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AddAnnotation *AddAnnotation*

Description

Add annotation to a FlowSOM plot

Usage

```
AddAnnotation(
  p,
  fsom,
  toAnnotate = NULL,
  prefix = list(metaclusters = "MCL ", clusters = "CL "),
  ...
)
```

Arguments

p	Plot to add annotation to. When using PlotStars, please use list_insteadof_ggarrange = TRUE.
fsom	FlowSOM object that goes with the plot.
toAnnotate	A named list with "metaclusters" and/or "clusters" as names and a vector with the (meta)clusters that need to be annotated. Names can be abbreviated. Use a named vector with the old names as values and new labels as names for custom labeling.
prefix	Prefix to be added to labels. Default is "MCL " and "CL " for metaclusters and clusters respectively.
...	Arguments passed to geom_text_repel.

Value

The updated plot

Examples

```
# Identify the files
fcs <- flowCore::read.FCS(system.file("extdata", "68983.fcs",
                                      package = "FlowSOM"))
# Build a FlowSOM object
flowSOM.res <- FlowSOM(fcs,
                       scale = TRUE,
                       compensate = TRUE,
                       transform = TRUE,
                       toTransform = 8:18,
                       colsToUse = c(9, 12, 14:18),
                       nClus = 10,
```

```
                      seed = 1)

p <- PlotStars(flowSOM.res, backgroundValues = flowSOM.res$metaclustering,
               list_insteadof_ggarrange = TRUE)
annotationList <- list("metaclusters" = c("CD8 T cells" = "1", "B cells" = "8"),
                   "clusters" = c(97))
AddAnnotation(p, flowSOM.res, toAnnotate = annotationList,
              prefix = list("metaclusters" = "", clusters = "CL "))
```

AddBackground *AddBackground*

Description

Function plots the background

Usage

```
AddBackground(
  p,
  backgroundValues,
  backgroundColors = NULL,
  backgroundLim = NULL,
  alpha = 0.4
)
```

Arguments

p	ggplot object
backgroundValues	Vector of values to be plotted as background for the nodes
backgroundColors	Color palette to be used for the background coloring. Can be either a function or an array specifying colors.
backgroundLim	Background limits (can be used to ensure consistent Color palette between plots). If NULL (default), will be automatically adapted to the data.
alpha	Transparency of the backgroundColor.

Value

Returns nothing, but plots the background

See Also

PlotFlowSOM, AddLabels, AddNodes, AddPies, AddStars

AddFlowFrame *Add a flowFrame to the data variable of the FlowSOM object*

Description

Add a flowFrame to the data variable of the FlowSOM object

Usage

```
AddFlowFrame(fsom, flowFrame)
```

Arguments

fsom	FlowSOM object, as constructed by the ReadInput function
flowFrame	flowFrame to add to the FlowSOM object

Value

FlowSOM object with data added

See Also

ReadInput

AddLabels *AddLabels*

Description

AddLabels

Usage

```
AddLabels(
  p,
  labels,
  hjust = 0.5,
  layout = NULL,
  textSize = 3.88,
  textColor = "black",
  ...
)
```

Arguments

p	ggplot object
labels	Labels to be added to each node
hjust	Horizontal adjust for labels. Default is centered.
layout	Dataframe with x and y columns. If null, the dataframe from the ggplot object will be reused.
textSize	Size for geom_text. Default (=3.88) is from geom_text.
textColor	Color for geom_text. Default = black.
...	Additional parameters to pass to geom_text

Value

Returns the ggplot object with labels added

See Also

PlotLabels, PlotNumbers

AddMST *AddMST*

Description

Function plots the MST

Usage

```
AddMST(p, fsom)
```

Arguments

p	ggplot object
fsom	FlowSOM object, as generated by FlowSOM

Value

Returns nothing, but plots the MST for FlowSOM MST view

See Also

PlotFlowSOM, ParseEdges, AddStarsPies, AddLabels, AddNodes, AddBackground, AddPies, AddStars

AddNodes *AddNodes*

Description

Function plots the nodes

Usage

```
AddNodes(
  p,
  nodeInfo = NULL,
  values = NULL,
  lim = NULL,
  colorPalette = NULL,
  fillColor = "white",
  showLegend = TRUE,
  label = "",
  ...
)
```

Arguments

p	ggplot object
nodeInfo	Dataframe with for every node an x, y and size value, if null the dataframe from the ggplot object will be reused.
values	Values used for coloring the nodes. Default = NULL, in which case all nodes are filled in fillColor.
lim	The limits of the color scale, not used if values = NULL.
colorPalette	Color palette for color in nodes, not used if values = NULL. A vector of colors or a color function.
fillColor	Fixed fill for node colors, default = white.
showLegend	Boolean, default = TRUE.
label	Title for the legend.
...	Additional arguments to pass to geom_circle

Value

Returns nothing, but plots the nodes

See Also

PlotFlowSOM, PlotMarker, PlotVariable, AddLabels, AddBackground, AddPies, AddStars, AddStarsPies

AddPies *AddPies*

Description

Function plots the pies

Usage

```
AddPies(p, fsom, cellLabels, layout = NULL, colorPalette = NULL)
```

Arguments

p	ggplot object
fsom	FlowSOM object, as generated by BuildMST
cellLabels	Array of factors indicating the cell labels
layout	Coordinates of nodes. Uses dataframe of the ggplot object if NULL.
colorPalette	Color palette to be used for colors. Can be either a function or an array specifying colors.

Value

ggplot object with the pies added

See Also

PlotFlowSOM, AddLabels, AddNodes, AddBackground, PlotPies, AddStars, ParseArcs

AddScale *AddScale*

Description

AddScale

Usage

```
AddScale(
  p,
  values = NULL,
  colors = NULL,
  limits = NULL,
  showLegend = TRUE,
  labelLegend = "",
  type = "fill"
)
```

Arguments

p	ggplot object
values	Values used for the fill
colors	Colors to use (can be a vector or a function)
limits	Limits to use in the scale
showLegend	Boolean on whether to show the legend
labelLegend	Label to show as title of the legend
type	fill (default) or color

Value

ggplot object with scale added

AddStars *AddStars*

Description

Function plots the stars

Usage

```
AddStars(p, fsom, markers = fsom$map$colsUsed, colorPalette = NULL)
```

Arguments

p	ggplot object
fsom	FlowSOM object, as generated by BuildMST
markers	Determines which markers to plot. Default = "fsommapcolsUsed"
colorPalette	Color palette to be used for colors. Can be either a function or an array specifying colors.

Value

ggplot object with the stars added

See Also

PlotFlowSOM, AddLabels, AddNodes, AddBackground, PlotStars, AddPies, ParseArcs

AddStarsPies *AddStarsPies*

Description

Function plots stars or pies

Usage

```
AddStarsPies(p, arcs, colorPalette, showLegend = TRUE)
```

Arguments

p	ggplot object
arcs	Dataframe that contains all the data for the plotting the pies or stars
colorPalette	A vector of colors or a color function
showLegend	Boolean on whether to show the legend

Value

Returns nothing, but plots the stars or pies

See Also

PlotFlowSOM, AddLabels, AddNodes, AddBackground, AddPies, AddStars, ParseArcs, PlotStars PlotPies

AggregateFlowFrames *Aggregate multiple FCS files together*

Description

Aggregate multiple FCS files to analyze them simultaneously. A new FCS file is written, which contains about cTotal cells, with ceiling(cTotal/nFiles) cells from each file. Two new columns are added: a column indicating the original file by index, and a noisy version of this for better plotting opportunities (index plus or minus a value between 0 and 0.1).

Usage

```
AggregateFlowFrames(
  fileNames,
  cTotal,
  channels = NULL,
  writeOutput = FALSE,
  outputFile = "aggregate.fcs",
  keepOrder = FALSE,
  silent = FALSE,
  sampleWithReplacement = FALSE,
  ...
)
```

Arguments

fileNames	Character vector containing full paths to the FCS files or a flowSet to aggregate
cTotal	Total number of cells to write to the output file
channels	Channels/markers to keep in the aggregate. Default NULL takes all channels of the first file.
writeOutput	Whether to write the resulting flowFrame to a file. Default FALSE
outputFile	Full path to output file. Default "aggregate.fcs"
keepOrder	If TRUE, the random subsample will be ordered in the same way as they were originally ordered in the file. Default = FALSE.
silent	If FALSE, prints an update every time it starts processing a new file. Default = FALSE.
sampleWithReplacement	If TRUE and more cells per file are requested than actually present, all cells will be included plus additional resampling. Otherwise, at most all cells will be included once. Default = FALSE.
...	Additional arguments to pass to read.FCS

Value

This function does not return anything, but will write a file with about `cTotal` cells to `outputFile`

See Also

ceiling

Examples

```
# Define filename
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
# This example will sample 2 times 500 cells.
ff_new <- AggregateFlowFrames(c(fileName, fileName), 1000)
```

AutoMaxNodeSize *AutoMaxNodeSize*

Description

Calculate node size

Usage

```
AutoMaxNodeSize(layout, overlap)
```

Arguments

layout	Coordinates of nodes
overlap	Parameter that determines how much overlap there will be. If negative the nodes will be smaller

Details

Function that calculates the minimum distance between the nodes to use this to adapt the maxNodeSize for better plotting

Value

Returns the maxNodeSize with some overlap

See Also

PlotFlowSOM, ScaleStarHeights, ParseNodeSize

BuildMST *BuildMST*

Description

Build Minimal Spanning Tree

Usage

```
BuildMST(fsom, silent = FALSE, tSNE = FALSE)
```

Arguments

fsom	FlowSOM object, as generated by BuildSOM
silent	If TRUE, no progress updates will be printed
tSNE	If TRUE, an alternative t-SNE layout is computed as well

Details

Add minimal spanning tree description to the FlowSOM object

Value

FlowSOM object containing MST description

See Also

BuildSOM, PlotStars

Examples

```
# Read from file, build self-organizing map
fileName <- system.file("extdata", "68983.fcs", package="FlowSOM")
flowSOM.res <- ReadInput(fileName, compensate=TRUE, transform = TRUE,
                         scale = TRUE)
flowSOM.res <- BuildSOM(flowSOM.res, colsToUse = c(9, 12, 14:18))

# Build the Minimal Spanning Tree
flowSOM.res <- BuildMST(flowSOM.res)
```

BuildSOM *Build a self-organizing map*

Description

Build a SOM based on the data contained in the FlowSOM object

Usage

```
BuildSOM(fsom, colsToUse = NULL, silent = FALSE, outlierMAD = 4, ...)
```

Arguments

fsom	FlowSOM object containing the data, as constructed by the ReadInput function
colsToUse	Markers, channels or indices to use for building the SOM
silent	if TRUE, no progress updates will be printed
outlierMAD	Number of MAD when a cell is considered an outlier. See also TestOutliers
...	options to pass on to the SOM function (xdim, ydim, rlen, mst, alpha, radius, init, distf, importance)

Value

FlowSOM object containing the SOM result, which can be used as input for the BuildMST function

References

This code is strongly based on the kohonen package. R. Wehrens and L.M.C. Buydens, Self- and Super-organising Maps in R: the kohonen package J. Stat. Softw., 21(5), 2007

See Also

ReadInput, BuildMST

Examples

```
# Read from file
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
flowSOM.res <- ReadInput(fileName, compensate = TRUE, transform = TRUE,
                         scale = TRUE)

# Build the Self-Organizing Map
# E.g. with gridsize 5x5, presenting the dataset 20 times,
# no use of MST in neighborhood calculations in between
flowSOM.res <- BuildSOM(flowSOM.res, colsToUse = c(9, 12, 14:18),
                        xdim = 5, ydim = 5, rlen = 20)

# Build the minimal spanning tree and apply metaclustering
flowSOM.res <- BuildMST(flowSOM.res)
metacl <- MetaClustering(flowSOM.res$map$codes,
                         "metaClustering_consensus", max = 10)
```

CountGroups *Calculate differences in cell counts between groups*

Description

Calculate differences in cell counts between groups

Usage

```
CountGroups(fsom, groups, plot = TRUE, silent = FALSE)
```

Arguments

fsom	FlowSOM object as generated by BuildSOM
groups	List containing an array with file names for each group
plot	Logical. If TRUE, make a starplot of each individual file
silent	Logical. If TRUE, print progress messages

Value

Distance matrix

See Also

GroupStats

Examples

```
set.seed(1)
fileName <-  system.file("extdata", "68983.fcs", package="FlowSOM")
flowSOM.res <- FlowSOM(fileName, compensate = TRUE, transform = TRUE,
                       scale = TRUE, colsToUse = c(9,12,14:18), nClus = 10)

ff <- flowCore::read.FCS(fileName)
# Make an additional file without cluster 7 and double amount of cluster 5
selection <- c(which(GetClusters(flowSOM.res) %in%
                                 which(flowSOM.res$metaclustering != 7)),
                 which(GetClusters(flowSOM.res) %in%
                                 which(flowSOM.res$metaclustering == 5)))
ff_tmp <- ff[selection,]
flowCore::write.FCS(ff_tmp, file="ff_tmp.fcs")

# Compare only the file with the double amount of cluster 10
features <- GetFeatures(flowSOM.res,
                        c(fileName, "ff_tmp.fcs"),
                        level = "clusters",
                        type = "percentages")
stats <- GroupStats(features$cluster_percentages,
                    groups = list("AllCells" = c(fileName),
                                  "Without_ydTcells" = c("ff_tmp.fcs")))
unlink("ff_tmp.fcs")
```

`Dist.MST` *Calculate distance matrix using a minimal spanning tree neighborhood*

Description

Calculate distance matrix using a minimal spanning tree neighborhood

Usage

```
Dist.MST(X)
```

Arguments

`X`	matrix in which each row represents a point

Value

Distance matrix

`FlowSOM` *Run the FlowSOM algorithm*

Description

Method to run general FlowSOM workflow. Will scale the data and uses consensus meta-clustering by default.

