

# Package ‘flowTime’

April 15, 2019

**Title** Annotation and analysis of biological dynamical systems using flow cytometry

**Version** 1.6.1

**Description** This package was developed for analysis of both dynamic and steady state experiments examining the function of gene regulatory networks in yeast (strain W303) expressing fluorescent reporter proteins using a BD Accuri C6 and SORP cytometers. However, the functions are for the most part general and may be adapted for analysis of other organisms using other flow cytometers. Functions in this package facilitate the annotation of flow cytometry data with experimental metadata, as is requisite for dissemination and general ease-of-use. Functions for creating, saving and loading gate sets are also included. In the past, we have typically generated summary statistics for each flowset for each timepoint and then annotated and analyzed these summary statistics. This method loses a great deal of the power that comes from the large amounts of individual cell data generated in flow cytometry, by essentially collapsing this data into a bulk measurement after subsetting. In addition to these summary functions, this package also contains functions to facilitate annotation and analysis of steady-state or time-lapse data utilizing all of the data collected from the thousands of individual cells in each sample.

**Depends** R (>= 3.4), flowCore, plyr

**License** Artistic-2.0

**LazyData** true

**biocViews** ImmunoOncology, FlowCytometry, TimeCourse, Visualization, DataImport, CellBasedAssays

**Suggests** knitr, rmarkdown, flowViz, ggplot2, BiocGenerics, moments, stats

**Imports** utils

**VignetteBuilder** knitr

**RoxygenNote** 6.0.1

**git\_url** <https://git.bioconductor.org/packages/flowTime>

**git\_branch** RELEASE\_3\_8

**git\_last\_commit** f25b1c3

**git\_last\_commit\_date** 2019-01-04

**Date/Publication** 2019-04-15

**Author** R. Clay Wright [aut, cre],  
Nick Bolten [aut],  
Edith Pierre-Jerome [aut]

**Maintainer** R. Clay Wright <wright.clay@gmail.com>

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addbs	<i>Add background subtraction to a summary data frame</i>
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### Description

Subtracts the background fluorescence of a given control strain from the chosen column.

### Usage

```
addbs(flowData, column = "FL3.Amean", baseline_column = "strain",
       baseline = "noYFP")
```

**Arguments**

flowData	the summary data frame of flowSet to be background subtracted
column	the column containing the fluorescent measurement to be background subtracted
baseline_column	the column containing the name of the strain representing background fluorescent values
baseline	character the name of the strain representing background fluorescent values

**Value**

A summary data frame with an additional column "column\_bs" containing the background subtracted fluorescent values

**Examples**

```
dat<-read.flowSet(path=system.file("extdata", "tc_example",
package = "flowTime"),alter.names = TRUE)
annotation <- read.csv(system.file("extdata", "tc_example.csv",
package = "flowTime"))
annotation[which(annotation$treatment == 0), 'strain'] <- 'background'
adat <- annotateFlowSet(dat, annotation)
loadGates(gatesFile = 'C6Gates')
dat_sum <- summarizeFlow(adat, ploidy = 'diploid', only = 'singlets',
channel = 'FL1.A')
dat_sum <- addbs(dat_sum, column = "FL1.Amean", baseline = "background")
```

---

addnorm

*Normalize fluorescence*


---

**Description**

Produces a normalized fluorescence column 'normed'. Expects the 'FL1.A\_bs' column to exist or a column to be specified. Has two different methods, version 1 and version 2, described in the script

**Usage**

```
addnorm(frame, factor_in = c("strain", "treatment"), method = 1,
column = "FL3.Amean_bs")
```

**Arguments**

frame	data frame of summary statistics to be normalized
factor_in	character vector containing the variables to split the data frame by
method	which normalization method to use, 1, 2 or 3.
column	character the column to apply the normalization to

## Details

Method 1, the default normalization method, takes the highest point in each dataset grouped by 'factor\_in' and normalizes all values in the group by this point. This method is default because it works regardless of whether the data is a time series. Method 2 finds the mean value of all time points with time values less than 0 for each group and normalizes each group by this respective value. Requires a time series with negative time values to work. Version 3 fits a linear model to the pre-zero time points for each groups, infers the y-intercept, and normalizes using this intercept. Method 3 also requires a time series with negative time values to work.

