read.FCSheader	. <b></b>		 		 		 •			 	
read.flowSet	. <b></b>		 		 					 	
rectangleGate-class			 		 					 	
sampleFilter-class			 		 					 	
scaleTransform											
setOperationFilter-class											
singleParameterTransfor											
sinht-class											
spillover											
split-methods											
splitscale-class											
splitScaleTransform											
squareroot-class	. <b></b>		 		 					 	
Subset	. <b></b>		 		 					 	
subsetting-class			 		 					 	
subsettingActionItem-cla											

4 flowCore-package

flow	Core-package	Provide. deal wit					ba	sic	in	fra	str	ис	tui	re	an	d	fur	ıct	ioi	ns	to
Index																					156
	%on%		 	 		 •	•				٠		•	٠					•		. 155
	write.flowSet																				
	write.FCS																				
	workFlow-class		 	 																	. 150
	view-class		 	 																	. 148
	validFilters		 	 																	. 147
	unitytransform-class																				
	truncateTransform .																				
	transformView-class																				
	transformReference-																				
	transformMap-class																				
	transformFilter-class transformList-class																				
	transformation-class																				
	transformActionIten																				
	transform-class																				
	timeFilter-class																				
	summarizeFilter-me																				

# 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
Type: Package
Version: 1.11.20
Date: 2009-09-16
License: Artistic-2.0

#### Author(s)

Maintainer: Florian Hahne <fhahne@fhcrc.org>

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

actionItem-class 5

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.

# **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

6 arcsinhTransform

## **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)
```

# **Arguments**

transformationId

character string to identify the transformation

- a positive double that corresponds to a shift about 0.
- b positive double that corresponds to a scale factor.
- c positive double

# Value

Returns an object of class transform.

# Author(s)

B. Ellis

#### See Also

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

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

asinht-class 7

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.

# 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

8 asinhtGml2-class

#### **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>
```

asinhtGml2-class

Class asinhtGml2

### **Description**

Inverse hyperbolic sin transformation as parameterized in Gating-ML 2.0 is defined by the following function:

```
bound(f, boundMin, boundMax) = max(min(f, boundMax), boundMin))
```

where

```
f(parameter, T, M, A) = (asinh(parameter*sinh(M*ln(10))/T) + A*ln(10))/((M+A)*ln(10))
```

This transformation is equivalent to Logicle(T, 0, M, A) (i.e., with W=0). It provides an inverse hyperbolic sine transformation that maps a data value onto the interval [0,1] such that:

- The top of scale value (i.e., T) is mapped to 1.
- Large data values are mapped to locations similar to an (M + A)-decade logarithmic scale.
- A decades of negative data are brought on scale.

In addition, if a boundary is defined by the boundMin and/or boundMax parameters, then the result of this transformation is restricted to the [boundMin,boundMax] interval. Specifically, should the result of the f function be less than boundMin, then let the result of this transformation be boundMin. Analogically, should the result of the f function be more than boundMax, then let the result of this transformation be boundMax. The boundMin parameter shall not be greater than the boundMax parameter.

# **Objects from the Class**

```
Objects can be created by calls to the constructor asinhtGml2(parameter, T, M, A, transformationId, boundMin, boundMax)
```

# Slots

.Data: Object of class function ~~

- T: Object of class numeric -positive constant (top of scale value)
- M: Object of class numeric -positive constant (desired number of decades)
- A: Object of class numeric -non-negative constant that is less than or equal to M (desired number of additional negative decades)

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

boundMin: Object of class numeric -lower bound of the transformation, default -Inf

boundMax: Object of class numeric -upper bound of the transformation, default Inf

#### **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 asinhtGml2 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)

Spidlen, J.

#### References

Gating-ML 2.0: International Society for Advancement of Cytometry (ISAC) standard for representing gating descriptions in flow cytometry. http://flowcyt.sourceforge.net/gating/20141009.pdf

#### See Also

```
asinht, transform-class, transform
```

# **Examples**

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

```
biexponentialTransform(transformationId="defaultBiexponentialTransform", a = 0.5, b = 1, c = 0.5,
```

# Arguments

		onId

	A name to assign to the transformation. Used by the transform/filter integration routines.
а	See the function description above. Defaults to 0.5
b	See the function description above. Defaults to 1.0
С	See the function description above. Defaults to 0.5 (the same as a)
d	See the function description above. Defaults to 1 (the same as b)
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

```
# Construct some "flow-like" data which tends to be hetereoscedastic.
data(GvHD)
biexp <- biexponentialTransform("myTransform")

after.1 <- transform(GvHD, transformList('FSC-H', biexp))

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

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 11

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")
```

#### **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.
```

12 boundaryFilter-class

#### **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-tol side: 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.

```
## 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.

#### Methods

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

#### References

~put references to the literature/web site here ~

#### **Examples**

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

checkOffset

Fix the offset when its values recorded in header and TEXT don't agree

# **Description**

Fix the offset when its values recorded in header and TEXT don't agree

# Usage

```
checkOffset(offsets, x, ignore.text.offset = FALSE, ...)
```

# **Arguments**

```
offsets the named vector returned by findOffsets

x the text segmented returned by readFCStext
ignore.text.offset
 whether to ignore the offset info stored in TEXT segment
... not used.
```

#### Value

the updated offsets

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.

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

### Author(s)

Florian Hahne

#### See Also

workFlow, actionItem, gateActionItem, transformActionItem, view

#### **Examples**

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

compensatedParameter-class

Class "compensatedParameter"

# 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**

 $Objects\ can\ be\ created\ by\ calls\ of\ the\ form\ compensated\ Parameter (parameters, spillRefId, transformationId, second the compensated). The compensated of the compensated of$ 

# **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)

compensateView-class 17

#### 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

# 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.

18 compensation-class

#### **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")
```

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, ...)
```

compensation-class 19

#### **Arguments**

spillover The spillover or compensation matrix.

 ${\tt compensationId} \ \ \, {\tt The identifier for the compensation object}.$ 

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.

...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.

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.

20 compensation-class

#### **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.
         Usage:
            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>
    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.
            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

concreteFilter-class 21

#### **Examples**

```
## Read sample data and a sample spillover matrix
      <- 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
## compensate using the spillover matrix directly
summary(samp)
samp <- compensate(samp, comp.mat)</pre>
summary(samp)
## create a compensation object and compensate using that
comp <- compensation(comp.mat)</pre>
compensate(samp, comp)
## demo the sample-specific compensation
## create a list of comps (each element could be a different compensation tailored for the specific sample)
comps <- sapply(sampleNames(samp), function(sn)comp, simplify = FALSE)</pre>
# the names of comps must be matched to sample names of the flowset
compensate(samp, comps)
```

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.

#### **Extends**

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

#### Author(s)

B. Ellis

# See Also

```
parameterFilter
```

22 dg1polynomial-class

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.

# 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

each\_col 23

## **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
```

```
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

### References

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

### See Also

hyperlog

ellipsoidGate-class 25

#### **Examples**

```
dat <- read.FCS(system.file("extdata","0877408774.B08",</pre>
                 package="flowCore"))
eh1<-EHtrans("FSC-H", a=1250, b=4, transformationId="eh1")
transOut<-eval(eh1)(exprs(dat))</pre>
```

```
ellipsoidGate-class
                         Class "ellipsoidGate"
```

### **Description**

Class and constructor for n-dimensional ellipsoidal filter objects.

#### Usage

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

# **Arguments**

An optional parameter that sets the filterId of this gate. filterId .gate A definition of the gate via a covariance matrix. Numeric vector of equal length as dimensions in .gate. mean 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 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.

# 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.
```

26 ellipsoidGate-class

#### **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
   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 = "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.

```
## 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,
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)</pre>
```

estimateMedianLogicle 27

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

##ellipsoidGate can be converted to polygonGate by interpolation
pg <- as(eg, "polygonGate")
pg</pre>
```

estimateMedianLogicle Estimates a common logicle transformation for a flowSet.

# Description

Of the negative values for each channel specified, the median of the specified quantiles are used.

# Usage

```
estimateMedianLogicle(flow_set, channels, m = 4.5, q = 0.05)
```

# **Arguments**

flow\_set object of class 'flowSet'
channels character vector of channels to transform

m TODO – default value from .lgclTrans
q quantile

# Value

TODO

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 constructor exponential (parameters, a, b).

28 exponential-class

#### **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

# Author(s)

Gopalakrishnan N, F.Hahne

#### References

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

#### See Also

logarithm

```
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 29

```
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.

# **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.

30 expressionFilter-class

#### 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.

```
## Loading example data
dat <- read.FCS(system.file("extdata","0877408774.B08",</pre>
package="flowCore"))
#Create the filter
ef <- expressionFilter(`FSC-H` > 200, filterId="myExpressionFilter")
## Filtering using sampeFilters
fres <- filter(dat, ef)</pre>
fres
summary(fres)
## The result of sample filtering is a logical subset
newDat <- Subset(dat, fres)</pre>
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)</pre>
cn <- paste("`", colnames(dat), "`", sep="")</pre>
exp <- paste(cn, ">", r[1,], "&", cn, "<", r[2,], collapse=" & ")
ef2 <- char2ExpressionFilter(exp, filterId="myExpressionFilter")</pre>
ef2
fres2 <- filter(dat, ef2)</pre>
fres2
summary(fres2)
```

fcReference-class 31

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="_"),
                                  env=new.env(parent=emptyenv()))
fcTransformReference(ID=paste("transRef",
                                          guid(), sep="_"),
                                 env=new.env(parent=emptyenv()))
fcNullReference(...)
assign(x, value, pos = -1, envir = as.environment(pos), inherits = FALSE,
    immediate = TRUE)
## S4 method for signature 'fcReference,missing,missing'
get(x, pos = -1, envir = as.environment(pos), mode = "any",
    inherits = TRUE)
```

32 fcReference-class

```
isNull(f)
Rm(symbol, envir, subSymbol, ...)
```

### **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:
```

fcReference-class 33

```
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
```

directly. Class "fcFilterResultReference", directly. Class "fcTransformReference", directly. Class "fcCompensateReference", directly. Class "fcTransformReference", directly. Class "fcNormalizationReference", directly. Class "fcTreeReference", directly. Class "fcAliasReference", directly. Class "fcReference", directly. Class "fcStructureReference", directly. Class "fcActionReference", by class "fcActionReference", distance 2. Class "fcReference", by class "fcActionReference", distance 3. Class "fcStructureReference", by class "fcViewReference", distance 2. Class "fcReference", by class "fcFilterResultReference", distance 2. Class "fcReference", by class "fcTransformReference", distance 2. Class "fcStructureReference", by class "fcTreeReference", distance 2. Class "fcReference", distance 2. Class "fcTreeReference", distance 2. Class "fcTreeReference", distance 2. Class "fcTreeReference", distance 2. 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.
```

34 FCSTransTransform

```
get signature(x = "fcNullReference", pos = "missing", envir = "missing", mode = "missing", inherits
         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")
  FCSTransTransform
                            Computes a transform using the 'iplogicle' function
```

### Description

Transforms FCS data using the iplogicle function from FCSTrans by Quian et al. The core functionality of FCSTrans has been imported to produce transformed FCS data rescaled and truncated as produced by FCSTrans. The w parameter is estimated by iplogicle automatically, then makes a call to iplogicore which in turn uses the logicle transform code of Wayne Moore.

For the details of the FCSTrans transformation, we recommend the excellent Supplementary File that accompanies Quian et al. (2012): http://onlinelibrary.wiley.com/doi/10.1002/cyto.a.22037/suppinfo

# Usage

FCSTransTransform(transformationId = "defaultFCSTransTransform", channelrange = 2^18, channeldeca

filter 35

### **Arguments**

transformationId

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

channelrange is the range of the data. By default,  $2^18 = 262144$ .

channeldecade is the number of logarithmic decades. By default, it is set to 4.5.

range the target resolution. The default value is  $2^12 = 4096$ .

cutoff a threshold below which the logicle transformation maps values to 0.

w the logicle width. This is estimated by iplogicle by default. Details can be

found in the Supplementary File from Quian et al.

rescale logical parameter whether or not the data should be rescaled to the number of

channels specified in range. By default, the value is TRUE but can be set to

FALSE if you want to work on the transformed scale.

### Author(s)

Wayne Moore, N Gopalakrishnan

#### References

Y Quian, Y Liu, J Campbell, E Thompson, YM Kong, RH Scheuermann; FCSTrans: An open source software system for FCS file conversion and data transformation. Cytometry A, 2012

#### See Also

 $inverse Logic le Transform, estimate Logic le \ , logic le Transform \\$ 

# **Examples**

```
data(GvHD)
samp <- GvHD[[1]]
## User defined logicle function
lgcl <- transformList(c('FL1-H', 'FL2-H'), FCSTransTransform())
after <- transform(samp, lgcl)</pre>
```

filter

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, method = c("convolution", "recursive"),
    sides = 2L, circular = FALSE, init = NULL)
```

36 filter

#### **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
```

```
## Filtering a flowFrame
samp <- read.FCS(system.file("extdata","0877408774.B08", package="flowCore"))
rectGate <- rectangleGate(filterId="nonDebris","FSC-H"=c(200,Inf))
fr <- filter(samp,rectGate)
class(fr)
summary(fr)

## filtering a flowSet
data(GvHD)
foo <- GvHD[1:3]
fr2 <- filter(foo, rectGate)
class(fr2)
summary(fr2)

## filtering a flowSet using different filters for each frame
rg2 <- rectangleGate(filterId="nonDebris","FSC-H"=c(300,Inf))
rg3 <- rectangleGate(filterId="nonDebris","FSC-H"=c(400,Inf))</pre>
```

filter-and-methods 37

```
flist <- list(rectGate, rg2, rg3)
names(flist) <- sampleNames(foo)
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.

