

Package ‘ncdfFlow’

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Title ncdfFlow: A package that provides ncdf based storage for flow cytometry data.

Version 2.10.30

Author Mike Jiang,Greg Finak,N. Gopalakrishnan

Description Provides netCDF storage based methods and functions for manipulation of flow cytometry data.

Maintainer Mike Jiang <wjiang2@fhcrc.org>

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Imports Biobase,flowCore,flowViz,methods,zlibbioc

Suggests testthat

License Artistic-2.0

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biocViews FlowCytometry

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as.flowSet	<i>convert from a ncdfFlowSet to a flowSet</i>
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Description

The main purpose of this API is to convert the archived data (stored in ncdfFlowSet) to flowSet when the speed is more concerned than memory efficiency. Although ncdfFlowSet is designed to minimize the disk-IO cost, so usually it is not necessary to do such coercion.

Usage

```
as.flowSet(from, top)
```

Arguments

from	a ncdfFlowSet
top	integer specifies a certain number of samples are evenly selected for the coercion. If this argument is missing, then coerce all the samples within the ncdfFlowSet. It is to be used with caution because it can incur the huge memory consumption given the flowSet is all-in-memory data structure.

Examples

```
data(GvHD)
nc1 <- ncdfFlowSet(GvHD[1:4])
fs <- as.flowSet(nc1)
```

clone.ncdfFlowSet	<i>Clone a ncdfFlowSet</i>
-------------------	----------------------------

Description

Create a new ncdfFlowSet object from an existing one

Usage

```
clone.ncdfFlowSet(ncfs, ncdfFile = NULL, isEmpty = TRUE, isNew = TRUE,
  dim = 2, compress = 0)
```

Arguments

ncfs	A ncdfFlowSet .
isNew	A logical scalar indicating whether the new cdf file should be created. If FALSE, the original cdf file is associated with the new ncdfFlowSet object.
ncdfFile	A character scalar giving the output file name. By default, It is NULL and the function will generate a random file name, potentially adding the .cdf suffix unless a file extension is already present. It is only valid when isNewNcFile=TRUE
isEmpty	A logical scalar indicating whether the raw data should also be copied.if FALSE, an empty cdf file is created with the same dimensions (sample*events*channels) as the original one.
dim	integer see details in read.ncdfFlowset .
compress	integer see details in read.ncdfFlowset .

Value

A ncdfFlowSet object

See Also

[read.ncdfFlowSet](#)

Examples

```
path<-system.file("extdata","compdata","data",package="flowCore")
files<-list.files(path,full.names=TRUE)[1:3]

#create ncdfFlowSet from fcs
nc1 <- read.ncdfFlowSet(files=files,ncdfFile="ncfsTest.nc",flowSetId="fs1",isWriteSlice= TRUE)

##clone the ncdfFlowSet object,by default the actual raw data is not added
nc2<-clone.ncdfFlowSet(nc1,"clone.nc")
nc2[[1]]

#add the actual raw data
fs1 <- read.flowSet(files=files)
nc2[[sampleNames(fs1)[1]]] <- fs1[[1]]
nc2[[1]]

#delete the cdf file associated with ncdfFlowSet before removing it from memory
unlink(nc2)
rm(nc2)

unlink(nc1)
rm(nc1)
```

getFileName	<i>get the cdf file name associated with ncdfFlowSet object</i>
-------------	---

Description

get the cdf file name associated with ncdfFlowSet object

Usage

```
getFileName(ncfs)
```

Arguments

ncfs	ncdfFlowSet
------	-------------

Value

character

getIndices,ncdfFlowSet,character-method	<i>extract the event indices of one or multiple samples from ncdfFlowSet</i>
---	--

Description

For internal use.

Usage

```
## S4 method for signature ncdfFlowSet,character
getIndices(obj, y)
```

Arguments

obj	ncdfFlowSet object
y	character sample name

Value

a logical vector.

