## Package 'ncdfFlow'

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

Version 1.4.3

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#### Description

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

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Depends R (>= 2.14.0), flowCore

Imports Biobase, flow Core, flow Viz, methods

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SystemRequirements netcdf 4.0.1, hdf5

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Collate AllGeneric.R AllClasses.R AllFunctions.R ncdfFlowSet-accessors.R ncdfFlowSet-split-methods.R ncdfFlowSet-Subset-methods.R ncdfFlowSet-rbind-methods.R bitVector.R ncdfFlowList-methods.R ncdfFlowSet-plot-methods.R

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ncdfFlow-package

#### Description

Define important flow cytometry data classes: ncdfFlowSet( a subclass of flowSet) and ncdf-FlowList(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.

#### Details

Package:	ncdfFlow
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#### References

http://www.rglab.org/

addFrame-methods add/replace the data in ncdfFlowSet

#### Description

Add one flowFrame to the ncdfFlowSet.

#### Usage

addFrame(ncfs,data,sampleName)

#### Arguments

ncfs	a ncdfFlowSet object
data	a flowFrame to be added
sampleName	a character object with the name of the target sample

#### clone.ncdfFlowSet

#### Details

The dimensions of the flowFrame to be added has to match the target sample data in ncdfFlowSet. It updates the target sample data if it already exists in ncdfFlowSet object.

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#### See Also

 ${\it read.ncdfFlowSet}$ 

#### Examples

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

##create empty ncdfFlowSet from fcs nc1 <- read.ncdfFlowSet(files=files,ncdfFile="ncfsTest.nc",flowSetId="fs1",isWriteSlice=FALSE) fs1<-read.flowSet(files); #add the actual data slices afterwards addFrame(nc1,fs1[[1]],sampleNames(fs1)[1]) nc1[[1]]##show the added flowFrame nc1[[2]]##show empty flowFrame

clone.ncdfFlowSet Clone a ncdfFlowSet

#### Description

Create a new ncdfFlowSet object from an existing one

#### Usage

```
clone.ncdfFlowSet(ncfs,fileName=NULL,isEmpty=TRUE, isNew=TRUE,isSaveMeta=FALSE)
```

#### 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.
fileName	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.
isSaveMeta	A logical scalar indicating whether the meta data other than raw data should be saved in cdf. It should be set as TRUE if the entire ncdfFlowSet is going to be loaded by ncdfFlowSet_open,character-method.

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A ncdfFlowSet object

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#### See Also

read.ncdfFlowSet

#### Examples

library(ncdfFlow)

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

##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)
addFrame(nc2,fs1[[1]],sampleNames(fs1)[1])
nc2[[1]]
```

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

