Package ‘FlowSOM’
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**Description**

Add annotation to a FlowSOM plot

**Usage**

```r
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**

```r
# 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

Description
Function plots the background

Usage
AddBackground(
  p, 
  backgroundValues, 
  backgroundColors = NULL, 
  backgroundLim = NULL
)

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.

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",
  ...
)
AddMST

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

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
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

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

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

Description

Function plots the stars

Usage

```r
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 = "fsom$map$colsUsed"
- **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

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 \( c_{\text{Total}} \) cells, with \( \text{ceiling}(c_{\text{Total}}/n_{\text{Files}}) \) 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

celling

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**

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**

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
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

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)
CountGroups

References


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
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**

```r
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
define.clustering <- GetMetaclusters(define.res)

## Description

This function 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

```r
FlowSOMmary(define, plotFile = "FlowSOMmary.pdf")
```

## Arguments

- `define`: FlowSOM object, as generated by `defineSOM`.
- `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

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

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

defineSOMmary(define.res)
```
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]])
**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

---

**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
GetChannels

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)

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

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

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

```r
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)

perc_pos <- GetClusterPercentagesPositive(flowSOM.res, cutoffs = c('CD4' = 5000))
```

Description

Get percentage-positive values for all clusters

Usage

```r
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

```r
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", "68903.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

Get counts of number of cells in clusters or metaclusters

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.
GetCVs

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)
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.

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,
GetFlowJoLabels

    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$flowFrame,
                       colsToUse = c(9, 12, 14:18),
                       nClus = 10,
# Plot pies indicating the percentage of cell types present in the nodes
PlotPies(flowSOM.res,
gatingResult$manual,
backgroundValues = flowSOM.res$metaclustering)

---

### GetMarkers

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**

```r
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**

```r
# 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

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

Description
Compute the median fluorescence intensities for the metaclusters

Usage
GetMetaclusterMFIs(fsom, colsUsed = FALSE, prettyColnames = FALSE)
GetMetaclusterPercentagesPositive

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
)
GetMetaclusters

Get metacluster label for all individual cells

### Description

Get metacluster label for all individual cells

### Usage

```r
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$somclustering will be used.

### Value

- vector label for every cell
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)
metacluster_labels <- GetMetaclusters(flowSOM.res)
metacluster_labels <- GetMetaclusters(flowSOM.res,
  meta = flowSOM.res$metaclustering)
mfis <- GetClusterMFIs(flowSOM.res)
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")

g_channels

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

Usage
g_channels(ff, markers)
get_markers

Arguments

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

Value

Corresponding marker names

See Also

get_channels

Examples

```r
# 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"))
```
Examples

```r
# 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"))
```

---

**Description**

Helper function to get the ggplot colors

**Usage**

```r
gg_color_hue(n)
```

**Arguments**

- `n` Number of colors

**Value**

array with hexadecimal color values

---

**GroupStats**

Calculate statistics between 2 groups based on the `GetFeatures` output

**Usage**

```r
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::logicileTransform())
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("Groups_foldchanges.pdf", p, width = 10)

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

---

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

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,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), nClus=10)
cvs <- GetMetaclusterCVs(flowSOM.res)

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

```r
# 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]]
```

Description

Cluster data using hierarchical consensus clustering with k clusters

Usage

```r
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
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")
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)

mfis <- GetMetaclusterMFIs(flowSOM.res)
**NClusters**

**Description**

Extracts the number of clusters from a FlowSOM object

**Usage**

\[
\text{NClusters}(\text{fsom})
\]

**Arguments**

- \text{fsom} FlowSOM object

**Value**

The number of clusters

**Examples**

```r
# 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**

**Description**

Map new data to a FlowSOM grid

**Usage**

\[
\text{NewData}(\text{fsom}, \text{input}, \text{madAllowed} = 4, \text{compensate} = \text{NULL}, \text{spillover} = \text{NULL}, \text{transform} = \text{NULL})
\]
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
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
NMetaclusters

See Also

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

Examples