Examples

```

data(GvHD)
nc <- ncdfFlowSet(GvHD[1:2])
sn <- sampleNames(nc)[1]
nrow(nc[[sn]])
getIndices(nc, sn) #initial index is NA
#subset with filter
morphGate <- norm2Filter("FSC-H", "SSC-H", filterId = "MorphologyGate",scale = 2)
nc1 <- Subset(nc, morphGate)
ind <- getIndices(nc1, sn)
all.equal(sum(ind), nrow(nc1[[sn]]))
initIndices(nc1)
getIndices(nc1, sn) #reset indices

```

```
initIndices,ncdfFlowSet-method
```

initialize the event indices for the entire ncdfFlowSet with NA

Description

For internal use.

Usage

```
## S4 method for signature ncdfFlowSet
initIndices(x)
```

Arguments

x ncdfFlowSet object

```
lapply,ncdfFlowList-method
```

lapply method for ncdfFlowList

Description

Depending on level parameter, loop either iterates through the list of ncdfFlowSet objects or everyflowFrame objects.

Usage

```
## S4 method for signature ncdfFlowList
lapply(X, FUN, level = 2, ...)
```

Arguments

X	ncdfFlowList object
FUN	function to apply
level	numeric. It controls whether loop at 'ncdfFlowSet' level or 'sample' level. when level = 2 (default value),FUN is applied to each sample. When level = 1, FUN is applied to each object stored in data slot.
...	other arguments passed to FUN

ncdfFlow	<i>ncdfFlow: A package that provides CDF storage based flow cytometry data analysis.</i>
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Description

ncdfFlow: A package that provides CDF storage based flow cytometry data analysis.

Details

Define important flow cytometry data classes: `ncdfFlowSet` (a subclass of `flowSet`) and `ncdfFlowList` (a list of `ncdfFlowSet` object) and their accessors.

Provide important compensation,transformation,filter,gating,subsetting,splitting functions for data analysis of large volumns of flow cytometry data that is too big to be held in memory.

Package:	ncdfFlow
Version:	2.9.24
Date:	2014-04-16
Depends:	R (>= 2.8.1), flowCore
License:	Artistic-2.0

Author(s)

Mike Jiang <wjiang2@fhcrc.org>, Greg Finak <gfinak@fhcrc.org>

Maintainer: Mike Jiang <wjiang2@fhcrc.org>

ncdfFlowList-class	<i>a class that stores multiple ncdfFlowSet objects</i>
--------------------	---

Description

It is a list of `ncdfFlowSet` objects

Usage

```
## S4 method for signature ncdfFlowList,filter
filter(x, filter, method = "missing",
       sides = "missing", circular = "missing", init = "missing")

## S4 method for signature ncdfFlowList,numeric
x[[i, j, ...]]

## S4 method for signature ncdfFlowList,logical
x[[i, j, ...]]

## S4 method for signature ncdfFlowList,character
x[[i, j, ...]]

## S4 method for signature ncdfFlowList
length(x)

## S4 method for signature ncdfFlowList
show(object)

## S4 method for signature ncdfFlowList
sampleNames(object)

## S4 method for signature ncdfFlowList,ANY
x[i, j, ..., drop = TRUE]

## S4 method for signature ncdfFlowList,factor
split(x, f, drop = FALSE, ...)

## S4 method for signature ncdfFlowList,character
split(x, f, drop = FALSE, ...)

## S4 method for signature ncdfFlowList
phenoData(object)

## S4 method for signature ncdfFlowList
pData(object)

## S4 method for signature ncdfFlowList
colnames(x)

## S4 method for signature formula,ncdfFlowList
xyplot(x, data, ...)

## S4 method for signature formula,ncdfFlowList
densityplot(x, data, ...)
```

Arguments

x	ncdfFlowList object
filter	filter to be applied
method	missing not used
sides	missing not used
circular	missing not used
init	missing not used
i	numeric index
j	column index
drop	For matrices and arrays. If TRUE the result is coerced to the lowest possible dimension (see the examples). This only works for extracting elements, not for the replacement. See drop for further details.
object	Any R object
...	further potential arguments passed to methods.
f	a 'factor' in the sense that as.factor(f) defines the grouping, or a list of such factors in which case their interaction is used for the grouping.
data	For the formula methods, a data frame (or more precisely, anything that is a valid <code>envir</code> argument in eval , e.g., a list or an environment) containing values for any variables in the formula, as well as groups and subset if applicable. If not found in data, or if data is unspecified, the variables are looked for in the environment of the formula. For other methods (where x is not a formula), data is usually ignored, often with a warning if it is explicitly specified.

