

# Package ‘cyanoFilter’

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**Type** Package

**Title** Phytoplankton Population Identification using Cell Pigmentation and/or Complexity

**Version** 1.12.0

**Description** An approach to filter out and/or identify phytoplankton cells from all particles measured via flow cytometry pigment and cell complexity information. It does this using a sequence of one-dimensional gates on pre-defined channels measuring certain pigmentation and complexity. The package is especially tuned for cyanobacteria, but will work fine for phytoplankton communities where there is at least one cell characteristic that differentiates every phytoplankton in the community.

**URL** <https://github.com/fomotis/cyanoFilter>

**BugReports** <https://github.com/fomotis/cyanoFilter/issues>

**Depends** R(>= 4.1.0)

**Imports** Biobase, flowCore, flowDensity, flowClust, cytometree, ggplot2, GGally, graphics, grDevices, methods, mrfDepth, stats, utils

**License** MIT + file LICENSE

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accTest	<i>tests the accuracy of several automated gating functions on monoculture flow cytometry experiments.</i>
---------	--

---

## Description

This function gates all flowFrames in the supplied flowSet to attach cluster labels. Then it mixes up the flowSet into one giant flowFrame and re-gates this to attach another label. These labels are used to examine if the gating algorithms can reproduce the earlier clusters before the mixing.

## Usage

```
accTest(
  fs,
  sfts = c("phytoFilter", "flowClust", "cytometree"),
  channels,
  nrun = 10000,
  ...
)
```

## Arguments

fs	flowSet with each flowFrame being a phytoplankton monoculture FCM experiment
sfts	character vector of gating function to test.
channels	channels to be used for gating
nrun	number of times the resampling should be done
...	extra options to be parsed to the gating function

## Value

a named list containing the following objects;

- **depth** - the multivariate-depth (median) of each flowFrame in the flowset supplied
- **accuracy** - computed accuracy based on resampling after joining the flowFrames together.

**Examples**

```

flowfile_path <- system.file("extdata", "B4_18_1.fcs",
package = "cyanoFilter",
  mustWork = TRUE)
flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE,
  transformation = FALSE,
  emptyValue = FALSE,
  dataset = 1)
flowfile_nona <- cyanoFilter::noNA(x = flowfile)
flowfile_noneg <- cyanoFilter::noNeg(x = flowfile_nona)
flowfile_logtrans <- cyanoFilter::lnTrans(x = flowfile_noneg,
  c('SSC.W', 'TIME'))
cells_nonmargin <- cellMargin(flowframe = flowfile_logtrans,
  Channel = 'SSC.W',
  type = 'estimate', y_toplot = "FSC.HLin")
cells_nodebris <- debrisNc(flowframe = reducedFlowframe(cells_nonmargin),
  ch_chlorophyll = "RED.B.HLin",
  ch_p2 = "YEL.B.HLin",
  ph = 0.05)

#phytoFilter specification
gateFunc(flowfile = reducedFlowframe(cells_nodebris),
  channels = c("RED.B.HLin", "YEL.B.HLin",
"RED.R.HLin", "FSC.HLin", "SSC.HLin"),
  sfts = "phytoFilter",
  list(ph = 0.1, proportion = 0.90)
)

```

accuracy

*samples two rows in a matrix and check if the samples are similar or different based on their cluster labels*

**Description**

This function

**Usage**

```
accuracy(mat, mono_clust, bi_clust, nrun = 10000)
```

**Arguments**

mat	matrix to be sampled from
mono_clust	monoculture cluster label
bi_clust	biculture cluster label
nrun	number of times the resampling should be carried out. Defaults to 10000

**Value**

a vector of integer values

**Examples**

```
x <- matrix(NA, nrow = 100, ncol = 3)
xx <- apply(x, 2, rnorm, 100)
xx <- cbind(xx, Mono = rep(1:2, each = 50),
            Bi = rep(1:2, times = 50))
accuracy(xx, "Mono", "Bi", nrun = 5000)
```

---

cellMargin	<i>Removes or assign indicators to margin events.</i>
------------	---

---

**Description**

The function identifies margin events, i.e. cells that are too large for the flow cytometer to measure.

**Usage**

```
cellMargin(
  flowframe,
  Channel = "SSC.W",
  type = c("manual", "estimate"),
  cut = NULL,
  y_toplot = "FSC,HLin"
)
```

**Arguments**

flowframe	Flowframe containing margin events to be filtered out
Channel	The channel on which margin events are. Defaults to SSC.W (side scatter width)
type	The method to be used in gating out the margin cells. Can either be 'manual' where user supplies a cut off point on the channel, 1 = not margin 0 = margin
cut	could not be NULL if type = 'manual'
y_toplot	channel on y-axis of plot with <i>Channel</i> used to gate out margin events

**Details**

Users can either supply a cut-off point along the channel describing particle width or allow the function to estimate the cut-off point using the [deGate](#) function from the *flowDensity* package. A plot of channel against "FSC.HLin" is provided with a vertical line showing the cut-off point separating margin events from other cells.

**Value**

an object of class MarginEvents class containing slots;

- **reducedflowframe** - flowframe without margin events

- **fullflowframe** - flowframe with an Margin.Indicator added as an extra column added to the expression matrix to indicate which particles are margin events. 1 = not margin event, 0 = margin event
- **N\_margin** - number of margin events recorded
- **N\_cell** - number of non-margin events
- **N\_particle** - is the number of particles in total, i.e. N\_cell + N\_margin

### Examples

```

flowfile_path <- system.file("extdata", "B4_18_1.fcs",
                             package = "cyanoFilter",
                             mustWork = TRUE)
flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE,
                              transformation = FALSE, emptyValue = FALSE,
                              dataset = 1)
flowfile_nona <- cyanoFilter::noNA(x = flowfile)
flowfile_nonneg <- cyanoFilter::noNeg(x = flowfile_nona)
flowfile_logtrans <- lnTrans(x = flowfile_nonneg, c('SSC.W', 'TIME'))
cellMargin(flowframe = flowfile_logtrans, Channel = 'SSC.W',
           type = 'estimate', y_toplot = "FSC.HLin")

```

---

clusterExtract	<i>extract clusters based on supplied cluster indicator</i>
----------------	---

---

### Description

extract clusters based on supplied cluster indicator

### Usage

```
clusterExtract(flowfile, cluster_var = "Clusters", cluster_val = NULL)
```

### Arguments

flowfile	flowframe containing cluster indicators as well
cluster_var	column name in expression matrix containing the cluster indicators, cannot be NULL.
cluster_val	cluster number, cannot be NULL.

### Value

flowFrame containing the clusters

**Examples**

```

flowfile_path <- system.file("extdata", "B4_18_1.fcs",
                             package = "cyanoFilter",
                             mustWork = TRUE)
flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE,
                              transformation = FALSE, emptyValue = FALSE,
                              dataset = 1)
flowfile_nona <- cyanoFilter::noNA(x = flowfile)
flowfile_noneg <- cyanoFilter::noNeg(x = flowfile_nona)
flowfile_logtrans <- cyanoFilter::lnTrans(x = flowfile_noneg,
                                         c('SSC.W', 'TIME'))
cells_nonmargin <- cellMargin(flowframe = flowfile_logtrans,
                              Channel = 'SSC.W',
                              type = 'estimate', y_toplot = "FSC.HLin")
fin <- phytoFilter(flowfile = reducedFlowframe(cells_nonmargin),
                  pig_channels = c("RED.B.HLin", "YEL.B.HLin", "RED.R.HLin"),
                  com_channels = c("FSC.HLin", "SSC.HLin"))

clusterExtract(flowfile = reducedFlowframe(fin),
               cluster_var = "Clusters",
               cluster_val = 1)

```

---

clusterExtractp	<i>takes a flowframe, name of cluster column and extracts part of flowframe that makes up proportion.</i>
-----------------	---

---

**Description**

takes a flowframe, name of cluster column and extracts part of flowframe that makes up proportion.

