

# Package ‘cytofast’

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**Title** cytofast - A quick visualization and analysis tool for CyTOF data

**Version** 1.0.0

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## Description

Multi-parametric flow and mass cytometry allows exceptional high-resolution exploration of the cellular composition of the immune system. Together with tools like FlowSOM and Cytosplore it is possible to identify novel cell types. By introducing cytofast we hope to offer a workflow for visualization and quantification of cell clusters for an efficient discovery of cell populations associated with diseases or other clinical outcomes.

**Depends** R (>= 3.6.0)

**Imports** flowCore, ggplot2, ggridges, RColorBrewer, reshape2, stats, grDevices, Rdpack, methods, grid, FlowSOM

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'cfList-methods.R' 'cytoBoxplots.R' 'cytoHeatmaps.R'  
'cytotest.R' 'drawTree.R' 'msiPlot.R' 'readCytosploreFCS.R'  
'spitzer-data.R'

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## R topics documented:

cellCounts . . . . .	2
cfList-class . . . . .	3
cytoBoxplots . . . . .	4
cytoHeatmaps . . . . .	5
cytottest . . . . .	6
drawTree . . . . .	7
msiPlot . . . . .	7
readCytosplereFCS . . . . .	8
spitzer . . . . .	9
[,cfList,ANY-method . . . . .	10

<b>Index</b>	<b>11</b>
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cellCounts	<i>Extract cell counts from cfList</i>
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### Description

A function to add the frequency (or abundance) of cell clusters per sample to a cfList.

### Usage

```
cellCounts(cfList, frequency = FALSE, scale = FALSE)
```

### Arguments

cfList	a cfList object. It should contain at least data in the 'expr' slot.
frequency	one of <ul style="list-style-type: none"> <li>a logical value. if FALSE, the abundance of the cell counts are used. If TRUE, the frequency of the total amount of given cells is used.</li> <li>a numeric vector of same length as amount of samples</li> </ul>
scale	a logical value. Do the cell frequencies need to be centered and scaled? The default <a href="#">scale</a> function is called.

### Value

Returns a cfList with a 'counts' slot.

### Examples

```
# Read Data
dirFCS <- system.file("extdata", package="cytofast")
cfData <- readCytosplereFCS(dir = dirFCS, colNames = "description")

# Add cell counts to cfList
cfData <- cellCounts(cfData)
```

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cfList-class	<i>Class cfList</i>
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## Description

This is an S4 class that contains all the data and results for the cytofast workflow. Most functions in this package will both use and return a `cfList`.

## Details

This class is used throughout the cytofast workflow, most functions in this package will both use and return a `cfList`. FCS files created by Cytosplore can be easily read in with `readCytosploreFCS` and will be returned as a `cfList`. It is also possible to manually create instances of this class if another clustering method is preferred. Below an example is shown for `FlowSOM`.

## Slots

`samples` data frame containing all meta information on each sample  
`expr` data frame containing the marker expression  
`counts` data frame containing (standardized) cell counts per cluster and per sample  
`results` list containing any results

## Methods

`samples` a method for obtaining the samples data  
`expr` a method for obtaining the marker expression  
`counts` a method for obtaining the cell counts  
`results` a method for obtaining any results from performed tests

## Examples

```
### manually create instance of cfList class
library(FlowSOM)

## Cluster with FlowSOM
dirFCS <- system.file("extdata", package="cytofast")
fSOM <- FlowSOM(input = dirFCS,
               transform = FALSE,
               scale = FALSE,
               colsToUse = c(9:11, 15:52),
               nClus = 10, # Note that this is an ambiguous choice
               seed = 123)

## expr slot
# retrieve clusters
clusterID <- as.factor(fSOM$FlowSOM$map$mapping[,1])
levels(clusterID) <- fSOM$metaclustering

# retrieve samples (As example, we assume each FCS file is its own sample)
sampleID <- lapply(fSOM$FlowSOM$metaData, function(x){rep(x[1], each = length(x[1]:x[2]))})
attr(sampleID, 'names') <- NULL
```

```

sampleID <- as.factor(unlist(sampleID))
levels(sampleID) <- paste("ID", 1:10, sep="_")

exprD <- data.frame(clusterID,
                    sampleID,
                    fSOM$FlowSOM$data[, c(9:11, 15:52)])

## samples slot
samplesD <- data.frame(sampleID = levels(sampleID),
                       group = rep(c("group1", "group1"), each=5))

## create cfList
cfList(samples = samplesD,
        expr = exprD)

```

---

cytoBoxplots

*Draw boxplots for cfList*


---

### Description

Draw boxplots for all given clusters. Values are based on the counts slot in the cfList.

### Usage

```
cytoBoxplots(cfList, group, stat)
```

### Arguments

cfList	a cfList object. It should contain at least data in the 'counts' slot.
group	one of: <ul style="list-style-type: none"> <li>a character referring to a column name in the samples slot of the cfList.</li> <li>a factor indicating the grouping (x-axis) for the boxplots.</li> </ul>
stat	ignore, will be implemented soon.