## Value

data frame containing the additional normalized variable

## Examples

```
dat <- read.flowSet(path=system.file("extdata", "tc_example",
package = "flowTime"), alter.names = TRUE)
annotation <- read.csv(system.file("extdata", "tc_example.csv",
package = "flowTime"))
adat <- annotateFlowSet(dat, annotation)
loadGates(gatesFile = 'C6Gates')
dat_sum <- summarizeFlow(adat, ploidy = "diploid", only = "singlets",
channel = "FL1.A")
dat_sum <- addnorm(dat_sum, c("strain", "treatment"), method = 1,
column = "FL1.Amean")
```

---

annotateFlowSet	<i>Annotate a flowSet with experimental metadata</i>
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---

## Description

Add annotations to a flowSets phenoData and plate numbers, strain names, and treatment also set T0

## Usage

```
annotateFlowSet(yourFlowSet, annotation_df, mergeBy = "name")
```

## Arguments

yourFlowSet	a flowSet with sampleNames of the format 'plate#_Well', we typically use the following code chunk to read data from individual plates as exported from BD Accuri C6 software.
annotation_df	A data frame with columns 'well', 'strain', 'treatment', containing all of the wells in the flowset labeled with the strain and treatment in that well.
mergeBy	the unique identifier column

## Value

An annotated flowSet

**Examples**

```
dat <- read.flowSet(path = system.file("extdata", "ss_example",
package = "flowTime"), alter.names = TRUE)
annotation <- read.csv(system.file("extdata", "ss_example.csv", package =
"flowTime"))
annotateFlowSet(dat, annotation, mergeBy = "name")
```

---

createAnnotation      *Create an annotation dataframe*

---

**Description**

Creates a data frame with rows containing the sample names of your flow set that can then be filled in with experimental metadata.

**Usage**

```
createAnnotation(yourFlowSet)
```

**Arguments**

yourFlowSet      the flowSet to create an annotation data frame for

**Value**

annotation\_df a data frame containing the sample names of your flow set

**Examples**

```
dat <- read.flowSet(path = system.file("extdata", "ss_example",
package = "flowTime"), alter.names = TRUE)
annotation <- createAnnotation(yourFlowSet = dat)
head(annotation)
```

---

dipdoubletGate      *A gate for the set of all diploid doublets*

---

**Description**

A gate for the set of all diploid doublets

**Usage**

```
data(dipdoubletGate)
```

**Format**

formal class polygonGate

---

dipsingletGate	<i>A gate for the set of all diploid singlet yeast cells</i>
----------------	--

---

**Description**

Typically set in FSC.A by FSC.H space Diploids are typically 5um x 6um ellipsoids while haploids are typically 4um x 4um spheroids. As a result, diploids are longer and you get a larger 'area/volume'.

**Usage**

```
data(dipsingletGate)
```

**Format**

formal class polygonGate

---

flsummary	<i>Get summary statistics for fluorescence or other data channels of a flowSet</i>
-----------	--

---

**Description**

Get summary statistics for fluorescence or other data channels of a flowSet

**Usage**

```
flsummary(flowset, channel = "FL3.A", moments = FALSE)
```

**Arguments**

flowset	the flowSet to create summary statistics for
channel	character the data channel to summarize
moments	boolean if TRUE then split each frame into early, middle, and late events

**Value**

A data frame containing summary statistics (mean, median, SD) for the specified fluorescent channel and time moments of the flowSet.