**Usage**

```
clusterExtractp(flowfile, cluster_var = "Clusters", proportion = 1)
```

**Arguments**

flowfile	flowframe after debris are removed.
cluster_var	column name in expression matrix containing the cluster indicators
proportion	value between 0 and 1 indicating percentage of the total particles wanted

**Value**

a list containing

- **particles\_per\_cluster**
- **clusters\_proportion**
- **flowfile\_proportion**

## Examples

```

flowfile_path <- system.file("extdata", "B4_18_1.fcs",
                             package = "cyanoFilter",
                             mustWork = TRUE)
flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE,
                              transformation = FALSE, emptyValue = FALSE,
                              dataset = 1)
flowfile_nona <- cyanoFilter::noNA(x = flowfile)
flowfile_noneg <- cyanoFilter::noNeg(x = flowfile_nona)
flowfile_logtrans <- cyanoFilter::lnTrans(x = flowfile_noneg,
                                         c('SSC.W', 'TIME'))
cells_nonmargin <- cyanoFilter::cellMargin(flowframe = flowfile_logtrans,
                                           Channel = 'SSC.W',
                                           type = 'estimate', y_toplot = "FSC.HLin")
fin <- phytoFilter(flowfile = reducedFlowframe(cells_nonmargin),
                  pig_channels = c("RED.B.HLin", "YEL.B.HLin", "RED.R.HLin"),
                  com_channels = c("FSC.HLin", "SSC.HLin"))

clusterExtractp(flowfile = reducedFlowframe(fin),
                 cluster_var = "Clusters",
                 proportion = 0.80)

```

---

cyanoFilter	<i>cyanoFilter: A package to identify and cluster phytoplankton cells contained in flow cytometry data.</i>
-------------	---

---

## Description

The package provides two categories of functions: *metafile* preprocessing functions and *fcsfile* processing functions.

### metafile preprocessing functions

This set of functions ([goodFcs](#) and [retain](#)) helps to identify the appropriate fcs file to read.

### fcsfile processing functions

These functions ([noNA](#) and [noNeg](#), [phytoFilter](#)) works on the fcs file to identify the phytoplankton populations contained in the fcs file.



---

DebrisFilter                    *the Debris class*

---

**Description**

the Debris class  
constructor for the DebrisFilter class

**Usage**

```
DebrisFilter(  
  fullflowframe,  
  reducedflowframe,  
  deb_pos,  
  syn_all_pos,  
  deb_cut,  
  ch_chlorophyll,  
  ch_p2  
)
```

```
DebrisFilter(  
  fullflowframe,  
  reducedflowframe,  
  deb_pos,  
  syn_all_pos,  
  deb_cut,  
  ch_chlorophyll,  
  ch_p2  
)
```

**Arguments**

fullflowframe	same as the input flowFrame
reducedflowframe	a partial flowframe containing non-margin events
deb_pos	number of margin particles measured
syn_all_pos	number of non-margin particles
deb_cut	estimated inflection point between debris and good cells
ch_chlorophyll	channel estimating chlorophyll level
ch_p2	plotting channel

**Value**

object of class DebrisFilter

**Slots**

fullflowframe object of class "flowFrame" same as the input flowFrame  
 reducedflowframe object of class "flowFrame" a partial flowframe containing a proportion of the measured particles  
 deb\_pos object of class "numeric" representing the proportion of particles in each cluster  
 syn\_all\_pos object of class "numeric" representing the number of particles in each cluster  
 deb\_cut object of class "numeric" representing the inflection point between debris and good cells.  
 ch\_chlorophyll object of class "character" representing the chlorophyll channel.  
 ch\_p2 object of class character to plot

---

debrisNc	<i>gates out or assign indicators to debris particle based on their chlorophyll expression.</i>
----------	---

---

**Description**

The function takes in a flowframe and identifies debris contained in the provided flowframe.

**Usage**

```
debrisNc(flowframe, ch_chlorophyll, ch_p2, ph = 0.09, n_sd = 2)
```

**Arguments**

flowframe	flowframe with debris and other cells.
ch_chlorophyll	first flowcytometer channel that can be used to separate debris from the rest, e.g. "RED.B.HLin".
ch_p2	second flowcytometer channel use for plotting from the rest, e.g. "YEL.B.HLin"
ph	the minimum peak height that should be considered. This aids the removal of tiny peaks. Defaults to 0.1
n_sd	number of standard deviations away from peak should be considered to filter out debris

**Details**

The function uses the [getPeaks](#) and [deGate](#) functions in the flowDensity package to identify peaks in ch\_chlorophyll, and identify cut-off points #between these peaks. A plot of both channels supplied with horizontal line separating debris from other cell populations is also returned.

**Value**

list containing;

- **syn** - flowframe containing non-debris particles
- **deb\_pos** - position of particles that are debris
- **syn\_pos** - position of particles that are not debris

**Examples**

```

flowfile_path <- system.file("extdata", "B4_18_1.fcs",
                             package = "cyanoFilter",
                             mustWork = TRUE)
flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE,
                              transformation = FALSE, emptyValue = FALSE,
                              dataset = 1)
flowfile_nona <- cyanoFilter::noNA(x = flowfile)
flowfile_noneg <- cyanoFilter::noNeg(x = flowfile_nona)
flowfile_logtrans <- cyanoFilter::lnTrans(x = flowfile_noneg,
c('SSC.W', 'TIME'))
cells_nonmargin <- cellMargin(flowframe = flowfile_logtrans,
Channel = 'SSC.W',
                             type = 'estimate', y_toplot = "FSC.HLin")
debrisNc(flowframe = reducedFlowframe(cells_nonmargin),
          ch_chlorophyll = "RED.B.HLin",
          ch_p2 = "YEL.B.HLin",
          ph = 0.05)

```

---

fullFlowframe

*generic function for extracting the full flowframe*


---

**Description**

generic function for extracting the full flowframe

**Usage**

```
fullFlowframe(x)
```

**Arguments**

x                    an object of either class PhytoFilter, MarginEvents or DebrisFilter

**Value**

generic to extract fullFlowframe

**Examples**

```

flowfile_path <- system.file("extdata", "B4_18_1.fcs",
                             package = "cyanoFilter",
                             mustWork = TRUE)
flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE,
                              transformation = FALSE,
                              emptyValue = FALSE,
                              dataset = 1)
flowfile_nona <- cyanoFilter::noNA(x = flowfile)

```

```

flowfile_noneg <- cyanoFilter::noNeg(x = flowfile_nona)
flowfile_logtrans <- cyanoFilter::lnTrans(x = flowfile_noneg,
                                          c('SSC.W', 'TIME'))
cells_nonmargin <- cellMargin(flowframe = flowfile_logtrans,
                              Channel = 'SSC.W',
                              type = 'estimate', y_toplot = "FSC.HLin")
reducedFlowframe(cells_nonmargin)

```

---

fullFlowframe,DebrisFilter-method

*accessor method for reduced flowframe (DebrisFilter class)*

---

### Description

accessor method for reduced flowframe (DebrisFilter class)

### Usage

```

## S4 method for signature 'DebrisFilter'
fullFlowframe(x)

```

### Arguments

x                    an object of class DebrisFilter

### Value

full flowFrame method for DebrisFilter

---

fullFlowframe,MarginEvents-method

*accessor method for the fullflowframe (MarginEvent class)*

---

### Description

accessor method for the fullflowframe (MarginEvent class)