Usage

```
FlowSOM(
  input,
  pattern = ".fcs",
  compensate = FALSE,
  spillover = NULL,
  transform = FALSE,
  toTransform = NULL,
  transformFunction = flowCore::logicleTransform(),
  transformList = NULL,
  scale = FALSE,
  scaled.center = TRUE,
  scaled.scale = TRUE,
  silent = TRUE,
  colsToUse = NULL,
  nClus = 10,
  maxMeta = NULL,
  importance = NULL,
  seed = NULL,
  ...
)
```

Arguments

`input`	a flowFrame, a flowSet, a matrix with column names or an array of paths to files or directories
`pattern`	if input is an array of file- or directorynames, select only files containing pattern
`compensate`	logical, does the data need to be compensated
`spillover`	spillover matrix to compensate with If NULL and compensate = TRUE, we will look for $SPILL description in FCS file.
`transform`	logical, does the data need to be transformed with the transformation given in `transformFunction`.
`toTransform`	column names or indices that need to be transformed. Will be ignored if `transformList` is given. If `NULL` and transform = `TRUE`, column names of `$SPILL` description in FCS file will be used.
`transformFunction`	Defaults to logicleTransform()

transformList	transformList to apply on the samples.
scale	logical, does the data needs to be rescaled. Default = FALSE
scaled.center	see scale
scaled.scale	see scale
silent	if TRUE, no progress updates will be printed
colsToUse	Markers, channels or indices to use for building the SOM. Default (NULL) is all the columns used to build the FlowSOM object.
nClus	Exact number of clusters for meta-clustering. Ignored if maxMeta is specified. Default = 10.
maxMeta	Maximum number of clusters to try out for meta-clustering. If NULL (default), only one option will be computed (nClus).
importance	array with numeric values. Parameters will be scaled according to importance
seed	Set a seed for reproducible results
...	options to pass on to the SOM function (xdim, ydim, rlen, mst, alpha, radius, init, distf)

Value

A list with two items: the first is the flowSOM object containing all information (see the vignette for more detailed information about this object), the second is the metaclustering of the nodes of the grid. This is a wrapper function for ReadInput, BuildSOM, BuildMST and MetaClustering. Executing them separately may provide more options.

See Also

scale, ReadInput, BuildSOM, BuildMST, MetaClustering

Examples

```
# Read from file
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
flowSOM.res <- FlowSOM(fileName, compensate = TRUE, transform = TRUE,
                       scale = TRUE, colsToUse = c(9, 12, 14:18), nClus = 10)
# Or read from flowFrame object
ff <- flowCore::read.FCS(fileName)
ff <- flowCore::compensate(ff, flowCore::keyword(ff)[["SPILL"]])
ff <- flowCore::transform(ff,
         flowCore::transformList(colnames(flowCore::keyword(ff)[["SPILL"]]),
                                 flowCore::logicleTransform()))
flowSOM.res <- FlowSOM(ff,
                       scale = TRUE,
                       colsToUse = c(9, 12, 14:18),
                       nClus = 10)

# Plot results
PlotStars(flowSOM.res,
          backgroundValues = flowSOM.res$metaclustering)
```

```
# Get metaclustering per cell
flowSOM.clustering <- GetMetaclusters(flowSOM.res)
```

`FlowSOM_colors` *FlowSOM default colors*

Description

FlowSOM default colors

Usage

```
FlowSOM_colors(n)
```

Arguments

`n`	Number of colors to generate

Value

array of n colors

`FlowSOMmary` *FlowSOMmary*

Description

This functions plots a summary of a flowSOM object. It includes a table of (meta)cluster data, the flowSOM trees and grid view, the (meta)cluster labels, the markers expression, the file distribution if present, the cluster per metacluster percentage, a t-SNE plot, and the MFI per metacluster.

Usage

```
FlowSOMmary(fsom, plotFile = "FlowSOMmary.pdf")
```

Arguments

`fsom`	FlowSOM object, as generated by `FlowSOM`
`plotFile`	Name of the pdf file that will be generated (default is FlowSOMmary.pdf). If `NULL`, a list of ggplots will be returned.

Value

Returns a summary of the FlowSOM object

Examples

```
# Identify the files
fcs <- flowCore::read.FCS(system.file("extdata", "68983.fcs",
                                      package = "FlowSOM"))

# Build a FlowSOM object
flowSOM.res <- FlowSOM(fcs,
                       scale = TRUE,
                       compensate = TRUE,
                       transform = TRUE,
                       toTransform = 8:18,
                       colsToUse = c(9, 12, 14:18),
                       nClus = 10,
                       seed = 1)

FlowSOMmary(flowSOM.res,
            plotFile = tempfile(fileext = ".pdf"))
```

`FlowSOMSubset` *FlowSOMSubset*

Description

FlowSOM subset

Usage

```
FlowSOMSubset(fsom, ids)
```

Arguments

`fsom`	FlowSOM object, as generated by `BuildMST`
`ids`	Array containing the ids to keep

Details

Take a subset from a FlowSOM object

Value

FlowSOM object containing updated data and median values, but with the same grid

See Also

`BuildMST`

Examples

```
# Read two files (Artificially, as we just split 1 file in 2 subsets)
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
ff1 <- flowCore::read.FCS(fileName)[1:1000, ]
flowCore::keyword(ff1)[["FIL"]] <- "File1"
ff2 <- flowCore::read.FCS(fileName)[1001:2000, ]
flowCore::keyword(ff2)[["FIL"]] <- "File2"

flowSOM.res <- FlowSOM(flowCore::flowSet(c(ff1, ff2)), compensate = TRUE,
                       transform = TRUE, scale = TRUE,
                       colsToUse = c(9, 12, 14:18), maxMeta = 10)

# see $metadata for subsets:
flowSOM.res$metaData

# Use only the second file, without changing the map
fSOM2 <- FlowSOMSubset(flowSOM.res,
                       (flowSOM.res$metaData[[2]][1]):
                        (flowSOM.res$metaData[[2]][2]))
```

FMeasure *F measure*

Description

Compute the F measure between two clustering results

Usage

```
FMeasure(realClusters, predictedClusters, silent = FALSE)
```

Arguments

realClusters	Array containing real cluster labels for each sample
predictedClusters	Array containing predicted cluster labels for each sample
silent	Logical, if FALSE (default), print some information about precision and recall

Value

F measure score

Examples

```
# Generate some random data as an example
realClusters <- sample(1:5,100,replace = TRUE)
predictedClusters <- sample(1:6, 100, replace = TRUE)

# Calculate the FMeasure
FMeasure(realClusters,predictedClusters)
```

`get_channels` *get_channels*

Description

Get channel names for an array of markers, given a flowFrame

Usage

```
get_channels(ff, markers)
```

Arguments

`ff`	The flowFrame of interest
`markers`	Vector with markers or channels of interest

Value

Corresponding channel names

See Also

`get_markers`

Examples

```
# Read the flowFrame
fileName <- system.file("extdata", "68983.fcs", package="FlowSOM")
ff <- flowCore::read.FCS(fileName)
GetChannels(ff, c("FSC-A", "CD3", "FITC-A"))
GetMarkers(ff, c("FSC-A", "CD3", "FITC-A"))
```

get_markers *get_markers*

Description

Get marker names, given a flowFrame. As available in "desc". If this is NA, defaults to channel name.

Usage

```
get_markers(ff, markers)
```

Arguments

ff	The flowFrame of interest
markers	Vector with markers or channels of interest

Value

Corresponding marker names

See Also

get_channels

Examples

```
# Read the flowFrame
fileName <- system.file("extdata", "68983.fcs", package="FlowSOM")
ff <- flowCore::read.FCS(fileName)
GetChannels(ff, c("FSC-A", "CD3", "FITC-A"))
GetMarkers(ff, c("FSC-A", "CD3", "FITC-A"))
```

GetChannels *GetChannels*

Description

Get channel names for an array of markers, given a flowFrame or a FlowSOM object. As available in "name". grep is used to look for the markers. Other regex can be added.

Usage

```
GetChannels(object, markers, exact = TRUE)
```

Arguments

object	The flowFrame or the FlowSOM object of interest
markers	Vector with markers or channels of interest. Also accepts the index of the marker found in the object.
exact	If TRUE (default), the grep pattern will be extended to start with ^\\Q and end with \\E$, so only exact matches are possible.

Value

Corresponding channel names

See Also

GetMarkers

Examples

```
# Read the flowFrame
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
ff <- flowCore::read.FCS(fileName)
GetChannels(ff, c("FSC-A", "CD3", "FITC-A"))
GetMarkers(ff, c("FSC-A", "CD3", "FITC-A"))
```

GetClusterCVs *Get CV values for all clusters*

Description

Get CV values for all clusters

Usage

```
GetClusterCVs(fsom)
```

Arguments

fsom	FlowSOM object as generated by the FlowSOM function or the BuildSOM function

Value

Matrix with coefficient of variation values for each marker

fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM") flowSOM.res <- FlowSOM(fileName, compensate = TRUE, transform = TRUE, scale = TRUE, colsToUse = c(9, 12, 14:18), nClus = 10) cvs <- GetClusterCVs(flowSOM.res)

`GetClusterMFIs` *Get MFI values for all clusters*

Description

Get MFI values for all clusters

Usage

```
GetClusterMFIs(fsom, colsUsed = FALSE, prettyColnames = FALSE)
```

Arguments

`fsom`	FlowSOM object as generated by the FlowSOM function or the BuildSOM function
`colsUsed`	logical. Should report only the columns used to build the SOM. Default = FALSE.
`prettyColnames`	logical. Should report pretty column names instead of standard column names. Default = FALSE.

Value

Matrix with median values for each marker

Examples

```
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
flowSOM.res <- FlowSOM(fileName, compensate = TRUE, transform = TRUE,
                       scale = TRUE, colsToUse = c(9, 12, 14:18), nClus = 10)
mfis <- GetClusterMFIs(flowSOM.res)
```

`GetClusterPercentagesPositive` *Get percentage-positive values for all clusters*

Description

Get percentage-positive values for all clusters

Usage

```
GetClusterPercentagesPositive(
  fsom,
  cutoffs,
  colsUsed = FALSE,
  prettyColnames = FALSE
)
```

Arguments

fsom	FlowSOM object as generated by the FlowSOM function or the BuildSOM function
cutoffs	named numeric vector. Upper bounds of negative population fluorescence-intensity values for each marker / channel.
colsUsed	logical. Should report only the columns used to build the SOM. Default = FALSE.
prettyColnames	logical. Should report pretty column names instead of standard column names. Default = FALSE.

Value

Matrix with percentages of cells that are positive in selected markers per each cluster

Examples

```
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
flowSOM.res <- FlowSOM(fileName, compensate = TRUE, transform = TRUE,
                       scale = TRUE, colsToUse = c(9, 12, 14:18), nClus = 10)
perc_pos <- GetClusterPercentagesPositive(flowSOM.res, cutoffs = c('CD4' = 5000))
```

GetClusters *Get cluster label for all individual cells*

Description

Get cluster label for all individual cells

Usage

```
GetClusters(fsom)
```

Arguments

fsom	FlowSOM object as generated by the FlowSOM function or the BuildSOM function

Value

vector label for every cell

Examples

```
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
flowSOM.res <- FlowSOM(fileName, compensate = TRUE, transform = TRUE,
                       scale = TRUE, colsToUse = c(9, 12, 14:18), nClus = 10)
cluster_labels <- GetClusters(flowSOM.res)
```

GetCounts *GetCounts*

Description

Get counts of number of cells in clusters or metaclusters

Usage

```
GetCounts(fsom, level = "metaclusters")
```

Arguments

fsom	FlowSOM object
level	Character string, should be either "clusters" or "metaclusters" (default) or abbreviations.

Value

A named vector with the counts

Examples

```
# Read from file, build self-organizing map and minimal spanning tree
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
ff <- flowCore::read.FCS(fileName)
ff <- flowCore::compensate(ff, flowCore::keyword(ff)[["SPILL"]])
ff <- flowCore::transform(ff, flowCore::estimateLogicle(ff,
                                                flowCore::colnames(ff)[8:18]))
flowSOM.res <- FlowSOM(ff,
                       scale = TRUE,
                       colsToUse = c(9, 12, 14:18),
                       nClus = 10,
                       seed = 1)
GetCounts(flowSOM.res)
GetCounts(flowSOM.res, level = "clusters")
```

GetCVs *Get CV values for all clusters*

Description

Get CV values for all clusters

Usage

```
GetCVs(fsom)
```

Arguments

fsom	FlowSOM object as generated by the FlowSOM function or the BuildSOM function

Value

Matrix with coefficient of variation values for each marker

fileName <- system.file("extdata", "68983.fcs", package="FlowSOM") flowSOM.res <- FlowSOM(fileName, compensate=TRUE,transform=TRUE, scale=TRUE,colsToUse=c(9,12,14:18),nClus=10) cvs <- GetClusterCVs(flowSOM.res)

GetFeatures *GetFeatures*

Description

Map FCS files on an existing FlowSOM object

Usage

```
GetFeatures(
  fsom,
  files,
  level = c("clusters", "metaclusters"),
  type = "counts",
  MFI = NULL,
  positive_cutoffs = NULL,
  filenames = NULL,
  silent = FALSE,
  ...
)
```

Arguments

fsom	FlowSOM object as generated by the FlowSOM function or the BuildSOM function
files	Either a vector of FCS files or paths to FCS files
level	Level(s) of interest. Default is c("clusters", "metaclusters"), but can also be only one of them. Can be abbreviated.
type	Type of features to extract. Default is "counts", can be a vector of "counts", "percentages", "MFIs" and/or "percentages_positive" or abbreviations.
MFI	Vector with channels / markers for which the MFI values must be returned when "MFIs" is in type

positive_cutoffs
: Named vector with fluorescence-intensity values per channel / marker that are the upper bounds for a negative population when "percentages_positive" is in type

filenames
: An optional vector with filenames that will be used as rownames in the count matrices. If NULL (default) either the paths will be used or a numerical vector.

silent
: Logical. If TRUE, print progress messages. Default = FALSE.

...
: Additional arguments to pass to NewData

Value

matrix with features per population - type combination

Examples

```
# Build FlowSom result
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
ff <- flowCore::read.FCS(fileName)
ff <- flowCore::compensate(ff, flowCore::keyword(ff)[["SPILL"]])
ff <- flowCore::transform(ff,
        flowCore::transformList(colnames(flowCore::keyword(ff)[["SPILL"]]),
                                flowCore::logicleTransform()))
flowSOM.res <- FlowSOM(ff[1:1000, ],
                       scale = TRUE,
                       colsToUse = c(9, 12, 14:18),
                       nClus = 10)

# Map new data
counts <- GetFeatures(fsom = flowSOM.res,
                      level = "clusters",
                      files = c(ff[1001:2000, ], ff[2001:3000, ]))
features <- GetFeatures(fsom = flowSOM.res,
                        files = c(ff[1001:2000, ], ff[2001:3000, ]),
                        type = c("counts", "percentages", "MFIs"),
                        MFI = "APC-A",
                        filenames = c("ff_1001-2000", "ff_2001-3000"))

# Get percentages of positive cells
positive_cutoffs <- c('CD8' = 1.5,
                      'CD4' = 0.3,
                      'CD19' = 1.3,
                      'CD3' = -0.3)

perc_pos <- GetFeatures(fsom = flowSOM.res,
                        files = c(ff[1001:2000, ], ff[2001:3000, ]),
                        type = c("percentages_positive"),
                        positive_cutoffs = positive_cutoffs,
                        filenames = c("ff_1001-2000", "ff_2001-3000"))
```

`GetFlowJoLabels` *Process a FlowJo workspace file*

Description

Reads a FlowJo workspace file using the flowWorkspace library and returns a list with a matrix containing gating results and a vector with a label for each cell from a set of specified gates

Usage

```
GetFlowJoLabels(
  files,
  wspFile,
  group = "All Samples",
  cellTypes = NULL,
  getData = FALSE,
  ...
)
```

Arguments

`files`	The FCS files of interest
`wspFile`	The FlowJo wsp file to read
`group`	The FlowJo group to parse. Default "All Samples".
`cellTypes`	Cell types to use for final labeling the cells. Should correspond with a subset of the gate names in FlowJo.
`getData`	If true, flowFrames are returned as well.
`...`	Extra arguments to pass to CytoML::flowjo_to_gatingset

Value

This function returns a list, which for every file contains a list in which the first element ("matrix") is a matrix containing filtering results for each specified gate and the second element ("manual") is a vector which assigns one label to each cell. If only one file is given, only one list is returned instead of a list of lists.