### Value

None

### Examples

```

# Read Data
dirFCS <- system.file("extdata", package="cytofast")
cfData <- readCytosploreFCS(dir = dirFCS, colNames = "description")

# Add cell counts to cfList and add meta data
cfData <- cellCounts(cfData, frequency = TRUE, scale = TRUE)
meta <- spitzer[match(row.names(cfData@samples), spitzer$CSPLR_ST),]
cfData@samples <- cbind(cfData@samples, meta)

# Remove unnecessary markers
cfData@expr <- cfData@expr[,-c(3:10, 13:16, 55:59, 61:63)]

```

```
# Draw boxplots
cytoBoxplots(cfData, group="group")
```

---

cytoHeatmaps

*Draw heatmaps for cfList*

---

## Description

Function to draw two heatmaps. They visualize the median intensity of the markers for the created clusters. The ordering of the clusters is based on the default hierarchical cluster analysis [hclust](#). Note that hclust takes the data after the median intensity is calculated per cluster, thus placing the most similar clusters next to each other.

## Usage

```
cytoHeatmaps(cfList, group, legend = FALSE)
```

## Arguments

cfList	a cfList object.
group	one of: <ul style="list-style-type: none"><li>• a character vector referring to a column name in the samples slot of the cfList.</li><li>• a factor indicating the grouping (x-axis) for the boxplots.</li></ul>
legend	logical, whether a legend should be added

## Value

None

## Examples

```
# Read Data
dirFCS <- system.file("extdata", package="cytofast")
cfData <- readCytosplereFCS(dir = dirFCS, colNames = "description")

# Add cell counts to cfList and add meta data
cfData <- cellCounts(cfData, frequency = TRUE, scale = TRUE)
meta <- spitzer[match(row.names(cfData@samples), spitzer[, "CSPLR_ST"]),]
cfData@samples <- cbind(cfData@samples, meta)

# Remove unnecessary markers
cfData@expr <- cfData@expr[,-c(3:10, 13:16, 55:59, 61:63)]

# Draw heatmaps
cytoHeatmaps(cfData, group = "group", legend = TRUE)
```

---

 cytottest

*perform t-test on cfList*


---

### Description

Performs a separate t-test on each cluster within a `cfList`. The output is added and can also be used by other functions

### Usage

```
cytottest(cfList, group, adjustMethod, ...)
```

### Arguments

`cfList` a `cfList` object. It should contain at least data in the 'counts' slot.

`group` one of:

- a character vector referring to a column name in the `samples` slot of the `cfList`.
- a factor indicating the grouping for the t.test.

`adjustMethod` character, correction method used in `p.adjust`, choose from `c("holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none")`. If omitted, no correction takes place.

`...` further arguments passed on to `t.test`.

### Value

Returns a `cfList` with a 'results' slot.

### Examples

```
# Read Data
dirFCS <- system.file("extdata", package="cytofast")
cfData <- readCytosplereFCS(dir = dirFCS, colNames = "description")

# relabeling of clusterID
levels(cfData@expr[, "clusterID"]) <- gsub("[^0-9]", "", levels(cfData@expr[, "clusterID"]))

# Add cell counts to cfList and add meta data
cfData <- cellCounts(cfData, frequency = TRUE, scale = TRUE)
meta <- spitzer[match(row.names(cfData@samples), spitzer$CSPLR_ST),]
cfData@samples <- cbind(cfData@samples, meta)

# Run t-test
cfData@samples$effect <- gsub("_D\\d", "", spitzer$group)
cfData <- cytottest(cfData, group = "effect", adjustMethod = "bonferroni")
cfData@results
```

---

drawTree	<i>Draw a dendrogram with ggplot</i>
----------	--------------------------------------

---

**Description**

Function to draw only a dendrogram with ggplot, the tree can be easily aligned to other graphs (e.g. heatmaps).

**Usage**

```
drawTree(hclust)
```

**Arguments**

hclust            an object of class [hclust](#).

**Value**

None

**Examples**

```
hc <- hclust(dist(data.frame(x1 = rnorm(10), x2 = rnorm(10), x3 = rnorm(10))))
drawTree(hc)
```

---

msiPlot	<i>Draw a density (median signal intensity) plot</i>
---------	--

---

**Description**

Function to plot the density (median signal intensity) for given markers.

**Usage**

```
msiPlot(cfList, markers, byGroup = NULL, byCluster = NULL, ...)
```

**Arguments**

cfList	a cfList object. It should contain at least data in the 'expr' and 'samples' slots.
markers	character vector with column names of the markers to be plotted. A numeric vector is also accepted, note that 1 starts after removing columns 'clusterID' and 'sampleID'.
byGroup	a character, referring to a column name in the samples slot of the cfList. This will be used as the grouping for the y-axis.
byCluster	character vector specifying which cluster to be plotted. This will be used as the grouping for the y-axis.
...	Additional arguments passed on to <a href="#">geom_density_ridges</a> .