## **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.

38 filter-in-methods

#### 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

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 39

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 de-
tails 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.

# Usage

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

## **Arguments**

x A list of filter objects.

filterId 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.

40 filterReference-class

#### **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,

## **Examples**

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

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
```

filterResult-class 41

### **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**

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

## Methods

```
== test equality
```

# Author(s)

B. Ellis, N. LeMeur

# See Also

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

## **Examples**

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

42 filterResultList-class

```
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.
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, filter Result, logical Filter Result, multiple Filter Result, random Filter Result\\
```

filters-class 43

#### **Examples**

```
## Not run:
library(flowStats)
## 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)
## End(Not run)
```

filters-class

Class "filters" and "filtersList"

## **Description**

The filters class is the container for a list of filter objects. The class mainly exists for displaying multiple filters/gates on one single panel(flowFrame) of xyplot. Note that it is different from filerList class which is to be applied to a flowSet. In other words, filter objects of a fliterList are to be applied to different flowFrames. However, all of filter objects of a filters are for one single flowFrame, more specifically for one pair of projections(parameters). So these filters should share the common parameters.

And filtersList is a list of filters objects, which are to be applied to a flowSet.

## Usage

```
filters(x)
filtersList(x)
```

## **Arguments**

Х

A list of filter or filters objects.

## Value

A filters or filtersList object for the constructor

44 filterSet-class

#### **Slots**

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

#### **Extends**

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

## **Objects from the Class**

Objects are created from regular lists using the constructors filters and filtersList.

### Author(s)

Mike Jiang

#### See Also

```
filter, filterList
```

filterSet-class

Class filterSet(deprecated)

## **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.

### **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.

filterSummary-class 45

#### **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

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.

46 filterSummary-class

#### **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).
```

#### 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 Filte

## **Examples**

```
## Not run:
library(flowStats)

## 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"]]</pre>
```

```
##accessing details
s$true
s$n
toTable(s)
## End(Not run)
```

```
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, ...)
```

## 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**

```
## Not run:
 library(flowStats)
 ## Loading example data, creating and applying a curv1Filter
 data(GvHD)
 dat <- GvHD[1:3]</pre>
 c1f <- curv1Filter(filterId="myCurv1Filter", x=list("FSC-H"), bwFac=2)</pre>
 fres <- filter(dat, c1f)</pre>
 ## creating and showing the summary
 summary(fres)
 s <- summary(fres)</pre>
 ## subsetting
 s[[1]]
 ##accessing details
 toTable(s)
 ## End(Not run)
flowFrame-class
                           'flowFrame': a class for storing observed quantitative properties for
```

### **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)

a population of cells from a FACS run

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 flowFrame, 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\$.

```
Usage:
   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<- . colnames and featureNames are synonyms, they extract parameter names (i.e., the colnames of the data matrix). For colnames there is also a replacement method. This will update the name column in the parameters slot as well. Usage: featureNames(flowFrame) colnames(flowFrame) colnames(flowFrame) <- value</pre> **names** Extract pretty formated names of the parameters including parameter descriptions. 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 instrument or actual data range of the flowFame. Note that instrument dynamic range is not necessarily the same as 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 forflowFrames. parameters: x: flowFrame object. type: Range type. either "instrument" or "data". Default is "instrument" range(x, type = "data") 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, translist, ...) 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.
     Usage:
        cbind2(flowFrame, matrix)
        cbind2(flowFrame, numeric)
compensate Apply a compensation matrix (or a compensation object) on a flowFrame object.
     This returns a compensated flowFrame.
     Usage:
        compensate(flowFrame, matrix)
                                              compensate(flowFrame, data.frame)
spillover Extract spillover matrix from description slot if present. It is equivalent to keyword(x, c("spillover", "SPI
     Thus will simply return a list of keywords value for "spillover" and "SPILL".
     Usage:
        spillover(flowFrame)
== Test equality between two flowFrames
<, >, <=, >= These operators basically treat the flowFrame as a numeric matrix.
```

initialize(flowFrame): Object instantiation, used by new; not to be called directly by the user.

#### Author(s)

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

## See Also

```
flowSet, read.FCS
```

#### **Examples**

```
## load example data
data(GvHD)
frame <- GvHD[[1]]

## 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,]
frame
exprs(frame) <- exprs(frame)[,1:6]
frame</pre>
```

```
## 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>
## range of a frame
range(frame) #instrument range
range(frame, type = "data") #actual data range
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, transformList(c("FL1.H", "FL2.H"), log))</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))</pre>
frame[rectGate,]
frame[fres,]
## accessing the spillover matrix
try(spillover(frame))
## check equality
frame2 <- frame</pre>
frame == frame2
exprs(frame2) <- exprs(frame)*2</pre>
frame == frame2
```

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>
```

 $\textbf{phenoData}, \textbf{phenoData} \textbf{<-} \ \, \textbf{Extract or replace the AnnotatedDataFrame from the phenoData slot}.$ 

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.

Usage:

```
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 documentation for details.

Usage:

```
compensate(flowSet, matrix)
```

**transform** Apply a transformation function on all frames of a flowSet object. See separate documentation 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
```

# **Examples**

```
## load example data and object creation
data(GvHD)
\hbox{\it \#\# subsetting to flowSet}
set <- GvHD[1:4]
GvHD[1:4,1:2]
sel <- sampleNames(GvHD)[1:2]</pre>
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$s5a02
## constructor
flowSet(GvHD[[1]], GvHD[[2]])
pd <- phenoData(GvHD)[1:2,]</pre>
flowSet(s5a01=GvHD[[1]], s5a02=GvHD[[2]],phenoData=pd)
## colnames
colnames(set)
colnames(set) <- make.names(colnames(set))</pre>
## object name
identifier(set)
identifier(set) <- "test"</pre>
## phenoData
pd <- phenoData(set)</pre>
pd$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"))
```

fsApply 59

```
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, transformList(c("FL1.H", "FL2.H"), log))</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]])
rectGate2 <- rectangleGate(filterId="nonDebris2", SSC.H=c(300,Inf))</pre>
fres2 <- filter(set, list(A=rectGate, B=rectGate2, C=rectGate, D=rectGate2))</pre>
## 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])
```

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)
```

60 gateActionItem-class

## **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
```

## **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)
```

gateActionItem-class 61

#### **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.

### **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. 62 gateView-class

#### Author(s)

Florian Hahne

#### See Also

```
workFlow, actionItem, transformActionItem, compensateActionItem, view
```

### **Examples**

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

gateView-class

Class "gateView"

## **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 A more human-readable name of the view.

data, action, 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.

gateView-class 63

#### **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.

#### **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

## **Examples**

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

64 getIndexSort

getChannelMarker	get channel and marker information from a flowFrame that matches to the given keyword
------------------	---

### **Description**

This function tries best to guess the flow parameter based on the keyword supplied by name It first does a complete word match(case insensitive) between name and flow channels and markers. If there are duplicated matches, throw the error. If no matches, it will try the partial match.

## Usage

```
getChannelMarker(frm, name, ...)
```

### **Arguments**

frm flowFrame object

name character the keyword to match

... other arguments: not used.

#### Value

an one-row data. frame that contains "name" (i.e. channel) and "desc" (i.e. stained marker) columns.

getIndexSort

Extract Index Sorted Data from an FCS File

## **Description**

Retrieve a data frame of index sorted data and sort indices from an FCS file.

## **Details**

The input FCS file should already be compensated. Index sorting permits association of cell-level fluorescence intensities with downstream data collection on the sorted cells. Cells are sorted into a plate with X,Y coordinates, and those coordinates are stored in the FCS file.

This function will extract the data frame of flow data and the X,Y coordinates for the cell-level data, which can be written to a text file, or concatenated with sample-level information and analyzed in R. The coordinates are names 'XLoc', 'YLoc', and a 'name' column is also prepended with the FCS file name.

### Value

Matrix of fluorescence intensities and sort indices for plate location. When no index sorting data is available, invisibly returns 0. Test for 0 to check success.

## Methods

**x** = "flowFrame" Return a matrix of fluorescence intensities and indices into the sorting plate for each cell.

GvHD 65

#### Author(s)

G. Finak

#### **Examples**

```
samp <- read.FCS(system.file("extdata","0877408774.B08", package="flowCore"))
# This will return a message that no index sorting data is available
getIndexSort(samp)</pre>
```

**GvHD** 

Extract of a Graft versus Host Disease monitoring experiment (Rizzieri et al., 2007)

### **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 http://www.ficcs.org/software.html#Data\_Files, 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

66 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

# References

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

hyperlogtGml2-class 67

### 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>
```

hyperlogtGml2-class

Class hyperlogtGml2

## **Description**

Hyperlog transformation parameterized according to Gating-ML 2.0 is defined by the following function:

bound(hyperlog, boundMin, boundMax) = max(min(hyperlog, boundMax), boundMin))

where

$$hyperlog(x, T, W, M, A) = root(EH(y, T, W, M, A) - x)$$

and EH is defined as:

$$EH(y, T, W, M, A) = ae^{by} + cy - f$$

where

- x is the value that is being transformed (an FCS dimension value). Typically, x is less than or equal to T, although the transformation function is also defined for x greater than T.
- y is the result of the transformation.
- T is greater than zero and represents the top of scale value.
- M is greater than zero and represents the number of decades that the true logarithmic scale approached at the high end of the Hyperlog scale would cover in the plot range.
- W is positive and not greater than half of M and represents the number of such decades in the approximately linear region.
- A is the number of additional decades of negative data values to be included. A shall be greater than or equal to -W, and less than or equal to M-2W
- root is a standard root finding algorithm (e.g., Newton's method) that finds y such as B(y, T, W, M, A) = x.

and a, b, c and f are defined by means of T, W, M, A, w, x0, x1, x2, e0, ca and fa as:

$$w = W/(M+A)$$

$$x2 = A/(M+A)$$

$$x1 = x2 + w$$

$$x0 = x2 + 2 * w$$

$$b = (M+A) * ln(10)$$

$$e0 = e^{b*x0}$$

$$ca = e0/w$$

$$fa = e^{b*x1} + ca * x1$$

$$a = T/(e^b + ca - fa)$$

$$c = ca * a$$

$$f = fa * a$$

In addition, if a boundary is defined by the boundMin and/or boundMax parameters, then the result of this transformation is restricted to the [boundMin,boundMax] interval. Specifically, should the result of the hyperlog function be less than boundMin, then let the result of this transformation be boundMin. Analogically, should the result of the hyperlog function be more than boundMax, then let the result of this transformation be boundMax. The boundMin parameter shall not be greater than the boundMax parameter.

## **Objects from the Class**

Objects can be created by calls to the constructor

hyperlogtGml2(parameter, T, M, W, A, transformationId, boundMin, boundMax)

#### **Slots**

.Data: Object of class function ~~

T: Object of class numeric -positive constant (top of scale value)

M: Object of class numeric -positive constant (desired number of decades)

W: Object of class numeric -positive constant that is not greater than half of M (the number of such decades in the approximately linear region)

A: Object of class numeric -a constant that is greater than or equal to -W, and also less than or equal to M-2W. (A represents the number of additional decades of negative data values to be included.)

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

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

boundMin: Object of class numeric -lower bound of the transformation, default -Inf boundMax: Object of class numeric -upper bound of the transformation, default Inf

## 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 hyperlogtGml2 in the signature.

identifier 69

#### Note

That hyperlogtGml2 transformation brings "reasonable" data values to the scale of [0,1]. The transformation is somewhat similar to logicletGml2. (See Gating-ML 2.0 for detailed comparison)

The hyperlog 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)

```
Spidlen, J., Moore, W.
```

#### References

Gating-ML 2.0: International Society for Advancement of Cytometry (ISAC) standard for representing gating descriptions in flow cytometry. http://flowcyt.sourceforge.net/gating/20141009.pdf

#### See Also

hyperlog, logicleTransform, transform-class, transform

### **Examples**

```
myDataIn <- read.FCS(system.file("extdata", "0877408774.B08",
    package="flowCore"))
myHyperLg <- hyperlogtGml2(parameters = "FSC-H", T = 1023, M = 4.5,
    W = 0.5, A = 0, transformationId="myHyperLg")
transOut <- eval(myHyperLg)(exprs(myDataIn))</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>
```

inverse Logic le Transform

Computes the inverse of the transform defined by the 'logicleTransform' function or the transformList generated by 'estimateLogicle' 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(trans,transformationId,...)
```