See Also

`PlotPies`

Examples

```
# Identify the files
fcs_file <- system.file("extdata", "68983.fcs", package = "FlowSOM")
wspFile <- system.file("extdata", "gating.wsp", package = "FlowSOM")
```

```
# Specify the cell types of interest for assigning one label per cell
cellTypes <- c("B cells",
               "gd T cells", "CD4 T cells", "CD8 T cells",
               "NK cells", "NK T cells")

# Parse the FlowJo workspace
gatingResult <- GetFlowJoLabels(fcs_file, wspFile,
                                cellTypes = cellTypes,
                                getData = TRUE)

# Check the number of cells assigned to each gate
colSums(gatingResult$matrix)

# Build a FlowSOM tree
flowSOM.res <- FlowSOM(gatingResult$flowFrame,
                       colsToUse = c(9, 12, 14:18),
                       nClus = 10,
                       seed = 1)

 # Plot pies indicating the percentage of cell types present in the nodes
 PlotPies(flowSOM.res,
          gatingResult$manual,
          backgroundValues = flowSOM.res$metaclustering)
```

GetMajorityLabels *GetMajorityLabels*

Description

Get majority cluster and metacluster labels from a vector of manual labels per cell in the FlowSOM object. Especially useful as starting point for UpdateMetaclusters.

Usage

```
GetMajorityLabels(fsom, labels)
```

Arguments

fsom	FlowSOM object, as generated by FlowSOM
labels	Array of celltypes per cell in fsom

Value

Named list with array of majority labels for the FlowSOM clusters and metaclusters.

See Also

UpdateMetaclusters

Examples

```
# Identify the files
fcs_file <- system.file("extdata", "68983.fcs", package = "FlowSOM")
gating_file <- system.file("extdata", "gatingResult.csv", package = "FlowSOM")

# Specify the cell types of interest for assigning one label per cell
cellTypes <- c("B cells",
               "gd T cells", "CD4 T cells", "CD8 T cells",
               "NK cells", "NK T cells")

# Load manual labels (e.g. GetFlowJoLabels can be used to extract labels from
# an fcs file)
gatingResult <- as.factor(read.csv(gating_file, header = FALSE)[, 1])


# Build a FlowSOM tree
flowSOM.res <- FlowSOM(fcs_file,
                       scale = TRUE,
                       compensate = TRUE,
                       transform = TRUE,
                       toTransform = 8:18,
                       colsToUse = c(9, 12, 14:18),
                       nClus = 10,
                       seed = 1)

# Get metacluster label based on the gatingResult
majorityLabels <- GetMajorityLabels(fsom = flowSOM.res,
                                    labels = gatingResult)
```

`GetMarkers` *GetMarkers*

Description

Get marker names for an array of channels, given a flowFrame or a FlowSOM object. As available in "desc". If this is NA, defaults to channel name. `grep` is used to look for the markers. Other regex can be added.

Usage

```
GetMarkers(object, channels, exact = TRUE)
```

Arguments

`object`	The flowFrame or the FlowSOM object of interest
`channels`	Vector with markers or channels of interest. Also accepts the index of the channel in the object.

exact	If TRUE (default), the grep pattern will be extended to start with ^\\Q and end with \\E$, so only exact matches are possible.

Value

Corresponding marker names

See Also

GetChannels

Examples

```
# Read the flowFrame
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
ff <- flowCore::read.FCS(fileName)
GetChannels(ff, c("FSC-A", "CD3", "FITC-A"))
GetMarkers(ff, c("FSC-A", "CD3", "FITC-A"))
```

GetMetaclusterCVs *GetMetaclusterCVs*

Description

Compute the coefficient of variation for the metaclusters

Usage

```
GetMetaclusterCVs(fsom, colsUsed = FALSE, prettyColnames = FALSE)
```

Arguments

fsom	Result of calling the FlowSOM function
colsUsed	Logical. Should report only the columns used to build the SOM. Default = FALSE.
prettyColnames	Logical. Should report pretty column names instead of standard column names. Default = FALSE.

Value

Metacluster CVs

Examples

```
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
ff <- flowCore::read.FCS(fileName)
ff <- flowCore::compensate(ff, flowCore::keyword(ff)[["SPILL"]])
ff <- flowCore::transform(ff,
         flowCore::transformList(colnames(flowCore::keyword(ff)[["SPILL"]]),
                                 flowCore::logicleTransform()))
flowSOM.res <- FlowSOM(ff,
                       scale = TRUE,
                       colsToUse = c(9, 12, 14:18),
                       nClus = 10)
cvs <- GetMetaclusterCVs(flowSOM.res)
```

`GetMetaclusterMFIs` *GetMetaclusterMFIs*

Description

Compute the median fluorescence intensities for the metaclusters

Usage

```
GetMetaclusterMFIs(fsom, colsUsed = FALSE, prettyColnames = FALSE)
```

Arguments

`fsom`	Result of calling the FlowSOM function
`colsUsed`	Logical. Should report only the columns used to build the SOM. Default = FALSE.
`prettyColnames`	Logical. Should report pretty column names instead of standard column names. Default = FALSE.

Value

Metacluster MFIs

Examples

```
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
ff <- flowCore::read.FCS(fileName)
ff <- flowCore::compensate(ff, flowCore::keyword(ff)[["SPILL"]])
ff <- flowCore::transform(ff,
         flowCore::transformList(colnames(flowCore::keyword(ff)[["SPILL"]]),
                                 flowCore::logicleTransform()))
flowSOM.res <- FlowSOM(ff,
                       scale = TRUE,
                       colsToUse = c(9, 12, 14:18),
                       nClus = 10)
mfis <- GetMetaclusterMFIs(flowSOM.res)
```

GetMetaclusterPercentagesPositive *Get percentage-positive values for all metaclusters*

Description

Get percentage-positive values for all metaclusters

Usage

```
GetMetaclusterPercentagesPositive(
  fsom,
  cutoffs,
  colsUsed = FALSE,
  prettyColnames = FALSE
)
```

Arguments

fsom	FlowSOM object as generated by the FlowSOM function or the BuildSOM function
cutoffs	named numeric vector. Upper bounds of negative population fluorescence-intensity values for each marker / channel.
colsUsed	logical. Should report only the columns used to build the SOM. Default = FALSE.
prettyColnames	logical. Should report pretty column names instead of standard column names. Default = FALSE.

Value

Matrix with percentages of cells that are positive in selected markers per each metacluster

Examples

```
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
flowSOM.res <- FlowSOM(fileName, compensate = TRUE, transform = TRUE,
                       scale = TRUE, colsToUse = c(9, 12, 14:18), nClus = 10)
perc_pos <- GetMetaclusterPercentagesPositive(flowSOM.res, cutoffs = c('CD4' = 5000))
```

GetMetaclusters *Get metacluster label for all individual cells*

Description

Get metacluster label for all individual cells

Usage

```
GetMetaclusters(fsom, meta = NULL)
```

Arguments

fsom	FlowSOM object as generated by the FlowSOM function or the BuildSOM function
meta	Metacluster label for each FlowSOM cluster. If this is NULL, the fsom argument should be as generated by the FlowSOM function, and fsom$metaclustering will be used.

Value

vector label for every cell

Examples

```
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
flowSOM.res <- FlowSOM(fileName, compensate = TRUE, transform = TRUE,
                       scale = TRUE, colsToUse = c(9, 12, 14:18), nClus = 10)
metacluster_labels <- GetMetaclusters(flowSOM.res)
metacluster_labels <- GetMetaclusters(flowSOM.res,
                                      meta = flowSOM.res$metaclustering)
```

GetMFIs *Get MFI values for all clusters*

Description

Get MFI values for all clusters

Usage

```
GetMFIs(fsom, colsUsed = FALSE, prettyColnames = FALSE)
```

Arguments

fsom	FlowSOM object as generated by the FlowSOM function or the BuildSOM function
colsUsed	logical. Should report only the columns used to build the SOM. Default = FALSE.
prettyColnames	logical. Should report pretty column names instead of standard column names. Default = FALSE.

Value

Matrix with median values for each marker

Examples

```
fileName <- system.file("extdata", "68983.fcs", package="FlowSOM")
flowSOM.res <- FlowSOM(fileName, compensate=TRUE,transform=TRUE,
                       scale=TRUE,colsToUse=c(9,12,14:18),nClus=10)
mfis <- GetClusterMFIs(flowSOM.res)
```

GetPercentages *GetPercentages*

Description

Get percentages of number of cells in clusters or metaclusters

Usage

```
GetPercentages(fsom, level = "metaclusters")
```

Arguments

fsom	FlowSOM object
level	Character string, should be either "clusters" or "metaclusters" (default) or abbreviations.

Value

A named vector with the percentages

Examples

```
# Read from file, build self-organizing map and minimal spanning tree
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
ff <- flowCore::read.FCS(fileName)
ff <- flowCore::compensate(ff, flowCore::keyword(ff)[["SPILL"]])
ff <- flowCore::transform(ff, flowCore::estimateLogicle(ff,
                                                flowCore::colnames(ff)[8:18]))
flowSOM.res <- FlowSOM(ff,
                       scale = TRUE,
                       colsToUse = c(9, 12, 14:18),
                       nClus = 10,
                       seed = 1)
GetPercentages(flowSOM.res)
GetPercentages(flowSOM.res, level = "clusters")
```

---

gg_color_hue *gg_color_hue*

Description

Helper function to get the ggplot colors

Usage

```
gg_color_hue(n)
```

Arguments

n	Number of colors

Value

array with hexadecimal color values

GroupStats *GroupStats*

Description

Calculate statistics between 2 groups based on the GetFeatures output

Usage

```
GroupStats(features, groups)
```

Arguments

features	Feature matrix as generated by GetFeatures, e.g. a percentages matrix
groups	Named list with file or patient IDs per group (should match with the rownames of the matrix).

Value

Matrix with the medians per group, the p-values (the raw, Benjamini Hochberg corrected one and the -log10) that resulted from a Wilcox test and the fold and log10 fold changes between the medians of the 2 groups

Examples

```
# Build FlowSom result
 fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
 ff <- flowCore::read.FCS(fileName)
 ff <- flowCore::compensate(ff, flowCore::keyword(ff)[["SPILL"]])
 ff <- flowCore::transform(ff,
         flowCore::transformList(colnames(flowCore::keyword(ff)[["SPILL"]]),
                                 flowCore::logicleTransform()))
 flowSOM.res <- FlowSOM(ff, scale = TRUE, colsToUse = c(9, 12, 14:18),
                        nClus = 10)

# Create new data
# To illustrate the output, we here generate new FCS files (with more
# cells in metaclusters 1 and 9).
# In practice you would not generate any new file but use your different
# files from your different groups
 flowCore::write.FCS(ff[sample(1:nrow(ff), 1000), ], file = "ff_tmp1.fcs")
 flowCore::write.FCS(ff[sample(1:nrow(ff), 1000), ], file = "ff_tmp2.fcs")
 flowCore::write.FCS(ff[sample(1:nrow(ff), 1000), ], file = "ff_tmp3.fcs")
 ff_tmp <- ff[c(1:1000,
                which(flowSOM.res$map$mapping[, 1] %in%
                      which(flowSOM.res$metaclustering == 9)),
                which(flowSOM.res$map$mapping[, 1] %in%
                      which(flowSOM.res$metaclustering == 1))), ]
 flowCore::write.FCS(ff_tmp[sample(1:nrow(ff_tmp), 1000), ],
                     file = "ff_tmp4.fcs")
 flowCore::write.FCS(ff_tmp[sample(1:nrow(ff_tmp), 1000), ],
                     file = "ff_tmp5.fcs")

# Get the count matrix
 percentages <- GetFeatures(fsom = flowSOM.res,
                            files = c("ff_tmp1.fcs",
                                      "ff_tmp2.fcs",
                                      "ff_tmp3.fcs",
                                      "ff_tmp4.fcs",
                                      "ff_tmp5.fcs"),
                            type = "percentages")
```

```
# Perform the statistics
groups <- list("Group 1" = c("ff_tmp1.fcs", "ff_tmp2.fcs", "ff_tmp3.fcs"),
               "Group 2" = c("ff_tmp4.fcs", "ff_tmp5.fcs"))
MC_stats <- GroupStats(percentages[["metacluster_percentages"]], groups)
C_stats <- GroupStats(percentages[["cluster_percentages"]], groups)

# Process the fold changes vector
fold_changes <- C_stats["fold changes", ]
fold_changes <- factor(ifelse(fold_changes < -3,
                              "Underrepresented compared to Group 1",
                              ifelse(fold_changes > 3,
                                     "Overrepresented compared to Group 1",
                                      "--")),
                       levels = c("--",
                                  "Underrepresented compared to Group 1",
                                  "Overrepresented compared to Group 1"))
fold_changes[is.na(fold_changes)] <- "--"

# Show in figure
## Fold change
gr_1 <- PlotStars(flowSOM.res,
                  title = "Group 1",
                  nodeSizes = C_stats["medians Group 1", ],
                  list_insteadof_ggarrange = TRUE)
gr_2 <- PlotStars(flowSOM.res, title = "Group 2",
            nodeSizes = C_stats["medians Group 2", ],
            backgroundValues = fold_changes,
            backgroundColors = c("white", "red", "blue"),
            list_insteadof_ggarrange = TRUE)
p <- ggpubr::ggarrange(plotlist = c(list(gr_1$tree), gr_2),
                       heights = c(3, 1))
ggplot2::ggsave(tempfile(fileext = ".png"), p, width = 10)

## p values
p <- PlotVariable(flowSOM.res, title = "Wilcox test group 1 vs. group 2",
variable = C_stats["p values", ])
ggplot2::ggsave(tempfile(fileext = ".png"), p)

## volcano plot
p <- ggplot2::ggplot(data.frame("-log10 p values" = c(C_stats[4, ],
                                                      MC_stats[4, ]),
                                "log10 fold changes" = c(C_stats[7, ],
                                                         MC_stats[7, ]),
check.names = FALSE), ggplot2::aes(x = `log10 fold changes`,
                                   y = `-log10 p values`)) +
ggplot2::xlim(-3, 3) +
ggplot2::ylim(0, 3) +
ggplot2::geom_point()

# Remove temporary files
unlink(c("ff_tmp1.fcs",
         "ff_tmp2.fcs",
         "ff_tmp3.fcs",
```

```
            "ff_tmp4.fcs",
            "ff_tmp5.fcs"))
```

`Initialize_KWSP` *Select k well spread points from X*

Description

Select k well spread points from X

Usage

```
Initialize_KWSP(X, xdim, ydim)
```

Arguments

`X`	matrix in which each row represents a point
`xdim`	x dimension of the grid
`ydim`	y dimension of the grid

Value

array containing the selected selected rows

Examples

```
points <- matrix(1:1000, ncol = 10)
selection <- Initialize_KWSP(points, 3, 3)
```

`Initialize_PCA` *Create a grid from first 2 PCA components*

Description

Create a grid from first 2 PCA components

Usage

```
Initialize_PCA(data, xdim, ydim)
```

Arguments

data	matrix in which each row represents a point
xdim	x dimension of the grid
ydim	y dimension of the grid

Value

array containing the selected selected rows

Examples

```
points <- matrix(1:1000, ncol = 10)
selection <- Initialize_PCA(points, 3, 3)
```

ManualVector — *Summarize the gating matrix into one vector, only including the cell types of interest*

Description

Extract the compensated and transformed data and all gate labels.