### Usage

```

## S4 method for signature 'MarginEvents'
fullFlowframe(x)

```

### Arguments

x                    an object of class MarginEvents

**Value**

full Flowframe method for MarginEvents

**Examples**

```

flowfile_path <- system.file("extdata", "B4_18_1.fcs",
                             package = "cyanoFilter",
                             mustWork = TRUE)
flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE,
                              transformation = FALSE, emptyValue = FALSE,
                              dataset = 1)
flowfile_nona <- cyanoFilter::noNA(x = flowfile)
flowfile_nonneg <- cyanoFilter::noNeg(x = flowfile_nona)
flowfile_logtrans <- cyanoFilter::lnTrans(x = flowfile_nonneg,
c('SSC.W', 'TIME'))
cells_nonmargin <- cellMargin(flowframe = flowfile_logtrans,
Channel = 'SSC.W',
                             type = 'estimate', y_toplot = "FSC.HLin")
fullFlowframe(cells_nonmargin)

```

---

fullFlowframe,PhytopFilter-method

*accessor method for full flowframe(PhytopFilter class)*

---

**Description**

accessor method for full flowframe(PhytopFilter class)

**Usage**

```

## S4 method for signature 'PhytopFilter'
fullFlowframe(x)

```

**Arguments**

x                    an object of class PhytopFilter

**Value**

fullFlowframe method for PhytopFilter

**Examples**

```

flowfile_path <- system.file("extdata", "B4_18_1.fcs",
                             package = "cyanoFilter",
                             mustWork = TRUE)
flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE,
                              transformation = FALSE,
                              emptyValue = FALSE,

```

```

                                dataset = 1)
flowfile_nona <- cyanoFilter::noNA(x = flowfile)
flowfile_noneg <- cyanoFilter::noNeg(x = flowfile_nona)
flowfile_logtrans <- cyanoFilter::lnTrans(x = flowfile_noneg,
                                         c('SSC.W', 'TIME'))
cells_nonmargin <- cellMargin(flowframe = flowfile_logtrans,
                             Channel = 'SSC.W',
                             type = 'estimate', y_toplot = "FSC.HLin")
cells_nodebris <- debrisNc(flowframe = reducedFlowframe(cells_nonmargin),
                          ch_chlorophyll = "RED.B.HLin",
                          ch_p2 = "YEL.B.HLin",
                          ph = 0.05)
phy1 <- phytoFilter(flowfile = reducedFlowframe(cells_nodebris),
                   pig_channels = c("RED.B.HLin", "YEL.B.HLin", "RED.R.HLin"),
                   com_channels = c("FSC.HLin", "SSC.HLin"))
fullFlowframe(phy1)

```

---

gateFunc	<i>tests the accuracy of several automated gating functions on monoculture flow cytometry experiments.</i>
----------	--

---

## Description

This function gates all flowFrames in the supplied flowSet to attach cluster labels. Then it mixes up the flowSet into one giant flowFrame and re-gates this to attach another label. These labels are used to examine if the gating algorithms can reproduce the earlier clusters before the mixing.

## Usage

```

gateFunc(
  flowfile,
  sfts = c("phytoFilter", "flowClust", "cytometree"),
  channels,
  funargs_list
)

```

## Arguments

flowfile	flowSet with each flowFrame being a phytoplankton monoculture FCM experiment
sfts	character vector of gating function to test.
channels	channels to be used for gating
funargs_list	additional options for the chosen gating function

## Value

a flowFrame with cluster indicator generated by the software used added to the expression matrix.

**Examples**

```

flowfile_path <- system.file("extdata", "B4_18_1.fcs",
package = "cyanoFilter",
  mustWork = TRUE)
flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE,
  transformation = FALSE,
  emptyValue = FALSE,
  dataset = 1)
flowfile_nona <- cyanoFilter::noNA(x = flowfile)
flowfile_noneg <- cyanoFilter::noNeg(x = flowfile_nona)
flowfile_logtrans <- cyanoFilter::lnTrans(x = flowfile_noneg,
  c('SSC.W', 'TIME'))
cells_nonmargin <- cellMargin(flowframe = flowfile_logtrans,
  Channel = 'SSC.W',
  type = 'estimate', y_toplot = "FSC.HLin")
cells_nodebris <- debrisNc(flowframe = reducedFlowframe(cells_nonmargin),
  ch_chlorophyll = "RED.B.HLin",
  ch_p2 = "YEL.B.HLin",
  ph = 0.05)

#phytoFilter specification
gateFunc(flowfile = reducedFlowframe(cells_nodebris),
  channels = c("RED.B.HLin", "YEL.B.HLin",
"RED.R.HLin", "FSC.HLin", "SSC.HLin"),
  sfts = "phytoFilter",
  list(ph = 0.1, proportion = 0.90)
)

#flowClust specification
gateFunc(flowfile = reducedFlowframe(cells_nodebris),
  channels = c("RED.B.HLin", "YEL.B.HLin",
"RED.R.HLin", "FSC.HLin", "SSC.HLin"),
  sfts = "flowClust",
  list(K = 1:4, B = 100)
)

#cytometree specification
gateFunc(flowfile = reducedFlowframe(cells_nodebris),
  channels = c("RED.B.HLin", "YEL.B.HLin",
"RED.R.HLin", "FSC.HLin", "SSC.HLin"),
  sfts = "cytometree",
  list(minleaf = 1, t = 0.10)
)

```

---

getChannel

*returns the channel with more than one peak present. It returns NA if there is only one peak present.*

---

**Description**

returns the channel with more than one peak present. It returns NA if there is only one peak present.

**Usage**

```
getChannel(flowfile, ch, ph)
```

**Arguments**

flowfile	flowframe after debris are removed.
ch	channel to be checked for multiple peaks.
ph	maximum peak height to be ignored. This allows ignoring of tiny peaks that could affect the gating process.

**Value**

name of channel with more than one peak

```
@examples flowfile_path <- system.file("extdata", "B4_18_1.fcs", package = "cyanoFilter", must-
Work = TRUE) flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE, transformation
= FALSE, emptyValue = FALSE, dataset = 1) flowfile_nona <- cyanoFilter::noNA(x = flowfile)
flowfile_noneg <- cyanoFilter::noNeg(x = flowfile_nona) flowfile_logtrans <- cyanoFilter::lnTrans(x
= flowfile_noneg, c('SSC.W', 'TIME')) getChannel(flowfile_logtrans, 'RED.B.HLin', 0.05)
```

---

ggpairsDens	<i>produces a scatter plot of the expression matrix of the flowframe. If a cluster variable is given, it assigns different colors to the clusters.</i>
-------------	--

---

**Description**

produces a scatter plot of the expression matrix of the flowframe. If a cluster variable is given, it assigns different colors to the clusters.

