

# ncdfFlow: Provides netCDF storage based methods and functions for manipulation of flow cytometry data

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## Abstract

**Background** The Bioconductor package `flowCore` is the object model and a collection of standard tools designed for flow cytometry data analysis. The related R packages including data analysis (`flowClust`, `flowMerge`, `flowMeans`, `flowTrans`, `flowStats`), visualization (`ggcyto`) and quality control (`QUALIFIER`) use the `flowCore` infrastructure to deal with flow cytometry data. However the `flowFrame` or `flowSet` which represent a single or a set of FCS files are memory-resident data structures and require the entire data elements to remain in memory in order to perform all kinds of the data manipulations. Hundreds or thousands of datasets generated by high throughput instruments can easily hit the memory limit if they are imported as the `flowSet` or `flowFrames` in R. It presents a challenge to scientists and bioinformaticians who use the R tools described above to perform statistical data analysis on a regular computer. We propose a new R object model and related functions to address this problem. The new model `ncdfFlowSet` inherit most of data structures from `flowSet`. It stores the large volume of event-level data on disk and only keeps the file handler and meta data in memory. Thus the memory consumption is significantly reduced. NetCDF is used as the data formats because it is self-describing, machine-independent and specifically optimized for storing and accessing array-oriented scientific data. With the compression and chunking features introduced by the new release of netCDF4, the new model is able to maintain high performance of data processing.

Most of the functions and methods including transformation, compensation, gating and subsetting methods for `flowSet` are extended to `ncdfFlowSet` (`spillover`, `normalize` and `workflow` methods of `flowCore` are currently not supported yet.). Thus the change of data structure is almost transparent to the users of `flowCore`-based R packages.

**keywords** Flow cytometry, high throughput, netCDF, `flowSet`, `ncdfFlowSet`

```
## Loading required package: flowCore
## Loading required package: RcppArmadillo
## Loading required package: BH
```

## 1 Representing Flow Cytometry Data with `ncdfFlowSet`

`ncdfFlow` represents a flow cytometry data model that is very similar to the `flowSet` structure. The only difference is that the event-based 2-D data matrices from multiple samples of the same experiment are stored as one single 3D data matrix on disk in netcdf format. Each sample can be accessed efficiently from the 3-D matrix as a data chunk or slice and further manipulated in memory.

The basic unit of manipulation in `ncdfFlow` is the *ncdfFlowSet*. It inherits all the slots from *flowSet*. However, the *flowFrame* objects stored in the "frames" slot of a *ncdfFlowSet* object do not host the data matrix. Instead, their "exprs" slots are kept empty and the actual data are stored in the 3-D data matrix on disk and only the file name is stored in "file" slot of *ncdfFlowSet*. Thus *ncdfFlowSet* reduces the memory requirements and meanwhile ensures the consistent data structure with *flowSet*.

## 2 Creating a *ncdfFlowSet*

We provide a function to read FCS files into a *ncdfFlowSet* object:

```
path<-system.file("extdata", "compdata", "data", package="flowCore")
files<-list.files(path, full.names=TRUE) [1:3]
nc1 <- read.ncdfFlowSet(files=files)

## All FCS files have the same following channels:
## FSC-H
## SSC-H
## FL1-H
## FL2-H
## FL3-H
## FL1-A
## FL4-H
## write 060909.001 to empty cdf slot...
## write 060909.002 to empty cdf slot...
## write 060909.003 to empty cdf slot...
## done!

nc1

## An ncdfFlowSet with 3 samples.
## NCDF file : D:\biocbuild\bbs-3.14-bioc\tmpdir\RtmpwfvQem\ncfs35f87b066342
## An object of class 'AnnotatedDataFrame'
##   rowNames: 060909.001 060909.002 060909.003
##   varLabels: name
##   varMetadata: labelDescription
##
##   column names:
##     FSC-H, SSC-H, FL1-H, FL2-H, FL3-H, FL1-A, FL4-H
```

As we see, the constructor function is very similar to the *flowSet* except that it requires a filename for the ncdf file.

```
fs1 <- read.flowSet(files=files)
```

Note that an ncdf file that stores the actual data is generated and saved on disk once a `ncdfFlowSet` is created. Users need to explicitly call the `unlink` method to remove the file before delete the object from memory by `rm`.

```
unlink(nc1)
rm(nc1)
```