```
ncfsUnlink(nc1)
rm(nc1)
```

ncdfFlowList-class 'ncdfFlowList': a class that stores multiple ncdfFlowSet objects

#### Description

It is a list of ncdfFlowSet objects

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

Objects can be created using new("ncdfFlowList", sampleNames = ...., #character of sample names datalist = .... #a list of ncdfFlowSet objects )

or visa the constructor, which takes a list of ncdfFlowSet objects: ncdfFlowList(ncdfList)

#### ncdfFlowSet-class

#### Slots

sampleNames: character of sample names datalist: a list of ncdfFlowSet objects

#### Methods

[ return a list of ncdfFlowSets

[[ return a specific ncdfFlowset object

show display object summary.

sampleNames Extract and replace sample names from the phenoData object.

colnames Extract or replace the colnames slot.

ncfsUnlink delete the cdf files associated with ncdfFlowSet objects contained in current ncdf-FlowList

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#### See Also

ncdfFlowSet

ncdfFlowSet-class 'ncdfFlowSet': a class for storing flow cytometry raw data in netCDF 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.

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

Objects can be created by using: read.ncdfFlowSet,clone.ncdfFlowSet

or ncdfFlowSet( x, #x is a flowSet fileName #the output cdf file name )

#### 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 TURE 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.

#### Methods

Most of the other flowSet methods are not listed here, but they all work as the same due to its inheritance from flowSet.Please see for more flowSetdetails for these methods.

addFrame add or replace the flowFrame in ncdfFlowSet.See addFrame for more details

Usage:

addFrame(ncfs,data,sampleName)

- [,[[ Subsetting. similar to ],[] for flowSet.
- **getIndices** extract the event indices of one or multiple samples from ncdfFlowSet,return a logical vector.

Usage:

getIndices(ncfs,sampleName)

initIndices initialize the event indices for the entire ncdfFlowSet with either an NA or TRUE/FALSE logical value

Usage:

initIndices(ncfs,y)

updateIndices update the event indices of the target sample in ncdfFlowSet

Usage:

updateIndices(ncfs,sampleName,y)

ncdfFlowSet\_open load the ncdfFlowSet object from the cdf file, return a ncdfFlowSet object

Usage:

ncdfFlowSet open(filename)

Note that in order to successfully recover the entire ncdfFlowSet object, the phenoData has to be already saved in cdf either by explicitly calling ncdfFlowSet\_sync or setting isSaveMeta as TRUE when ncdfFlowSet is created by link{read.ncdfFlowSet} or link{clone.ncdfFlowSet}

ncdfFlowSet create ncdfFlowSet from a flowSet object

Usage:

ncdfFlowSet(fs1)

Note that only flowSet can be coerced to ncdfFlowSet,attempt to apply this method to flowFrame will get an error message.

#### ncdfFlowSet\_sync save phenoData to cdf file.

Usage:

ncdfFlowSet sync(filename)

ncfsUnlink delete the netCDF file associated with the ncdfFlowSet object

Usage:

ncfsUnlink(ncfs)

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

**ncdfExprs** Extract or replace the raw data intensities, equivalent to exprs. It reads the data matrix from cdf file instead of memory.

Usage:

ncdfExprs(ncfs,sampleName, channel,subByIndice)

NcdfFlowSetToFlowSet convert a subset of ncdfFlowSet to flowSet.

Usage:

NcdfFlowSetToFlowSet(ncfs,top)

Argument top specifies the number of samples evenly selected from ncdfFlowSet.

**ncfsApply** equivalent to fsApply., which could cause memory issue due to the. So ncfsApply will return a ncdfFlowSet object.

Usage:

ncfsApply(x="ncdfFlowSet",FUN="ANY")

Note that the function given by argument "FUN" should return an entire flowFrame object with the same size of the original one(such as compensate,transform...) Otherwise,fsApply should be used instead.

- **rbind2** similar to flowCore:rbind2. It returns a new ncdfFlowSet with a new cdf file that combines two raw datasets. It is recommended to construct a ncdfFlowList and apply rbind2 to it directly when combining more than two ncdfFlowSets. Because using "do.call" on a list ncdfFlowSets will create one cdf file for every two ncdfFlowSets, which is not efficient.
- **split** equivalent to flowCore:split. It returns a new ncdfFlowSet object without creating new cdf raw data file but assigning logical indices to subset the original raw data.
- **Subset** equivalent to flowCore:Subset. It returns a new ncdfFlowSet object without creating new cdf raw data file but assigning logical indices to subset the original raw data.
- **densityplot,xyplot** equivalent to flowViz:densityplot and flowViz:xyplot. User need to be careful about applying these plot methods to ncdfFlowSet because it could be slow for large number of flow data.

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#### See Also

flowSet, read.ncdfFlowSet, ncdfFlowList

read.ncdfFlowSet

#### Description

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

#### Usage

```
read.ncdfFlowSet(files = NULL, ncdfFile, flowSetId, isWriteSlice=TRUE, isSaveMeta=FALSE, phenoData)
```

#### Arguments

files	A character vector giving the source FCS raw file paths.
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
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.
isSaveMeta	A logical scalar indicating whether the meta data other than raw data should be saved in cdf. It should be set as TRUE if the entire ncdfFlowSet is going to be loaded by ncdfFlowSet_open,character-method.
phenoData	An object of AnnotatedDataFrame providing a way to manually set the phenotyoic data for the whole data set in ncdfFlowSet.

#### Value

A ncdfFlowSet object

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#### See Also

#### ${\it clone.ncdf} FlowSet$

#### Examples

library(ncdfFlow)

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

```
\label{eq:constraint} \begin{array}{l} \# create \ ncdfFlowSet \ from \ fcs \ with \ the \ actual \ raw \ data \ written \ in \ cdf \\ nc1 \ <- \ read.ncdfFlowSet(isSaveMeta=FALSE, files=files, ncdfFile="ncfsTest.nc", flowSetId="fs1", isWriteSlice=TRUE) \\ nc1 \end{array}
```

#### read.ncdfFlowSet

nc1[[1]] ncfsUnlink(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)
addFrame(nc1,fs1[[1]],sampleNames(fs1)[1])
nc1[[1]]
nc1[[2]]
```

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