**Examples**

```
plate1 <- read.flowSet(path = system.file("extdata",
"ss_example", package = "flowTime"), alter.names = TRUE)
flsummary(plate1)
```

---

getTime	<i>Get the time at which at flowFrame began collection</i>
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---

**Description**

Get the time at which at flowFrame began collection

**Usage**

```
getTime(flowframe)
```

**Arguments**

flowframe      The flowFrame for which you would like the initial time

**Value**

numeric time value in minutes

**Examples**

```
plate1<-read.flowSet(path = system.file("extdata", "ss_example", package =  
"flowTime"),alter.names = TRUE)  
getTime(plate1$A01.fcs)
```

---

hapdoubletGate	<i>A gate for the set of all haploid doublets</i>
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---

**Description**

A gate for the set of all haploid doublets

**Usage**

```
data(hapdoubletGate)
```

**Format**

formal class polygonGate

---

hapsingletGate	<i>A gate for the set of all haploid singlets</i>
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---

**Description**

A gate for the set of all haploid singlets

**Usage**

```
data(hapsingletGate)
```

**Format**

formal class polygonGate

---

loadGates	<i>Load a yeast gate file</i>
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**Description**

Loads a set of yeast gates into active memory to be used in analysis functions

**Usage**

```
loadGates(gatesFile = NULL, path = NULL, envir = environment())
```

**Arguments**

gatesFile	the gates file to be loaded into memory, or path to the gates file
path	The path to the gates file. If 'NULL' this will look through lazy loaded data for the gatesFile
envir	The environment in which to load the gates

**Value**

gate objects created in the current environment

**Examples**

```
loadGates(system.file("extdata/SORPGates.RData", package = "flowTime"))
```



---

ploidy	<i>Guess the ploidy of a given flowframe</i>
--------	--

---

**Description**

Use the FSC.A/FSC.H ratio. Diploids are typically 5um x 6um ellipsoids while haploids are typically 4um x 4um spheroids. As a result, diploids are longer and you get a larger 'area/volume' FSC.A. 'Width' might also be useful on certain cytometers.

**Usage**

```
ploidy(flowframe)
```

**Arguments**

flowframe      the flowFrame you would like to identify the ploidy of

**Value**

"Diploid" or "Haploid" and the mean FSC.A/FSC.H quotient

**Examples**

```
dat <- read.flowSet(path = system.file("extdata", "ss_example",  
package = "flowTime"), alter.names = TRUE)  
ploidy(dat$A01.fcs)
```

---

polyGate	<i>Create a polygon gate</i>
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**Description**

Create a polygon gate

**Usage**

```
polyGate(x, y, filterID = "newGate", channels = c("FSC.A", "FSC.H"))
```

**Arguments**

x                      a vector of x coordinates  
y                      a vector of y coordinates  
filterID              name of the gate  
channels              vector containing the channels matching the x and y coordinates above

**Value**

a polygon gate object

**Examples**

```
polyGate(x = c(1,1,10000,10000), y = c(1,10000, 10000, 1), )
```

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qaGating	<i>Quality assurance check</i>
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---

**Description**

Check whether a flowSet (or a single flowFrame) contains empty values, in which case normalization may fail (divide by zero). This is particularly useful for removing wash wells from a flowSet.

**Usage**

```
qaGating(x, threshold = 100)
```

**Arguments**

x	flowSet or flowFrame to be checked
threshold	flowFrames with fewer events than this threshold will be identified.

**Value**

A vector containing the flowFrames with fewer events than the threshold.

**Examples**

```
plate1<-read.flowSet(path = system.file("extdata", "ss_example", package =
"flowTime"), alter.names = TRUE)
qaGating(plate1)
```

---

read.plateSet	<i>Read a FCS files from set of plates</i>
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---

**Description**

Reads all folders within the specified path containing the specified pattern in the folder names. Each folder contains a set a plate of FCS files. These folders typically make up a whole experiment. Plates are numbered according to the standard lexicographical ordering of your operating system.

**Usage**

```
read.plateSet(path = getwd(), pattern = "", ...)
```

**Arguments**

path	The path to search for folders containing FCS files
pattern	The <a href="#">regex</a> pattern used to identify the folders of FCS files to be read
...	Additional arguments passed to read.flowSet. Note that 'alter.names' is forced to be TRUE in this implementation.