## **Arguments**

trans

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

transformationId

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

... not used.

invsplitscale-class 71

#### 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

### **Examples**

```
data(GvHD)
samp <- GvHD[[1]]

##########inverse the transform object###############################
logicle <- logicleTransform(t = 10000, w = 0.5, m = 4.5 , a =0 ,"logicle")
## transform FL1-H parameter using logicle transformation
after <- transform(samp, transformList('FL1-H', logicle))

## Inverse transform the logicle transformed data to retrieve the original data
invLogicle <- inverseLogicleTransform(trans = logicle)
before <- transform (after, transformList('FL1-H', invLogicle))

###############
translist <- estimateLogicle(samp, c("FL1-H", "FL2-H"))
after <- transform(samp, translist)
## Inverse
invLogicle <- inverseLogicleTransform(translist)
before <- transform (after, invLogicle)</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,

$$b = \frac{transitionChannel}{2}$$
 
$$d = \frac{2*log_{10}(e)*r}{transitionChannel} + log_{10}(maxValue)$$

72 invsplitscale-class

$$t = 10^{log_{10}t}$$

$$a = \frac{transitionChannel}{2*t}$$

$$log_{10}ct = \frac{(a*t+b)*d}{r}$$

$$c = 10^{log_{10}ct}$$

## **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

## 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

keyword-methods 73

## **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.

... compact: logical scaler to indicate whether to hide all the cytometer instrument

and laser settings from keywords.

## **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.

74 kmeansFilter-class

```
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

### **Examples**

```
samp <- read.FCS(system.file("extdata","0877408774.B08", package="flowCore"))
keyword(samp)
keyword(samp, compact = TRUE)
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>
```

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-class 75

```
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.
```

#### 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
   evaluate the filter on data.
```

Usage:

This is usually not called directly by the user, but internally by the filter methods.

76 linearTransform

```
show signature(object = "kmeansFilter"): Print information about the filter.
Usage:
```

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.

# **Examples**

```
## Loading example data
dat <- read.FCS(system.file("extdata","0877408774.B08",</pre>
package="flowCore"))
## Create the filter
kf <- kmeansFilter("FSC-H"=c("Pop1","Pop2","Pop3"), filterId="myKmFilter")</pre>
## Filtering using kmeansFilters
fres <- filter(dat, kf)</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="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)
```

lintGml2-class 77

## **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
```

#### **Examples**

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

lintGml2-class

Class lintGml2

## **Description**

Linear transformation as parameterized in Gating-ML 2.0 is defined by the following function:

```
bound(f,boundMin,boundMax) = max(min(f,boundMax),boundMin))
```

where

$$f(parameter, T, A) = (parameter + A)/(T + A)$$

This transformation provides a linear display that maps scale values from the [-A, T] interval to the [0, 1] interval. However, it is defined for all xinR including outside of the [-A, T] interval.

In addition, if a boundary is defined by the boundMin and/or boundMax parameters, then the result of this transformation is restricted to the [boundMin,boundMax] interval. Specifically, should the result of the f function be less than boundMin, then let the result of this transformation be boundMin. Analogically, should the result of the f function be more than boundMax, then let the result of this transformation be boundMax. The boundMin parameter shall not be greater than the boundMax parameter.

# **Objects from the Class**

```
Objects can be created by calls to the constructor
```

```
lintGml2(parameter, T, A, transformationId, boundMin, boundMax)
```

78 lintGml2-class

#### **Slots**

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

T: Object of class numeric -positive constant (top of scale value)

A: Object of class numeric -non-negative constant that is less than or equal to T; it is determining the bottom end of the transformation

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

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

boundMin: Object of class numeric -lower bound of the transformation, default -Inf boundMax: Object of class numeric -upper bound of the transformation, default Inf

#### **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 lintGml2 in the signature.

#### Note

The linear 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)

Spidlen, J.

#### References

Gating-ML 2.0: International Society for Advancement of Cytometry (ISAC) standard for representing gating descriptions in flow cytometry. http://flowcyt.sourceforge.net/gating/20141009.pdf

#### See Also

linearTransform, transform-class, transform

```
myDataIn <- read.FCS(system.file("extdata", "0877408774.B08",
    package="flowCore"))
myLinTr1 <- lintGml2(parameters = "FSC-H", T = 1000, A = 0,
    transformationId="myLinTr1")
transOut <- eval(myLinTr1)(exprs(myDataIn))</pre>
```

InTransform 79

lnTransform	Create the definition of a ln transformation function (natural logarthim) to be applied on a data set
2	

# 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, transformList('FSC-H', lnTrans))

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

80 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

# **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")
```

82 logicletGml2-class

logicletGml2-class

Class logicletGml2

### **Description**

Logicle transformation as published by Moore and Parks is defined by the following function:

bound(logicle, boundMin, boundMax) = max(min(logicle, boundMax), boundMin))

where

$$logicle(x, T, W, M, A) = root(B(y, T, W, M, A) - x)$$

and B is a modified biexponential function:

$$B(y, T, W, M, A) = ae^{by} - ce^{-dy} - f$$

where

- x is the value that is being transformed (an FCS dimension value). Typically, x is less than or equal to T, although the transformation function is also defined for x greater than T.
- y is the result of the transformation.
- T is greater than zero and represents the top of scale value.
- M is greater than zero and represents the number of decades that the true logarithmic scale approached at the high end of the Logicle scale would cover in the plot range.
- W is non-negative and not greater than half of M and represents the number of such decades in the approximately linear region. The choice of W=M/2 specifies a scale that is essentially linear over the whole range except for a small region of large data values. For situations in which values of W approaching M/2 might be chosen, ordinary linear display scales will usually be more appropriate. The choice of W=0 gives essentially the hyperbolic sine function.
- A is the number of additional decades of negative data values to be included. A shall be greater than or equal to -W, and less than or equal to M-2W
- root is a standard root finding algorithm (e.g., Newton's method) that finds y such as B(y, T, W, M, A) = x.

and a, b, c, d and f are defined by means of T, W, M, A, w, x0, x1, x2, ca and fa as:

$$w = W/(M+A)$$

$$x2 = A/(M+A)$$

$$x1 = x2 + w$$

$$x0 = x2 + 2 * w$$

$$b = (M+A) * ln(10)$$

and d is a constant so that

$$2 * (ln(d) - ln(b)) + w * (d + b) = 0$$

given b and w, and

$$ca = e^{x0*(b+d)}$$

logicletGml2-class 83

$$fa = e^{b*x1} - (ca/(e^{d*x1}))$$

$$a = T/(e^b - fa - (ca/e^d))$$

$$c = ca*a$$

$$f = fa*a$$

The Logicle scale is the inverse of a modified biexponential function. It provides a Logicle display that maps scale values onto the [0,1] interval such that the data value T is mapped to 1, large data values are mapped to locations similar to an (M+A)-decade logarithmic scale, and A decades of negative data are brought on scale. For implementation purposes, it is recommended to follow guidance in Moore and Parks publication.

In addition, if a boundary is defined by the boundMin and/or boundMax parameters, then the result of this transformation is restricted to the [boundMin,boundMax] interval. Specifically, should the result of the logicle function be less than boundMin, then let the result of this transformation be boundMin. Analogically, should the result of the logicle function be more than boundMax, then let the result of this transformation be boundMax. The boundMin parameter shall not be greater than the boundMax parameter.

### **Objects from the Class**

Objects can be created by calls to the constructor

logicletGml2(parameter, T, M, W, A, transformationId, boundMin, boundMax)

### **Slots**

.Data: Object of class function ~~

T: Object of class numeric -positive constant (top of scale value)

M: Object of class numeric -positive constant (desired number of decades)

W: Object of class numeric -non-negative constant that is not greater than half of M (the number of such decades in the approximately linear region)

A: Object of class numeric -a constant that is greater than or equal to -W, and also less than or equal to M-2W. (A represents the number of additional decades of negative data values to be included.)

 $\verb|parameters: Object of class "transformation" - flow parameter to be transformed$ 

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

boundMin: Object of class numeric -lower bound of the transformation, default -Inf

boundMax: Object of class numeric -upper bound of the transformation, default Inf

## 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 logicletGml2 in the signature.

84 logicleTransform

#### Note

Please note that logicletGml2 and logicleTransform are similar transformations; however, the Gating-ML 2.0 compliant logicletGml2 brings "reasonable" data values to the scale of [0,1] while the logicleTransform scales these values to [0,M].

The logicle 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)

```
Spidlen, J., Moore, W.
```

#### References

Gating-ML 2.0: International Society for Advancement of Cytometry (ISAC) standard for representing gating descriptions in flow cytometry. http://flowcyt.sourceforge.net/gating/20141009.pdf

Moore, WA and Parks, DR. Update for the logicle data scale including operational code implementations. Cytometry A., 2012:81A(4):273-277.

Parks, DR and Roederer, M and Moore, WA. A new "Logicle" display method avoids deceptive effects of logarithmic scaling for low signals and compensated data. Cytometry A., 2006:69(6):541-551.

#### See Also

logicleTransform, transform-class, transform

## **Examples**

logicle Transform

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,...)
```

logicleTransform 85

### **Arguments**

			_			٠		-	- 1
+	ra	nc.	tΛ	rma	+ ב	1	$\alpha$ n	١I	М

A name to assign to the transformation. Used by the transform/filter routines. w is the linearization width in asymptotic decades. w should be > 0 and deter-W mines 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 Top of the scale data value, e.g., 10000 for common 4 decade data or 262144 for t a 18 bit data range. t should be greater than zero m is the full width of the transformed display in asymptotic decades. m should m be greater than zero Additional negative range to be included in the display in asymptotic decades. а 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. Input flow frame for which the logicle transformations are to be estimated. channels channels or markers for which the logicle transformation is to be estimated. other arguments:

# 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.

q: a numeric type specifying quantile value, default is 0.05

#### See Also

inverseLogicleTransform, estimateLogicle

```
data(GvHD)
samp <- GvHD[[1]]
## User defined logicle function
lgcl <- logicleTransform( w = 0.5, t= 10000, m =4.5)
trans <- transformList(c("FL1-H", "FL2-H"), lgcl)
after <- transform(samp, trans)
invLgcl <- inverseLogicleTransform(trans = lgcl)
trans <- transformList(c("FL1-H", "FL2-H"), invLgcl)
before <- transform (after, trans)

## 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>
```

86 logtGml2-class

logtGml2-class

Class logtGml2

# **Description**

Log transformation as parameterized in Gating-ML 2.0 is defined by the following function:

bound(f, boundMin, boundMax) = max(min(f, boundMax), boundMin))

where

$$f(parameter, T, M) = (1/M) * log10(x/T) + 1$$

This transformation provides a logarithmic display that maps scale values from the (0,T] interval to the (-Inf,1] interval such that the data value T is mapped to 1 and M decades of data are mapped into the interval. Also, the limit for x going to 0 is -Inf.

In addition, if a boundary is defined by the boundMin and/or boundMax parameters, then the result of this transformation is restricted to the [boundMin,boundMax] interval. Specifically, should the result of the f function be less than boundMin, then let the result of this transformation be boundMin. Analogically, should the result of the f function be more than boundMax, then let the result of this transformation be boundMax. The boundMin parameter shall not be greater than the boundMax parameter.

### **Objects from the Class**

Objects can be created by calls to the constructor

logtGml2(parameter, T, M, transformationId, boundMin, boundMax)

#### **Slots**

.Data: Object of class function ~~

T: Object of class numeric -positive constant (top of scale value)

M: Object of class numeric -positive constant (number of decades)

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

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

boundMin: Object of class numeric -lower bound of the transformation, default -Inf

boundMax: Object of class numeric -upper bound of the transformation, default Inf

#### **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 logtGml2 in the signature.

logTransform 87

#### Note

The log 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)

Spidlen, J.

#### References

Gating-ML 2.0: International Society for Advancement of Cytometry (ISAC) standard for representing gating descriptions in flow cytometry. http://flowcyt.sourceforge.net/gating/20141009.pdf

#### See Also

logTransform, transform-class, transform

# **Examples**

```
myDataIn <- read.FCS(system.file("extdata", "0877408774.B08",
    package="flowCore"))
myLogTr1 <- logtGml2(parameters = "FSC-H", T = 1023, M = 4.5,
    transformationId="myLogTr1")
transOut <- eval(myLogTr1)(exprs(myDataIn))</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)
trans <- transformList('FSC-H', logTrans)
dataTransform <- transform(samp, trans)</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.

markernames 89

# Author(s)

B. Ellis

# See Also

```
filterResult
```

# **Examples**

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

markernames

get or update the marker names

# **Description**

marker names corresponds to the 'desc' column of the phenoData of the flowFrame.

# Usage

```
markernames(object, ...)
## S4 method for signature 'flowFrame'
markernames(object)

markernames(object) <- value

## S4 replacement method for signature 'flowFrame'
markernames(object) <- value

## S4 method for signature 'flowSet'
markernames(object)

## S4 replacement method for signature 'flowSet'
markernames(object) <- value</pre>
```

# **Arguments**

object flowFrame or flowSet

... not used

value a named list or character vector. the names corresponds to the name(channel)

and actual values are the desc(marker).

# **Details**

When extract marker names from a flowSet, it throws the warning if the marker names are not all the same across samples.

#### Value

marker names as a character vector. The marker names for FSC,SSC and Time channels are automatically excluded in the returned value. When object is a flowSet and the marker names are not consistent across flowFrames, it returns a list of unique marker sets.

# **Examples**

```
data(GvHD)
fr <- GvHD[[1]]
markernames(fr)

chnls <- c("FL1-H", "FL3-H")
markers <- c("CD15", "CD14")
names(markers) <- chnls
markernames(fr) <- markers
markernames(fr)

fs <- GvHD[1:3]
markernames(fs)</pre>
```

```
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.

norm2Filter-class 91

## Author(s)

B. Ellis

#### See Also

filterResult

# **Examples**

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

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**

x,y	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.

