

# 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`

## 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)
> nc1
```

```
An ncdfFlowSet with 3 samples.
NCDF file : /tmp/Rtmp94rq58/ncfsaa4142b90e8.nc
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)
> 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]]
> 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]]
> nc2[[1]]
```

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

```
> data(GvHD)
> GvHD <- GvHD[pData(GvHD)$Patient %in% 6:7][1:4]
> nc1<-ncdfFlowSet(GvHD)
```

Or coerce a `ncdfFlowSet` to `flowSet`

```
> fs1<-as.flowSet(nc1,top=2)
```

Note that `ncdfFlowSet` is designed to store large datasets and it is not recommended to coerce the entire `ncdfFlowSet` to `flowSet`. Usually users want to select a subset from `ncdfFlowSet` by `[]` and convert the subsetting data. Sometimes it is helpful to randomly select a certain number of `flowFrames` from the entire datasets represented by `ncdfFlowSet` to have a preview of the data. The argument `"top"` can be used here for this purpose.

### 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

150 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

150 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 `RobjectncdfFlowSet`:

```
> 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)
> 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`))
```

```

> 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				

```

> nc2 <- Subset(nc1, fr)
> 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

```
> rm(nc2)
> 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

150 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

150 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 : /tmp/Rtmp94rq58/ncfsaa4d0c2b53.nc
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 : /tmp/Rtmp94rq58/ncfsaa4d0c2b53.nc
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 : /tmp/Rtmp94rq58/ncfsaa4d0c2b53.nc
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 : /tmp/Rtmp94rq58/ncfsaa4d0c2b53.nc
```

```
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

150 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

150 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

150 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.

```
> ncList_new<-split(nc1,fr,isNew=TRUE)
```