Objects from the Class

Objects can be created by coercing a list of `ncdfFlowSet` objects as(`"ncdfFlowList", nclist = #a list of ncdfFlowSet objects`)

See Also

[ncdfFlowSet](#)

Examples

```
data(GvHD)
nc1 <- ncdfFlowSet(GvHD[1])
nc2 <- ncdfFlowSet(GvHD[2])
nc3 <- ncdfFlowSet(GvHD[3])
list1 <- list(nc1, nc2, nc3)
#coerce from list to ncdfFlowList
nclist <- as(list1, "ncdfFlowList")
nclist
#coerce(collapse) from ncdfFlowList to a single flowFrame
collapsedData <- as(nclist, "flowFrame")
collapsedData
```

```
ncdfFlowSet,flowFrame-method
```

```
create ncdfFlowSet from flowFrame (not supported)
```

Description

create ncdfFlowSet from flowFrame (not supported)

Normally the ncdfFlowSet is constructed by loading raw FCS files using `read.ncdfFlowSet`. In case there is a legacy `flowSet` object, we can convert it to ncdfFlowSet with this constructor.

Usage

```
## S4 method for signature flowFrame
ncdfFlowSet(x, ncdfFile)

## S4 method for signature flowSet
ncdfFlowSet(x, ncdfFile, dim = 2, compress = 0)
```

Arguments

x	flowSet
ncdfFile	character specifies the file name of cdf file
dim	integer see details in read.ncdfFlowset .
compress	integer see details in read.ncdfFlowset .

Examples

```
data(GvHD)
fs <- GvHD[1:2]
ncfs <- ncdfFlowSet(fs)
```

```
ncdfFlowSet-class      a class for storing flow cytometry raw data in HDF5 format
```

Description

This class is a subclass of `flowSet`. It stores the raw data in cdf file instead of memory so that the analysis tools provided by flowCore based packages can be used in the study that produces hundreds or thousands FCS files.

Usage

```
## S4 method for signature ncdfFlowSet,ANY
compensate(x, spillover)

## S4 method for signature ncdfFlowSet
transform(_data, ...)

## S4 method for signature ncdfFlowSet
show(object)

## S4 method for signature formula,ncdfFlowSet
densityplot(x, data, ...)

## S4 method for signature formula,ncdfFlowSet
xyplot(x, data, ...)
```

Arguments

x	An object of class <code>flowFrame</code> or <code>flowSet</code> .
spillover	The spillover or compensation matrix.
...	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 <code>transform</code> objects and the colnames in <code>spillover</code> match to these parameter names. The first element in ... is a character vector of parameter names or a list of character scalars or <code>transform</code> objects and the colnames in <code>spillover</code> match to these parameter names. Argument <code>spillover</code> 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 <code>spillover</code> .
_data	The object to be transformed
object	Any R object
data	For the <code>formula</code> method, an optional data source (usually a data frame) in which variables are to be evaluated (see <code>xyplot</code> for details). <code>data</code> should not be specified for the other methods, and is ignored with a warning if it is.

Slots

`file`: A character containing the ncdf file name.

`maxEvents`: An integer containing the maximum number of events of all samples stored in this `ncdfFlowSet` object

`flowSetId`: A character for the id of `ncdfFlowSet` object

indices: Object of class "environment" containing events indices of each sample stored as "raw" vector. Each index value is either TRUE or FALSE and the entire indices vector is used to subset the raw data. the indices vector of each sample is NA by default when the ncdfFlowSet first created. It is assigned with actual value when ncdfFlowSet object is subsetted by [Subset](#) or other subsetting methods.

origSampleVector: A character vector containing the sample names, which indicates the original order of samples physically stored in cdf format

origColnames: A character vector containing the flow channel names, which indicates the original order of columns physically stored in cdf format

frames: Object of class "environment", which replicates the "frame" slot in [flowSet](#), except that [exprs](#) matrix is empty and the actual data is stored in cdf file.

phenoData: see [phenoData](#)

colnames: see [colnames](#). Here it serves as the current data view which does not reflect the actual number and order of columns stored in cdf file.