92 norm2Filter-class

#### **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).

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
   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 = "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",
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</pre>
```

normalization-class 93

```
n2f <- norm2Filter(filterId="myNorm2Filter", x=list("FSC-H", "SSC-H"),
scale.factor=2)
n2f <- norm2Filter(filterId="myNorm2Filter", x=c("FSC-H", "SSC-H"),
scale.factor=2)

## Filtering using norm2Filter
fres <- filter(dat, n2f)
fres
summary(fres)

## The result of norm2 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>
```

normalization-class

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.

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.

... other arguments: see normalize-methodsfor details.

### **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

94 normalization-class

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.

### **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
    workFlow.
```

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 95

```
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.

96 normalizeView-class

#### **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

#### 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.

parameterFilter-class 97

#### **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.
```

#### **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.

98 parameters

#### **Slots**

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

### **Extends**

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

### Methods

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

#### Author(s)

B. Ellis

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**

object Object 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.

parameters-class 99

### Author(s)

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

# **Examples**

```
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.

100 polygonGate-class

#### **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.
```

#### 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.

polygonGate-class 101

#### 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.
```

#### **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 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 = "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",
package="flowCore"))

## Defining the gate
sqrcut <- matrix(c(300,300,600,600,50,300,300,50),ncol=2,nrow=4)
colnames(sqrcut) <- c("FSC-H","SSC-H")
pg <- polygonGate(filterId="nonDebris", boundaries= sqrcut)
pg</pre>
```

102 polytopeGate-class

```
## Filtering using polygonGates
fres <- filter(dat, pg)
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
split(dat, fres)</pre>
```

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.

quadGate-class 103

#### 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

quadGate-class

Class "quadGate"

# **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.

104 quadGate-class

#### **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.
```

### **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",
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>
```

quadratic-class 105

```
fres <- filter(dat, qg)
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+")))</pre>
```

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)

106 quadraticTransform

#### 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

# **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>
```

quadraticTransform

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
```

randomFilterResult-class 107

#### **Examples**

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

```
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**

 $Objects\ can\ be\ created\ by\ calls\ to\ the\ constructor\ ratio (parameter 1, parameter 2, transformation Id)$ 

.

108 ratiotGml2-class

### **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
```

#### **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

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

ratiotGml2-class 109

## **Description**

Ratio transformation as parameterized in Gating-ML 2.0 is defined by the following function:

bound(f, boundMin, boundMax) = max(min(f, boundMax), boundMin))

where

$$f(p1, p2, A, B, C) = A * (p1 - B)/(p2 - C)$$

If a boundary is defined by the boundMin and/or boundMax parameters, then the result of this transformation is restricted to the [boundMin,boundMax] interval. Specifically, should the result of the f function be less than boundMin, then let the result of this transformation be boundMin. Analogically, should the result of the f function be more than boundMax, then let the result of this transformation be boundMax. The boundMin parameter shall not be greater than the boundMax parameter.

# **Objects from the Class**

Objects can be created by calls to the constructor

ratiotGml2(p1, p2, A, B, C, transformationId, boundMin, boundMax)

#### **Slots**

.Data: Object of class function ~~

numerator: Object of class "transformation" -flow parameter to be used as numerator in the transformation function

denominator: Object of class "transformation" -flow parameter to be used as denominator in the transformation function

pA: Object of class numeric constant A

pB: Object of class numeric constant B

pC: Object of class numeric constant C

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

boundMin: Object of class numeric -lower bound of the transformation, default -Inf

boundMax: Object of class numeric -upper bound of the transformation, default Inf

### **Extends**

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

## Methods

No methods defined with class "ratiotGml2" in the signature.

#### Note

The ratiotGml2 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)

Spidlen, J.

110 read.FCS

#### References

Gating-ML 2.0: International Society for Advancement of Cytometry (ISAC) standard for representing gating descriptions in flow cytometry. http://flowcyt.sourceforge.net/gating/20141009.pdf

### See Also

```
ratio, transform-class, transform
```

### **Examples**

read.FCS

Read an FCS file

### Description

Check validity and Read Data File Standard for Flow Cytometry

# Usage

# **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), linearize-with-PnG-scaling, or scale. The linearize transformation applies the appropriate power transform to the data. The linearize-with-PnG-scalit transformation applies the appropriate power transform for parameters stored on log scale, and also a linear scaling transformation based on the 'gain' (FCS \PnG keywords) for parameters stored on a linear scale. 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(or NULL) corresponds to no transformation. Also when the transformation keyword of the FCS header is set to "custom" or "applied", no transformation will be used.

read.FCS 111

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.

invert.pattern logical. By default, FALSE. If TRUE, inverts the regular expression specified in

column.pattern. This is useful for indicating the channel names that we do not want to read. If column.pattern is set to NULL, this argument is ignored.

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

ncdf Deprecated. Please use 'ncdfFlow' package for cdf based storage.

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.

truncate\_max\_range

logical type. Default is TRUE, can be optionally turned off to avoid truncating

the extreme positive value to the instrument measurement range .i.e.'\$PnR'.

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 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.

channel\_alias a data.frame used to provide the alias of the channels to standardize and solve

the discrepancy across FCS files.It is expected to contain 'alias' and 'channels' column. Each row/entry specifies the common alias name for a collection of

channels (comma separated). See examples for details.

ignore.text.offset: whether to ignore the keyword values in TEXT segment when they don't agree with the HEADER. Default is FALSE, which throws the error

they don't agree with the HEADER. Default is FALSE, which throws the error when such discrepancy is found. User can turn it on to ignore TEXT segment when he is sure of the accuracy of HEADER so that the file still can be read.

**Details** 

The function is FCS file determines whether its arguments are valid FCS files.

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.

112 read.FCS

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 which.lines 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. It is mainly used when there is not enough memory to read one single FCS (which probably will not happen). It will probably take more time than reading the entire FCS (due to the multiple disk IO).

### 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

```
## a sample file
fcsFile <- system.file("extdata", "0877408774.B08", package="flowCore")</pre>
## read file and linearize values
samp <- read.FCS(fcsFile, transformation="linearize")</pre>
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")</pre>
exprs(subset)
## Read in a random sample of 100 lines
subset <- read.FCS(fcsFile, which.lines=100, transformation="linearize")</pre>
nrow(subset)
#manually supply the alias vs channel options mapping as a data.frame
map <- data.frame(alias = c("A", "B")</pre>
                   , channels = c("FL2", "FL4")
fr <- read.FCS(fcsFile, channel_alias = map)</pre>
fr
```

read.FCSheader 113

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.

# Usage

```
read.FCSheader(files, path=".", keyword=NULL, emptyValue = TRUE)
```

### **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.

emptyValue see link[flowCore]{read.FCS}

### **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}
```

```
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>
```

114 read.flowSet

read.flowSet	Read a set of FCS files	
--------------	-------------------------	--

## **Description**

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

### **Usage**

```
read.flowSet(files=NULL, path=".", pattern=NULL, phenoData,
             descriptions, name.keyword, alter.names=FALSE,
             transformation = "linearize", which.lines=NULL,
            column.pattern = NULL, invert.pattern = FALSE, decades=0, sep="\t",
             as.is=TRUE, name, ncdf=FALSE, dataset=NULL, min.limit=NULL,
             truncate_max_range = TRUE, emptyValue=TRUE,
             ignore.text.offset = FALSE, channel_alias = NULL, ...)
```

### **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.

An object of class AnnotatedDataFrame, character or a list of values to be phenoData

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. invert.pattern see read.FCS for details. decades see read. FCS for details.

sep Separator character that gets passed on to read. AnnotatedDataFrame.

Logical that gets passed on to read. AnnotatedDataFrame. This controls the as.is

automatic coercion of characters to factors in the phenoDataslot.

An optional character scalar used as name of the object. name

ncdf Deprecated. Please refer to 'ncdfFlow' package for cdf based storage.

dataset see read. FCS for details. see read. FCS for details. min.limit truncate.max.range

see link[flowCore]{read.FCS} for details.

see read. FCS for details. emptyValue

rectangleGate-class 115

#### **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. row names of the underlying data.frame). Also column name will be generated based on sample names if it is not there. This column is mainly used by visualization methods in flowViz. 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.

# 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.

116 rectangleGate-class

### 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.

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.

rectangleGate-class 117

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
    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 = "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

## 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.

```
## Loading example data
dat <- read.FCS(system.file("extdata","0877408774.B08",
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),
"SSC-H"=c(0, 400)))
mat <- matrix(c(200, 600, 0, 400), ncol=2, dimnames=list(c("min", "max"),
c("FSC-H", "SSC-H")))
rg <- rectangleGate(filterId="myRectGate", .gate=mat)

## Filtering using rectangleGates
fres <- filter(dat, rg)
fres</pre>
```

118 sampleFilter-class

```
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"]

##2d rectangleGate can be coerced to polygonGate
as(rg, "polygonGate")</pre>
```

sampleFilter-class

Class "sampleFilter"

## **Description**

This non-parameter filter selects a number of events from the primary flowFrame.

# Usage

```
sampleFilter(size, filterId="defaultSampleFilter")
```

## **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.
```

sampleFilter-class 119

### **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)
sf

## 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>
```

120 scaleTransform

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
```

```
samp <- read.FCS(system.file("extdata",
    "0877408774.B08", package="flowCore"))
scaleTrans <- scaleTransform(transformationId="Truncate-transformation", a=1, b=10^4)
dataTransform <- transform(samp, transformList('FSC-H', scaleTrans))</pre>
```

setOperationFilter-class 121

```
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

```
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.

## **Extends**

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

122 sinht-class

#### 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)

### Author(s)

Gopalakrishnan N, F.Hahne

spillover 123

#### 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.

When matching stain channels in x with the compensation controls, we provide a few options. If ordered, we assume the ordering of the channels in the flowSet object is the same as the ordering of the compensation-control samples. IF regexpr, we use a regular expression to match the channel names with the filenames of the compensation controls. By default, we must "guess" based on the largest statistic for the compensation control (i.e., the row).

# 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.
stain_match	Determines how the stain channels are matched with the compensation controls. See details.
useNormFilt	Logical Indicating whether to apply a norm2Filter first before computing the spillover
pregate	logical. Should we pregate each channel before computing the spillover matrix? By default, no.

124 split-methods

plot logical. Plots the kernel density for each channel when pregating. Displays the

gate used. If pregate is set to FALSE, this argument is ignored.

... Additional arguments passed to rangeGate.

### **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.

#### 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).

split-methods 125

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 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.

126 split-methods

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.
- 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

```
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]]</pre>
fres <- filter(samp, qGate)</pre>
split(samp, qGate)
## return a flowSet rather than a list
split(samp, fres, flowSet=TRUE)
## only keep one population
##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 127

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

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

$$log_{10}(c*parameter)*\frac{r}{d}$$
 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

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.

128 splitScaleTransform

#### 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)

### 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, transitionChan

# Arguments

r

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

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. squareroot-class 129

#### 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)

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, transformList('FSC-H', ssTransform))

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
```

Subset

### **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 "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.
```

subsetting-class 131

#### **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

134 subsetting View-class

### 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 subsettingView 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.

summarizeFilter-methods 135

#### **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 = "parameterFilter" see above
```

136 timeFilter-class

timeFilter-class Class "timeFilter"
-------------------------------------

# **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.

timeFilter-class 137

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.

```
## 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>
```

138 transform-class

```
## 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

```
cosTransform <- function(transformId, a=1, b=1){
  t = new("transform", .Data = function(x) cos(a*x+b))
  t@transformationId = transformId
  t
}</pre>
```

transformActionItem-class 139

```
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.

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.

 $\label{lem:constructor} A \ \text{transformActionItem object for the constructor.}$ 

## **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.

140 transformation-class

#### **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-class 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 141

### 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
```

142 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 143

## **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
```

```
new("transformMap", input="FSC-H", output="FSC-H", f=log)
```

144 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 145

#### **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")
```

146 unitytransform-class

truncateTransform	Create the definition of a truncate transformation function to be ap-
	plied 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**

transformationId

character string to identify the transformation

a 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,transformList('FSC-H', truncateTrans))</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).

validFilters 147

#### **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>
```

validFilters

Check if all filters in a filters matches same paramters

## **Description**

Check if all filters in a filters matches same paramters

## Usage

```
validFilters(flist)
```

## **Arguments**

flist a filters object

## Value

TRUE or FALSE

148 view-class

Class "view"
--------------

## **Description**

Class and method to capture the results of standard operations (called "views" here) in a flow cytometry workflow.