Users can also create an empty `ncdfFlowSet` first and add data slices later by assigning argument `isWriteSlice` as `FALSE`.

```
nc1 <- read.ncdfFlowSet(files=files, isWriteSlice= FALSE)

## All FCS files have the same following channels:
## FSC-H
## SSC-H
## FL1-H
## FL2-H
## FL3-H
## FL1-A
## FL4-H
## done!

nc1[[1]]

## flowFrame object 'anonymous'
## with 0 cells and 7 observables:
##
```

	name	desc	range	minRange	maxRange
## \$P1	FSC-H	FSC-Height	1024	-111	1023
## \$P2	SSC-H	SSC-Height	1024	-111	1023
## \$P3	FL1-H	NA	1024	-111	1023
## \$P4	FL2-H	NA	1024	-111	1023
## \$P5	FL3-H	NA	1024	-111	1023
## \$P6	FL1-A	NA	1024	-111	1023
## \$P7	FL4-H	NA	1024	-111	1023

```
## 1 keywords are stored in the 'description' slot
```

As we see here, before writing the actual `flowFrame` by `[[<-`, the `flowFrame` object returned by `[[` has 0 events.

```
targetSampleName<-sampleNames(fs1)[1]
nc1[[targetSampleName]] <- fs1[[1]]

## write 060909.001 to empty cdf slot...

nc1[[1]]
```

```
## flowFrame object '060909.001'
## with 10000 cells and 7 observables:
##           name      desc      range  minRange  maxRange
## $P1      FSC-H    FSC-Height    1024         0      1023
## $P2      SSC-H    SSC-Height    1024         0      1023
## $P3      FL1-H           NA    1024         1     10000
## $P4      FL2-H           NA    1024         1     10000
## $P5      FL3-H           NA    1024         1     10000
## $P6      FL1-A           NA    1024         0      1023
## $P7      FL4-H           NA    1024         1     10000
## 141 keywords are stored in the 'description' slot

nc1[[2]]

## flowFrame object 'anonymous'
## with 0 cells and 7 observables:
##           name      desc      range  minRange  maxRange
## $P1      FSC-H    FSC-Height    1024        -111     1023
## $P2      SSC-H    SSC-Height    1024        -111     1023
## $P3      FL1-H           NA    1024        -111     1023
## $P4      FL2-H           NA    1024        -111     1023
## $P5      FL3-H           NA    1024        -111     1023
## $P6      FL1-A           NA    1024        -111     1023
## $P7      FL4-H           NA    1024        -111     1023
## 1 keywords are stored in the 'description' slot
```

Note that it is important to always use sample name to specify the target position in the data matrix where the actual is added. Because the sample name is the identifier used to index the data matrix.

Sometime it is helpful to copy the structure from an existing `ncdfFlow` object and then add the data slice to the empty `ncdfFlow` cloned by `clone.ncdfFlowSet`.

```
nc2 <- clone.ncdfFlowSet(nc1, isEmpty = TRUE)
nc2[[1]]
nc2[[sampleNames(fs1)[1]]] <- fs1[[1]]

## write 060909.001 to empty cdf slot...

nc2[[1]]
```

We also provide the `coerce` function to convert the `flowSet` to a `ncdfFlowSet`.

```
data(GvHD)

## Warning in load(zfile, envir = tmp_env): strings not representable
## in native encoding will be translated to UTF-8
```

[illegible]



### 3 Working with metadata

Like *flowSet*, *ncdfFlowSet* has an associated *AnnotatedDataFrame* that provides metadata of experiments. This data frame is accessed and modified via the same methods of *flowCore*. :

```
phenoData(nc1)
pData(nc1)
varLabels(nc1)
varMetadata(nc1)
sampleNames(nc1)
keyword(nc1, "FILENAME")
identifier(nc1)
colnames(nc1)
colnames(nc1, prefix="s6a01")
length(nc1)
getIndices(nc1, "s6a01")
```