Extends

Class "[flowSet](#)", directly.

ncfsApply,ncdfFlowSet-method

apply method for ncdfFlowSet (for internal use)

Description

It is equivalent to [fsApply](#). But the latter could cause memory issue when FUN returns a flowFrame. ncdfApply writes to a new cdf file instead of memory. Thus it will return a ncdfFlowSet object.

Usage

```
## S4 method for signature ncdfFlowSet
ncfsApply(x, FUN, ..., use.exprs = FALSE,
          newNcFile = NULL)
```

Arguments

x	ncdfFlowSet
FUN	function to apply
...	other arguments to pass to FUN
use.exprs	logical see fsApply
newNcFile	logical whether to create a new hdf file or simply overwrite the existing file.

Details

When the function given by argument "FUN" does not return the entire flowFrame object with the same size of the original one (such as [compensate](#),[transform](#)...), [fsApply](#) should be used instead.

Examples

```

data(GvHD)
nc <- ncdfFlowSet(GvHD[1:2])

#use fsApply when FUN does not return a flowFrame
fsApply(nc, nrow)
fsApply(nc, range)

#use ncfsApply when FUN returns a flowFrame
lgcl <- logicleTransform( w = 0.5, t= 10000, m =4.5)
nc1 <- ncfsApply(nc, transform, FL1-H = lgcl(FL1-H), FL2-H = lgcl(FL2-H))

```

```
rbind2,ncdfFlowList,ANY-method
```

combine multiple ncdfFlowSet objects into one

Description

Similar to [flowCore:rbind2](#). But one needs to first construct a `ncdfFlowList` and then apply `rbind2` to it instead of merging them pairwise

Usage

```

## S4 method for signature ncdfFlowList,ANY
rbind2(x, ncdfFile = tempfile(pattern = "ncfs"),
       dim = 2, compress = 0)

```

Arguments

<code>x</code>	<code>ncdfFlowList</code>
<code>ncdfFile</code>	character see details in read.ncdfFlowset
<code>dim</code>	integer see details in read.ncdfFlowset .
<code>compress</code>	integer see details in read.ncdfFlowset .

Value

a new `ncdfFlowSet` with a new cdf file that combines multiple raw datasets.

Examples

```

data(GvHD)

nc1 <- ncdfFlowSet(GvHD[1:2])
nc2 <- ncdfFlowSet(GvHD[3:4])
nc3 <- ncdfFlowSet(GvHD[5:6])
ncslist <- as(list(nc1,nc2,nc3),"ncdfFlowList")
nc4 <- rbind2(ncslist)
nc4

```

read.ncdfFlowSet *create ncdfFlowSet from FCS files*

Description

read FCS files from the disk and load them into a ncdfFlowSet object

Usage

```
read.ncdfFlowSet(files = NULL, ncdfFile, flowSetId = "",
  isWriteSlice = TRUE, phenoData, channels = NULL, dim = 2,
  compress = 0, ...)
```