## Usage

## **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.

view-class 149

#### **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.
```

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")
```

150 workFlow-class

workFlow-class	Class "workFlow"(deprecated)

## **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 workFlow.
wf	Object of class workFlow.
n	The number of operations to undo.

#### 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.

workFlow-class 151

#### **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 = "missing
  "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 = "missing"): see above
- assign signature(x = "missing", value = "ANY", pos = "missing", envir = "workFlow", inherits = "missing"): same as above, but provide custom symbol for the assignment.
- assign signature(x = "character", value = "ANY", pos = "workFlow", envir = "missing", inherits =
   "missing"): see above
- [ signature(x = "workFlow", i = "ANY"): Cast a useful error message.
- [[ signature(x = "workFlow", i = "ANY"): Treat the workFlow object as a regular environment. 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 considered to be internal.
- get signature(x = "character", pos = "workFlow", envir = "missing", mode = "missing", inherits = "m
  Get an object identified by symbol x from the environment in the workFlow.
- get signature(x = "character", pos = "missing", envir = "workFlow", mode = "missing", inherits = "m
   see above
- ls signature(name = "workFlow", pos = "missing", envir = "missing", all.names = "missing", pattern
  List the content of the environment in the workFlow.
- ls signature(name = "workFlow", pos = "missing", envir = "missing", all.names = "missing", pattern
  see above

152 workFlow-class

```
mget signature(x = "character", envir = "workFlow", mode = "missing", ifnotfound = "missing", inher
         "missing"): Get multiple objects identified by the symbols in x from the environment in the
         workFlow.
    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
See Also
```

# Examples

showClass("view")

"view", "actionItem"

write.FCS 153

write.FCS Write an FCS file
-----------------------------

## Description

Write FCS file from a flowFrame

#### Usage

```
write.FCS(x, filename, what="numeric", delimiter = "|", endian="big")
```

# **Arguments**

x A flowFrame.

filename A character scalar giving the output file name.

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.

delimiter a single character to separate the FCS keyword/value pairs. Default is: "I" endian a character, either "little" or "big" (default), specifying the most significant or

#### **Details**

The function write.FCS creates FCS 3.0 standard file from an object of class flowFrame.

least significant byte is stored first in a 32 bit word.

For specifications of FCS 3.0 see <a href="http://www.isac-net.org">http://www.isac-net.org</a> 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}
```

## **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>
```

154 write.flowSet

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\_ 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.