```r
# 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, ])
```

<table>
<thead>
<tr>
<th>NMetaclusters</th>
<th>NMetaclusters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Description

Extracts the number of metaclusters from a FlowSOM object

Usage

NMetaclusters(fsom)

Arguments

fsom FlowSOM object

Value

The number of metaclusters

Examples

```r
# 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

**Description**

Parses stars

**Usage**

```r
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

**Description**

Parses edges

**Usage**

```r
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

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

Description

Parses node size

Usage

ParseNodeSize(nodeSizes, maxSize, 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*

---

**Description**

Parses query

**Usage**

```r
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
**ParseSD**

*ParseSD Parses SD in FlowSOM object*

---

**Description**

Calculates the standard deviation of a FlowSOM object

**Usage**

```r
calculateSD(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, QueryStarPlot, ParseSD`

---

**Plot2DScatters**

*Plot2DScatters*

---

**Description**

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

**Usage**

```r
plot2DScatters(
  fsom,
  channelpairs,
  clusters = NULL,
  metaclusters = NULL,
  maxBgPoints = 3000,
  sizeBgPoints = 0.5,
  maxPoints = 1000,
)```

**See Also**

`PlotFlowSOM, ParseEdges, ParseNodeSize, ParseArcs, ParseQuery, PlotSD`
sizePoints = 0.5,
xLim = NULL,
yLim = NULL,
xyLabels = c("marker"),
density = TRUE,
centers = TRUE,
colors = NULL,
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 indices of metaclusters of interest
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 abbreviations. 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.
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)

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")))

Description

Plot cluster centers on a 2D plot

Usage

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

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>fsom</td>
<td>FlowSOM object, as generated by <code>BuildMST</code></td>
</tr>
<tr>
<td>marker1</td>
<td>Marker to show on the x-axis</td>
</tr>
<tr>
<td>marker2</td>
<td>Marker to show on the y-axis</td>
</tr>
<tr>
<td>MST</td>
<td>Type of visualization, if 1 plot tree, else plot grid</td>
</tr>
</tbody>
</table>
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

```r
# 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)
```

Description

Plot nodes on scatter plot

Usage

PlotClusters2D(
  fsom,
  marker1,
  marker2,
  nodes,
  col = "#FF0000",
  maxBgPoints = 10000,
  pchBackground = ".",
  pchCluster = ".",
  main = "",
  xlab = fsom$prettyColnames[marker1],
)
y.lab = fsom$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)
PlotDimRed <- BuildSOM(flowSOM.res, colsToUse = c(9, 12, 14:18))
flowSOM.res <- BuildMST(flowSOM.res)

# Plot cells
## Not run:
Plot2DScatters(flowSOM.res, c(1, 2), clusters = 91)
## End(Not run)

---

**PlotDimRed**

**Usage**

PlotDimRed(
  fsom,
  colsToUse = fsom$max$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 = `fsom$max$colsUsed`.
- **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

**Description**

Plot a dimensionality reduction
The total amount of cells to be used in the dimensionality reduction. Default is all the cells.

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).

A function to extract the coordinates from the results of the dimred default = function(dimred)dimred$Y.

If label = TRUE (default), labels are added to plot.

If TRUE, this function returns a dataframe with the layout of dimred and the original IDs and the plot. Default = FALSE.

A seed for reproducibility.

A title for the plot.

Additional arguments to pass to dimred.

Plot a dimensionality reduction of fsom$data

A dimensionality reduction plot made in ggplot2

Examples

```r
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")
```

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. If 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
### Description

Base layer to plot a FlowSOM result

### Examples

```r
# 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
  channels = c("Pacific Blue-A",
    "Alexa Fluor 700-A",
    "PE-Cy7-A"),
  maxPoints = 1000)
```

### Value

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

`PlotFlowSOM(
  fsom,
  view = "MST",
  nodeSizes = fsom$map$pctgs,
  maxNodeSize = 1,
  refNodeSize = max(nodeSizes),
  equalNodeSize = FALSE,
  backgroundValues = NULL,
  backgroundColors = NULL,
  backgroundLim = NULL,
  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 = fsom$MST$sizes
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.
backgroundLim Only used when backgroundValues are numerical. Defaults to min and max of the backgroundValues.
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))

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

---

**PlotLabels**

**Description**

Plot labels for each cluster

**Usage**