Arguments

files	A character vector giving the source FCS raw file paths.
ncdfFile	A character scalar giving the output file name. Default is NULL and the function will generate a random file in the temporary folder, potentially adding the .cdf suffix unless a file extension is already present. It is sometimes useful to specify this file path to avoid the failure of writing large flow data set to cdf file due to the the shortage of disk space in system temporary folder. It is only valid when isNewNcFile=TRUE
flowSetId	A character scalar giving the unique ncdfFlowSet ID.
isWriteSlice	A logical scalar indicating whether the raw data should also be copied.if FALSE, an empty cdf file is created with the dimensions (sample*events*channels) supplied by raw FCS files.
phenoData	An object of AnnotatedDataFrame providing a way to manually set the phenotypic data for the whole data set in ncdfFlowSet.
channels	A character vector specifying which channels to extract from FCS files. It can be useful when FCS files do not share exactly the same channel names. Thus this argument is used to select those common channels that are of interests. Default value is NULL and the function will try to scan the FCS headers of all files and determine the common channels.
dim	integer the number of dimensions that specifies the physical storage format of hdf5 dataset. Default is 2, which stores each FCS data as a seperate 2d dataset. Normally, user shouldn't need to change this but dim can also be set to 3, which stores all FCS data as one single 3d dataset.
compress	integer the HDF5 compression ratio (from 0 to 9). Default is 0, which does not compress the data and is recommended (especially for 2d format) because the speed loss usually outweighs the disk saving.
...	extra arguments to be passed to read.FCS .

Value

A ncdfFlowSet object

See Also[clone.ncdfFlowSet](#)**Examples**

```

library(ncdfFlow)

path<-system.file("extdata","compdata","data",package="flowCore")
files<-list.files(path,full.names=TRUE)[1:3]

#create ncdfFlowSet from fcs with the actual raw data written in cdf
nc1 <- read.ncdfFlowSet(files=files,ncdfFile="ncfsTest.nc",flowSetId="fs1",isWriteSlice= TRUE)
nc1
nc1[[1]]
unlink(nc1)
rm(nc1)

#create empty ncdfFlowSet from fcs and add data slices afterwards
nc1 <- read.ncdfFlowSet(files=files,ncdfFile="ncfsTest.nc",flowSetId="fs1",isWriteSlice= FALSE)
fs1<-read.flowSet(files)
nc1[[1]] <- fs1[[1]]
nc1[[1]]
nc1[[2]]

```

replacement method for `ncdfFlowSet`

write the flow data from a flowFrame to ncdfFlowSet

Description

`flowFrame` can have less channels than `ncdfFlowSet`, which is used for partial updating (useful for normalization)

Arguments

<code>x</code>	a <code>ncdfFlowSet</code>
<code>i</code>	a numeric or character used as sample index of <code>ncdfFlowSet</code>
<code>j</code>	not used
<code>only.exprs</code>	a logical Default is FALSE. which will update the parameters and descriptions slot as well as the raw data. Sometime it is more efficient to set it to TRUE skip the overhead of colnames matching and updating when user is only concerned about raw data instead of the entire <code>flowFrame</code> .
<code>compress</code>	integer It is only relevant to writing slice to '2d' format because the compression is set during the creation of hdf5 file for '3d' format. see details in read.ncdfFlowset .

Examples

```

data(GvHD)
nc <- ncdfFlowSet(GvHD[1:2])
samples <- sampleNames(nc)
sn <- samples[1]
#return the entire flowFrame
fr <- nc[[sn]]

apply(exprs(nc[[sn]]), 2, range)

#transform the data
lgcl <- logicleTransform( w = 0.5, t= 10000, m =4.5)
fr_trans <- transform(fr, FL1-H = lgcl(FL1-H), FL2-H = lgcl(FL2-H))

#update the data
nc[[sn]] <- fr_trans
apply(exprs(nc[[sn]]), 2, range)

#subset on channels
nc1 <- nc[,2:3]
#only write the channels of interest (reduce disk IO)
nc1[[sn]] <- fr_trans[,2:3]

#chanel colnames
colnames(fr_trans)[3:4] <- c("<FL1-H>", "<FL2-H>")

#write data without matching up the colnames
nc[[sn, only.exprs = TRUE]] <- fr_trans

```

```

split,ncdfFlowSet,filter-method
      split a ncdfFlowSet object.

```

Description

Equivalent to split method for flowSet object.

Usage

```

## S4 method for signature ncdfFlowSet,filter
split(x, f, drop = FALSE, population = NULL,
      prefix = NULL, ...)

## S4 method for signature ncdfFlowSet,filterResultList
split(x, f, drop = FALSE,
      population = NULL, prefix = NULL, ...)

## S4 method for signature ncdfFlowSet,list
split(x, f, isNew = FALSE, drop = FALSE,

```

```

    population = NULL, prefix = NULL, ...)

## S4 method for signature ncdfFlowSet,factor
split(x, f, isNew = FALSE, drop = FALSE, ...)

## S4 method for signature ncdfFlowSet,character
split(x, f, drop = FALSE, ...)