## Author(s)

F. Hahne

#### See Also

```
link[flowCore]{write.FCS}
```

%on%

### **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>
```

# Index

I Silter method (Silter aless) 27	laniaalfiltamDaawlt alaaa 01
!, filter-method (filter-class), 37	logicalFilterResult-class, 81
*Topic IO	logicletGml2-class, 82
read FCShooden 112	logtGml2-class, 86
read.FCSheader, 113 read.flowSet, 114	manyFilterResult-class, 88
write.FCS, 153	<pre>multipleFilterResult-class, 90 norm2Filter-class, 91</pre>
write.flowSet, 154	normalization-class, 93
*Topic <b>classes</b>	normalizeActionItem-class, 95
actionItem-class, 5	normalizeView-class, 96
asinht-class, 7	parameterFilter-class, 97
asinhtGml2-class, 8	parameters-class, 99
boundaryFilter-class, 11	parameterTransform-class, 99
characterOrTransformation-class,	quadGate-class, 103
13	quadratic-class, 105
compensateActionItem-class, 14	randomFilterResult-class, 107
compensatedParameter-class, 16	ratio-class, 107
compensateView-class, 17	ratiotGml2-class, 108
compensation-class, 18	rectangleGate-class, 115
concreteFilter-class, 21	sampleFilter-class, 118
dg1polynomial-class, 22	setOperationFilter-class, 121
EHtrans-class, 24	singleParameterTransform-class,
exponential-class, 27	121
expressionFilter-class, 29	sinht-class, 122
fcReference-class, 31	splitscale-class, 127
filter-class, 37	squareroot-class, 129
filterList-class, 39	subsetting-class, 131
filterReference-class, 40	subsettingActionItem-class, 132
filterResult-class, 41	subsettingView-class, 134
filterResultList-class, 42	timeFilter-class, 136
filters-class, 43	transform-class, 138
filterSet-class,44	transformActionItem-class, 139
filterSummary-class, 45	transformation-class, 140
filterSummaryList-class, 47	transformFilter-class, 141
flowFrame-class, 48	transformList-class, 142
flowSet-class, 54	transformMap-class, 143
<pre>gateActionItem-class, 60</pre>	transformReference-class, 144
gateView-class, 62	transformView-class, 144
hyperlog-class, 66	unitytransform-class, 146
hyperlogtGml2-class, 67	view-class, 148
invsplitscale-class, 71	workFlow-class, 150
kmeansFilter-class, 74	*Topic datasets
lintGml2-class, 77	GvHD, 65
logarithm-class, $80$	*Topic <b>iteration</b>

fsApply, 59	==,filterResult,flowFrame-method
*Topic <b>manip</b>	(filterResult-class), 41
Subset, 130	==,flowFrame,filterResult-method
*Topic <b>methods</b>	(flowFrame-class), 48
%on%, 155	==,flowFrame,flowFrame-method
arcsinhTransform, 6	(flowFrame-class), 48
biexponentialTransform, 9	>,flowFrame,ANY-method
boundaryFilter-class, 11	(flowFrame-class), 48
coerce, 14	>=,flowFrame,ANY-method
compensation-class, 18	(flowFrame-class), 48
each_col, 23	[,filterResultList,ANY-method
ellipsoidGate-class, 25	(filterResultList-class), 42
expressionFilter-class, 29	[,filterSet,character-method
FCSTransTransform, 34	(filterSet-class), 44
filter, 35	[,flowFrame,ANY-method
filter-and-methods, 37	(flowFrame-class), 48
filter-in-methods, 38	[,flowFrame,filter-method
filterDetails-methods, 39	(flowFrame-class), 48
getIndexSort, 64	[,flowFrame,filterResult-method
identifier, 69	(flowFrame-class), 48
inverseLogicleTransform, 70	[,flowSet,ANY-method(flowSet-class),5
keyword-methods, 73	[,flowSet-method(flowSet-class),54
kmeansFilter-class, 74	[,multipleFilterResult,ANY-method
linearTransform, 76	(multipleFilterResult-class),
InTransform, 79	90
logicleTransform, 84	[,rectangleGate,ANY-method
logTransform, 87	(rectangleGate-class), 115
norm2Filter-class, 91	[,rectangleGate,character-method
normalization-class, 93	(rectangleGate-class), 115
parameters, 98	[,workFlow,ANY-method(workFlow-class).
polygonGate-class, 100	150
polytopeGate-class, 102	[[,filterResult,ANY-method
quadGate-class, 103	(filterResult-class), 41
quadraticTransform, 106	[[,filterResultList,ANY-method
rectangleGate-class, 115	(filterResultList-class), 42
sampleFilter-class, 118	[[,filterSet,character-method
scaleTransform, 120	(filterSet-class), 44
spillover, 123	[[,filterSummary,character-method
split-methods, 124	(filterSummary-class), 45
splitScaleTransform, 128	[[,filterSummary,numeric-method
subsetting-class, 131	(filterSummary-class), 45
summarizeFilter-methods, 135	[[,flowSet,ANY-method(flowSet-class),
timeFilter-class, 136	54
truncateTransform, 146	[[,flowSet-method(flowSet-class),54
*Topic package	[[,logicalFilterResult,ANY-method
flowCore-package, 4	(logicalFilterResult-class), 81
*,rectangleGate,rectangleGate-method	[[,manyFilterResult,ANY-method
(rectangleGate-class), 115	(manyFilterResult-class), 88
<pre>&lt;,flowFrame,ANY-method</pre>	[[,manyFilterResult-method
(flowFrame-class), 48	(manyFilterResult-class), 88
<=,flowFrame,ANY-method	[[,multipleFilterResult,ANY-method
(flowFrame-class), 48	(multipleFilterResult-class),
(1 10wi i dille C1033), 40	(martiple: free Nesart Class),

90	%in%,flowFrame,filterResult-method
[[,multipleFilterResult-method	(filter-in-methods), 38
<pre>(multipleFilterResult-class),</pre>	%in%,flowFrame,intersectFilter-method
90	(filter-in-methods), 38
[[,workFlow,ANY-method	%in%,flowFrame,kmeansFilter-method
(workFlow-class), 150	(filter-in-methods), 38
[[<-,filterSet,ANY,ANY,filterReference-metho	
(filterSet-class), 44	(filter-in-methods), 38
[[<-,filterSet,NULL,ANY,filter-method	%in%,flowFrame,polygonGate-method
(filterSet-class), 44	(filter-in-methods), 38
[[<-,filterSet,NULL,ANY,formula-method	*
	%in%,flowFrame,polytopeGate-method
(filterSet-class), 44	(filter-in-methods), 38
[[<-,filterSet,character,ANY,filter-method	%in%,flowFrame,quadGate-method
(filterSet-class), 44	(filter-in-methods), 38
[[<-,filterSet,character,ANY,formula-method	%in%,flowFrame,rectangleGate-method
(filterSet-class), 44	(filter-in-methods), 38
<pre>[[&lt;-,filterSet,missing,ANY,filter</pre>	%in%,flowFrame,sampleFilter-method
(filterSet-class), 44	(filter-in-methods), 38
<pre>[[&lt;-,filterSet,missing,ANY,filter-method</pre>	%in%,flowFrame,subsetFilter-method
(filterSet-class), 44	(filter-in-methods), 38
<pre>[[&lt;-,flowFrame-method(flowSet-class),</pre>	%in%,flowFrame,timeFilter-method
54	(filter-in-methods), 38
<pre>[[&lt;-,flowSet,ANY,ANY,flowFrame-method</pre>	%in%,flowFrame,transformFilter-method
(flowSet-class), 54	(filter-in-methods), 38
<pre>[[&lt;-,flowSet-method(flowSet-class), 54</pre>	%in%,flowFrame,unionFilter-method
<pre>\$,filterSummary-method</pre>	(filter-in-methods), 38
(filterSummary-class), 45	%in%-methods (filter-in-methods), 38
\$,flowSet-method(flowSet-class),54	%on%, ANY, flowSet-method (%on%), 155
\$, workFlow-method (workFlow-class), 150	%on%,filter,parameterTransform-method
\$.flowFrame (flowFrame-class), 48	(%on%), 155
%&% (filter-and-methods), 37	%on%, filter, transform-method (%on%), 155
%&%, ANY-method (filter-and-methods), 37	%on%, filter, transformList-method
	(%on%), 155
%&%, filter, filter-method	
(filter-and-methods), 37	%on%,parameterTransform,flowFrame-method
%&%-methods (filter-and-methods), 37	(%on%), 155
%in% (filter-in-methods), 38	%on%, transform, flowFrame-method (%on%),
%in%,ANY,filterReference-method	155
(filter-in-methods), 38	%on%, transformList, flowFrame-method
%in%,ANY,filterResult-method	(%on%), 155
(filter-in-methods), 38	%on%,transformList,flowSet-method
%in%,ANY,manyFilterResult-method	(%on%), 155
(filter-in-methods), 38	%on%-methods (%on%), 155
%in%,ANY,multipleFilterResult-method	%subset%(filter-and-methods), 37
(filter-in-methods), 38	%subset%,ANY-method
%in%,flowFrame,boundaryFilter-method	(filter-and-methods), 37
(filter-in-methods), 38	%subset%,filter,filter-method
%in%,flowFrame,complementFilter-method	(filter-and-methods), 37
(filter-in-methods), 38	%subset%,filterSet,filter-method
%in%,flowFrame,ellipsoidGate-method	(filterSet-class), 44
(filter-in-methods), 38	%subset%,list,filter-method
%in%, flowFrame, expressionFilter-method	(filter-and-methods), 37
(filter-in-methods), 38	&,filter,filter-method

(filter-and-methods), 37	asinhtGml2(asinhtGml2-class), $8$
&,filter,list-method	asinhtGml2-class,8
(filter-and-methods), 37	assign (fcReference-class), 31
&,list,filter-method	assign, ANY, ANY, missing, workFlow, missing, missing-method
(filter-and-methods), 37	(workFlow-class), 150
%on%, <i>51</i> , <i>141</i> , 155	assign,character,ANY,workFlow,missing,missing,missing-r
	(workFlow-class), 150
action (view-class), 148	assign,fcReference,ANY,workFlow,missing,missing,missing
action, view-method (view-class), 148	(workFlow-class), 150
actionItem, <i>15–18</i> , <i>32</i> , <i>61–63</i> , <i>96</i> , <i>97</i> ,	assign, missing, ANY, missing, workFlow, missing, missing-me
133–135, 140, 145, 148, 149, 152	(workFlow-class), 150
actionItem-class, 5	assign, missing, ANY, workFlow, missing, missing, missing-me
actionItems, 15, 96, 133, 140, 145, 149, 150,	(workFlow-class), 150
152	, , , , , , , , , , , , , , , , , , , ,
actions (workFlow-class), 150	biexponentialTransform, 9, 84, 138
actions,workFlow-method	booleanGate,filter-class
(workFlow-class), 150	(filter-class), 37
add (workFlow-class), 150	<pre>boundaryFilter (boundaryFilter-class),</pre>
add,workFlow,character-method	11
(subsetting-class), 131	boundaryFilter-class, 11
add,workFlow,compensation-method	
(workFlow-class), 150	c,transformList-method
add,workFlow,concreteFilter-method	(transformList-class), 142
(workFlow-class), 150	call, filter-method (filter), 35
add,workFlow,filterList-method	<pre>cbind2,flowFrame,matrix-method</pre>
(workFlow-class), 150	(flowFrame-class), 48
add,workFlow,logical-method	cbind2,flowFrame,numeric-method
(subsetting-class), 131	(flowFrame-class), 48
add,workFlow,normalization-method	char2ExpressionFilter
(normalization-class), 93	(expressionFilter-class), 29
add,workFlow,numeric-method	character, filter-method (filter), 35
(subsetting-class), 131	characterOrTransformation, 7, 9, 16, 22,
add,workFlow,subsetting-method	24, 28, 66, 68, 72, 78, 80, 83, 86,
(subsetting-class), 131	105, 108, 109, 121, 122, 127, 130,
add,workFlow,transformList-method	140, 144, 147
(workFlow-class), 150	characterOrTransformation-class, 13
alias (view-class), 148	checkOffset, 13
alias,actionItem-method	cleanup (read. FCS), 110
(actionItem-class), 5	coerce, 14
alias,environment-method	coerce, call, filter-method (coerce), 14
(workFlow-class), 150	coerce, character, filter-method
alias, view-method (view-class), 148	(coerce), 14
alias,workFlow-method(workFlow-class),	coerce,complementFilter,call-method
150	(coerce), 14
AnnotatedDataFrame, 49, 50, 54, 55, 98, 115	coerce,complementFilter,logical-method
AnnotatedDataFrames, $50$	(coerce), 14
apply, 23, 60	coerce,ellipsoidGate,polygonGate-method
arcsinhTransform, 6, 138	(coerce), 14
as.data.frame.manyFilterResult	coerce, environment, flowSet-method
(manyFilterResult-class), 88	(coerce), 14
asinht, 9	coerce,factor,filterResult-method
asinht (asinht-class), 7	(coerce), 14
asinht-class, 7	coerce, filter, call-method (coerce), 14

<pre>coerce, filter, logical-method (coerce),</pre>	(coerce), 14
14	<pre>coerce, ratio, character-method (coerce),</pre>
coerce,filterReference,call-method	14
(coerce), 14	<pre>coerce,rectangleGate,polygonGate-method</pre>
<pre>coerce,filterReference,concreteFilter-method</pre>	(coerce), 14
(coerce), 14	coerce, subsetFilter, call-method
coerce, filterResult, logical-method	(coerce), 14
(coerce), 14	<pre>coerce, subsetFilter, logical-method</pre>
coerce, filterResultList, list-method	(coerce), 14
(coerce), 14	coerce, transform, character-method
<pre>coerce, filterSet, list-method (coerce),</pre>	(coerce), 14
14	coerce, unionFilter, call-method
coerce, filterSummary, data.frame-method	(coerce), 14
(filterSummary-class), 45	coerce,unionFilter,logical-method
coerce, flowFrame, filterSet-method	(coerce), 14
(coerce), 14	coerce, unitytransform, character-method
coerce, flowFrame, flowSet-method	(coerce), 14
(coerce), 14	colnames, flowFrame-method
coerce,flowSet,flowFrame-method	(flowFrame-class), 48
(coerce), 14	colnames, flowSet-method
coerce, flowSet, list-method (coerce), 14	(flowSet-class), 54
coerce, formula, filter-method (coerce),	colnames, transformList-method
14	(transformList-class), 142
coerce,gateView,filterResult-method	colnames<- (flowFrame-class), 48
(coerce), 14	colnames<-,flowFrame-method
coerce,intersectFiler,call-method	(flowFrame-class), 48
(coerce), 14	colnames<-,flowSet-method
coerce,intersectFilter,call-method	(flowSet-class), 54
(filter-and-methods), 37	
	compensate, 123, 124
coerce,intersectFilter,logical-method (coerce), 14	compensate (compensation-class), 18
	compensate, flowFrame, compensation-method
coerce, list, filterResultList-method	(flowFrame-class), 48
(coerce), 14	compensate, flowFrame, data. frame-method
coerce, list, filterSet-method (coerce),	(flowFrame-class), 48
14	compensate, flowFrame, matrix-method
coerce, list, flowSet-method (coerce), 14	(flowFrame-class), 48
coerce, list, transformList-method	compensate, flowSet, ANY-method
(coerce), 14	(flowSet-class), 54
coerce, logical, filterResult-method	compensate, flowSet, data.frame-method
(coerce), 14	(flowSet-class), 54
coerce,logicalFilterResult,logical-method	compensate, flowSet, list-method
(coerce), 14	(flowSet-class), 54
coerce, matrix, filterResult-method	compensateActionItem, <i>5</i> , <i>62</i> , <i>96</i> , <i>134</i> , <i>140</i> ,
(coerce), 14	151
coerce, name, filter-method (coerce), 14	compensateActionItem
coerce, nullParameter, character-method	<pre>(compensateActionItem-class),</pre>
(coerce), 14	14
coerce, numeric, filterResult-method	compensateActionItem-class, 14
(coerce), 14	compensatedParameter
coerce, parameters, character-method	$({\tt compensatedParameter-class}),$
(coerce), 14	16
coerce,randomFilterResult,logical-method	compensatedParameter-class, 16

compensateView, 63, 97, 135, 145, 148, 149,	estimateMedianLogicle, 27
151	eval,asinht,missing-method
<pre>compensateView (compensateView-class),</pre>	(asinht-class), 7
17	eval,asinhtGml2,missing-method
compensateView-class, 17	(asinhtGml2-class), 8
compensation, 15, 18, 32, 52, 151	$\verb eval,compensatedParameter,missing-method \\$
compensation (compensation-class), 18	<pre>(compensatedParameter-class),</pre>
compensation-class, 18	16
complementFilter-class	eval,dg1polynomial,missing-method
(setOperationFilter-class), 121	(dg1polynomial-class), 22