**Usage**

```
ggpairsDens(flowfile, channels = NULL, group = NULL, notToPlot = NULL, ...)
```

**Arguments**

flowfile	flowframe to be plotted
channels	a character vector of length 2 or more. It must contain channel names in the flowfile.
group	cluster groups. It must be equal to the number of particles in the flowfile. If group is null cluster boundaries are not drawn.
notToPlot	columns not to plot. This is especially useful for for plotting all columns in a
...	not used at the moment
	@return a ggplot object

**Value**

a ggplot object



**Examples**

```
# example without clustering
flowfile_path <- system.file("extdata", "B4_18_1.fcs",
                             package = "cyanoFilter",
                             mustWork = TRUE)
flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE,
                              transformation = FALSE, emptyValue = FALSE,
                              dataset = 1)
flowfile_nona <- cyanoFilter::noNA(x = flowfile)
flowfile_noneg <- cyanoFilter::noNeg(x = flowfile_nona)
flowfile_logtrans <- lnTrans(x = flowfile_noneg, c('SSC.W', 'TIME'))
ggpairsDens(flowfile = flowfile_logtrans,
            channels = c("FSC.HLin", "RED.R.HLin", "RED.B.HLin",
                        "NIR.R.HLin"))
```

---

ggplotDens

*plots two channels of a flowframe.*


---

**Description**

plots two channels of a flowframe.

**Usage**

```
ggplotDens(flowfile, channels, ...)
```

**Arguments**

flowfile	flowframe to be plotted
channels	a character vector of length 2, must contain channel names in the flowfile.
...	not used at the moment

**Value**

a ggplot object

**Examples**

```
flowfile_path <- system.file("extdata", "B4_18_1.fcs",
                             package = "cyanoFilter",
                             mustWork = TRUE)
flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE,
                              transformation = FALSE, emptyValue = FALSE,
                              dataset = 1)
flowfile_nona <- cyanoFilter::noNA(x = flowfile)
flowfile_noneg <- cyanoFilter::noNeg(x = flowfile_nona)
flowfile_logtrans <- cyanoFilter::lnTrans(x = flowfile_noneg,
                                         c('SSC.W', 'TIME'))
ggplotDens(flowfile_logtrans,
           channels = c("FSC.HLin", "RED.R.HLin"))
```

---

ggplotDens2	<i>plots two channels of a flowframe with different colors for clusters identified.</i>
-------------	---

---

### Description

plots two channels of a flowframe with different colors for clusters identified.

### Usage

```
ggplotDens2(flowfile, channels, group, ...)
```

### Arguments

flowfile	flowframe to be plotted
channels	a character vector of length 2, must contain channel names in the flowfile.
group	cluster groups. must be equal to the number of particles in the flow cytometer.
...	not used at the moment

### Value

a ggplot object

### Examples

```
flowfile_path <- system.file("extdata", "B4_18_1.fcs",
  package = "cyanoFilter",
  mustWork = TRUE)
flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE,
  transformation = FALSE,
  emptyValue = FALSE,
  dataset = 1)
flowfile_nona <- cyanoFilter::noNA(x = flowfile)
flowfile_noneg <- cyanoFilter::noNeg(x = flowfile_nona)
flowfile_logtrans <- cyanoFilter::lnTrans(x = flowfile_noneg,
  c('SSC.W', 'TIME'))
cells_nonmargin <- cellMargin(flowframe = flowfile_logtrans,
  Channel = 'SSC.W',
  type = 'estimate', y_toplot = "FSC.HLin")
cells_nodebris <- debrisNc(flowframe = reducedFlowframe(cells_nonmargin),
  ch_chlorophyll = "RED.B.HLin",
  ch_p2 = "YEL.B.HLin",
  ph = 0.05)
cct <- phytoFilter(flowfile = reducedFlowframe(cells_nodebris),
  pig_channels = c("RED.B.HLin", "YEL.B.HLin", "RED.R.HLin"),
  com_channels = c("FSC.HLin", "SSC.HLin"))
ggplotDens2(reducedFlowframe(cct),
  c("RED.B.HLin", "YEL.B.HLin"),
  group = "Clusters")
```

---

goodFcs	<i>indicates if measurement from a flowfile is good or bad.</i>
---------	---

---

### Description

This function examines the column containing *cells/μL* and determines if the measurement can be used for further analysis or not based on a supplied range.

### Usage

```
goodFcs(metafile, col_cpml = "CellspML", mxd_cellpML = 1000, mnd_cellpML = 50)
```

### Arguments

metafile	associated metafile to the supplied fcsfile. This is a csv file containing computed stats from the flow cytometer.
col_cpml	column name or column number in metafile containing cell per microlitre measurements.
mxd_cellpML	maximal accepted cell per microlitre. Flowfiles with larger cell per microlitre are termed bad. Defaults to 1000.
mnd_cellpML	minimum accepted cell per microlitre. Flowfiles with lesser cell per microlitre are termed bad. Defaults to 50.

### Details

Most flow cytometer makers will always inform clients within which range can measurements from the machine be trusted. The machines normally stores the amount of *cells/μL* it counted in a sample. Too large value could mean possible doublets and too low value could mean too little cells.

### Value

character vector with length same as the number of rows in the metafile whose entries are **good** for good files and **bad** for bad files.

### Examples

```
require("stringr")
metadata <- system.file("extdata", "2019-03-25_Rstarted.csv",
  package = "cyanoFilter",
  mustWork = TRUE)
metafile <- read.csv(metadata, skip = 7, stringsAsFactors = FALSE,
  check.names = TRUE, encoding = "UTF-8")
metafile <- metafile[, seq_len(65)] #first 65 columns contains useful information
#extract the part of the Sample.ID that corresponds to BS4 or BS5
metafile$Sample.ID2 <- stringr::str_extract(metafile$Sample.ID, "BS*[4-5]")
#clean up the Cells.muL column
names(metafile)[which(stringr::str_detect(names(metafile),
  "Cells."))] <- "CellspML"
```

```
goodFcs(metafile = metafile, col_cpml = "CellspML", mxd_cellpML = 1000,  
mnd_cellpML = 50)
```

---

is.DebrisFilter      *function to check if object is of class cyanoFilter(DebrisFilter)*

---

**Description**

function to check if object is of class cyanoFilter(DebrisFilter)

**Usage**

```
is.DebrisFilter(x)
```

**Arguments**

x                    any R object

**Value**

TRUE if object is of class DebrisFilter. FALSE otherwise

**Examples**

```
x <- c(1, 5, 4)  
is.DebrisFilter(x)
```

---

is.flowFrame      *function to check if object is a flowFrame*

---

**Description**

function to check if object is a flowFrame

**Usage**

```
is.flowFrame(x)
```

**Arguments**

x                    any R object

**Value**

TRUE if object is a flowFrame. FALSE otherwise

**Examples**

```
x <- c(1, 5, 4)
is.flowFrame(x)
```

---

is.flowSet                    *function to check if object is a flowSet*

---

**Description**

function to check if object is a flowSet

**Usage**

```
is.flowSet(x)
```

**Arguments**

x                    any R object

**Value**

TRUE if object is a flowSet. FALSE otherwise

**Examples**

```
x <- c(1, 5, 4)
is.flowSet(x)
```

---

is.MarginEvents                *function to check if object is of class cyanoFilter(MarginEvents)*

---

**Description**

function to check if object is of class cyanoFilter(MarginEvents)

**Usage**

```
is.MarginEvents(x)
```

**Arguments**

x                    any R object

**Value**

TRUE if object is of class MarginEvents. FALSE otherwise

**Examples**

```
x <- c(1, 5, 4)
is.MarginEvents(x)
```

---

is.PhytopFilter	<i>function to check if object is of class cyanoFilter(PhytoFilter)</i>
-----------------	---

---

**Description**

function to check if object is of class cyanoFilter(PhytoFilter)

**Usage**

```
is.PhytopFilter(x)
```

**Arguments**

x                    any R object

**Value**

TRUE if object is of class PhytoFilter. FALSE otherwise

**Examples**

```
x <- c(1, 5, 4)
is.PhytopFilter(x)
```

---

lnTrans	<i>log transforms the expression matrix of a flowframe</i>
---------	--

---

**Description**

log transforms the expression matrix of a flowframe

**Usage**

```
lnTrans(x, notToTransform = c("SSC.W", "TIME"))
```

**Arguments**

x                    flowframe to be transformed  
notToTransform    columns not to be transformed