### 4 Manipulating a *ncdfFlowSet*

You can extract a *flowFrame* from a *ncdfFlowSet* object in the same way as *flowSet* by using the `[]` or `$` extraction operators. Note that using the `[]` extraction operator returns a new *ncdfFlowSet* that points to the same ncdf file. SO the original ncdf file serves as a data repository and the *ncdfFlowSet* works as view of the data in this sense.

```
nm<-sampleNames(nc1)[1]
expr1<-paste("nc1$", nm, "'", sep="")
eval(parse(text=expr1))

## flowFrame object 's6a01'
## with 2205 cells and 8 observables:
##           name           desc      range  minRange  maxRange
## $P1      FSC-H       FSC-Height    1024         0       1023
## $P2      SSC-H       SSC-Height    1024         0       1023
## $P3      FL1-H       CD15 FITC     1024         1      10000
## $P4      FL2-H       CD45 PE       1024         1      10000
## $P5      FL3-H       CD14 PerCP    1024         1      10000
## $P6      FL2-A              NA     1024         0       1023
## $P7      FL4-H       CD33 APC      1024         1      10000
## $P8      Time Time (102.40 sec.)  1024         0       1023
## 166 keywords are stored in the 'description' slot

nc1[[nm]]

## flowFrame object 's6a01'
```

```
## with 2205 cells and 8 observables:
##           name           desc      range  minRange  maxRange
## $P1      FSC-H          FSC-Height    1024         0      1023
## $P2      SSC-H          SSC-Height    1024         0      1023
## $P3      FL1-H          CD15 FITC     1024         1     10000
## $P4      FL2-H          CD45 PE       1024         1     10000
## $P5      FL3-H          CD14 PerCP    1024         1     10000
## $P6      FL2-A          NA            1024         0      1023
## $P7      FL4-H          CD33 APC       1024         1     10000
## $P8      Time Time (102.40 sec.)    1024         0      1023
## 166 keywords are stored in the 'description' slot

nm<-sampleNames(nc1)[c(1,3)]
nc2<-nc1[nm]
summary(nc2)

## $s6a01
##           FSC-H      SSC-H      FL1-H      FL2-H      FL3-H      FL2-A
## Min.        60.0000    0.0000    1.000000    1.00000    1.000000    0.00000
## 1st Qu.     159.0000   48.0000    1.046045   35.34981    1.000000    6.00000
## Median     196.0000   65.0000    2.644158  160.42741    1.382810   36.00000
## Mean       220.7642  108.8853   57.543711  210.07988    7.366665   48.69569
## 3rd Qu.     264.0000   97.0000    7.054802  320.88828    2.460406   75.00000
## Max.       1023.0000 1023.0000 3781.922363 1637.10388  326.718719 516.00000
##           FL4-H      Time
## Min.        1.000000 11.00000
## 1st Qu.      1.000000 40.00000
## Median       5.288867 57.00000
## Mean       16.243151 51.90476
## 3rd Qu.     20.782274 66.00000
## Max.       503.335175 80.00000
##
## $s6a03
##           FSC-H      SSC-H      FL1-H      FL2-H      FL3-H      FL2-A
## Min.        59.0000    0.000    1.000000    1.0000    1.000000    0.0000
## 1st Qu.     147.0000   49.000    1.000000   341.7625    1.000000   79.0000
## Median     192.0000   71.000    1.144593   526.5112    1.069867  124.0000
## Mean       188.4942  116.008    62.174581   543.7355    5.400462  127.8592
## 3rd Qu.     226.0000  119.000   10.204639   702.3116    2.208442  164.0000
## Max.       1023.0000 1023.000 10000.000000 8503.9121 7564.633301 1023.0000
##           FL4-H      Time
## Min.        1.000000    0.0000
## 1st Qu.      1.165390 105.0000
## Median       2.228415 215.5000
## Mean        8.351631 233.9134
```



```
## 3rd Qu.    4.833503 353.0000
## Max.      665.379456 567.0000
```

*flowSet*-specific iterator `fsApply` can also be applied to `RobjncdfFlowSet`:

```
fsApply(nc1, range)
fsApply(nc1, each_col, median)
```

However, we recommend to use another iterator `ncfsApply` designed for the function that returns a `flowFrame` (such as `compensate`, `transform`...). `ncfsApply` works the same as `fsApply` except that it returns a `ncdfFlowSet` object with the actual data stored in `cdf` to avoid the huge memory consumption. Note that the return value of the function applied in `ncfsApply` must be a `flowFrame` object and it should have the same dimensions (channels and events) as the original data.

## 5 Compensation, Transformation and Gating

`transform` and `compensate` for `ncdfFlowSet` work the same as `flowSet`.