```r
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

```r
# 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)
```

**Description**

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

**Usage**

```r
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("PlotManualBars.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()
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 \texttt{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

\texttt{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")
PlotNode

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
PlotNumbers

- `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**

```r
# 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**

**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.
PlotOutliers

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)

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)

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) color-brewer "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 marker pair 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

```r
## 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("FSC-A", "SSC-A"),
    c("CD3", "CD19"),
    c("TCRb", "TCRyd"),
    c("CD4", "CD8"))
metaclusters_of_interest = 1:10

# Recommended to write to png

## Not run:
png("Markeroverview.png",
    width = 500 * length(markers_of_interest),
    height = 500 * length(metaclusters_of_interest))
Plot2DScatters(flowSOM.res,
    channelpairs = markers_of_interest,
    metaclusters = metaclusters_of_interest)
dev.off()

## End(Not run)
```

Description

Plot comparison with other clustering
PlotPies

Usage

PlotPies(
  fsom,
  cellTypes,
  colorPalette = grDevices::colorRampPalette(c("white", "#00007F", "blue", "#007FFF",
                                              "cyan", "#7FFF7F", "yellow", "#F7F000", "red", "#7F0000")),
  ...     
)

Arguments

fsom        FlowSOM object, as generated by FlowSOM

cellTypes   Array of factors indicating the cell types

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**

**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)
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))
PlotStar

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.
...

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)

ewLayout <- 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

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.
lm 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
print.FlowSOM

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**

```r
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**

```r
# 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)
```

---

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**

```r
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, "query_multiple.pdf")
```

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.

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

```r
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, 
  backgroundColors = cellTypes, 
  backgroundColors = c("#FFFFFF00", "#ca0020aa"))
```
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)
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")
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,
  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
scaled.center see scale
scaled.scale see scale
silent if TRUE, no progress updates will be printed. Default = FALSE
Description

Write FlowSOM clustering results to the original FCS files

Usage

```
SaveClustersToFCS(
  fsom,  # FlowSOM object containing the data, which can be used as input for the BuildSOM function
  originalFiles,
  preprocessedFiles = NULL,
  selectionColumn = NULL,
  silent = FALSE,
)```

Value

`Value` contains information on the parameters and behavior of the `SaveClustersToFCS` function. It is used to ensure that the necessary data is correctly managed and processed. The `Value` section can be further explained as follows:

- **Value**: This parameter specifies the value to be used in the function. It is often used to control the output or behavior of the function. In the context of `SaveClustersToFCS`, it might refer to the data or settings that need to be saved to the original FCS files.

See Also

`scale`, `BuildSOM`

Examples

```r
# 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]]
```
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

```r
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)
```

---

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)
**SOM**

Build a self-organizing map

**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**

```r
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` Height 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


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
)
TestOutliers

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>fsom</td>
<td>FlowSOM object</td>
</tr>
<tr>
<td>madAllowed</td>
<td>Number of median absolute deviations allowed. Default = 4.</td>
</tr>
<tr>
<td>fsomReference</td>
<td>FlowSOM object to use as reference. If NULL (default), the original fsom object is used.</td>
</tr>
<tr>
<td>plotFile</td>
<td>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</td>
</tr>
<tr>
<td>channels</td>
<td>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.</td>
</tr>
</tbody>
</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

```r
# 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$channel_specific != 0))
outlier_type <- paste(GetClusters(flowSOM.res), 
 apply(outlier_report$channel_specific, 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**

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

**Usage**
```r
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**

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

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?", "8+9"), 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

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)

---

Pipe operator

Description

See magrittr::%>% for details.

Usage

lhs %>% rhs
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