**Value**

**flowframe** with log transformed expression matrix

**Examples**

```

flowfile_path <- system.file("extdata", "B4_18_1.fcs",
                             package = "cyanoFilter",
                             mustWork = TRUE)
flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE,
                               transformation = FALSE, emptyValue = FALSE,
                               dataset = 1)
flowfile_nona <- cyanoFilter::noNA(x = flowfile)
flowfile_nonneg <- cyanoFilter::noNeg(x = flowfile_nona)
lnTrans(x = flowfile_nonneg, c('SSC.W', 'TIME'))

```

---

MarginEvents	<i>the marginEvent class</i>
--------------	------------------------------

---

**Description**

the marginEvent class

constructor for the MarginEvents class

**Usage**

```

MarginEvents(
  fullflowframe,
  reducedflowframe,
  N_margin,
  N_nonmargin,
  N_particle,
  Channel,
  y_toplot,
  cut
)

```

```

MarginEvents(
  fullflowframe,
  reducedflowframe,
  N_margin,
  N_nonmargin,
  N_particle,
  Channel,
  y_toplot,
  cut
)

```

**Arguments**

`fullflowframe` same as the input `flowFrame`  
`reducedflowframe`  
                   a partial flowframe containing non-margin events  
`N_margin`          number of margin particles measured  
`N_nonmargin`      number of non-margin particles  
`N_particle`       total number of particles measured  
`Channel`          channel measuring the width of the particles  
`y_toplot`        another channel to use in a bivariate plot  
`cut`              the cut-off point estimated or supplied.

**Value**

object of class `MarginEvents`

**Slots**

`fullflowframe` object of class "flowFrame" same as the input flowFrame  
`reducedflowframe` object of class "flowFrame" a partial flowframe containing a proportion of the measured particles  
`N_margin` object of class "numeric" representing the proportion of particles in each cluster  
`N_nonmargin` object of class "integer" representing the number of particles in each cluster  
`N_particle` object of class "integer" representing the labels for each cluster  
`Channel` object of class character representing channel measuring cell width  
`y_toplot` object of class character representing plot variable  
`cut` object of class numeric representing estimated inflection point or supplied cut-off point

**Examples**

```

flowfile_path <- system.file("extdata", "B4_18_1.fcs",
                             package = "cyanoFilter",
                             mustWork = TRUE)
flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE,
                               transformation = FALSE, emptyValue = FALSE,
                               dataset = 1)
flowfile_nona <- cyanoFilter::noNA(x = flowfile)
flowfile_nonneg <- cyanoFilter::noNeg(x = flowfile_nona)
flowfile_logtrans <- lnTrans(x = flowfile_nonneg, c('SSC.W', 'TIME'))
cellMargin(flowframe = flowfile_logtrans, Channel = 'SSC.W',
            type = 'estimate', y_toplot = "FSC.HLin")
  
```



---

newFlowframe	<i>takes a flowframe, a group indicator and formulates another flowframe with group indicator as part of the expression matrix of the new flowframe.</i>
--------------	--

---

## Description

takes a flowframe, a group indicator and formulates another flowframe with group indicator as part of the expression matrix of the new flowframe.

## Usage

```
newFlowframe(flowfile, group = NULL, togate = NULL)
```

## Arguments

flowfile	flowframe after debris are removed.
group	cluster group to be added to the expression matrix
togate	channel detected to have more than one peak

## Value

flowframe with indicators for particle cluster

## Examples

```
flowfile_path <- system.file("extdata", "B4_18_1.fcs",  
                             package = "cyanoFilter",  
                             mustWork = TRUE)  
flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE,  
                              transformation = FALSE, emptyValue = FALSE,  
                              dataset = 1)  
flowfile_nona <- cyanoFilter::noNA(x = flowfile)  
flowfile_nonneg <- cyanoFilter::noNeg(x = flowfile_nona)  
flowfile_logtrans <- cyanoFilter::lnTrans(x = flowfile_nonneg,  
c('SSC.W', 'TIME'))  
oneDgate(flowfile, 'RED.B.HLin')
```

---

noNA *Removes NA values from the expression matrix of a flow cytometer file.*

---

**Description**

Removes NA values from the expression matrix of a flow cytometer file.

**Usage**

```
noNA(x)
```

**Arguments**

x flowframe with expression matrix containing NAs.

**Value**

flowframe with expression matrix rid of NAs.

**Examples**

```
flowfile_path <- system.file("extdata", "B4_18_1.fcs",  
package = "cyanoFilter",  
mustWork = TRUE)  
flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE,  
transformation = FALSE, emptyValue = FALSE,  
dataset = 1)  
noNA(x = flowfile)
```

---

noNeg *Removes negative values from the expression matrix*

---

**Description**

Removes negative values from the expression matrix

**Usage**

```
noNeg(x)
```

**Arguments**

x is the flowframe whose expression matrix contains negative values

**Value**

flowframe with non-negative values in its expression matrix

**Examples**

```
flowfile_path <- system.file("extdata", "B4_18_1.fcs",
  package = "cyanoFilter",
  mustWork = TRUE)
flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE,
  transformation = FALSE, emptyValue = FALSE,
  dataset = 1)
flowfile_nona <- cyanoFilter::noNA(x = flowfile)
noNeg(x = flowfile_nona)
```

---

oneDgate

*returns the labels stating the cluster of each row in a flowfile.*

---

**Description**

returns the labels stating the cluster of each row in a flowfile.

**Usage**

```
oneDgate(flowfile, togate)
```

**Arguments**

flowfile            flowframe after debris are removed.  
 togate            channels detected to have more than one peak present. Provide by the [getChannel](#) function.

**Value**

list of indicators for cells above and below an estimated threshold

**Examples**

```
flowfile_path <- system.file("extdata", "B4_18_1.fcs",
  package = "cyanoFilter",
  mustWork = TRUE)
flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE,
  transformation = FALSE, emptyValue = FALSE,
  dataset = 1)
flowfile_nona <- cyanoFilter::noNA(x = flowfile)
flowfile_noneg <- cyanoFilter::noNeg(x = flowfile_nona)
flowfile_logtrans <- cyanoFilter::lnTrans(x = flowfile_noneg,
  c('SSC.W', 'TIME'))
```

```
oneDgate(flowfile, 'RED.B.HLin')
```

---

pairsPlot	<i>produces a scatter plot of the expression matrix of a flowframe. Note that, it takes some time to display the plot.</i>
-----------	--

---

### Description

produces a scatter plot of the expression matrix of a flowframe. Note that, it takes some time to display the plot.

### Usage

```
pairsPlot(x, notToPlot = c("TIME"), ...)
```

### Arguments

x	flowframe to be plotted
notToPlot	column in expression matrix not to be plotted
...	other arguments. Not used at the moment

### Value

a plot object

### Examples

```
flowfile_path <- system.file("extdata", "B4_18_1.fcs",
                             package = "cyanoFilter",
                             mustWork = TRUE)
flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE,
                              transformation = FALSE, emptyValue = FALSE,
                              dataset = 1)
flowfile_nona <- cyanoFilter::noNA(x = flowfile)
flowfile_noneg <- cyanoFilter::noNeg(x = flowfile_nona)
flowfile_logtrans <- cyanoFilter::lnTrans(x = flowfile_noneg,
                                         c('SSC.W', 'TIME'))
pairsPlot(flowfile_logtrans,
          notToPlot = c("TIME", "SSC.W",
                       "SSC.HLin", "NIR.R.HLin",
                       "FSC.HLin"))
```

---

phytoFilter	<i>gates out and assign indicators to phytoplankton cells based on the expression of measured cell complexity channels.</i>
-------------	---

---

## Description

This function takes in a flowframe with debris removed and identifies the different phytoplankton cell population based on cell pigmentation and/or complexity.