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

#compensation
summary(nc1)[[1]]
nc2<-compensate(nc1, comp)

## write s6a01 to empty cdf slot...
## write s6a02 to empty cdf slot...
## write s6a03 to empty cdf slot...
## write s6a04 to empty cdf slot...

summary(nc2)[[1]]
unlink(nc2)
rm(nc2)

#transformation
asinhTrans <- arcsinhTransform(transformationId="ln-transformation", a=1, b=1, c=1)
nc2 <- transform(nc1, `FL1-H`=asinhTrans(`FL1-H`))

## write s6a01 to empty cdf slot...
## write s6a02 to empty cdf slot...
## write s6a03 to empty cdf slot...
## write s6a04 to empty cdf slot...

summary(nc1)[[1]]
summary(nc2)[[1]]
unlink(nc2)
rm(nc2)
```

Note that compensation/transformation return the `ncdfFlowSet` objects that point to the new cdf file containing the compensated/transformed data.

`filter` for `flowSet` also works for `ncdfFlowSet`:

```
rectGate <- rectangleGate(filterId="nonDebris", "FSC-H"=c(200, Inf))
fr <- filter(nc1, rectGate)
summary(fr)

rg2 <- rectangleGate(filterId="nonDebris", "FSC-H"=c(300, Inf))
rg3 <- rectangleGate(filterId="nonDebris", "FSC-H"=c(400, Inf))
flist <- list(rectGate, rg2, rg3)
names(flist) <- sampleNames(nc1[1:3])
fr3 <- filter(nc1[1:3], flist)
summary(fr3[[3]])
```

## 6 Subsetting

The `Subset` and `split` methods for `ncdfFlowSet`:

```
nc2 <- Subset(nc1, rectGate)
summary(nc2[[1]])
```

```
##           FSC-H      SSC-H      FL1-H      FL2-H      FL3-H      FL2-A
## Min.      200.0000      0.0000      1.000000      1.00000      1.000000      0.00000
## 1st Qu.    230.0000     69.0000      1.333897     22.33436      1.000000      3.00000
## Median    266.0000     87.0000      3.371780     77.36830      1.499523     16.00000
## Mean      296.9887    141.0131     91.895427    165.68587     10.830258     38.24038
## 3rd Qu.    316.0000    122.0000     18.992949    223.84692      2.550628     53.00000
## Max.     1023.0000  1023.0000    1942.529175   1637.10388    326.718719    516.00000
##           FL4-H      Time
## Min.        1.000000    11.00000
## 1st Qu.      2.692201    40.00000
## Median     12.896131    57.00000
## Mean       22.021327    51.81972
## 3rd Qu.     29.791735    66.00000
## Max.      464.158875    80.00000
```

```
library(flowStats)
morphGate <- norm2Filter("FSC-H", "SSC-H", filterId = "MorphologyGate", scale = 2)
smaller <- Subset(nc1[c(1, 3)], morphGate, c("FSC-H", "SSC-H"))
smaller[[1]]

## flowFrame object 's6a01'
```

```
## with 1647 cells and 2 observables:
##           name      desc      range  minRange  maxRange
## $P1      FSC-H    FSC-Height    1024        0    1023
## $P2      SSC-H    SSC-Height    1024        0    1023
## 166 keywords are stored in the 'description' slot

nc1[[1]]

## flowFrame object 's6a01'
## with 2205 cells and 8 observables:
##           name      desc      range  minRange  maxRange
## $P1      FSC-H    FSC-Height    1024        0    1023
## $P2      SSC-H    SSC-Height    1024        0    1023
## $P3      FL1-H    CD15 FITC    1024        1   10000
## $P4      FL2-H    CD45 PE    1024        1   10000
## $P5      FL3-H    CD14 PerCP    1024        1   10000
## $P6      FL2-A    NA    1024        0    1023
## $P7      FL4-H    CD33 APC    1024        1   10000
## $P8      Time Time (102.40 sec.)    1024        0    1023
## 166 keywords are stored in the 'description' slot

rm(smaller)
```

Note that Subset does not create the new ncdf file (the same as extraction operator []). So we need to be careful about using unlink to delete the subsetted data since it points to the same ncdf file that is also used by the original ncdfFlowSet object.

split returns a ncdfFlowList object which is a container of multiple ncdfFlowSet objects.