```

Arguments

x ncdfFlowSet
 f,drop,population,prefix,...
 see [split-methods](#)

isNew logical whether to create a new hdf file or using existing hdf file.

Value

a list of ncdfFlowSet objects that may not may not share the same hdf file depending on isNew argument.

Subset,ncdfFlowSet,filterResultList-method
subset a ncdfFlowSet by filter

Description

Equivalent to Subset method for flowSet.

Usage

```

## S4 method for signature ncdfFlowSet,filterResultList
Subset(x, subset, select, ...)

## S4 method for signature ncdfFlowList,filterResultList
Subset(x, subset, select, ...)

## S4 method for signature ncdfFlowSet,filter
Subset(x, subset, ...)

## S4 method for signature ncdfFlowList,filter
Subset(x, subset, ...)

## S4 method for signature ncdfFlowSet,list
Subset(x, subset, select, ...)

```

Arguments

x ncdfFlowSet or ncdfFlowList
subset,select,...
 see [Subset-methods](#)

Value

one or more ncdfFlowSet objects which share the same hdf5 file with the original one.

unlink,ncdfFlowSet-method

delete the cdf file associated with the ncdfFlowSet object

Description

ncdfFlowSet object is unrecoverable after cdf is deleted. So this method is usually called when ncdfFlowSet object is no longer in need.

Usage

```
## S4 method for signature ncdfFlowSet
unlink(x, recursive = FALSE, force = FALSE)
```

Arguments

x ncdfFlowSet
recursive see [unlink](#)
force see [unlink](#)

Examples

```
data(GvHD)
nc <- ncdfFlowSet(GvHD[1:2])
nc[[1]] # data is loaded from cdf file
unlink(nc)
```

updateIndices,ncdfFlowSet,character,logical-method
update the event indices of the target sample in ncdfFlowSet

Description

For internal use.

Usage

```
## S4 method for signature ncdfFlowSet,character,logical
updateIndices(x, y, z)
```

Arguments

x	ncdfFlowSet object
y	character sample name
z	logical vector to be assigned.

[,ncdfFlowSet,ANY-method
subsetting by sampleNames,channels(not for events) methods

Description

similar to [\[](#).

Usage

```
## S4 method for signature ncdfFlowSet,ANY
x[i, j, ..., drop = FALSE]
```

Arguments

x	ncdfFlowSet
i	sample index(or name)
j	column(or channel) index (or name)
...	other arguments not used
drop	logical not used.

Examples

```

data(GvHD)
nc <- ncdfFlowSet(GvHD[1:2])
samples <- sampleNames(nc)
nc[1]
nc1 <- nc[samples[1]]
#nc1 and nc share the cdf file
all.equal(getFileName(nc1), getFileName(nc))

```

```
[[,ncdfFlowSet,ANY-method
```

extract a flowFrame object from ncdfFlowSet

Description

Similiar to `[[`, and there are cerntain ways to reduce the disk IO and optimize the speed.

Usage

```

## S4 method for signature ncdfFlowSet,ANY
x[[i, j, use.exprs = TRUE, ...]]

```

Arguments

<code>x</code>	a <code>ncdfFlowSet</code>
<code>i</code>	a numeric or character used as sample index
<code>j</code>	a numeric or character used as channel index
<code>use.exprs</code>	a logical scalar indicating whether to read the actual data from cdf
<code>...</code>	other arguments. not used.

Examples

```

data(GvHD)
nc <- ncdfFlowSet(GvHD[1:2])
samples <- sampleNames(nc)
sn <- samples[1]
#return the entire flowFrame
fr <- nc[[sn]]

#access the flowFrame meta data without loading the raw event data from disk
nc[[sn, use.exprs = FALSE]]

#only read a subset of channels (more efficient than reading entire data set)
nc[[sn, 1:2]]

```

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