**Value**

None

**Examples**

```

# Read Data
dirFCS <- system.file("extdata", package="cytofast")
cfData <- readCytosploreFCS(dir = dirFCS, colNames = "description")

# relabeling of clusterID
levels(cfData@expr[, "clusterID"]) <- gsub("[^0-9]", "", levels(cfData@expr[, "clusterID"]))

# Add cell counts to cfList and add meta data
cfData <- cellCounts(cfData, frequency = TRUE, scale = TRUE)
meta <- spitzer[match(row.names(cfData@samples), spitzer$CSPLR_ST),]
cfData@samples <- cbind(cfData@samples, meta)

# Remove unnecessary markers
cfData@expr <- cfData@expr[,-c(3:10, 13:16, 55:59, 61:63)]

# Draw median signal intensity plot, by group
msiPlot(cfData, markers = c("MHC.II", "CD45", "CD4"), byGroup = 'group')

# Or by cluster
msiPlot(cfData, markers = c("MHC.II", "CD45", "CD4"), byCluster = c("1", "6", "10"))

```

---

readCytosploreFCS      *Read .fcs files created by cytosplore*

---

**Description**

This function reads and combines .FCS files created specifically by Cytosplore.

**Usage**

```
readCytosploreFCS(dir = NULL, colNames = c("names", "description"))
```

**Arguments**

dir	directory containing the .FCS files created by Cytosplore
colNames	character string that specifies which label should be used as the column names. This could be the name of the markers 'names' or the description for the markers 'description'.

**Value**

The function returns an object of class `data.frame`. It includes both `clusterID` and `sampleID` as variables.



**Note**

This function is a wrapper around [read.FCS](#). For more flexibility see their help page.

**Examples**

```
dirFCS <- system.file("extdata", package="cytofast")
cfData <- readCytosploreFCS(dir = dirFCS, colNames = "description")
```

---

spitzer

*Spitzer meta file*

---

**Description**

A dataset containing information on the samples from the Spitzer study

**Usage**

```
spitzer
```

**Format**

A data frame with 26 observations and 3 variables:

**CSPLR\_ST** sample tag, corresponds with .fcs files created by Cytosplore

**group** grouping variable, effected vs. ineffectd, and day 3 vs. day 8

**sample\_name** original sampleID

**Source**

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5312823/>

**References**

Spitzer MH, Carmi Y, Reticker-Flynn NE, Kwek SS, Madhireddy D, Martins MM, Gherardini PF, Prestwood TR, Chabon J, Bendall SC, Fong L, Nolan GP, Engleman EG (2017). "Systemic Immunity is Required for Effective Cancer Immunotherapy HHS Public Access." *Cell*, **26**(168(3)), 487–502. doi: [10.1016/j.cell.2016.12.022](https://doi.org/10.1016/j.cell.2016.12.022), <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5312823/pdf/nihms842747.pdf>.

---

[,cfList,ANY-method] *Extract parts of cfList*

---

**Description**

Extract parts of cfList

**Usage**

```
## S4 method for signature 'cfList,ANY'  
x[i, j]
```

**Arguments**

x	a cfList object from which to extract from
i	index specifying which samples to extract. The index is either a numeric or character vector.
j	index specifying which clusters to extract. The index is either a numeric or character vector.

**Value**

Returns the specified clusters or samples from a cfList.

# Index

- \*Topic **FCS**,
  - readCytosploreFCS, 8
- \*Topic **FCS**
  - cytoHeatmaps, 5
- \*Topic **boxplot**,
  - cytoBoxplots, 4
- \*Topic **cells**
  - cellCounts, 2
- \*Topic **counts**,
  - cellCounts, 2
- \*Topic **cytosplore**
  - readCytosploreFCS, 8
- \*Topic **data**,
  - readCytosploreFCS, 8
- \*Topic **datasets**
  - spitzer, 9
- \*Topic **dendrogram**
  - drawTree, 7
- \*Topic **density**
  - msiPlot, 7
- \*Topic **frequency**,
  - cellCounts, 2
- \*Topic **heatmap**,
  - cytoHeatmaps, 5
- \*Topic **heatmap**
  - drawTree, 7
- \*Topic **intensity**
  - msiPlot, 7
- \*Topic **markers**,
  - cytoHeatmaps, 5
- \*Topic **median**
  - msiPlot, 7
- \*Topic **read**,
  - readCytosploreFCS, 8
- \*Topic **signal**
  - msiPlot, 7
- \*Topic **tree**
  - drawTree, 7
- [, cfList, ANY-method, 10
  
- cellCounts, 2
- cfList (cfList-class), 3
- cfList-class, 3
- counts (cfList-class), 3
  
- counts, cfList-method (cfList-class), 3
- cytoBoxplots, 4
- cytoHeatmaps, 5
- cytotest, 6
  
- data.frame, 8
- drawTree, 7
  
- expr (cfList-class), 3
- expr, cfList-method (cfList-class), 3
  
- FlowSOM, 3
  
- geom\_density\_ridges, 7
  
- hclust, 5, 7
  
- msiPlot, 7
  
- p.adjust, 6
  
- read.FCS, 9
- readCytosploreFCS, 3, 8
- results (cfList-class), 3
- results, cfList-method (cfList-class), 3
  
- samples (cfList-class), 3
- samples, cfList-method (cfList-class), 3
- scale, 2
- show, cfList-method (cfList-class), 3
- spitzer, 9
  
- t.test, 6