## Usage

```
phytoFilter(  
  flowfile,  
  pig_channels = NULL,  
  com_channels = NULL,  
  ph = 0.05,  
  proportion = 0.8  
)
```

## Arguments

flowfile	flowframe after debris are removed.
pig_channels	flowcytometer channels measuring cell pigments.
com_channels	flowcytometer channels measuring cell complexity.
ph	maximum peak height to be ignored. This allows ignoring of tiny peaks that could affect the gating process.
proportion	proportion of cell count to be returned.

## Details

The function uses the [getPeaks](#) and [deGate](#) functions in the *flowDensity* package to identify peaks and identify cut-off points between these peaks.

## Value

object of class [PhytopFilter](#) containing;

- **fullflowframe** - flowframe containing all phytoplankton cells with added columns indicating cluster
- **flowframe\_proportion** - a part of fullflowframe containing proportion of cell count.
- **clusters\_proportion** - proportion of cells in each cluster
- **particles\_per\_cluster** - number of particles per cluster
- **Cluster\_ind** - indicator for each cluster
- **gated\_channels** - channels with multiple peaks

**Examples**

```

flowfile_path <- system.file("extdata", "B4_18_1.fcs",
package = "cyanoFilter",
  mustWork = TRUE)
flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE,
  transformation = FALSE,
  emptyValue = FALSE,
  dataset = 1)
flowfile_nona <- cyanoFilter::noNA(x = flowfile)
flowfile_noneg <- cyanoFilter::noNeg(x = flowfile_nona)
flowfile_logtrans <- cyanoFilter::lnTrans(x = flowfile_noneg,
  c('SSC.W', 'TIME'))
cells_nonmargin <- cellMargin(flowframe = flowfile_logtrans,
  Channel = 'SSC.W',
  type = 'estimate', y_toplot = "FSC.HLin")
cells_nodebris <- debrisNc(flowframe = reducedFlowframe(cells_nonmargin),
  ch_chlorophyll = "RED.B.HLin",
  ch_p2 = "YEL.B.HLin",
  ph = 0.05)
phytoFilter(flowfile = reducedFlowframe(cells_nodebris),
  pig_channels = c("RED.B.HLin", "YEL.B.HLin", "RED.R.HLin"),
  com_channels = c("FSC.HLin", "SSC.HLin"))

```

---

PhytopFilter

*the phytofilter class*


---

**Description**

the phytofilter class

constructor for the PhytoFilter class

**Usage**

```

PhytopFilter(
  fullflowframe,
  flowframe_proportion,
  clusters_proportion,
  particles_per_cluster,
  Cluster_ind,
  gated_channels,
  channels
)

```

```

PhytopFilter(
  fullflowframe,

```

```

    flowframe_proportion,
    clusters_proportion,
    particles_per_cluster,
    Cluster_ind,
    gated_channels,
    channels
  )

```

### Arguments

**fullflowframe** same as the input flowFrame  
**flowframe\_proportion** a partial flowframe containing containing a proportion of the measured particles  
**clusters\_proportion** number of margin particles measured  
**particles\_per\_cluster** number of particles in each cluster  
**Cluster\_ind** labels for each cluster  
**gated\_channels** channels used for gating  
**channels** all channels supplied

### Value

object of class PhytoFilter

### Slots

**fullflowframe** object of class "flowFrame" same as the input flowFrame  
**flowframe\_proportion** object of class "flowFrame" a partial flowframe containing a proportion of the measured particles  
**clusters\_proportion** object of class "numeric" representing the proportion of particles in each cluster  
**particles\_per\_cluster** object of class "data.frame" representing the number of particles in each cluster  
**Cluster\_ind** object of class "integer" representing the labels for each cluster  
**gated\_channels** object of class "character" representing the names of channels with multiple peaks  
**channels** object of class "character" representing the names of the channels

### Examples

```

flowfile_path <- system.file("extdata", "B4_18_1.fcs",
                             package = "cyanoFilter",
                             mustWork = TRUE)
flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE,
                              transformation = FALSE, emptyValue = FALSE,

```

```

                                dataset = 1)
flowfile_nona <- cyanoFilter::noNA(x = flowfile)
flowfile_noneg <- cyanoFilter::noNeg(x = flowfile_nona)
flowfile_logtrans <- lnTrans(x = flowfile_noneg, c('SSC.W', 'TIME'))
cellMargin(flowframe = flowfile_logtrans, Channel = 'SSC.W',
            type = 'estimate', y_toplot = "FSC.HLin")

flowfile_path <- system.file("extdata", "B4_18_1.fcs",
                             package = "cyanoFilter",
                             mustWork = TRUE)
flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE,
                              transformation = FALSE, emptyValue = FALSE,
                              dataset = 1)
flowfile_nona <- cyanoFilter::noNA(x = flowfile)
flowfile_noneg <- cyanoFilter::noNeg(x = flowfile_nona)
flowfile_logtrans <- lnTrans(x = flowfile_noneg, c('SSC.W', 'TIME'))
cellMargin(flowframe = flowfile_logtrans, Channel = 'SSC.W',
            type = 'estimate', y_toplot = "FSC.HLin")

```

---

pigmentGate

*gates out or assign indicators to phytoplankton cells based on the expression of the measured pigments.*

---

## Description

This function takes in a flowframe with debris removed and identifies phytoplankton cell population in the provided frame.

## Usage

```
pigmentGate(flowfile, pig_channels, ph = 0.05)
```

## Arguments

flowfile	flowframe after debris are removed.
pig_channels	flowcytometer channels measuring phytoplankton pigmentations.
ph	maximum peak height to be ignored. This allows ignoring of tiny peaks that could affect the gating process.

## Details

The function uses the [getPeaks](#) and [deGate](#) functions in the *flowDensity* package to identify peaks and identify cut-off points between these peaks.



**Value**

list containing;

- **full\_flowframe** - flowframe containing only phytoplankton cells
- **phy\_ind** - indicator for phytoplankton clusters found
- **gated\_channels** - pigment channels with more than one peak

**Examples**

```
flowfile_path <- system.file("extdata", "B4_18_1.fcs",
                             package = "cyanoFilter",
                             mustWork = TRUE)
flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE,
                              transformation = FALSE, emptyValue = FALSE,
                              dataset = 1)
flowfile_nona <- cyanoFilter::noNA(x = flowfile)
flowfile_noneg <- cyanoFilter::noNeg(x = flowfile_nona)
flowfile_logtrans <- cyanoFilter::lnTrans(x = flowfile_noneg,
c('SSC.W', 'TIME'))
cyanoFilter::pigmentGate(flowfile = flowfile_logtrans,
pig_channels = c("RED.B.HLin", "YEL.B.HLin",
                 "FSC.HLin", "RED.R.HLin"),
ph = 0.06)
```