Usage

```
ManualVector(manualMatrix, cellTypes)
```

Arguments

manualMatrix	Matrix containing boolean values, indicating for every gate (column) whether the cell (row) is part of it or not.
cellTypes	Cell types to use in the summary vector. All others will be ignored and cells which do not fall in one of these gates will get the label "Unknown". Order is important!

Value

A factor with one label for every cell

`MapDataToCodes` *Assign nearest node to each datapoint*

Description

Assign nearest node to each datapoint

Usage

```
MapDataToCodes(codes, newdata, distf = 2)
```

Arguments

`codes`	matrix with nodes of the SOM
`newdata`	datapoints to assign
`distf`	Distance function (1 = manhattan, 2 = euclidean, 3 = chebyshev, 4 = cosine)

Value

Array with nearest node id for each datapoint

`MetaclusterCVs` *MetaclusterCVs*

Description

Compute the coefficient of variation for the metaclusters

Usage

```
MetaclusterCVs(fsom)
```

Arguments

`fsom`	Result of calling the FlowSOM function

Value

Metacluster CVs

Examples

```
fileName <- system.file("extdata", "68983.fcs", package="FlowSOM")
ff <- flowCore::read.FCS(fileName)
ff <- flowCore::compensate(ff,flowCore::keyword(ff)$SPILL)
ff <- flowCore::transform(ff,
         flowCore::transformList(colnames(flowCore::keyword(ff)$SPILL),
                                 flowCore::logicleTransform()))
flowSOM.res <- FlowSOM(ff,scale=TRUE,colsToUse=c(9,12,14:18), nClus=10)
cvs <- GetMetaclusterCVs(flowSOM.res)
```

`MetaClustering` *MetaClustering*

Description

Cluster data with automatic number of cluster determination for several algorithms

Usage

```
MetaClustering(data, method, max = 20, seed = NULL, ...)
```

Arguments

`data`	Matrix containing the data to cluster
`method`	Clustering method to use
`max`	Maximum number of clusters to try out
`seed`	Seed to pass on to given clustering method
`...`	Extra parameters to pass along

Value

Numeric array indicating cluster for each datapoint

See Also

`metaClustering_consensus`

Examples

```
# Read from file, build self-organizing map and minimal spanning tree
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
flowSOM.res <- ReadInput(fileName, compensate = TRUE,transform = TRUE,
                         scale = TRUE)
flowSOM.res <- BuildSOM(flowSOM.res,colsToUse = c(9, 12, 14:18))
flowSOM.res <- BuildMST(flowSOM.res)

# Apply metaclustering
```

```
metacl <- MetaClustering(flowSOM.res$map$codes,
                         "metaClustering_consensus",
                         max = 10)

# Get metaclustering per cell
flowSOM.clustering <- metacl[flowSOM.res$map$mapping[, 1]]
```

`metaClustering_consensus` *MetaClustering*

Description

Cluster data using hierarchical consensus clustering with k clusters

Usage

```
metaClustering_consensus(data, k = 7, seed = NULL)
```

Arguments

`data`	Matrix containing the data to cluster
`k`	Number of clusters
`seed`	Seed to pass to consensusClusterPlus

Value

Numeric array indicating cluster for each datapoint

See Also

`MetaClustering`

Examples

```
# Read from file, build self-organizing map and minimal spanning tree
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
flowSOM.res <- ReadInput(fileName, compensate = TRUE,transform = TRUE,
                         scale = TRUE)
flowSOM.res <- BuildSOM(flowSOM.res,colsToUse = c(9, 12, 14:18))
flowSOM.res <- BuildMST(flowSOM.res)

# Apply consensus metaclustering
metacl <- metaClustering_consensus(flowSOM.res$map$codes, k = 10)
```

MetaclusterMFIs *MetaclusterMFIs*

Description

Compute the median fluorescence intensities for the metaclusters

Usage

```
MetaclusterMFIs(fsom)
```

Arguments

fsom	Result of calling the FlowSOM function

Value

Metacluster MFIs

Examples

```
fileName <- system.file("extdata", "68983.fcs", package="FlowSOM")
ff <- flowCore::read.FCS(fileName)
ff <- flowCore::compensate(ff,ff@description$SPILL)
ff <- flowCore::transform(ff,
         flowCore::transformList(colnames(ff@description$SPILL),
                                 flowCore::logicleTransform()))
flowSOM.res <- FlowSOM(ff,scale=TRUE,colsToUse=c(9,12,14:18),maxMeta=10)
mfis <- GetMetaclusterMFIs(flowSOM.res)
```

NClusters *NClusters*

Description

Extracts the number of clusters from a FlowSOM object

Usage

```
NClusters(fsom)
```

Arguments

fsom	FlowSOM object

Value

The number of clusters

Examples

```
# Build FlowSom result
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
ff <- flowCore::read.FCS(fileName)
flowSOM.res <- FlowSOM(ff,
                       compensate = TRUE, transform = TRUE, scale = TRUE,
                       colsToUse = c(9, 12, 14:18),
                       maxMeta = 10)
NClusters(flowSOM.res)
```

NewData *NewData*

Description

Map new data to a FlowSOM grid

Usage

```
NewData(
  fsom,
  input,
  madAllowed = 4,
  compensate = NULL,
  spillover = NULL,
  transform = NULL,
  toTransform = NULL,
  transformFunction = NULL,
  transformList = NULL,
  scale = NULL,
  scaled.center = NULL,
  scaled.scale = NULL,
  silent = FALSE
)
```

Arguments

fsom	FlowSOM object
input	A flowFrame, a flowSet or an array of paths to files or directories
madAllowed	A warning is generated if the distance of the new data points to their closest cluster center is too big. This is computed based on the typical distance of the points from the original dataset assigned to that cluster, the threshold being set to median + madAllowed * MAD. Default is 4.
compensate	logical, does the data need to be compensated. If NULL, the same value as in the original FlowSOM call will be used.

spillover	spillover matrix to compensate with. If NULL, the same value as in the original FlowSOM call will be used.
transform	logical, does the data need to be transformed. If NULL, the same value as in the original FlowSOM call will be used.
toTransform	column names or indices that need to be transformed. If NULL, the same value as in the original FlowSOM call will be used.
transformFunction	If NULL, the same value as in the original FlowSOM call will be used.
transformList	If NULL, the same value as in the original FlowSOM call will be used.
scale	Logical, does the data needs to be rescaled. If NULL, the same value as in the original FlowSOM call will be used.
scaled.center	See scale. If NULL, the same value as in the original FlowSOM call will be used.
scaled.scale	See scale. If NULL, the same value as in the original FlowSOM call will be used.
silent	Logical. If TRUE, print progress messages. Default = FALSE.

Details

New data is mapped to an existing FlowSOM object. The input is similar to the ReadInput function. A new FlowSOM object is created, with the same grid, but a new mapping, node sizes and mean values. The same preprocessing steps (compensation, transformation and scaling) will happen to this file as was specified in the original FlowSOM call. The scaling parameters from the original grid will be used.

Value

A new FlowSOM object

See Also

FlowSOMSubset if you want to get a subset of the current data instead of a new dataset

Examples

```
# Build FlowSom result
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
ff <- flowCore::read.FCS(fileName)
ff <- flowCore::compensate(ff, flowCore::keyword(ff)[["SPILL"]])
ff <- flowCore::transform(ff,
        flowCore::transformList(colnames(flowCore::keyword(ff)[["SPILL"]]),
                                flowCore::logicleTransform()))
  flowSOM.res <- FlowSOM(ff[1:1000, ],
                         scale = TRUE,
                         colsToUse = c(9, 12, 14:18),
                         nClus = 10)

  # Map new data
```

```
fSOM2 <- NewData(flowSOM.res, ff[1001:2000, ])
```

NMetaclusters *NMetaclusters*

Description

Extracts the number of metaclusters from a FlowSOM object

Usage

```
NMetaclusters(fsom)
```

Arguments

fsom	FlowSOM object

Value

The number of metaclusters

Examples

```
# Build FlowSom result
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
ff <- flowCore::read.FCS(fileName)
flowSOM.res <- FlowSOM(ff,
                       compensate = TRUE, transform = TRUE, scale = TRUE,
                       colsToUse = c(9, 12, 14:18),
                       maxMeta = 10)
NMetaclusters(flowSOM.res)
```

ParseArcs *ParseArcs*

Description

Parses stars

Usage

```
ParseArcs(x, y, arcValues, arcHeights)
```

Arguments

x	x coordinate of node
y	y coordinate of node
arcValues	A named vector with the frequency of how the node should be divided
arcHeights	The heights of the arcs

Details

Function that parses the FlowSOM object into a dataframe for the star values for ggplot

Value

A dataframe ready to use with ggplot, consisting of the coordinates of centers, the radius and angles of the star values

See Also

PlotFlowSOM, ParseEdges, ParseNodeSize, ParseQuery, ParseSD

ParseEdges *ParseEdges*

Description

Parses edges

Usage

```
ParseEdges(fsom)
```

Arguments

fsom	FlowSOM object, as generated by FlowSOM

Details

Function that parses the graph edges of the FlowSOM object into a dataframe

Value

A dataframe consisting of start and end coordinates of edges

See Also

PlotFlowSOM, ParseNodeSize, ParseArcs, ParseQuery, ParseSD, AddMST

`ParseLayout` *ParseLayout*

Description

ParseLayout

Usage

```
ParseLayout(fsom, layout)
```

Arguments

`fsom`	FlowSOM object
`layout`	"MST", "grid" or a matrix/dataframe with 2 columns and 1 row per cluster

Value

dataframe with 2 columns and 1 row per cluster

`ParseNodeSize` *ParseNodeSize*

Description

Parses node size

Usage

```
ParseNodeSize(nodeSizes, maxNodeSize, refNodeSize)
```

Arguments

`nodeSizes`	A vector with node sizes
`maxNodeSize`	Determines the maximum node size.
`refNodeSize`	Reference for node size against which the nodeSizes will be scaled. Default = max(nodeSizes)

Details

Function that parses the mapping of the FlowSOM object into node sizes relative to the abundances of cells per cluster

Scales node size relative to the abundances of cells per cluster

Value

A vector is returned consisting of node sizes

See Also

PlotFlowSOM, ParseEdges, AutoMaxNodeSize, ParseArcs, ParseQuery, ParseSD

ParseQuery *ParseQuery*

Description

Parses query

Usage

```
ParseQuery(fsom, query)
```

Arguments

fsom	FlowSOM object, as generated by FlowSOM
query	Array containing "high" or "low" for the specified column names of the FlowSOM data

Details

Identify nodes in the tree which resemble a certain profile of "high" or "low" marker expressions.

Value

A list, containing the ids of the selected nodes, the individual scores for all nodes and the scores for each marker for each node

See Also

PlotFlowSOM, ParseEdges, ParseNodeSize, ParseArcs, QueryStarPlot, ParseSD

ParseSD *ParseSD Parses SD in FlowSOM object*

Description

Calculates the standard deviation of a FlowSOM object

Usage

```
ParseSD(fsom, marker = NULL)
```

Arguments

fsom	FlowSOM object, as generated by FlowSOM
marker	If a marker is given, the standard deviation for this marker is shown. Otherwise, the maximum ratio is used.

Value

A vector containing the SDs

See Also

PlotFlowSOM, ParseEdges, ParseNodeSize, ParseArcs, ParseQuery, PlotSD

Plot1DScatters *Plot1DScatters*

Description

Function to draw 1D scatter plots of FlowSOM (meta)clusters

Usage

```
Plot1DScatters(
  fsom,
  channels = fsom$map$colsUsed,
  clusters = NULL,
  metaclusters = NULL,
  maxBgPoints = 3000,
  sizeBgPoints = 0.5,
  colBgPoints = "gray",
  maxPoints = 1000,
  sizePoints = 0.5,
  yLim = NULL,
  xLabels = c("marker"),
```

```
  centers = FALSE,
  colors = NULL,
  ncol = NULL,
  nrow = NULL,
  width = NULL,
  height = NULL,
  plotFile = "1DScatterPlots.png"
)
```

Arguments

fsom	FlowSOM object, as created by FlowSOM
channels	Vector in which each element is a channel or a marker
clusters	Vector with indices of clusters of interest
metaclusters	Vector with metaclusters of interest. Can be either a character vector with metacluster names or a vector with metacluster indices.
maxBgPoints	Maximum number of background cells to plot
sizeBgPoints	Size of the background cells
colBgPoints	Color of background cells
maxPoints	Maximum number of (meta)cluster cells to plot
sizePoints	Size of the (meta)cluster cells
yLim	Optional vector of a lower and upper limit of the y-axis
xLabels	Determines the label of the x-axis. Can be "marker" and\or "channel" or abbreviations. Default = "marker".
centers	Add cluster centers to plot. Default = FALSE.
colors	Vector of colors for all the cells in the selected nodes. First the clusters are colored, then the metaclusters. If NULL, the default ggplot colors, are used.
ncol	Number of columns in the final plot, optional
nrow	Number of rows in the final plot, optional
width	Width of png file. By default NULL the width parameter is estimated based on the input.
height	Height of png file. By default NULL the width parameter is estimated based on the input.
plotFile	If a filepath for a png is given (default = 1DScatterPlots.png), the plots will be plotted in the corresponding png file. If NULL, a list of ggplot objects will be returned

Details

Plot multiple 1D scatter plots in a png file. A subset of fsom$data is plotted in gray, and those of the selected clusters and metaclusters are plotted in color.