```
##splitting by a gate
qGate <- quadGate(filterId="qg", "FSC-H"=200, "SSC-H"=400)
fr<-filter(nc1,qGate)
ncList<-split(nc1,fr)
ncList

## $`FSC-Height+SSC-Height+`
## An ncdfFlowSet with 4 samples.
## NCDF file : D:\biocbuild\bbs-3.14-bioc\tmpdir\RtmpwfvQem\ncfs35f83378763d
## An object of class 'AnnotatedDataFrame'
##   rowNames: s6a01 s6a02 s6a03 s6a04
##   varLabels: Patient Visit ... population (6 total)
##   varMetadata: labelDescription
##
##   column names:
##     FSC-H, SSC-H, FL1-H, FL2-H, FL3-H, FL2-A, FL4-H, Time
```

```
##
##
## $`FSC-Height-SSC-Height+`
## An ncdfFlowSet with 4 samples.
## NCDF file : D:\biocbuild\bbs-3.14-bioc\tmpdir\RtmpwfvQem\ncfs35f83378763d
## An object of class 'AnnotatedDataFrame'
##   rowNames: s6a01 s6a02 s6a03 s6a04
##   varLabels: Patient Visit ... population (6 total)
##   varMetadata: labelDescription
##
##   column names:
##     FSC-H, SSC-H, FL1-H, FL2-H, FL3-H, FL2-A, FL4-H, Time
##
##
## $`FSC-Height+SSC-Height-`
## An ncdfFlowSet with 4 samples.
## NCDF file : D:\biocbuild\bbs-3.14-bioc\tmpdir\RtmpwfvQem\ncfs35f83378763d
## An object of class 'AnnotatedDataFrame'
##   rowNames: s6a01 s6a02 s6a03 s6a04
##   varLabels: Patient Visit ... population (6 total)
##   varMetadata: labelDescription
##
##   column names:
##     FSC-H, SSC-H, FL1-H, FL2-H, FL3-H, FL2-A, FL4-H, Time
##
##
## $`FSC-Height-SSC-Height-`
## An ncdfFlowSet with 4 samples.
## NCDF file : D:\biocbuild\bbs-3.14-bioc\tmpdir\RtmpwfvQem\ncfs35f83378763d
## An object of class 'AnnotatedDataFrame'
##   rowNames: s6a01 s6a02 s6a03 s6a04
##   varLabels: Patient Visit ... population (6 total)
##   varMetadata: labelDescription
##
##   column names:
##     FSC-H, SSC-H, FL1-H, FL2-H, FL3-H, FL2-A, FL4-H, Time

nc1[[1]]

## flowFrame object 's6a01'
## with 2205 cells and 8 observables:
##
```

	name	desc	range	minRange	maxRange
## \$P1	FSC-H	FSC-Height	1024	0	1023
## \$P2	SSC-H	SSC-Height	1024	0	1023
## \$P3	FL1-H	CD15 FITC	1024	1	10000

```
## $P4      FL2-H      CD45 PE      1024      1      10000
## $P5      FL3-H      CD14 PerCP    1024      1      10000
## $P6      FL2-A      NA           1024      0      1023
## $P7      FL4-H      CD33 APC      1024      1      10000
## $P8      Time Time (102.40 sec.)  1024      0      1023
## 166 keywords are stored in the 'description' slot

ncList[[2]][[1]]

## flowFrame object 's6a01'
## with 36 cells and 8 observables:
##      name      desc      range  minRange  maxRange
## $P1      FSC-H      FSC-Height    1024      0      1023
## $P2      SSC-H      SSC-Height    1024      0      1023
## $P3      FL1-H      CD15 FITC      1024      1      10000
## $P4      FL2-H      CD45 PE      1024      1      10000
## $P5      FL3-H      CD14 PerCP    1024      1      10000
## $P6      FL2-A      NA           1024      0      1023
## $P7      FL4-H      CD33 APC      1024      1      10000
## $P8      Time Time (102.40 sec.)  1024      0      1023
## 166 keywords are stored in the 'description' slot

ncList[[1]][[1]]

## flowFrame object 's6a01'
## with 74 cells and 8 observables:
##      name      desc      range  minRange  maxRange
## $P1      FSC-H      FSC-Height    1024      0      1023
## $P2      SSC-H      SSC-Height    1024      0      1023
## $P3      FL1-H      CD15 FITC      1024      1      10000
## $P4      FL2-H      CD45 PE      1024      1      10000
## $P5      FL3-H      CD14 PerCP    1024      1      10000
## $P6      FL2-A      NA           1024      0      1023
## $P7      FL4-H      CD33 APC      1024      1      10000
## $P8      Time Time (102.40 sec.)  1024      0      1023
## 166 keywords are stored in the 'description' slot
```

Note that the `ncdfFlowSet` objects contained in `ncdfFlowList` by default share the same `ncdf` file as the original `ncdfFlowSet`. In order to keep its own data copy, try to set argument `isNew` to "TRUE" in `split` method.