---

plot,DebrisFilter,ANY-method

*plot method for DebrisFilter objects*

---

**Description**

plot method for DebrisFilter objects

**Usage**

```
## S4 method for signature 'DebrisFilter,ANY'
plot(x)
```

**Arguments**

x                    an object of class DebrisFilter

**Value**

object of class ggplot

---

`plot,MarginEvents,ANY-method`  
*plot method for MarginEvents objects*

---

**Description**

plot method for MarginEvents objects

**Usage**

```
## S4 method for signature 'MarginEvents,ANY'  
plot(x)
```

**Arguments**

`x` an object of class MarginEvents

**Value**

object of class ggplot

---

`plot,PhytopFilter,ANY-method`  
*plot method for PhytoFilter objects*

---

**Description**

plot method for PhytoFilter objects

**Usage**

```
## S4 method for signature 'PhytopFilter,ANY'  
plot(x)
```

**Arguments**

`x` an object of class PhytoFilter

**Value**

object of class ggplot

---

reducedFlowframe	<i>generic function for extracting the full flowframe</i>
------------------	---

---

**Description**

generic function for extracting the full flowframe

**Usage**

```
reducedFlowframe(x)
```

**Arguments**

x                    an object of either class PhytoFilter, MarginEvents or DebrisFilter

**Value**

generic to extract fullFlowframe

**Examples**

```
flowfile_path <- system.file("extdata", "B4_18_1.fcs",
  package = "cyanoFilter",
  mustWork = TRUE)
flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE,
  transformation = FALSE,
  emptyValue = FALSE,
  dataset = 1)
flowfile_nona <- cyanoFilter::noNA(x = flowfile)
flowfile_noneg <- cyanoFilter::noNeg(x = flowfile_nona)
flowfile_logtrans <- cyanoFilter::lnTrans(x = flowfile_noneg,
  c('SSC.W', 'TIME'))
cells_nonmargin <- cellMargin(flowframe = flowfile_logtrans,
  Channel = 'SSC.W',
  type = 'estimate', y_toplot = "FSC.HLin")
reducedFlowframe(cells_nonmargin)
```

---

reducedFlowframe,DebrisFilter-method	<i>accessor method for reduced flowframe (DebrisFilter class)</i>
--------------------------------------	---

---

**Description**

accessor method for reduced flowframe (DebrisFilter class)

**Usage**

```
## S4 method for signature 'DebrisFilter'
reducedFlowframe(x)
```

**Arguments**

x                    an object of class DebrisFilter

**Value**

reduced flowFrame method for DebrisFilter

---

reducedFlowframe,MarginEvents-method

*accessor method for reduced flowframe (MarginEvent class)*

---

**Description**

accessor method for reduced flowframe (MarginEvent class)

**Usage**

```
## S4 method for signature 'MarginEvents'
reducedFlowframe(x)
```

**Arguments**

x                    an object of class MarginEvents

**Value**

reduced Flowframe method for MarginEvents

**Examples**

```
flowfile_path <- system.file("extdata", "B4_18_1.fcs",
                             package = "cyanoFilter",
                             mustWork = TRUE)
flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE,
                              transformation = FALSE, emptyValue = FALSE,
                              dataset = 1)
flowfile_nona <- cyanoFilter::noNA(x = flowfile)
flowfile_nonneg <- cyanoFilter::noNeg(x = flowfile_nona)
flowfile_logtrans <- cyanoFilter::lnTrans(x = flowfile_nonneg,
c('SSC.W', 'TIME'))
cells_nonmargin <- cellMargin(flowframe = flowfile_logtrans,
Channel = 'SSC.W',
                             type = 'estimate', y_toplot = "FSC.HLin")
reducedFlowframe(cells_nonmargin)
```

---

```
reducedFlowframe,PhytopFilter-method
      accessor method for reduced flowframe(PhytopFilter class)
```

---

**Description**

accessor method for reduced flowframe(PhytopFilter class)

**Usage**

```
## S4 method for signature 'PhytopFilter'
reducedFlowframe(x)
```

**Arguments**

x                    an object of class PhytopFilter

**Value**

```
reduced flowFrame method for PhytopFilter #' @examples flowfile_path <- system.file("extdata",
"B4_18_1.fcs", package = "cyanoFilter", mustWork = TRUE) flowfile <- flowCore::read.FCS(flowfile_path,
alter.names = TRUE, transformation = FALSE, emptyValue = FALSE, dataset = 1) flowfile_nona
<- cyanoFilter::noNA(x = flowfile) flowfile_noneg <- cyanoFilter::noNeg(x = flowfile_nona) flow-
file_logtrans <- cyanoFilter::lnTrans(x = flowfile_noneg, c('SSC.W', 'TIME')) cells_nonmargin
<- cellMargin(flowframe = flowfile_logtrans, Channel = 'SSC.W', type = 'estimate', y_toplot =
"FSC.HLin") cells_nodebris <- debrisNc(flowframe = reducedFlowframe(cells_nonmargin), ch_chlorophyll
= "RED.B.HLin", ch_p2 = "YEL.B.HLin", ph = 0.05) phy1 <- phytoFilter(flowfile = reduced-
Flowframe(cells_nodebris), pig_channels = c("RED.B.HLin", "YEL.B.HLin", "RED.R.HLin"), com_channels
= c("FSC.HLin", "SSC.HLin")) reducedFlowframe(phy1)
```

---

```
retain                    Decides if a file should be retained or removed based on its status.
```

---

**Description**

Function to determine what files to retain and finally read from the flow cytometer FCS file.

**Usage**

```
retain(
  meta_files,
  make_decision = c("maxi", "mini", "unique"),
  Status = "Status",
  CellspML = "CellspML"
)
```

## Arguments

<code>meta_files</code>	dataframe from meta file that has been preprocessed by the <code>goodFcs</code> function.
<code>make_decision</code>	decision to be made should more than one <i>cells/μL</i> be good.
<code>Status</code>	column name in <code>meta_files</code> containing status obtained from the <code>goodFcs</code> function.
<code>CellspML</code>	column name in <code>meta_files</code> containing <i>cells/μL</i> measurements.

## Details

It is typically not known in advance which dilution level would result in the desired *cells/μL*, therefore the samples are ran through the flow cytometer at two or more dilution levels. Out of these, one has to decide which to retain and finally use for further analysis. This function and `goodFcs` are to help you decide that. If more than one of the dilution levels are judged good, the option `make_decision = "maxi"` will give "Retain" to the row with the maximum *cells/μL* while the opposite occurs for `make_decision = "mini"`. `make_decision = "unique"` i there is only one measurement for that particular sample, while `make_decision = "maxi"` and `make_decision = "mini"` should be used for files with more than one measurement for the sample in question.

## Value

a character vector with entries "Retain" for a file to be retained or "No!" for a file to be discarded.

## See Also

[goodFcs](#)

## Examples

```
require("stringr")
metadata <- system.file("extdata", "2019-03-25_Rstarted.csv",
  package = "cyanoFilter",
  mustWork = TRUE)
metafile <- read.csv(metadata, skip = 7, stringsAsFactors = FALSE,
  check.names = TRUE, encoding = "UTF-8")
metafile <- metafile[, seq_len(65)] #first 65 columns contain useful information
#extract the part of the Sample.ID that corresponds to BS4 or BS5
metafile$Sample.ID2 <- stringr::str_extract(metafile$Sample.ID, "BS*[4-5]")
#clean up the Cells.muL column
names(metafile)[which(stringr::str_detect(names(metafile), "Cells."))] <-
  "CellspML"
metafile$Status <- cyanoFilter::goodFcs(metafile = metafile, col_cpml =
  "CellspML",
  mxd_cellpML = 1000, mnd_cellpML = 50)
metafile$Retained <- NULL
# first 3 rows contain BS4 measurements at 3 dilution levels
metafile$Retained[seq_len(3)] <-
  cyanoFilter::retain(meta_files = metafile[seq_len(3),],
  make_decision = "maxi",
  Status = "Status", CellspML = "CellspML")
# last 3 rows contain BS5 measurements at 3 dilution levels as well
```

```

metafile$Retained[seq(4, 6, by = 1)] <-
  cyanoFilter::retain(meta_files = metafile[seq(4, 6, by = 1),],
make_decision = "maxi",
Status = "Status", CellspML = "CellspML")