Value

If `plot` is `TRUE`, nothing is returned and a plot is drawn in which background cells are plotted in gray and the cells of the selected nodes in color. If `plot` is `FALSE`, a ggplot objects list is returned.

Examples

```
# Identify the files
fcs <- flowCore::read.FCS(system.file("extdata", "68983.fcs",
                                      package = "FlowSOM"))
# Build a FlowSOM object
flowSOM.res <- FlowSOM(fcs,
                       scale = TRUE,
                       compensate = TRUE,
                       transform = TRUE,
                       toTransform = 8:18,
                       colsToUse = c(9, 12, 14:18),
                       nClus = 10,
                       seed = 1)

# Make the 1D scatter plots of the clusters and metaclusters of interest
Plot1DScatters(fsom = flowSOM.res,
               channels = c("PE-Cy7-A", "PE-Cy5-A"),
               clusters = c(1, 48, 49, 82, 95),
               metaclusters = list(c(1, 4), 9),
               xLabels = c("marker", "channel"),
               plotFile = tempfile(fileext = ".png"))
Plot1DScatters(flowSOM.res,
               metaclusters = 4,
               clusters = c(1, 2),
               colors = c("red", "green", "blue"),
               plotFile = tempfile(fileext = ".png"))
```

`Plot2DScatters` *Plot2DScatters*

Description

Function to draw 2D scatter plots of FlowSOM (meta)clusters

Usage

```
Plot2DScatters(
  fsom,
  channelpairs,
  clusters = NULL,
  metaclusters = NULL,
  maxBgPoints = 3000,
  sizeBgPoints = 0.5,
```

```
  maxPoints = 1000,
  sizePoints = 0.5,
  xLim = NULL,
  yLim = NULL,
  xyLabels = c("marker"),
  density = TRUE,
  centers = TRUE,
  colors = NULL,
  fontsize_axes = 12,
  fontsize_title = 12,
  plotFile = "2DScatterPlots.png"
)
```

Arguments

fsom	FlowSOM object, as created by FlowSOM
channelpairs	List in which each element is a pair of channel or marker names
clusters	Vector or list (to combine multiple clusters in one plot) with indices of clusters of interest
metaclusters	Vector or list (to combine multiple metaclusters in one plot) with metaclusters of interest. Can be either a character vector with metacluster names or a vector with metacluster indices.
maxBgPoints	Maximum number of background cells to plot
sizeBgPoints	Size of the background cells
maxPoints	Maximum number of (meta)cluster cells to plot
sizePoints	Size of the (meta)cluster cells
xLim	Optional vector of a lower and upper limit of the x-axis
yLim	Optional vector of a lower and upper limit of the y-axis
xyLabels	Determines the label of the x- and y-axis. Can be "marker" and\or "channel" or abbrevations. Default = "marker".
density	Default is TRUE to color the (meta)cluster points according to density. Set to FALSE to use a plain color
centers	Default is TRUE to show the cluster centers
colors	Colors for all the cells in the selected nodes (ordered list). First the clusters are colored, then the metaclusters. If NULL, the default ggplot colors, indexed by metacluster number, are used.
fontsize_axes	Font size for the x and y axes labels. Default is 12.
fontsize_title	Font size for the title. Default is 12. If NULL, no title is shown.
plotFile	If a filepath for a png is given (default = 2DScatterPlots.png), the plots will be plotted in the corresponding png file. If NULL, a list of ggplot objects will be returned

Details

Plot multiple 2D scatter plots in a png file. A subset of fsom$data is plotted in gray, and those of the selected clusters and metaclusters are plotted in color.

Value

If plot is TRUE, nothing is returned and a plot is drawn in which background cells are plotted in gray and the cells of the selected nodes in color. If plot is FALSE, a ggplot objects list is returned.

Examples

```
# Identify the files
fcs <- flowCore::read.FCS(system.file("extdata", "68983.fcs",
                                      package = "FlowSOM"))

# Build a FlowSOM object
flowSOM.res <- FlowSOM(fcs,
                       scale = TRUE,
                       compensate = TRUE,
                       transform = TRUE,
                       toTransform = 8:18,
                       colsToUse = c(9, 12, 14:18),
                       nClus = 10,
                       seed = 1)

# Make the 2D scatter plots of the clusters and metaclusters of interest
Plot2DScatters(fsom = flowSOM.res,
               channelpairs = list(c("PE-Cy7-A", "PE-Cy5-A"),
                                   c("PE-Texas Red-A", "Pacific Blue-A")),
               clusters = c(1, 48, 49, 82, 95),
               metaclusters = list(c(1, 4), 9),
               density = FALSE,
               plotFile = tempfile(fileext = ".png"))

Plot2DScatters(fsom = flowSOM.res,
               channelpairs = list(c("PE-Texas Red-A", "Pacific Blue-A")),
               metaclusters = list(c(1, 4)),
               density = FALSE,
               colors = list(c("red", "green")),
               plotFile = tempfile(fileext = ".png"))
```

PlotCenters *PlotCenters*

Description

Plot cluster centers on a 2D plot

Usage

```
PlotCenters(fsom, marker1, marker2, MST = TRUE)
```

Arguments

fsom	FlowSOM object, as generated by BuildMST
marker1	Marker to show on the x-axis
marker2	Marker to show on the y-axis
MST	Type of visualization, if 1 plot tree, else plot grid

Details

Plot FlowSOM nodes on a 2D scatter plot of the data

Value

Nothing is returned. A 2D scatter plot is drawn on which the nodes of the grid are indicated

See Also

PlotStars,PlotPies, PlotMarker,BuildMST

Examples

```
# Read from file, build self-organizing map and minimal spanning tree
fileName <- system.file("extdata", "68983.fcs", package="FlowSOM")
flowSOM.res <- ReadInput(fileName, compensate=TRUE,transform=TRUE,
                         scale=TRUE)
flowSOM.res <- BuildSOM(flowSOM.res,colsToUse=c(9,12,14:18))
flowSOM.res <- BuildMST(flowSOM.res)

# Plot centers
plot <- Plot2DScatters(flowSOM.res,
               channelpairs = list(c("FSC-A","SSC-A")),
               clusters = list(seq_len(NClusters(flowSOM.res))),
               maxPoints = 0,
               plotFile = NULL)
```

PlotClusters2D *PlotClusters2D*

Description

Plot nodes on scatter plot

Usage

```
PlotClusters2D(
  fsom,
  marker1,
  marker2,
  nodes,
  col = "#FF0000",
  maxBgPoints = 10000,
  pchBackground = ".",
  pchCluster = ".",
  main = "",
  xlab = fsom$prettyColnames[marker1],
  ylab = fsom$prettyColnames[marker2],
  xlim = c(min(fsom$data[, marker1]), max(fsom$data[, marker1])),
  ylim = c(min(fsom$data[, marker2]), max(fsom$data[, marker2])),
  ...
)
```

Arguments

fsom	FlowSOM object, as generated by BuildMST
marker1	Marker to plot on the x-axis
marker2	Marker to plot on the y-axis
nodes	Nodes of which the cells should be plotted in red
col	Colors for all the cells in the selected nodes (ordered array)
maxBgPoints	Maximum number of background points to plot
pchBackground	Character to use for background cells
pchCluster	Character to use for cells in cluster
main	Title of the plot
xlab	Label for the x axis
ylab	Label for the y axis
xlim	Limits for the x axis
ylim	Limits for the y axis
...	Other parameters to pass on to plot

Details

Plot a 2D scatter plot. All cells of fsom$data are plotted in black, and those of the selected nodes are plotted in red. The nodes in the grid are indexed starting from the left bottom, first going right, then up. E.g. In a 10x10 grid, the node at top left will have index 91.

Value

Nothing is returned. A plot is drawn in which all cells are plotted in black and the cells of the selected nodes in red.

See Also

PlotNumbers, PlotCenters, BuildMST

Examples

```
## Deprecated - use Plot2DScatters instead ##

# Read from file, build self-organizing map and minimal spanning tree
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
flowSOM.res <- ReadInput(fileName, compensate = TRUE, transform = TRUE,
                         scale = TRUE)
flowSOM.res <- BuildSOM(flowSOM.res, colsToUse = c(9, 12, 14:18))
flowSOM.res <- BuildMST(flowSOM.res)

# Plot cells

Plot2DScatters(flowSOM.res, list(c(1, 2)), clusters = 91,
               plotFile = tempfile(fileext = ".png"))
```

PlotDimRed *PlotDimRed*

Description

Plot a dimensionality reduction

Usage

```
PlotDimRed(
  fsom,
  colsToUse = fsom$map$colsUsed,
  colorBy = "metaclusters",
  colors = NULL,
  lim = NULL,
  cTotal = NULL,
  dimred = Rtsne::Rtsne,
  extractLayout = function(dimred) {
     dimred$Y
 },
  label = TRUE,
  returnLayout = FALSE,
  seed = NULL,
  title = NULL,
  ...
)
```

Arguments

fsom	FlowSOM object, as generated by BuildMST
colsToUse	The columns used for the dimensionality reduction. Default = fsommapcolsUsed.
colorBy	Defines how the dimensionality reduction will be colored. Can be "metaclusters" (default), "clusters" (or abbreviations) or a marker/channel/index.
colors	A vector of custom colors. Default returns ggplot colors for categorical variables and the FlowSOM colors for continuous variables. When using a categorical variable, the vector must be as long as the levels of the categorical variable.
lim	Limits for the colorscale
cTotal	The total amount of cells to be used in the dimensionality reduction. Default is all the cells.
dimred	A dimensionality reduction function. Default = Rtsne::Rtsne. Alternatively, a data.frame or matrix with either equal number of rows to the fsom or an OriginalID column. Recommended to put cTotal to NULL when providing a matrix (or ensuring that the dimred corresponds to subsampling the flowSOM data for cTotal cells with the same seed).
extractLayout	A function to extract the coordinates from the results of the dimred default = function(dimred){dimred$Y}.
label	If label = TRUE (default), labels are added to plot.
returnLayout	If TRUE, this function returns a dataframe with the layout of dimred and the original IDs and the plot. Default = FALSE.
seed	A seed for reproducibility.
title	A title for the plot.
...	Additional arguments to pass to dimred.

Details

Plot a dimensionality reduction of fsom$data

Value

A dimensionality reduction plot made in ggplot2

Examples

```
file <- system.file("extdata", "68983.fcs", package="FlowSOM")
flowSOM.res <- FlowSOM(file, compensate = TRUE, transform = TRUE,
               scale = TRUE,
               colsToUse = c(9, 12, 14:18), nClus = 10, silent = FALSE,
               xdim = 7, ydim = 7)
PlotDimRed(flowSOM.res, cTotal = 5000, seed = 1, title = "t-SNE")
PlotDimRed(flowSOM.res, cTotal = 5000, colorBy = "CD3", seed = 1,
           title = "t-SNE")
```

PlotFileScatters *PlotFileScatters*

Description

Make a scatter plot per channel for all provided files

Usage

```
PlotFileScatters(
  input,
  fileID = "File",
  channels = NULL,
  yLim = NULL,
  yLabel = "marker",
  quantiles = NULL,
  names = NULL,
  groups = NULL,
  color = NULL,
  legend = FALSE,
  maxPoints = 50000,
  ncol = NULL,
  nrow = NULL,
  width = NULL,
  height = NULL,
  silent = FALSE,
  plotFile = "FileScatters.png",
  ...
)
```

Arguments

input	Either a flowSet, a flowFrame with a file ID column (e.g. output from the AggregateFlowFrames includes a "File" column) or a vector of paths pointing to FCS files
fileID	Name of the file ID column when the input is a flowFrame, default to "File" (File ID column in the AggregateFlowFrames flowFrame output).
channels	Vector of channels or markers that need to be plotted, if NULL (default), all channels from the input will be plotted
yLim	Optional vector of a lower and upper limit of the y-axis
yLabel	Determines the label of the y-axis. Can be "marker" and\or "channel" or abbreviations. Default = "marker".
quantiles	If provided (default NULL), a numeric vector with values between 0 and 1. These quantiles are indicated on the plot
names	Optional parameter to provide filenames. If NULL (default), the filenames will be numbers. Duplicated filenames will be made unique.

groups	Optional parameter to specify groups of files, should have the same length as the input. Id NULL (default), all files will be plotted in the same color
color	Optional parameter to provide colors. Should have the same lengths as the number of groups (or 1 if groups is NULL)
legend	Logical parameter to specify whether the group levels should be displayed. Default is FALSE
maxPoints	Total number of data points that will be plotted per channel, default is 50000
ncol	Number of columns in the final plot, optional
nrow	Number of rows in the final plot, optional
width	Width of png file. By default NULL the width parameter is estimated based on the input.
height	Height of png file. By default NULL the width parameter is estimated based on the input.
silent	If FALSE, prints an update every time it starts processing a new file. Default = FALSE.
plotFile	Path to png file, default is "FileScatters.png". If NULL, the output will be a list of ggplots
...	Arguments for read.FCS (e.g. truncate_max_range)

Value

List of ggplot objects if plot is FALSE, otherwise filePlot with plot is created.