concreteFilter, 11, 25, 29, 42, 75, 92, 98,	eval,EHtrans,missing-method
101, 104, 116, 118, 136	(EHtrans-class), 24
concreteFilter-class, 21	eval,exponential,missing-method
cov.rob, 92	(exponential-class), 27
CovMcd, 92	eval,filterReference,missing-method
Data (view-class) 149	(filterReference-class), 40
Data (view-class), 148	eval,hyperlog,missing-method
Data, view-method (view-class), 148 data.frames, 49	(hyperlog-class), 66
decisionTreeGate, filter-class	eval,hyperlogtGml2,missing-method
(filter-class), 37	(hyperlogtGml2-class), 67
description, 73, 74	eval,invsplitscale,missing-method
description (flowFrame-class), 48	(invsplitscale-class), 71
description, flowFrame-method	eval,lintGml2,missing-method
(flowFrame-class), 48	(lintGml2-class), 77
description<-,flowFrame,ANY-method	eval,logarithm,missing-method
(flowFrame-class), 48	(logarithm-class), $80$
description<-,flowFrame,list-method	eval,logicletGml2,missing-method
(flowFrame-class), 48	(logicletGml2-class), 82
dg1polynomial (dg1polynomial-class), 22	eval,logtGml2,missing-method
dg1polynomial-class, 22	(logtGml2-class), 86
dim (flowFrame-class), 48	eval,quadratic,missing-method
dim, flowFrame-method (flowFrame-class),	(quadratic-class), 105
48	eval,ratio,missing-method
dir, 114	(ratio-class), 107
do.call, 94	eval,ratiotGml2,missing-method
	(ratiotGml2-class), 108
each_col, 23, 51	eval, sinht, missing-method
each_col, flowFrame-method (each_col), 23	(sinht-class), 122
each_col-methods (each_col), 23	eval,splitscale,missing-method
each_row(each_col), 23	(splitscale-class), 127
each_row,flowFrame-method(each_col),23	eval,squareroot,missing-method
each_row-methods (each_col), 23	(squareroot-class), 129
EHtrans (EHtrans-class), 24	eval,transformReference,missing-method
EHtrans-class, 24	(transformReference-class), 144
ellipsoidGate, 25, 101, 117	eval,unitytransform,missing-method
ellipsoidGate (ellipsoidGate-class), 25	(unitytransform-class), 146
ellipsoidGate,filter-class	exponential (exponential-class), 27
(filter-class), 37	exponential-class, 27
ellipsoidGate-class, 25	expressionFilter, 29
environment, 55, 57	expressionFilter
estimateLogicle, 35, 85	(expressionFilter-class), 29
estimateLogicle(logicleTransform), 84	expressionFilter-class, 29

exprs(flowFrame-class),48	fcSubsettingReference
exprs,flowFrame-method	(fcReference-class), 31
(flowFrame-class),48	fcSubsettingReference-class
exprs<-(flowFrame-class),48	(fcReference-class), 31
exprs<-,flowFrame,ANY-method	fcTransformReference, 33
(flowFrame-class), 48	fcTransformReference
exprs<-,flowFrame,matrix-method	(fcReference-class), 31
(flowFrame-class), 48	fcTransformReference-class
	(fcReference-class), 31
fcActionReference, 33	fcTreeReference, 33
fcActionReference (fcReference-class),	fcTreeReference (fcReference-class), 31
31	fcTreeReference-class
fcActionReference-class	(fcReference-class), 31
(fcReference-class), 31	fcViewReference, 33
fcAliasReference, 33	fcViewReference (fcReference-class), 31
fcAliasReference (fcReference-class), 31	fcViewReference-class
fcAliasReference-class	(fcReference-class), 31
(fcReference-class), 31	featureNames (flowFrame-class), 48
fcCompensateReference, 33	featureNames, flowFrame-method
fcCompensateReference	(flowFrame-class), 48
(fcReference-class), 31	filerList, 43
fcCompensateReference-class	filter, 11, 12, 21, 25, 26, 29, 30, 32, 35,
(fcReference-class), 31	36–44, 49, 51, 56, 57, 61, 63, 69, 70
fcDataReference, 33	75, 76, 81, 88, 90–92, 98, 100–104,
fcDataReference (fcReference-class), 31	107, 115–119, 121, 125, 131, 136,
fcDataReference-class	137, 141, 151
(fcReference-class), 31	filter, filter-method (filter-class), 37
fcFilterReference, 33	filter,flowFrame,filter-method
fcFilterReference (fcReference-class),	(filter), 35
31	filter,flowFrame,filterSet-method
fcFilterReference-class	(filter), 35
(fcReference-class), 31	filter, flowFrame, norm2Filter (filter),
fcFilterResultReference, 33	35
fcFilterResultReference	<pre>filter,flowFrame,polygonGate(filter),</pre>
(fcReference-class), 31	35
fcFilterResultReference-class	filter,flowFrame,rectangleGate
(fcReference-class), 31	(filter), 35
fcNormalizationReference, 33	filter, flowFrame-method (filter), 35
fcNormalizationReference	filter, flowSet, filter-method (filter),
(fcReference-class), 31	35
fcNormalizationReference-class	filter,flowSet,filterList-method
(fcReference-class), 31	(filter), 35
fcNullReference (fcReference-class), 31	filter,flowSet,filterSet-method
fcNullReference-class	(filter), 35
(fcReference-class), 31	filter, flowSet, list-method (filter), 35
fcReference, 32, 33, 151	filter-and-methods, 37
fcReference (fcReference-class), 31	filter-class, 37
fcReference-class, 31	filter-in-methods, 38
FCSTransTransform, 34	filter-on-methods (%on%), 155
fcStructureReference, 32, 33	filterDetails (filterDetails-methods),
fcStructureReference-class	39
(fcReference-class), 31	filterDetails,filterResult,ANY-method
( . 55. 5. 555 61455), 51	

(filterDetails-methods), 39	30, 32, 36, 37, 46, 48, 54–57, 60, 69,
filterDetails,filterResult,missing-method	70, 73, 75, 76, 88, 91, 92, 98, 101,
(filterDetails-methods), 39	103, 104, 112, 114, 116–119,
filterDetails-methods, 39	124–126, 130, 131, 136, 137, 141,
filterDetails<-	142, 150, 153
(filterDetails-methods), 39	flowFrame (flowFrame-class), 48
filterDetails<-,filterResult,character,ANY-m	efthowFrame-class,48
(filterDetails-methods), 39	flowFrames, 42, 75
filterDetails<-,filterResult,character,filte	rfinews to 4, 12, 19, 32, 36, 42, 51, 52, 60, 74,
(filterDetails-methods), 39	76, 92–94, 103, 104, 114, 115,
filterDetails<-,filterResult,character,setOp	
(filterDetails-methods), 39	flowSet (flowSet-class), 54
filtergate, filter-class (filter-class),	flowSet-class, 54
37	flowSets, 19, 47
filterList, 44, 151	flowViz, 26, 50, 76, 92, 101, 104, 117, 137
filterList (filterList-class), 39	formula, filter-method (filter), 35
filterList-class, 39	fsApply, 59, 131
filterReference, 21, 70	fsApply,flowSet,ANY(fsApply),59
filterReference	<pre>fsApply,flowSet-method(flowSet-class),</pre>
(filterReference-class), 40	54
filterReference, environment, character-method	function, 100
(filterReference-class), 40	
filterReference, filterSet, character-method	gate (gateActionItem-class), 60
(filterSet-class), 44	<pre>gate,gateActionItem-method</pre>
filterReference-class, 40	(gateActionItem-class), 60
filterResult, 11, 32, 36, 38, 42, 45–49, 51,	gateActionItem, 5, 16, 96, 134, 140, 151
56, 57, 61–63, 69, 70, 75, 81, 88–91,	<pre>gateActionItem(gateActionItem-class),</pre>
107, 125, 126, 131, 135	60
filterResult (filterResult-class), 41	<pre>gateActionItem-class, 60</pre>
	gateView, 18, 97, 135, 145, 148, 149, 151
filterResult-class, 41	<pre>gateView(gateView-class), 62</pre>
filterResultList, <i>36, 47, 48</i> filterResultList	gateView-class, 62
	get,character,missing,workFlow-method
(filterResultList-class), 42	(workFlow-class), 150
filterResultList-class, 42	<pre>get,character,workFlow,missing-method</pre>
filterResults, 63	(workFlow-class), 150
filters (filters-class), 43	${\tt get,fcNullReference,missing,missing-method}$
filters-class, 43	(fcReference-class), 31
filterSet, 40, 41, 44, 45, 56, 88, 125	<pre>get,fcReference,missing,missing-method</pre>
filterSet (filterSet-class), 44	(fcReference-class), 31
filterSet-class, 44	getChannelMarker, 64
filtersList (filters-class), 43	getIndexSort, 64
filtersList-class (filters-class), 43	getIndexSort,flowFrame-method
filterSummary, 48, 61	(getIndexSort), 64
filterSummary (filterSummary-class), 45	graphNEL, <i>32</i> , <i>151</i>
filterSummary-class, 45	GvHD, 65
filterSummaryList, 42, 46	
filterSummaryList	head,flowFrame-method
(filterSummaryList-class), 47	(flowFrame-class), 48
filterSummaryList-class,47	here, <i>37</i>
flowCore(flowCore-package),4	histogram, $50$
flowCore-package,4	hyperlog, 69
flowFrame, 4, 11, 12, 19, 20, 23, 25, 26, 29,	hyperlog (hyperlog-class), 66

hyperlog-class, 66	identifier<-,subsetting,character-method
hyperlogtGml2(hyperlogtGml2-class), 67	(subsetting-class), 131
hyperlogtGml2-class, 67	<pre>identifier&lt;-,transformList,character-method</pre>
	(transformList-class), 142
identifier, <i>51</i> , 69	initialize,dg1polynomial-method
identifier,actionItem-method	(dg1polynomial-class), 22
<pre>(actionItem-class), 5</pre>	initialize, flowFrame-method
identifier, compensation-method	(flowFrame-class), 48
(compensation-class), 18	initialize, parameterFilter-method
identifier,fcReference-method	(parameterFilter-class), 97
(fcReference-class), 31	initialize, ratio-method (ratio-class),
identifier, filter-method (identifier),	107
69	initialize,ratiotGml2-method
identifier,filterList-method	
(filterList-class), 39	(ratiotGml2-class), 108
identifier, filterReference-method	initialize, singleParameterTransform-method
	<pre>(singleParameterTransform-class),</pre>
(identifier), 69	121
identifier, filterResult-method	intersectFilter-class
(identifier), 69	(setOperationFilter-class), 121
identifier, filterSet, character-method	intersectFilter-method
(filterSet-class), 44	(filter-and-methods), 37
identifier,filterSet-method	inverseLogicleTransform, $35, 70, 85$
(filterSet-class), 44	invsplitscale (invsplitscale-class), 71
identifier,flowFrame-method	invsplitscale-class, 71
(identifier), 69	isFCSfile (read.FCS), 110
identifier,flowSet-method	isNull (fcReference-class), 31
(flowSet-class), 54	isNull, fcReference-method
identifier, normalization-method	(fcReference-class), 31
(normalization-class), 93	(
identifier, NULL-method (view-class), 148	journal (workFlow-class), 150
identifier, subsetting-method	journal, environment-method
(subsetting-class), 131	(workFlow-class), 150
identifier, transform-method	journal, workFlow-method
(identifier), 69	(workFlow-class), 150
identifier,transformList-method	(101111 1011 11435); 150
(transformList-class), 142	keyword, <i>50</i> , <i>56</i>
identifier, view-method (view-class), 148	keyword (keyword-methods), 73
identifier-methods (identifier), 69	keyword, flowFrame, character-method
identifier<- (identifier), 69	(keyword-methods), 73
	keyword, flowFrame, function-method
identifier<-,compensation,character-method	
(compensation-class), 18	(keyword-methods), 73
identifier<-,filter,character-method	keyword, flowFrame, list-method
(filter), 35	(keyword-methods), 73
identifier<-,filterList,character-method	keyword, flowFrame, missing-method
(filterList-class), 39	(keyword-methods), 73
identifier<-,flowFrame,ANY-method	keyword,flowSet,ANY-method
(identifier), 69	(keyword-methods), 73
identifier<-,flowFrame-method	keyword,flowSet,list-method
(identifier), 69	(keyword-methods), 73
identifier<-,flowSet,ANY-method	keyword-methods, 73
(flowSet-class), 54	keyword<- (keyword-methods), 73
<pre>identifier&lt;-,normalization,character-method</pre>	keyword<-,flowFrame,ANY-method
(normalization-class), 93	(keyword-methods), 73

keyword<-,flowFrame,character-method	make.names, ///
(keyword-methods), 73	manyFilterResult, 125
keyword<-,flowFrame,list-method	manyFilterResult
(keyword-methods), 73	(manyFilterResult-class), 88
<pre>keyword&lt;-,flowSet,list-method</pre>	manyFilterResult-class, 88
(keyword-methods), 73	markernames, 89
kmeansFilter, 37	markernames, flowFrame-method
kmeansFilter (kmeansFilter-class), 74	(markernames), 89
kmeansFilter(), 37	markernames, flowSet-method
kmeansFilter-class, 74	(markernames), 89
,	markernames <- (markernames), 89
length, filter-method (filter), 35	markernames<-,flowFrame-method
length, filterReference-method	(markernames), 89
(filterReference-class), 40	markernames<-,flowSet-method
length, filterSummary-method	(markernames), 89
(filterSummary-class), 45	mget, character, workFlow-method
length, flowSet-method (flowSet-class),	(workFlow-class), 150
54	multipleFilterResult, <i>41</i> , <i>42</i> , <i>46</i> , <i>48</i> , <i>75</i> ,
	103, 125
length, kmeansFilter-method	multipleFilterResult
(kmeansFilter-class), 74	<pre>(multipleFilterResult-class),</pre>
length,logicalFilterResult-method	90
(logicalFilterResult-class), 81	multipleFilterResult-class, 90
length, manyFilterResult-method	multipleFilterResults, 46, 62
(manyFilterResult-class), 88	, , , , , , , , , , , , , , , , , , ,
length, multipleFilterResult-method	name, filter-method (filter), 35
<pre>(multipleFilterResult-class),</pre>	names (flowFrame-class), 48
90	names,actionItem-method
linearTransform, 76, 78, 138	<pre>(actionItem-class), 5</pre>
lintGml2 (lintGml2-class), 77	names, filterResultList-method
lintGml2-class, 77	(filterResultList-class), 42
list, 40, 42, 44, 47, 99	names, filterSet-method
InTransform, 79, <i>138</i>	(filterSet-class), 44
logarithm(logarithm-class), 80	names, filterSummary-method
logarithm-class, $80$	(filterSummary-class), 45
logicalFilterResult, 41, 42, 46, 48, 91,	names, flowFrame-method
116, 125	(flowFrame-class), 48
logicalFilterResult	names,logicalFilterResult-method
(logicalFilterResult-class), 81	(logicalFilterResult-class), 81
logicalFilterResult-class, 81	names, manyFilterResult-method
logicalFilterResults, 62	(manyFilterResult-class), 88
logicletGml2, 69	names, multipleFilterResult-method
<pre>logicletGml2 (logicletGml2-class), 82</pre>	(multipleFilterResult-class),
logicletGml2-class, 82	90
logicleTransform, 35, 69, 71, 84, 84, 138	names, view-method (view-class), 148
logtGml2(logtGml2-class), 86	names, workFlow-method (workFlow-class),
logtGml2-class, 86	150
logTransform, <i>87</i> , <i>87</i> , <i>138</i>	names<-,multipleFilterResult,ANY-method
ls (workFlow-class), 150	(multipleFilterResult-class),
ls,workFlow,missing,missing,missing,character	•
(workFlow-class), 150	names<-,multipleFilterResult-method
ls,workFlow,missing,missing,missing,missing-	
(workFlow-class), 150	90

ncol, flowFrame-method	parameters, ratiotymiz-method
(flowFrame-class), 48	(ratiotGml2-class), 108
<pre>nodes,workFlow-method(workFlow-class),</pre>	parameters, setOperationFilter-method
150	(parameters), 98
norm2Filter, <i>37</i> , <i>38</i> , <i>91</i> , <i>123</i>	parameters, singleParameterTransform-method
norm2Filter (norm2Filter-class), 91	<pre>(singleParameterTransform-class),</pre>
norm2Filter,filter-class	121
(filter-class), 37	parameters, transform-method
norm2Filter-class, 91	(parameters), 98