```

---

rowNumbers	<i>returns the position of the cells below, above or between estimated gates</i>
------------	--

---

### Description

returns the position of the cells below, above or between estimated gates

### Usage

```
rowNumbers(flowframe, gates, ch)
```

### Arguments

flowframe	after debris are removed.
gates	cut point between the identified clusters
ch	gated channel

### Value

a numeric vector

### Examples

```

flowfile_path <- system.file("extdata", "B4_18_1.fcs",
  package = "cyanoFilter",
  mustWork = TRUE)
flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE,
  transformation = FALSE, emptyValue = FALSE,
  dataset = 1)
flowfile_nona <- cyanoFilter::noNA(x = flowfile)
flowfile_noneg <- cyanoFilter::noNeg(x = flowfile_nona)
flowfile_logtrans <- cyanoFilter::lnTrans(x = flowfile_noneg,
c('SSC.W', 'TIME'))
oneDgate(flowfile, 'RED.B.HLin')

```

---

summaries	<i>takes a flowframes, a vector of channels, cluster indicator and return desired summaries per cluster</i>
-----------	---

---

## Description

takes a flowframes, a vector of channels, cluster indicator and return desired summaries per cluster

## Usage

```
summaries(object, channels, cluster_var, summary)
```

## Arguments

object	An object of class cyanoFilter to be summarised.
channels	channels whose summaries are to be computed
cluster_var	column name in expression matrix containing the cluster indicators
summary	summary statistic of interest. Only mean and variance-covariance matrix supported at the moment.

## Value

list containing computed summaries

## Examples

```
flowfile_path <- system.file("extdata", "B4_18_1.fcs",
                             package = "cyanoFilter",
                             mustWork = TRUE)
flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE,
                              transformation = FALSE, emptyValue = FALSE,
                              dataset = 1)
flowfile_nona <- cyanoFilter::noNA(x = flowfile)
flowfile_nonneg <- cyanoFilter::noNeg(x = flowfile_nona)
flowfile_logtrans <- cyanoFilter::lnTrans(x = flowfile_nonneg,
c('SSC.W', 'TIME'))
cells_nonmargin <- cellMargin(flowframe = flowfile_logtrans,
Channel = 'SSC.W',
                             type = 'estimate', y_toplot = "FSC.HLin")
cells_nodebris <- debrisNc(flowframe = reducedFlowframe(cells_nonmargin),
                           ch_chlorophyll = "RED.B.HLin",
                           ch_p2 = "YEL.B.HLin",
                           ph = 0.05)
```



---

summaries,DebrisFilter-method

*takes a flowframes, a vector of channels, cluster indicator and return desired summaries per cluster*

---

## Description

takes a flowframes, a vector of channels, cluster indicator and return desired summaries per cluster

## Usage

```
## S4 method for signature 'DebrisFilter'  
summaries(object, channels = NULL)
```

## Arguments

**object**            An object of class MarginEvents to be summarised.  
**channels**        channels whose summaries are to be computed

## Value

list containing the required summaries

## Examples

```
flowfile_path <- system.file("extdata", "B4_18_1.fcs",  
                             package = "cyanoFilter",  
                             mustWork = TRUE)  
flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE,  
                              transformation = FALSE, emptyValue = FALSE,  
                              dataset = 1)  
flowfile_nona <- cyanoFilter::noNA(x = flowfile)  
flowfile_noneg <- cyanoFilter::noNeg(x = flowfile_nona)  
flowfile_logtrans <- cyanoFilter::lnTrans(x = flowfile_noneg,  
c('SSC.W', 'TIME'))  
cells_nonmargin <- cellMargin(flowframe = flowfile_logtrans,  
Channel = 'SSC.W',  
                             type = 'estimate', y_toplot = "FSC.HLin")  
summaries(cells_nonmargin,  
c("RED.B.HLin", "YEL.B.HLin", "RED.R.HLin"))
```

---

summaries,MarginEvents-method

*takes a flowframes, a vector of channels, cluster indicator and return desired summaries per cluster*

---

## Description

takes a flowframes, a vector of channels, cluster indicator and return desired summaries per cluster

## Usage

```
## S4 method for signature 'MarginEvents'
summaries(object, channels = NULL)
```

## Arguments

object	An object of class MarginEvents to be summarised.
channels	channels whose summaries are to be computed

## Value

list containing the required summaries

## Examples

```
flowfile_path <- system.file("extdata", "B4_18_1.fcs",
                             package = "cyanoFilter",
                             mustWork = TRUE)
flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE,
                              transformation = FALSE, emptyValue = FALSE,
                              dataset = 1)
flowfile_nona <- cyanoFilter::noNA(x = flowfile)
flowfile_noneg <- cyanoFilter::noNeg(x = flowfile_nona)
flowfile_logtrans <- cyanoFilter::lnTrans(x = flowfile_noneg,
c('SSC.W', 'TIME'))
cells_nonmargin <- cellMargin(flowframe = flowfile_logtrans,
Channel = 'SSC.W',
                             type = 'estimate', y_toplot = "FSC.HLin")
summaries(cells_nonmargin,
c("RED.B.HLin", "YEL.B.HLin", "RED.R.HLin"))
```

---

 summaries,PhytopFilter-method

*takes a flowframes, a vector of channels, cluster indicator and return desired summaries per cluster*

---

## Description

takes a flowframes, a vector of channels, cluster indicator and return desired summaries per cluster

## Usage

```
## S4 method for signature 'PhytopFilter'
summaries(
  object,
  channels = NULL,
  cluster_var = "Clusters",
  summary = c("mean", "median", "cov", "n")
)
```

## Arguments

object	An object of class cyanoFilter to be summarised.
channels	channels whose summaries are to be computed
cluster_var	column name in expression matrix containing the cluster indicators
summary	summary statistic of interest. Only mean and variance-covariance matrix supported at the moment.

## Value

list containing computed summaries

## Examples

```
flowfile_path <- system.file("extdata", "B4_18_1.fcs",
  package = "cyanoFilter",
  mustWork = TRUE)
flowfile <- flowCore::read.FCS(flowfile_path, alter.names = TRUE,
  transformation = FALSE, emptyValue = FALSE,
  dataset = 1)
flowfile_nona <- cyanoFilter::noNA(x = flowfile)
flowfile_noneg <- cyanoFilter::noNeg(x = flowfile_nona)
flowfile_logtrans <- cyanoFilter::lnTrans(x = flowfile_noneg,
  c('SSC.W', 'TIME'))
cells_nonmargin <- cellMargin(flowframe = flowfile_logtrans,
  Channel = 'SSC.W',
  type = 'estimate', y_toplot = "FSC.HLin")
cells_nodebris <- debrisNc(flowframe = reducedFlowframe(cells_nonmargin),
  ch_chlorophyll = "RED.B.HLin",
```

```
        ch_p2 = "YEL.B.HLin",
        ph = 0.05)
fin <- phytoFilter(flowfile = reducedFlowframe(cells_nodebris),
  pig_channels = c("RED.B.HLin", "YEL.B.HLin", "RED.R.HLin"),
  com_channels = c("FSC.HLin", "SSC.HLin"))

summaries(object = fin,
  channels = c("RED.B.HLin", "YEL.B.HLin", "RED.R.HLin"),
  cluster_var = "Clusters",
  summary = 'mean')
```

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