Examples

```
# Preprocessing
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
ff <- flowCore::read.FCS(fileName)
ff <- flowCore::compensate(ff, flowCore::keyword(ff)[["SPILL"]])
ff <- flowCore::transform(ff,
         flowCore::transformList(colnames(flowCore::keyword(ff)[["SPILL"]]),
                                 flowCore::logicleTransform()))

flowCore::write.FCS(ff[1:1000, ], file = "ff_tmp1.fcs")
flowCore::write.FCS(ff[1001:2000, ], file = "ff_tmp2.fcs")
flowCore::write.FCS(ff[2001:3000, ], file = "ff_tmp3.fcs")

# Make plot
PlotFileScatters(input = c("ff_tmp1.fcs", "ff_tmp2.fcs", "ff_tmp3.fcs"),
                 channels = c("Pacific Blue-A",
                              "Alexa Fluor 700-A",
                              "PE-Cy7-A"),
                 maxPoints = 1000,
                 plotFile = tempfile(fileext = ".png"))

unlink(c("ff_tmp1.fcs", "ff_tmp2.fcs", "ff_tmp3.fcs"))
```

PlotFlowSOM *PlotFlowSOM*

Description

Base layer to plot a FlowSOM result

Usage

```
PlotFlowSOM(
  fsom,
  view = "MST",
  nodeSizes = fsom$map$pctgs,
  maxNodeSize = 1,
  refNodeSize = max(nodeSizes),
  equalNodeSize = FALSE,
  backgroundValues = NULL,
  backgroundColors = NULL,
  backgroundSize = 1.5,
  equalBackgroundSize = FALSE,
  backgroundLim = NULL,
  alpha = 0.4,
  title = NULL
)
```

Arguments

fsom	FlowSOM object, as created by FlowSOM
view	Preferred view, options: "MST", "grid" or "matrix" with a matrix/dataframe consisting of coordinates. Default = "MST"
nodeSizes	A vector containing node sizes. These will automatically be scaled between 0 and maxNodeSize and transformed with a sqrt. Default = fsomMSTsizes
maxNodeSize	Determines the maximum node size. Default is 1.
refNodeSize	Reference for node size against which the nodeSizes will be scaled. Default = max(nodeSizes)
equalNodeSize	If TRUE, the nodes will be equal to maxNodeSize. If FALSE (default), the nodes will be scaled to the number of cells in each cluster
backgroundValues	Values to be used for background coloring, either numerical values or something that can be made into a factor (e.g. a clustering)
backgroundColors	Color palette to be used for the background coloring. Can be either a function or an array specifying colors.
backgroundSize	The size of the background. Will be multiplied by the size of the nodes.

equalBackgroundSize
: If you want the background sizes to be the same size.

backgroundLim
: Only used when backgroundValues are numerical. Defaults to min and max of the backgroundValues.

alpha
: Transparency of the backgroundColor.

title
: Title of the plot

Details

Base layer of the FlowSOM plot, where you can choose layout (MST, grid or coordinates of your own choosing), background colors and node size. Can then be extended by e.g. AddStars, AddLabels, AddPies, ...

Value

A ggplot object with the base layer of a FlowSOM plot

See Also

PlotStars, PlotVariable, PlotMarker, PlotLabels, PlotNumbers, PlotPies, QueryStarPlot, PlotSD

Examples

```
# Locate file on file system
fcs_file <- system.file("extdata", "68983.fcs", package = "FlowSOM")

# Build FlowSOM model
flowSOM.res <- FlowSOM(fcs_file,
                       scale = TRUE,
                       compensate = TRUE,
                       transform = TRUE,
                       toTransform = 8:18,
                       colsToUse = c(9, 12, 14:18),
                       nClus = 10,
                       seed = 1)

# Plot with background coloring
PlotFlowSOM(flowSOM.res,
            backgroundValues = flowSOM.res$metaclustering) %>%
            AddLabels(seq(100))
```

PlotGroups *PlotGroups*

Description

Plot differences between groups

Usage

```
PlotGroups(fsom, groups, threshold = NULL, pThreshold = 0.05, ...)
```

Arguments

fsom	FlowSOM object, as generated by BuildMST
groups	Groups result as generated by CountGroups
threshold	Relative difference in groups before the node is colored
pThreshold	Threshold on p-value from wilcox-test before the node is colored. If this is not NULL, threshold will be ignored.
...	Additional arguments to pass to PlotFlowSOM

Details

Plot FlowSOM trees, where each node is represented by a star chart indicating mean marker values, the size of the node is relative to the mean percentage of cells present in each

Value

A vector containing the labels assigned to the nodes for all groups except the first

See Also

PlotStars,PlotVariable, PlotFlowSOM,PlotLabels,PlotNumbers, PlotMarker,PlotPies,QueryStarPlot, PlotSD

Examples

```
#Run FlowSOM
fileName <-  system.file("extdata", "68983.fcs", package="FlowSOM")
fsom <- FlowSOM(fileName, compensate = TRUE, transform = TRUE,
                      scale = TRUE, colsToUse = c(9,12,14:18), nClus = 10)

ff <- flowCore::read.FCS(fileName)
# Make an additional file without cluster 7 and double amount of cluster 5
selection <- c(which(GetClusters(fsom) %in% which(fsom$metaclustering != 7)),
                which(GetClusters(fsom) %in% which(fsom$metaclustering == 5)))
ff_tmp <- ff[selection,]
flowCore::write.FCS(ff_tmp, file="ff_tmp.fcs")
```

```
# Compare only the file with the double amount of cluster 10
features <- GetFeatures(fsom,
                        c(fileName, "ff_tmp.fcs"),
                        level = "clusters",
                        type = "percentages")
stats <- GroupStats(features$cluster_percentages,
                    groups = list("AllCells" = c(fileName),
                                  "Without_ydTcells" = c("ff_tmp.fcs")))

fold_changes <- stats["fold changes", ]
fold_changes_label <- factor(ifelse(fold_changes < -1.5,
                           "Underrepresented compared to Group 1",
                           ifelse(fold_changes > 1.5,
                                  "Overrepresented compared to Group 1",
                                  "--")),
                            levels = c("--",
                               "Underrepresented compared to Group 1",
                               "Overrepresented compared to Group 1"))
fold_changes_label[is.na(fold_changes_label)] <- "--"
gr_1 <- PlotStars(fsom,
                  title = "All Cells",
                  nodeSizes = stats["medians AllCells", ],
                  list_insteadof_ggarrange = TRUE)
gr_2 <- PlotStars(fsom, title = "Group 2",
                nodeSizes = stats["medians Without_ydTcells", ],
                 backgroundValues = fold_changes_label,
                backgroundColors = c("white", "red", "blue"),
                list_insteadof_ggarrange = TRUE)
p <- ggpubr::ggarrange(plotlist = c(list(gr_1$tree), gr_2),
                    heights = c(3, 1))
p

unlink("ff_tmp.fcs")
```

PlotLabels *PlotLabels*

Description

Plot labels for each cluster

Usage

```
PlotLabels(
  fsom,
  labels,
  maxNodeSize = 0,
  textSize = 3.88,
```

```
  textColor = "black",
  ...
)
```

Arguments

fsom	FlowSOM object, as generated by FlowSOM
labels	A vector of labels for every node.
maxNodeSize	Determines the maximum node size. Default is 0.
textSize	Size for geom_text. Default (=3.88) is from geom_text.
textColor	Color for geom_text. Default = black.
...	Additional arguments to pass to PlotFlowSOM

Details

Plot FlowSOM grid or tree, with in each node a label. Especially useful to show metacluster numbers

Value

Nothing is returned. A plot is drawn in which each node is represented by a label.

See Also

PlotStars, PlotVariable, PlotFlowSOM, PlotMarker, PlotNumbers, PlotPies, QueryStarPlot, PlotSD

Examples

```
# Read from file, build self-organizing map and minimal spanning tree
fileName <- system.file("extdata", "68983.fcs", package="FlowSOM")
ff <- flowCore::read.FCS(fileName)
ff <- flowCore::compensate(ff, flowCore::keyword(ff)[["SPILL"]])
ff <- flowCore::transform(ff,
        flowCore::transformList(colnames(flowCore::keyword(ff)[["SPILL"]]),
                                flowCore::logicleTransform()))
flowSOM.res <- FlowSOM(ff,
                       scale = TRUE,
                       colsToUse = c(9, 12, 14:18),
                       nClus = 10,
                       seed = 1)

# Plot the node IDs
PlotLabels( flowSOM.res,
            flowSOM.res$metaclustering)
```

PlotManualBars *PlotManualBars*

Description

Function to plot the manual labels per FlowSOM (meta)cluster in a barplot

Usage

```
PlotManualBars(
  fsom,
  fcs = NULL,
  manualVector,
  manualOrder = NULL,
  colors = NULL,
  list_insteadof_plots = FALSE
)
```

Arguments

fsom	FlowSOM object, as generated by FlowSOM or by NewData. The clusters and metaclusters will be plotted in the order of the factor levels.
fcs	FCS file that should be mapped on the FlowSOM object. Default is NULL.
manualVector	Vector with cell labels, e.g. obtained by manual gating
manualOrder	Optional vector with unique cell labels to fix in which order the cell labels should be shown
colors	Optional color vector, should have the same length as the number of unique cell labels
list_insteadof_plots	If FALSE (default), it returns multiple plots. If TRUE, it returns a list of ggplot objects

Value

Either a plot or a ggplot objects list is returned.

Examples

```
# Identify the files
fcs_file <- system.file("extdata", "68983.fcs", package = "FlowSOM")
gating_file <- system.file("extdata", "gatingResult.csv", package = "FlowSOM")

# Specify the cell types of interest for assigning one label per cell
cellTypes <- c("B cells",
               "gd T cells", "CD4 T cells", "CD8 T cells",
               "NK cells", "NK T cells")
```

```
# Load manual labels (e.g. GetFlowJoLabels can be used to extract labels from
# an fcs file)

gatingResult <- as.factor(read.csv(gating_file, header = FALSE)[, 1])

# Build a FlowSOM object
flowSOM.res <- FlowSOM(fcs_file,
                       scale = TRUE,
                       compensate = TRUE,
                       transform = TRUE,
                       toTransform = 8:18,
                       colsToUse = c(9, 12, 14:18),
                       nClus = 10,
                       seed = 1)

# Make the barplot of the manual labels
pdf(tempfile(fileext = ".pdf"))
PlotManualBars(fsom = flowSOM.res,
               fcs = fcs_file,
               manualVector = gatingResult,
               manualOrder = c(cellTypes, "Unlabeled"),
               colors = c("#F8766D", "#B79F00", "#00BA38", "#00BFC4",
                          "#619CFF", "#F564E3", "#D3D3D3"))
dev.off()
```

PlotMarker *PlotMarker*

Description

Plot comparison with other clustering

Usage

```
PlotMarker(
  fsom,
  marker,
  refMarkers = fsom$map$colsUsed,
  title = GetMarkers(fsom, marker),
  colorPalette = FlowSOM_colors,
  lim = NULL,
  ...
)
```

Arguments

fsom	FlowSOM object
marker	A vector of markers/channels to plot.

refMarkers	Is used to determine relative scale of the marker that will be plotted. Default are all markers used in the clustering.
title	A vector with custom titles for the plot. Default is the marker name.
colorPalette	Color palette to use. Can be a function or a vector.
lim	Limits for the scale
...	Additional arguments to pass to PlotFlowSOM, e.g. view, backgroundValues, equalNodeSize ...

Details

Plot FlowSOM grid or tree, colored by node values for a specific marker

Value

A ggplot figure is returned in which every cluster is colored according to the MFI value for the specified marker

See Also

PlotStars, PlotVariable

Examples

```
# Build FlowSOM model
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
flowSOM.res <- FlowSOM(fileName,
                       compensate = TRUE, transform = TRUE, scale = FALSE,
                       colsToUse = c(9, 12, 14:18),
                       nClus = 10,
                       seed = 1)
# Plot one marker
PlotMarker(flowSOM.res,
           "CD19")

PlotMarker(flowSOM.res,
           "CD19",
           colorPalette = c("gray", "red"))

# Plot all markers
PlotMarker(flowSOM.res,
           c(9, 12, 14:18))

# Use specific limits if the ones from the columns used for clustering
# are not relevant for  your marker of choice
PlotMarker(flowSOM.res,
           "FSC-A",
            lim = c(55000, 130000))

# Example with additional FlowSOM plotting options
PlotMarker(flowSOM.res,
```

```
            "CD19",
            view = "grid",
            equalNodeSize = TRUE,
            backgroundValues = 1:100 == 27,
            backgroundColors = c("white", "red"))
```

`PlotMFIHeatmap` *PlotMFIHeatmap*

Description

Plots a heatmap of the cluster medians and metacluster medians

Usage

```
PlotMFIHeatmap(
  fsom,
  level = c("clusters", "metaclusters"),
 heatmapColors = c("#313695", "#4575B4", "#74ADD1", "#ABD9E9", "#E0F3F8", "#FEE090",
    "#FDAE61", "#F46D43", "#D73027", "#A50026"),
  annotationColors = gg_color_hue,
  cluster_rows = FALSE,
  cluster_columns = FALSE,
  labels = FALSE
)
```

Arguments

`fsom`	FlowSOM object, as created by `FlowSOM`
`level`	A vector with "clusters" and/or "metaclusters (or abbreviations) to show clusters and/or metaclusters. Default shows both.
`heatmapColors`	Vector of colors for the heatmap. The default colors are RdYlBu
`annotationColors`	A color function to color the metacluster column annotation. The default colors are the ggplot colors
`cluster_rows`	Whether or not to cluster the rows. Default = FALSE
`cluster_columns`	Whether or not to cluster the columns Default = FALSE
`labels`	Whether or not to display the MFIs. Default = FALSE

Value

Plot

Examples

```
# Identify the files
fcs <- flowCore::read.FCS(system.file("extdata", "68983.fcs",
                                      package = "FlowSOM"))
# Build a FlowSOM object
flowSOM.res <- FlowSOM(fcs,
                       scale = TRUE,
                       compensate = TRUE,
                       transform = TRUE,
                       toTransform = 8:18,
                       colsToUse = c(9, 12, 14:18),
                       nClus = 10,
                       seed = 1)
p <- PlotMFIHeatmap(flowSOM.res)
```

PlotNode *PlotNode Plot star chart*

Description

Plot a star chart indicating median marker values of a single node

Usage

```
PlotNode(
  fsom,
  id,
  markers = fsom$map$colsUsed,
 colorPalette = grDevices::colorRampPalette(c("#00007F", "blue", "#007FFF", "cyan",
    "#7FFF7F", "yellow", "#FF7F00", "red", "#7F0000")),
  main = paste0("Cluster ", id)
)
```

Arguments

fsom	FlowSOM object, as generated by BuildMST or the first element of the list returned by FlowSOM
id	Id of the node to plot (check PlotNumbers to get the ids)
markers	Array of markers to use. Default: the markers used to build the tree
colorPalette	Color palette to be used for the markers
main	Title of the plot

Value

Nothing is returned. A plot is drawn in which the node is represented by a star chart indicating the median fluorescence intensities.

See Also

PlotStars,PlotNumbers, FlowSOM

Examples

```
# Read from file, build self-organizing map and minimal spanning tree
fileName <- system.file("extdata", "68983.fcs", package="FlowSOM")
flowSOM.res <- FlowSOM(fileName, compensate=TRUE,transform=TRUE,
                       scale=TRUE,colsToUse=c(9,12,14:18),nClus=10)

# Deprecated, it is currently not possible anymore to plot an individual
# node alone. If necessary, zooming in on a node can be approximated by
# exagerating the size of the node.
PlotStars(flowSOM.res, nodeSizes = c(100, rep(0,99)), maxNodeSize = 10)
```

PlotNumbers *PlotNumbers*

Description

Plot cluster ids for each cluster

Usage

```
PlotNumbers(fsom, level = "clusters", maxNodeSize = 0, ...)
```

Arguments

fsom	FlowSOM object
level	Character string, should be either "clusters" or "metaclusters". Can be abbreviated.
maxNodeSize	Determines the maximum node size. Default is 0. See PlotFlowSOM for more options.
...	Additional arguments to pass to PlotLabels and to PlotFlowSOM

Details

Plot FlowSOM grid or tree, with in each node the cluster id.