**Value**

A single flowSet containing all FCS files within the identified folders. The index of each folder in the list according to lexicographical ordering (1,2,...) is prepended to the sampleNames.

**Examples**

```
# Read in both of the example data sets as a single flowSet
plate1<-read.plateSet(path = system.file("extdata", package = "flowTime"),
pattern = "")
```

---

saveGates	<i>Save a yeast gate set</i>
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**Description**

Save a yeast gate set

**Usage**

```
saveGates(yeastGate = "yeastGate", dipsingletGate = "dipsingletGate",
dipdoubletGate = "dipdoubletGate", hapsingletGate = "hapsingletGate",
hapdoubletGate = "hapdoubletGate", path = getwd(),
fileName = "defaultGates.RData")
```

**Arguments**

yeastGate	a gate object defining the population of yeast cells
dipsingletGate	a gate object defining the population of diploid singlet cells
dipdoubletGate	a gate object defining the population of diploid doublet cells
hapsingletGate	a gate object defining the population of haploid singlet cells
hapdoubletGate	a gate object defining the population of haploid doublet cells
path	path to the folder in which you would like to save the gates
fileName	name of the .Rdata file you would like to save these gates within

**Value**

a .RData file in the "extdata" folder of the package containing the specified gates

**Examples**

```
loadGates("SORPGates")
saveGates()
```

---

steadyState	<i>Analysis of steady state fluorescence flow cytometry</i>
-------------	---

---

**Description**

Generates a data frame which can be used to visualize and analyze steady state flow cytometry data. Steady state in this case means that

**Usage**

```
steadyState(flowset, gated = FALSE, ploidy = "diploid", only = "singlets")
```

**Arguments**

flowset	your flowSet to be analyzed
gated	boolean is the data already gated?
ploidy	character gate to subset your flowset based on the ploidy of you strains
only	character which population of events to analyze, 'yeast', 'singlets', or 'doublets'?

**Value**

a data frame containing all of the selected subset of events from the original flowSet

**Examples**

```
dat <- read.flowSet(path = system.file("extdata", "ss_example",
package = "flowTime"), alter.names = TRUE)
annotation <- read.csv(system.file("extdata", "ss_example.csv",
package = "flowTime"))
dat <- annotateFlowSet(dat, annotation, mergeBy = "name")
loadGates(gatesFile = 'SORPGates')
steadyState(dat, gated = FALSE, ploidy = "diploid", only = "singlets")
```

---

summarizeFlow	<i>Generate summary statistics for a flowSet</i>
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---

**Description**

Gates a sample to all yeast, then singlet, then doublets. Also calculates singlet to doublet ratio. Returns a list of data frames, e.g. output\$singlets, output\$doublets, etc.

**Usage**

```
summarizeFlow(flowset, channel = "FL1.A", gated = FALSE, ploidy = FALSE,
moments = FALSE, only = FALSE)
```

**Arguments**

flowset	the flowSet to be summarized
channel	character which data channel should be summarized
gated	boolean is the data already appropriately gated?
ploidy	character does the flowSet contain haploid or diploid cells?
moments	boolean split the data into early, middle, and late moments?
only	character summarize only "singlet", "doublet", or all "yeast" cells, FALSE will return all

**Value**

data frame containing the specified summary statistics of the specified cell populations for each frame

**Examples**

```
plate1 <- read.flowSet(path = system.file("extdata", "ss_example",
package = "flowTime"), alter.names = TRUE)
summarizeFlow(plate1, channel = "FL1.A", gated = TRUE,
ploidy = "diploid", moments = FALSE, only = "yeast")
```

---

yeastGate

*A gate for the set of all yeast cells*

---

**Description**

Typically set in FSC.A by SSC.A space to excluded any debris

**Usage**

```
data(yeastGate)
```

**Format**

formal class polygonGate

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