normalization, <i>95</i> , <i>97</i> , <i>133</i>	parameters, transformReference-method
normalization (normalization-class), 93	(transformReference-class), 144
normalization-class, 93	parameters-class, 99
normalize (normalization-class), 93	parameters<- (parameters), 98
normalize, flowSet, normalization-method	parameters<-,dg1polynomial,character-method
(normalization-class), 93	(dg1polynomial-class), 22
normalizeActionItem	parameters<-,dg1polynomial,parameters-method
<pre>(normalizeActionItem-class), 95</pre>	(dg1polynomial-class), 22
normalizeActionItem-class, 95	parameters<-,dg1polynomial,transform-method
normalizeView, 18	(parameters), 98
normalizeView (normalizeView-class), 96	parameters<-,flowFrame,AnnotatedDataFrame-method
normalizeView-class, 96	(parameters), 98
nrow,flowFrame-method	parameters<-,parameterFilter,character-method
(flowFrame-class), 48	(parameters), 98
	parameters<-,parameterFilter,list-method
parameterFilter, 11, 21, 25, 37, 75, 92, 98,	(parameters), 98
101, 104, 116, 136	parameters<-,parameterFilter,transform-method
parameterFilter-class, 97	(parameters), 98
parameters, 50, 75, 98	parameters<-,singleParameterTransform,character-method
parameters, compensation-method	(parameters), 98
(compensation-class), 18	parameters<-,singleParameterTransform,transform-method
parameters, filter-method (parameters),	(parameters), 98
98	parameterTransform-class, 99
parameters, filterReference-method	parent (view-class), 148
(parameters), $98$	parent,actionItem-method
parameters, filterResult-method	(actionItem-class), 5
(parameters), $98$	
parameters, filterResultList-method	parent, NULL-method (view-class), 148
(filterResultList-class), 42	parent, view-method (view-class), 148
parameters,flowFrame,missing-method	pData, flowSet-method (flowSet-class), 54
(parameters), $98$	pData<-,flowSet,data.frame-method
parameters,flowFrame-method	(flowSet-class), 54
(parameters), 98	phenoData, flowSet-method
parameters,manyFilterResult-method	(flowSet-class), 54
(manyFilterResult-class), 88	phenoData<-,flowSet,ANY-method
parameters, normalization-method	(flowSet-class), 54
(normalization-class), 93	phenoData<-,flowSet,phenoData-method
parameters, nullParameter-method	(flowSet-class), 54
(parameters), $98$	plot, flowFrame, ANY-method
parameters,parameterFilter-method	(flowFrame-class), 48
(parameters), $98$	plot, flowFrame-method
parameters,parameterTransform-method	(flowFrame-class), 48
(parameters), $98$	plot,flowSet,ANY-method
parameters, ratio-method (parameters), 98	(flowSet-class), 54

plot, flowSet-method (flowSet-class), 54	<pre>rbind2,flowSet,missing(flowSet-class),</pre>
plot,workFlow,missing-method	54
(workFlow-class), 150	rbind2,flowSet,missing-method
polygonGate, 26, 101, 103, 117	(flowSet-class), 54
polygonGate (polygonGate-class), 100	read.AnnotatedDataFrame, 114, 115
polygonGate, filter-class	read.FCS, 49, 52, 110, 114, 115
(filter-class), 37	read.FCSheader, 113
polygonGate-class, 100	read.flowSet, 55, 57, 112, 114, 154
polytopeGate, 26, 101, 117	rectangleGate, 26, 101, 103, 116
polytopeGate (polytopeGate-class), 102	rectangleGate (rectangleGate-class), 115
polytopeGate-class, 102	rectangleGate(), 37
<pre>print,compensateActionItem-method</pre>	rectangleGate,filter-class
<pre>(compensateActionItem-class),</pre>	(filter-class), 37
14	rectangleGate-class, 115
print, filterSummary-method	Rm (fcReference-class), 31
(filterSummary-class), 45	Rm,actionItem,workFlow,character-method
<pre>print,gateActionItem-method</pre>	<pre>(actionItem-class), 5</pre>
(gateActionItem-class), 60	Rm,character,workFlow,character-method
<pre>print,normalizeActionItem-method</pre>	(workFlow-class), 150
<pre>(normalizeActionItem-class), 95</pre>	Rm,compensateActionItem,workFlow,character-method
<pre>print,subsettingActionItem-method</pre>	<pre>(compensateActionItem-class),</pre>
<pre>(subsettingActionItem-class),</pre>	14
132	Rm,compensateView,workFlow,character-method
<pre>print,transformActionItem-method</pre>	(compensateView-class), 17
<pre>(transformActionItem-class),</pre>	Rm,fcNullReference,missing,character-method
139	(fcReference-class), 31
print, view-method (view-class), 148	Rm,fcReference,missing,character-method
	(fcReference-class), 31
quadGate (quadGate-class), 103	Rm, fcReference, workFlow, character-method
quadGate-class, 103	(fcReference-class), 31
quadratic (quadratic-class), 105	${\tt Rm,gateActionItem,workFlow,character-method}$
quadratic-class, 105	(gateActionItem-class), $60$
quadraticTransform, 106, 138	Rm,gateView,workFlow,character-method
	(gateView-class), 62
randomFilterResult, 41, 42	${\tt Rm, normalize Action Item, work Flow, character-method}$
randomFilterResult-class, 107	<pre>(normalizeActionItem-class), 95</pre>
range (flowFrame-class), 48	${\tt Rm, normalize View, work Flow, character-method}$
range, flowFrame-method	(normalizeView-class), 96
(flowFrame-class), 48	${\tt Rm, subsettingActionItem, workFlow, character-method}$
rangeGate, 124	<pre>(subsettingActionItem-class),</pre>
ratio, 110	132
ratio (ratio-class), 107	Rm, subsettingView, workFlow, character-method
ratio-class, 107	(subsettingView-class), 134
ratiotGml2 (ratiotGml2-class), 108	${\tt Rm, transformActionItem, workFlow, character-method}$
ratiotGml2-class, 108	<pre>(transformActionItem-class),</pre>
rbind2,flowFrame,flowSet-method	139
(flowSet-class), 54	${\tt Rm, transform View, work Flow, character-method}$
rbind2, flowSet, flowFrame-method	(transformView-class), 144
(flowSet-class), 54	Rm,view,workFlow,character-method
rbind2, flowSet, flowSet, missing-method	(view-class), 148
(flowSet-class), 54	3
rbind2, flowSet, flowSet-method	sampleFilter(sampleFilter-class), 118
(flowSet-class), 54	sampleFilter-class, 118

sampleNames,flowSet-method	<pre>(multipleFilterResult-class),</pre>
(flowSet-class), 54	90
<pre>sampleNames&lt;-,flowSet,ANY-method</pre>	show,norm2Filter-method
(flowSet-class), 54	(norm2Filter-class), 91
sapply, 56, 60	show,normalizeActionItem-method
scaleTransform, 120	(normalizeActionItem-class), 95
setOperationFilter-class, 121	show,polygonGate-method
show, boundaryFilter-method	(polygonGate-class), 100
(boundaryFilter-class), 11	show,polytopeGate-method
show, compensateActionItem-method	(polytopeGate-class), 102
(compensateActionItem-class),	<pre>show, quadGate-method (quadGate-class),</pre>
14	103
show, compensation-method	show,rectangleGate-method
(compensation-class), 18	(rectangleGate-class), 115
show, complementFilter-method	show, sampleFilter-method
(setOperationFilter-class), 121	(sampleFilter-class), 118
show,ellipsoidGate-method	show,subsetFilter-method
(ellipsoidGate-class), 25	(setOperationFilter-class), 121
show, expressionFilter-method	show, subsetting-method
(expressionFilter-class), 29	(subsetting-class), 131
show, fcNullReference-method	show, subsettingActionItem-method
(fcReference-class), 31	(subsettingActionItem-class),
show, fcReference-method	132
(fcReference-class), 31	show,timeFilter-method
show, filter-method (filter), 35	(timeFilter-class), 136
show, filterList-method	show, transform-method
(filterList-class), 39	(transform-class), 138
show, filterReference-method	show,transformActionItem-method
(filterReference-class), 40	(transformActionItem-class),
show, filterResult-method	139
(filterResult-class), 41	show,transformFilter-method
show, filterResultList-method	(transformFilter-class), 141
(filterResultList-class), 42	show, transformMap-method
show, filters-method (filters-class), 43	(transformMap-class), 143
show, filterSet-method	show, unionFilter-method
(filterSet-class), 44	(setOperationFilter-class), 121
show, filtersList-method	show, unitytransform-method
(filters-class), 43	(unitytransform-class), 146
show, filterSummary-method	show, view-method (view-class), 148
(filterSummary-class), 45	show, workFlow-method (workFlow-class),
show, flowFrame-method	150
(flowFrame-class), 48	singleParameterTransform, 7, 9, 24, 28, 66,
show, flowSet-method (flowSet-class), 54	68, 72, 78, 80, 83, 86, 105, 122, 127,
show, gateActionItem-method	130
(gateActionItem-class), 60	singleParameterTransform-class, 121
show, intersectFilter-method	sinht (sinht-class), 122
	sinht-class, 122
(setOperationFilter-class), 121	
show, kmeansFilter-method	smoothScatter, 50
(kmeansFilter-class), 74	sort, filterSet-method
show, manyFilterResult-method	(filterSet-class), 44
(manyFilterResult-class), 88	spillover, 19, 20, 123
show, multipleFilterResult-method	spillover,flowFrame-method

(flowFrame-class), 48	Subset,flowFrame,logical-method
spillover,flowSet-method(spillover),	(Subset), 130
123	Subset, flowFrame-method (Subset), 130
split, 26, 30, 42, 51, 56, 75, 76, 92, 101, 104,	Subset, flowSet, ANY (Subset), 130
117, 119, 131, 137	Subset, flowSet, ANY-method (Subset), 130
split(split-methods), 124	Subset, flowSet, filterResultList-method
split,flowFrame,ANY-method	(Subset), 130
(split-methods), 124	Subset, flowSet, list-method (Subset), 130
split,flowFrame,character-method	subsetFilter-class
(split-methods), 124	<pre>(setOperationFilter-class), 121</pre>
split,flowFrame,factor-method	subsetFilter-method
(split-methods), 124	(filter-and-methods), 37
split,flowFrame,filter-method	subsetting, <i>133</i> , <i>134</i>
(split-methods), 124	subsetting (subsetting-class), 131
split,flowFrame,filterSet-method	subsetting-class, 131
(split-methods), 124	subsettingActionItem
split,flowFrame,logicalFilterResult-method	(subsettingActionItem-class),
(split-methods), 124	132
split,flowFrame,manyFilterResult-method	subsettingActionItem-class, 132
(split-methods), 124	subsettingView (subsettingView-class),
split,flowFrame,multipleFilterResult-method	134
(split-methods), 124	subsettingView-class, 134
split,flowFrame,numeric-method	summarizeFilter
(split-methods), 124	(summarizeFilter-methods), 135
split,flowSet,ANY-method	summarizeFilter, filterResult, filter-method
(split-methods), 124	(summarizeFilter-methods), 135
split,flowSet,character-method	summarizeFilter, filterResult, filterReference-method
(split-methods), 124	(summarizeFilter-methods), 135
split,flowSet,factor-method	summarizeFilter, filterResult, parameterFilter-method
(split-methods), 124	(summarizeFilter-methods), 135
split,flowSet,filter-method	summarizeFilter, filterResult, subsetFilter-method
(split-methods), 124	(summarizeFilter-methods), 135
split,flowSet,filterResult-method	summarizeFilter,logicalFilterResult,norm2Filter-method
(split-methods), 124	(summarizeFilter-methods), 135
split,flowSet,filterResultList-method	summarizeFilter,logicalFilterResult,parameterFilter-met
(filterResultList-class), 42	(summarizeFilter-methods), 135
split,flowSet,list-method	summarizeFilter, multipleFilterResult, parameterFilter-me
(split-methods), 124	(summarizeFilter-methods), 135
split,flowSet,numeric-method	summarizeFilter-methods, 135
(split-methods), 124	·
split-methods, 124	summary, 37
splitscale (splitscale-class), 127	summary, filter-method (filter), 35
splitscale (splitscale-class), 127	summary, filterReference-method
splitScaleTransform, 128	(filterReference-class), 40
	summary, filterResult-method
splom, 50	(filterSummary-class), 45
squareroot (squareroot-class), 129	summary, filterResultList-method
squareroot-class, 129	(filterResultList-class), 42
Subset, 12, 26, 30, 36, 92, 101, 117, 119, 130,	summary, flowFrame-method
137	(flowFrame-class), 48
subset, 130, 131	<pre>summary,flowSet-method(flowSet-class),</pre>
Subset, flowFrame, filter-method	54
(Subset), 130	summary,gateActionItem-method

(gateActionItem-class), 60	transformFilter
summary,gateView-method	(transformFilter-class), 141
(gateView-class), 62	transformFilter-class, 141
summary,logicalFilterResult-method	transformList, 38, 141, 143
(logicalFilterResult-class), 81	transformList(transformList-class), 142
summary,manyFilterResult-method	transformList-class, 142
(manyFilterResult-class), 88	transformMap, 142
summary,multipleFilterResult-method	transformMap(transformMap-class), 143
<pre>(multipleFilterResult-class),</pre>	transformMap-class, 143
90	transformReference
summary,rectangleGate-method	(transformReference-class), 144
(rectangleGate-class), 115	transformReference-class, 144
summary,subsetFilter-method	transformReferences, 20
(setOperationFilter-class), 121	transforms, 20
summary,transform-method	transformView, 18, 63, 97, 135, 148, 149, 151
(transform-class), 138	transformView(transformView-class), 144
summary,workFlow-method	transformView-class, 144
(workFlow-class), 150	tree (workFlow-class), 150
	tree, environment-method
tail,flowFrame-method	(workFlow-class), 150
(flowFrame-class), 48	tree, workFlow-method (workFlow-class),
timeFilter, <i>136</i>	150
timeFilter(timeFilter-class), 136	truncateTransform, 146
timeFilter-class, 136	
timeLinePlot, <i>137</i>	undo (workFlow-class), 150
toTable(filterSummary-class),45	unionFilter-class
toTable,filterSummary-method	(setOperationFilter-class), 121
(filterSummary-class), 45	uniroot, 10
toTable,filterSummaryList-method	unitytransform (unitytransform-class),
(filterSummaryList-class), 47	146
transform, 6, 7, 9, 10, 16, 19, 20, 22, 24, 28,	unitytransform-class, 146
32, 37, 38, 51, 66, 68–70, 72, 75,	unity transform-class, 140
77–80, 83, 84, 86–88, 100, 105, 106,	
108–110, 120–122, 127, 129, 130,	validFilters, 147
139–147, 151	varLabels, flowSet-method
transform(transform-class), 138	(flowSet-class), 54
transform,flowFrame-method	varLabels<-,flowSet,ANY-method
(flowFrame-class), 48	(flowSet-class), 54
transform,flowSet-method	varLabels<-,flowSet-method
(flowSet-class), 54	(flowSet-class), 54
transform, missing-method	varMetadata, flowSet-method
(transform-class), 138	(flowSet-class), 54
transform-class, 138	varMetadata<-,flowSet,ANY-method
transformActionItem, 5, 16, 62, 96, 134, 151	(flowSet-class), 54
transformActionItem	vector, 99
<pre>(transformActionItem-class), 139</pre>	view, 5, 15, 16, 18, 32, 61–63, 95–97, 133–135, 139, 140, 145, 150, 152
transformActionItem-class, 139	view(view-class), 148
transformation, 7, 9, 16, 22, 24, 28, 66, 68,	view-class, 148
72, 75, 78, 80, 83, 86, 105, 108, 109,	views, <i>5</i> , <i>151</i> , <i>152</i>
121, 122, 127, 130, 144, 147	views (workFlow-class), 150
transformation-class, 140	<pre>views,workFlow-method(workFlow-class),</pre>
transformFilter, <i>143</i>	150