Value

Nothing is returned. A plot is drawn in which each node is labeled by its cluster id.

See Also

PlotStars, PlotVariable, PlotFlowSOM, PlotLabels, PlotMarker, PlotPies, QueryStarPlot, PlotSD

Examples

```
# Read from file, build self-organizing map and minimal spanning tree
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
ff <- flowCore::read.FCS(fileName)
ff <- flowCore::compensate(ff, flowCore::keyword(ff)[["SPILL"]])
ff <- flowCore::transform(ff, flowCore::estimateLogicle(ff,
                                                flowCore::colnames(ff)[8:18]))
flowSOM.res <- FlowSOM(ff,
                       scale = TRUE,
                       colsToUse = c(9, 12, 14:18),
                       nClus = 10,
                       seed = 1)

# Plot the node IDs
PlotNumbers(flowSOM.res)
PlotNumbers(flowSOM.res, "metaclusters")

PlotNumbers(flowSOM.res,
            view = "grid")

PlotNumbers(flowSOM.res,
            maxNodeSize = 1,
            equalNodeSize = TRUE)
```

`PlotOutliers` *PlotOutliers*

Description

Visual overview of outliers

Usage

```
PlotOutliers(fsom, outlierReport)
```

Arguments

`fsom`	FlowSOM object.
`outlierReport`	Outlier overview as generated by TestOutliers()

Value

Plot

Examples

```
# Identify the files
fcs <- flowCore::read.FCS(system.file("extdata", "68983.fcs",
                                      package = "FlowSOM"))
# Build a FlowSOM object
flowSOM.res <- FlowSOM(fcs,
                       scale = TRUE,
                       compensate = TRUE,
                       transform = TRUE,
                       toTransform = 8:18,
                       colsToUse = c(9, 12, 14:18),
                       nClus = 10,
                       seed = 1)
outlierReport <- TestOutliers(flowSOM.res)
p <- PlotOutliers(flowSOM.res, outlierReport)
```

`PlotOverview2D` *PlotOverview2D*

Description

Plot metaclusters on scatter plots

Usage

```
PlotOverview2D(fsom, markerlist, metaclusters, colors = NULL, ff, ...)
```

Arguments

`fsom`	FlowSOM object, as generated by `FlowSOM`. If using a FlowSOM object as generated by `BuildMST`, it needs to be wrapped in a list, list(FlowSOM = fsom, metaclustering = metaclustering).
`markerlist`	List in which each element is a pair of marker names
`metaclusters`	Metaclusters of interest
`colors`	Named vector with color value for each metacluster. If NULL (default) colorbrewer "paired" is interpolated
`ff`	flowFrame to use as reference for the marker names
`...`	Other parameters to pass on to PlotClusters2D

Details

Write multiple 2D scatter plots to a png file. All cells of fsom$data are plotted in black, and those of the selected metaclusters are plotted in color.

Value

Nothing is returned, but a plot is drawn for every markerpair and every metacluster. The individual cells are colored, and the center of each FlowSOM cluster is indicated with a blue cross.

See Also

PlotClusters2D

Examples

```
## Deprecated - use Plot2DScatters instead ##

# Read from file, build self-organizing map and minimal spanning tree
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
flowSOM.res <- FlowSOM(fileName,
                       compensate = TRUE, transform = TRUE, scale = TRUE,
                       colsToUse = c(9, 12, 14:18),
                       nClus = 10,
                       seed = 1)

# Plot cells
markers_of_interest = list(c("FSC-A", "SSC-A"),
                           c("CD3", "CD19"),
                           c("TCRb", "TCRyd"),
                           c("CD4", "CD8"))
metaclusters_of_interest = 1:10

# Recommended to write to png


  Plot2DScatters(flowSOM.res,
                 channelpairs = markers_of_interest,
                 metaclusters = metaclusters_of_interest,
                 plotFile = tempfile(fileext = ".png"))
```

PlotPies *PlotPies*

Description

Plot comparison with other clustering

Usage

```
PlotPies(
  fsom,
  cellTypes,
```

```
 colorPalette = grDevices::colorRampPalette(c("white", "#00007F", "blue", "#007FFF",
    "cyan", "#7FFF7F", "yellow", "#FF7F00", "red", "#7F0000")),
  ...
)
```

Arguments

fsom	FlowSOM object, as generated by FlowSOM
cellTypes	Array of factors indicating the celltypes
colorPalette	Color palette to use.
...	Additional arguments to pass to PlotFlowSOM

Details

Plot FlowSOM grid or tree, with pies indicating another clustering or manual gating result

Value

ggplot plot

See Also

PlotStars, PlotVariable, PlotFlowSOM, PlotLabels, PlotNumbers, PlotMarker, QueryStarPlot, PlotSD

Examples

```
# Identify the files
fcs_file <- system.file("extdata", "68983.fcs", package = "FlowSOM")
gating_file <- system.file("extdata", "gatingResult.csv", package = "FlowSOM")

# Specify the cell types of interest for assigning one label per cell
cellTypes <- c("B cells",
               "gd T cells", "CD4 T cells", "CD8 T cells",
               "NK cells", "NK T cells")

# Load manual labels (e.g. GetFlowJoLabels can be used to extract labels from
# an fcs file)

gatingResult <- as.factor(read.csv(gating_file, header = FALSE)[, 1])


# Build a FlowSOM tree
flowSOM.res <- FlowSOM(fcs_file,
                       scale = TRUE,
                       compensate = TRUE,
                       transform = TRUE,
                       toTransform = 8:18,
                       colsToUse = c(9, 12, 14:18),
                       nClus = 10,
                       seed = 1)
```

```
# Plot pies indicating the percentage of cell types present in the nodes
PlotPies(flowSOM.res,
         gatingResult,
         backgroundValues = flowSOM.res$metaclustering)
```

PlotSD *PlotSD*

Description

Plot FlowSOM grid or tree, colored by standard deviation.

Usage

```
PlotSD(fsom, marker = NULL, ...)
```

Arguments

fsom	FlowSOM object, as generated by FlowSOM
marker	If a marker/channel is given, the sd for this marker is shown. Otherwise, the maximum ratio is used.
...	Additional arguments to pass to PlotFlowSOM

Value

Nothing is returned. A plot is drawn in which each node is colored depending on its standard deviation

See Also

PlotStars, PlotVariable, PlotFlowSOM, PlotLabels, PlotNumbers, PlotMarker, PlotPies, QueryStarPlot

Examples

```
# Read from file, build self-organizing map and minimal spanning tree
fileName <- system.file("extdata", "68983.fcs", package  = "FlowSOM")
flowSOM.res <- ReadInput(fileName, compensate  = TRUE, transform  = TRUE,
                         scale  = TRUE)
flowSOM.res <- BuildSOM(flowSOM.res, colsToUse  = c(9, 12, 14:18))
flowSOM.res <- BuildMST(flowSOM.res)

PlotSD(flowSOM.res)
```

PlotStarLegend *PlotStarLegend*

Description

Plots star legend

Usage

```
PlotStarLegend(markers, colors, starHeight = 1)
```

Arguments

markers	Vector of markers used in legend
colors	Color palette for the legend. Can be a vector or a function.
starHeight	Star height. Default = 1.

Details

Function makes the legend of the FlowSOM star plot

Value

Returns nothing, but plots a legend for FlowSOM star plot

See Also

PlotFlowSOM

Examples

```
PlotStarLegend(c("CD3", "CD4", "CD8"),
               FlowSOM_colors(3))
```

PlotStars *PlotStars*

Description

Plot star charts

Usage

```
PlotStars(
  fsom,
  markers = fsom$map$colsUsed,
  colorPalette = FlowSOM_colors,
  list_insteadof_ggarrange = FALSE,
  ...
)
```

Arguments

fsom	FlowSOM object, as generated by BuildMST
markers	Markers to plot (will be parsed by GetChannels)
colorPalette	Color palette to use
list_insteadof_ggarrange	If FALSE (default), the plot and the legend are combined by ggarrange. If TRUE, the separate elements are returned in a list, to allow further customization.
...	Additional arguments to pass to PlotFlowSOM

Details

Plot FlowSOM grid or tree, where each node is represented by a star chart indicating median marker values

Value

Nothing is returned. A plot is drawn in which each node is represented by a star chart indicating the median fluorescence intensities. Resets the layout back to 1 plot at the end.

See Also

PlotMarker, PlotVariable, PlotFlowSOM, PlotLabels, PlotNumbers, PlotPies, QueryStarPlot, PlotSD

Examples

```
# Read from file, build self-organizing map and minimal spanning tree
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
flowSOM.res <- FlowSOM(fileName, compensate = TRUE, transform = TRUE,
                       scale = TRUE, colsToUse = c(9, 12, 14:18))

# Plot stars indicating the MFI of the cells present in the nodes
PlotStars(flowSOM.res, backgroundValues = flowSOM.res$metaclustering)

newLayout <- igraph::layout_with_fr(flowSOM.res[["MST"]][["graph"]])
PlotStars(flowSOM.res, backgroundValues = flowSOM.res$metaclustering,
          view = newLayout)
```

```
PlotStars(flowSOM.res, backgroundValues = flowSOM.res$metaclustering,
          view = "grid")
```

PlotVariable *PlotVariable*

Description

Plot a variable for all nodes

Usage

```
PlotVariable(
  fsom,
  variable,
  variableName = "",
  colorPalette = FlowSOM_colors,
  lim = NULL,
  ...
)
```

Arguments

fsom	FlowSOM object
variable	A vector containing a value for every cluster
variableName	Label to show on the legend
colorPalette	Color palette to use. Can be a function or a vector.
lim	Limits for the scale
...	Additional arguments to pass to PlotFlowSOM, e.g. view, backgroundValues, equalNodeSize ...

Details

Plot FlowSOM grid or tree, colored by node values given in variable

See Also

PlotStars, QueryStarPlot, PlotFlowSOM, PlotLabels, PlotNumbers, PlotMarker, PlotPies, PlotSD

Examples

```
# Build FlowSOM model
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
flowSOM.res <- FlowSOM(fileName,
                       compensate = TRUE, transform = TRUE, scale = FALSE,
                       colsToUse = c(9, 12, 14:18),
                       nClus = 10,
                       seed = 1)

# Plot some random values
rand <- runif(flowSOM.res$map$nNodes)
PlotVariable(flowSOM.res,
             variable = rand,
             variableName = "Random")

PlotVariable(flowSOM.res,
             variable = flowSOM.res$metaclustering,
             variableName = "Metaclustering") %>%
  AddLabels(labels = flowSOM.res$metaclustering)
```

---

print.FlowSOM *Print FlowSOM object*

Description

Print FlowSOM object

Usage

```
## S3 method for class 'FlowSOM'
print(x, ...)
```

Arguments

x	FlowSOM object to print information about
...	Further arguments, not used

Examples

```
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
flowSOM.res <- FlowSOM(fileName, compensate = TRUE, transform = TRUE,
                       scale = TRUE, colsToUse = c(9, 12, 14:18), nClus = 10)
print(flowSOM.res)
```

Purity *Calculate mean weighted cluster purity*

Description

Calculate mean weighted cluster purity

Usage

```
Purity(realClusters, predictedClusters, weighted = TRUE)
```

Arguments

realClusters	array with real cluster values
predictedClusters	array with predicted cluster values
weighted	logical. Should the mean be weighted depending on the number of points in the predicted clusters

Value

Mean purity score, worst score, number of clusters with score < 0.75

Examples

```
# Generate some random data as an example
realClusters <- sample(1:5, 100, replace = TRUE)
predictedClusters <- sample(1:6, 100, replace = TRUE)

# Calculate the FMeasure
Purity(realClusters, predictedClusters)
```

query_multiple *query_multiple*

Description

Function which takes a named list of multiple cell types, where every item is a named vector with values "high"/"low" and the names correspond to the markers or channels (e.g. as generated by parse_markertable).

Usage

```
query_multiple(fsom, cell_types, pdf_name = "query_multiple.pdf", ...)
```

Arguments

fsom	FlowSOM object
cell_types	Description of the cell types. Named list, with one named vector per cell type containing "high"/"low" values
pdf_name	Path to a pdf file to save figures
...	Additional arguments to pass to QueryStarPlot

Value

A label for every FlowSOM cluster (Unknown or one of the celltype names of the list, if selected by QueryStarPlot)

See Also

QueryStarPlot

Examples

```
file <- system.file("extdata", "68983.fcs", package="FlowSOM")
ff <- flowCore::read.FCS(file)
# Use the wrapper function to build a flowSOM object (saved in flowSOM.res)
# and a metaclustering (saved in flowSOM.res[["metaclustering"]])
flowSOM.res <- FlowSOM(ff,compensate = TRUE, transform = TRUE,scale = TRUE,
               colsToUse = c(9,12,14:18), nClus = 10, silent = FALSE,
               xdim=7, ydim=7)
cell_types <- list("CD8 T cells" = c("PE-Cy7-A" = "high",
                                     "APC-Cy7-A" = "high",
                                     "Pacific Blue-A" = "high"),
                   "B cells" = c("PE-Cy5-A" = "high"),
                   "NK cells" = c("PE-A" = "high",
                                  "PE-Cy7-A" = "low",
                                  "APC-Cy7-A" = "low"))
query_res <- QueryMultiple(flowSOM.res, cell_types, "query_multiple.pdf")
unlink("query_multiple.pdf")
```

QueryMultiple *QueryMultiple*

Description

Function which takes a named list of multiple cell types, where every item is a named vector with values "high"/"low" and the names correspond to the markers or channels (e.g. as generated by parse_markertable).

Usage

```
QueryMultiple(fsom, cellTypes, plotFile = "queryMultiple.pdf", ...)
```

Arguments

fsom	FlowSOM object
cellTypes	Description of the cell types. Named list, with one named vector per cell type containing "high"/"low" values
plotFile	Path to a pdf file to save the plots (default is queryMultiple.pdf). If NULL, no plots will be generated
...	Additional arguments to pass to QueryStarPlot

Value

A label for every FlowSOM cluster (Unknown or one of the celltype names of the list, if selected by QueryStarPlot)

Examples

```
file <- system.file("extdata", "68983.fcs", package = "FlowSOM")
ff <- flowCore::read.FCS(file)
# Use the wrapper function to build a flowSOM object (saved in flowSOM.res)
# and a metaclustering (saved in flowSOM.res[["metaclustering"]])
flowSOM.res <- FlowSOM(ff, compensate = TRUE, transform = TRUE, scale = TRUE,
                colsToUse = c(9, 12, 14:18), nClus = 10, silent = FALSE,
                xdim = 7, ydim = 7)
cellTypes <- list("CD8 T cells" = c("PE-Cy7-A" = "high",
                                    "APC-Cy7-A" = "high",
                                    "Pacific Blue-A" = "high"),
                  "B cells" = c("PE-Cy5-A" = "high"),
                  "NK cells" = c("PE-A" = "high",
                                 "PE-Cy7-A" = "low",
                                 "APC-Cy7-A" = "low"))
query_res <- QueryMultiple(flowSOM.res, cellTypes,
                           plotFile = tempfile(fileext = ".pdf"))
```

QueryStarPlot *QueryStarPlot*

Description

Query a certain cell type

Usage

```
QueryStarPlot(
  fsom,
  query,
  plot = TRUE,
  colorPalette = FlowSOM_colors,
```

```
  backgroundColors = "#CA0020",
  ...
)
```

Arguments

fsom	FlowSOM object, as generated by BuildMST
query	Array containing "high" or "low" (or abbreviations) for the specified column names of the FlowSOM data.
plot	If true, a plot with a gradient of scores for the nodes is shown.
colorPalette	Color palette to be used for colors for "stars", "pies" or "marker". Can be either a function or an array specifying colors.
backgroundColors	Color to use for nodes with a high score in the plot. Default is red.
...	Additional arguments to pass to PlotFlowSOM

Details

Identify nodes in the tree which resemble a certain profile of "high" or "low" marker expressions.

Value

A list, containing the ids of the selected nodes, the individual scores for all nodes and the scores for each marker for each node

See Also

PlotStars, PlotVariable, PlotFlowSOM, PlotLabels, PlotNumbers, PlotMarker, PlotPies, PlotSD

Examples

```
file <- system.file("extdata", "68983.fcs", package="FlowSOM")
flowSOM.res <- FlowSOM(file, compensate = TRUE, transform = TRUE,
               scale = TRUE, colsToUse = c(9, 12, 14:18), nClus = 10,
               silent = FALSE, xdim = 7, ydim = 7)
query <- c("CD3" = "high", #CD3
           "CD4" = "low", #TCRb
           "CD8" = "high") #CD8
query_res <- QueryStarPlot(flowSOM.res, query, equalNodeSize = TRUE)

cellTypes <- factor(rep("Unlabeled", 49),
                    levels = c("Unlabeled", "CD8 T cells"))
cellTypes[query_res$selected] <- "CD8 T cells"
PlotStars(flowSOM.res,
          backgroundValues = cellTypes,
          backgroundColors = c("#FFFFFF00", "#ca0020aa"))
```

`ReadInput` *Read FCS-files or flowFrames*

Description

Take some input and return FlowSOM object containing a matrix with the preprocessed data (compensated, transformed, scaled)

Usage

```
ReadInput(
  input,
  pattern = ".fcs",
  compensate = FALSE,
  spillover = NULL,
  transform = FALSE,
  toTransform = NULL,
  transformFunction = flowCore::logicleTransform(),
  transformList = NULL,
  scale = FALSE,
  toScale = NULL,
  scaled.center = TRUE,
  scaled.scale = TRUE,
  silent = FALSE,
  ...
)
```

Arguments

`input`	a flowFrame, a flowSet, a matrix with column names or an array of paths to files or directories
`pattern`	if input is an array of file- or directorynames, select only files containing pattern
`compensate`	logical, does the data need to be compensated
`spillover`	spillover matrix to compensate with If `NULL` and compensate = `TRUE`, we will look for `$SPILL` description in FCS file.
`transform`	logical, does the data need to be transformed
`toTransform`	column names or indices that need to be transformed. Will be ignored if `transformList` is given. If `NULL` and transform = `TRUE`, column names of `$SPILL` description in FCS file will be used.
`transformFunction`	Defaults to logicleTransform()
`transformList`	transformList to apply on the samples.
`scale`	logical, does the data needs to be rescaled
`toScale`	column names or indices that need to be scaled If `NULL` and scale = `TRUE`, all columns will be scaled.

scaled.center	see scale
scaled.scale	see scale
silent	if TRUE, no progress updates will be printed. Default = FALSE
...	Additional arguments for read.FCS function

Value

FlowSOM object containing the data, which can be used as input for the BuildSOM function

See Also

scale, BuildSOM

Examples

```
# Read from file
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
flowSOM.res <- ReadInput(fileName, compensate = TRUE, transform = TRUE,
                         scale = TRUE)

# Or read from flowFrame object
ff <- flowCore::read.FCS(fileName)
ff <- flowCore::compensate(ff, flowCore::keyword(ff)[["SPILL"]])
ff <- flowCore::transform(ff,
         flowCore::transformList(colnames(flowCore::keyword(ff)[["SPILL"]]),
                                 flowCore::logicleTransform()))
flowSOM.res <- ReadInput(ff, scale = TRUE)

# Build the self-organizing map and the minimal spanning tree
flowSOM.res <- BuildSOM(flowSOM.res, colsToUse = c(9, 12, 14:18))
flowSOM.res <- BuildMST(flowSOM.res)

# Apply metaclustering
metacl <- MetaClustering(flowSOM.res$map$codes,
                         "metaClustering_consensus", max = 10)

# Get metaclustering per cell
flowSOM.clustering <- metacl[flowSOM.res$map$mapping[, 1]]
```

SaveClustersToFCS *Write FlowSOM clustering results to the original FCS files*

Description

Write FlowSOM clustering results to the original FCS files

Usage

```
SaveClustersToFCS(
  fsom,
  originalFiles,
  preprocessedFiles = NULL,
  selectionColumn = NULL,
  silent = FALSE,
  outputDir = ".",
  suffix = "_FlowSOM.fcs",
  ...
)
```

Arguments

fsom	FlowSOM object as generated by BuildSOM
originalFiles	FCS files that should be extended
preprocessedFiles	FCS files that correspond to the input of FlowSOM, If NULL (default), the originalFiles are used.
selectionColumn	Column of the FCS file indicating the original cell ids. If NULL (default), no selection is made.
silent	If FALSE (default), print some extra output
outputDir	Directory to save the FCS files. Default to the current working directory (".")
suffix	Suffix added to the filename. Default _FlowSOM.fcs
...	Options to pass on to the read.FCS function (e.g. truncate_max_range)

Value

Saves the extended FCS file as [originalName]_FlowSOM.fcs

Examples

```
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
flowSOM.res <- FlowSOM(fileName, compensate = TRUE, transform = TRUE,
                       scale = TRUE, colsToUse = c(9, 12, 14:18), nClus = 10)
SaveClustersToFCS(flowSOM.res, fileName)

# Remove file again for clean example
unlink("68983_FlowSOM.fcs")
```

ScaleStarHeights *ScaleStarHeights*

Description

Scales starheights

Usage

```
ScaleStarHeights(data, nodeSizes)
```

Arguments

data	Median values of relevant markers extracted from FlowSOM object
nodeSizes	A vector that is returned from ParseNodeSize

Details

Function that scales the star values between 0 and the node size

Value

A dataframe consisting of the scaled values of the stars. The stars are scaled between 0 and the maximum of all stars

See Also

PlotFlowSOM, ParseNodeSize, AutoMaxNodeSize

SOM *Build a self-organizing map*

Description

Build a self-organizing map

Usage

```
SOM(
  data,
  xdim = 10,
  ydim = 10,
  rlen = 10,
  mst = 1,
  alpha = c(0.05, 0.01),
  radius = stats::quantile(nhbrdist, 0.67) * c(1, 0),
```

```
  init = FALSE,
  initf = Initialize_KWSP,
  distf = 2,
  silent = FALSE,
  map = TRUE,
  codes = NULL,
  importance = NULL
)
```

Arguments

data	Matrix containing the training data
xdim	Width of the grid
ydim	Hight of the grid
rlen	Number of times to loop over the training data for each MST
mst	Number of times to build an MST
alpha	Start and end learning rate
radius	Start and end radius
init	Initialize cluster centers in a non-random way
initf	Use the given initialization function if init == T (default: Initialize_KWSP)
distf	Distance function (1 = manhattan, 2 = euclidean, 3 = chebyshev, 4 = cosine)
silent	If FALSE, print status updates
map	If FALSE, data is not mapped to the SOM. Default TRUE.
codes	Cluster centers to start with
importance	array with numeric values. Parameters will be scaled according to importance

Value

A list containing all parameter settings and results

References

This code is strongly based on the kohonen package. R. Wehrens and L.M.C. Buydens, Self- and Super-organising Maps in R: the kohonen package J. Stat. Softw., 21(5), 2007

See Also

BuildSOM

TestOutliers *TestOutliers*

Description

Test if any cells are too far from their cluster centers

Usage

```
TestOutliers(
  fsom,
  madAllowed = 4,
  fsomReference = NULL,
  plotFile = NULL,
  channels = NULL
)
```

Arguments

fsom	FlowSOM object
madAllowed	Number of median absolute deviations allowed. Default = 4.
fsomReference	FlowSOM object to use as reference. If NULL (default), the original fsom object is used.
plotFile	If NULL (default), no plot will be created. If a filepath is given for a pdf, the plot will be written in the corresponding file
channels	If channels are given, the number of outliers in the original space for those channels will be calculated and added to the final results table.

Details

For every cluster, the distance from the cells to the cluster centers is used to label cells which deviate too far as outliers. The threshold is chosen as the median distance + madAllowed times the median absolute deviation of the distances.

Value

An outlier report

See Also

FlowSOMSubset if you want to get a subset of the current data instead of a new dataset

Examples

```
# Build FlowSom result
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
ff <- flowCore::read.FCS(fileName)
flowSOM.res <- FlowSOM(ff,
                       compensate = TRUE, transform = TRUE, scale = TRUE,
                       colsToUse = c(9, 12, 14:18),
                       nClus = 10)

# Map new data
outlier_report <- TestOutliers(flowSOM.res,
                               madAllowed = 5,
                               channels = flowSOM.res$map$colsUsed)

# Number of cells which is an outlier for x channels
outlier_on_multiple_markers <- table(rowSums(outlier_report$perCell != 0))
outlier_type <- paste(GetClusters(flowSOM.res),
                      apply(outlier_report$perCell, 1, paste0, collapse = ""))
outlier_counts <- table(grep(" .*1.*", outlier_type, value = TRUE))
outliers_of_interest <- names(which(outlier_counts > 10))
outlier_boolean <- outlier_type %in% outliers_of_interest
```

`UpdateFlowSOM` *UpdateFlowSOM*

Description

Update old FlowSOM object to a new one and checks if it is a flowSOM object

Usage

```
UpdateFlowSOM(fsom)
```

Arguments

`fsom`	FlowSOM object, as generated by `BuildMST` or `FlowSOM`

Details

Determines whether or not the fsom input is of class "FlowSOM" and returns the FlowSOM object and metaclustering object inside fsom

Value

A FlowSOM object

See Also

`PlotFlowSOM`

UpdateMetaclusters *UpdateMetaclusters*

Description

Adapt the metacluster levels. Can be used to rename the metaclusters, split or merge existing metaclusters, add a metaclustering and/or reorder the levels of the metaclustering.

Usage

```
UpdateMetaclusters(
  fsom,
  newLabels = NULL,
  clusterAssignment = NULL,
  levelOrder = NULL
)
```

Arguments

fsom	Result of calling the FlowSOM function.
newLabels	Optional. Named vector, with the names the original metacluster names and the values the replacement. Can be used to rename or merge metaclusters.
clusterAssignment	Optional. Either a named vector, with the names the cluster numbers (characters) or a vector of length NClusters(fsom). Can be used to assign clusters to existing or new metaclusters.
levelOrder	Optional. Vector showing the preferred order of the fsom metacluster levels.

Value

Updated FlowSOM object

Examples

```
fileName <- system.file("extdata", "68983.fcs", package = "FlowSOM")
ff <- flowCore::read.FCS(fileName)
ff <- flowCore::compensate(ff, flowCore::keyword(ff)[["SPILL"]])
ff <- flowCore::transform(ff,
         flowCore::transformList(colnames(flowCore::keyword(ff)[["SPILL"]]),
                                 flowCore::logicleTransform()))
flowSOM.res <- FlowSOM(ff,
                       scale = TRUE,
                       colsToUse = c(9, 12, 14:18),
                       nClus = 10,
                       seed = 1)

PlotStars(flowSOM.res, backgroundValues = flowSOM.res$metaclustering)
GetCounts(flowSOM.res)
```

```
# Merge MC8 and MC9
flowSOM.res <- UpdateMetaclusters(flowSOM.res, newLabels = c("8" = "8+9",
                                                             "9" = "8+9"))
PlotStars(flowSOM.res, backgroundValues = flowSOM.res$metaclustering)
GetCounts(flowSOM.res)

# Split cluster 24 from metacluster 2 and order the metacluster levels
flowSOM.res <- UpdateMetaclusters(flowSOM.res,
                                  clusterAssignment = c("24" = "debris?"),
                                  levelOrder = c("debris?", as.character(c(1:7)),
                                                 "8+9", "10"))
PlotStars(flowSOM.res, backgroundValues = flowSOM.res$metaclustering)
PlotNumbers(flowSOM.res, level = "metaclusters")

GetCounts(flowSOM.res)
```

`UpdateNodeSize` *UpdateNodeSize*

Description

Update nodesize of FlowSOM object

Usage

```
UpdateNodeSize(
  fsom,
  count = NULL,
  reset = FALSE,
  transform = sqrt,
  maxNodeSize = 15,
  shift = 0,
  scale = NULL
)
```

Arguments

`fsom`	FlowSOM object, as generated by `BuildMST`
`count`	Absolute cell count of the sample
`reset`	Logical. If `TRUE`, all nodes get the same size
`transform`	Transformation function. Use e.g. square root to let counts correspond with area of node instead of radius
`maxNodeSize`	Maximum node size after rescaling. Default: 15
`shift`	Shift of the counts, defaults to 0
`scale`	Scaling of the counts, defaults to the maximum of the value minus the shift. With shift and scale set as default, the largest node will be maxNodeSize and an empty node will have size 0

Details

Add size property to the graph based on cellcount for each node

Value

Updated FlowSOM object

See Also

BuildMST

Examples

```
# Read from file, build self-organizing map and minimal spanning tree
fileName <- system.file("extdata", "68983.fcs", package="FlowSOM")
flowSOM.res <- ReadInput(fileName, compensate=TRUE,transform=TRUE,
                         scale=TRUE)
flowSOM.res <- BuildSOM(flowSOM.res,colsToUse=c(9,12,14:18))
flowSOM.res <- BuildMST(flowSOM.res)

# Give all nodes same size
PlotStars(flowSOM.res, equalNodeSize = TRUE)

# Node sizes relative to amount of cells assigned to the node
PlotStars(flowSOM.res)
```

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