

Package ‘FlowSOM’

April 15, 2020

Version 1.18.0

Date 2019-01-18

Title Using self-organizing maps for visualization and interpretation of cytometry data

Depends R (>= 3.6), igraph

Imports stats, utils, flowCore, ConsensusClusterPlus, BiocGenerics, tsne, flowWorkspace, CytoML, XML, RColorBrewer

Suggests BiocStyle

Description FlowSOM offers visualization options for cytometry data, by using Self-Organizing Map clustering and Minimal Spanning Trees.

License GPL (>= 2)

LazyData true

URL <http://www.r-project.org>, <http://dambi.ugent.be>

biocViews CellBiology, FlowCytometry, Clustering, Visualization, Software, CellBasedAssays

RoxygenNote 6.1.1

git_url <https://git.bioconductor.org/packages/FlowSOM>

git_branch RELEASE_3_10

git_last_commit 850e5b7

git_last_commit_date 2019-10-29

Date/Publication 2020-04-14

Author Sofie Van Gassen [aut, cre],
Britt Callebaut [aut],
Yvan Saeys [aut]

Maintainer Sofie Van Gassen <sofie.vangassen@ugent.be>

R topics documented:

AddFlowFrame	2
AggregateFlowFrames	3
BuildMST	4
BuildSOM	5
computeBackgroundColor	6

CountGroups	6
Dist.MST	7
FlowSOM	8
FlowSOMSubset	9
FMeasure	10
GetClusters	11
GetCVs	11
GetFlowJoLabels	12
GetMetaclusters	13
GetMFIs	14
get_channels	14
get_markers	15
Initialize_KWSP	16
Initialize_PCA	16
MapDataToCodes	17
MetaclusterCVs	17
MetaClustering	18
metaClustering_consensus	19
MetaclusterMFIs	19
NewData	20
PlotCenters	21
PlotClusters2D	22
PlotGroups	23
PlotLabels	24
PlotMarker	25
PlotNode	27
PlotNumbers	28
PlotOverview2D	29
PlotPies	30
PlotSD	31
plotStarLegend	33
PlotStars	33
PlotVariable	35
Purity	36
QueryStarPlot	37
ReadInput	38
SaveClustersToFCS	39
SOM	40
TestOutliers	41
UpdateNodeSize	42
Index	43

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](#)

AggregateFlowFrames *Aggregate multiple fcs files together*

Description

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

Usage

```
AggregateFlowFrames(fileNames, cTotal, writeOutput = FALSE,
  outputFile = "aggregate.fcs", writeMeta = FALSE, keepOrder = FALSE,
  verbose = FALSE)
```

Arguments

fileNames	Character vector containing full paths to the fcs files to aggregate
cTotal	Total number of cells to write to the output file
writeOutput	Whether to write the resulting flowframe to a file
outputFile	Full path to output file
writeMeta	If TRUE, files with the indices of the selected cells are generated
keepOrder	If TRUE, the random subsample will be ordered in the same way as they were originally ordered in the file. Default = FALSE.
verbose	If TRUE, prints an update every time it starts processing a new file. Default = FALSE.

Value

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

See Also[ceiling](#)**Examples**

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

BuildMST*Build Minimal Spanning Tree*

Description

Add minimal spanning tree description to the FlowSOM object

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 tSNE layout is computed as well

Value

FlowSOM object containing MST description

See Also[BuildSOM](#), [PlotStars](#)**Examples**

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

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

```
computeBackgroundColor
```

Internal function for computing background nodes

Description

Internal function for computing background nodes

Usage

```
computeBackgroundColor(backgroundValues, backgroundColor,
  backgroundLim = NULL, backgroundBreaks = NULL)
```

Arguments

backgroundValues

Values to be used for background coloring, either numerical values or something that can be made into a factor (e.g. a clustering)

backgroundColor

Colorpalette 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.

backgroundBreaks

Breaks to pass on to `cut`, to split numerical background values. If NULL, the length of backgroundColor will be used (default 100).

```
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

Examples

```

set.seed(1)

# Build the FlowSOM tree on the example 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)

# Have a look at the resulting tree
PlotStars(flowSOM.res[[1]], backgroundValues = as.factor(flowSOM.res[[2]]))

# Select all cells except the branch that corresponds with automated
# cluster 7 (CD3+ TCRyd +) and write to another file for the example
# In practice you would not generate any new file but use your different
# files from your different groups
ff <- flowCore::read.FCS(fileName)
ff_tmp <- ff[flowSOM.res[[1]]$map$mapping[,1] %in%
          which(flowSOM.res[[2]] != 7),]
flowCore::write.FCS(ff_tmp, file="ff_tmp.fcs")
# Make an extra file without cluster 7 and double amount of cluster 10
ff_tmp <- ff[c(which(flowSOM.res[[1]]$map$mapping[,1] %in%
                    which(flowSOM.res[[2]] != 7)),
              which(flowSOM.res[[1]]$map$mapping[,1] %in%
                    which(flowSOM.res[[2]] == 5))),]
flowCore::write.FCS(ff_tmp, file="ff_tmp2.fcs")

# Compare the original file with the two new files we made
groupRes <- CountGroups(flowSOM.res[[1]],
                       groups=list("AllCells"=c(fileName),
                                   "Without_ydTcells"=c("ff_tmp.fcs", "ff_tmp2.fcs")))
PlotGroups(flowSOM.res[[1]], groupRes)

# Compare only the file with the double amount of cluster 10
groupRes <- CountGroups(flowSOM.res[[1]],
                       groups=list("AllCells"=c(fileName),
                                   "Without_ydTcells"=c("ff_tmp2.fcs")))
PlotGroups(flowSOM.res[[1]], groupRes)

```

Dist.MST

*Calculate distance matrix using a minimal spanning tree neighbourhood***Description**

Calculate distance matrix using a minimal spanning tree neighbourhood

Usage

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

Arguments

X matrix in which each row represents a point

Value

Distance matrix

FlowSOM

*Run the FlowSOM algorithm***Description**

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

Usage

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

Arguments

input	a flowFrame, a flowSet 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 a logicle transform
toTransform	column names or indices that need to be transformed. If NULL and transform = TRUE, column names of \$SPILL description in fcs file will be used.
transformFunction	Defaults to logicleTransform()
scale	logical, does the data need to be rescaled
scaled.center	see scale
scaled.scale	see scale
silent	if TRUE, no progress updates will be printed
colsToUse	column names or indices to use for building the SOM
nClus	Exact number of clusters for meta-clustering. If NULL, several options will be tried (1:maxMeta)
maxMeta	Maximum number of clusters to try out for meta-clustering. Ignored if nClus is specified
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, 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)

# Plot results
PlotStars(flowSOM.res[[1]])

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

FlowSOMSubset

FlowSOM subset

Description

Take a subset from a FlowSOM object

Usage

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

Arguments

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

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,]
ff1@description$FIL <- "File1"
ff2 <- flowCore::read.FCS(fileName)[1001:2000,]
ff2@description$FIL <- "File2"

flowSOM.res <- FlowSOM(flowCore::flowSet(c(ff1,ff2)), compensate=TRUE,
                      transform=TRUE, scale=TRUE,
                      colsToUse=c(9,12,14:18), maxMeta=10)
fSOM <- flowSOM.res[[1]]

# see $metadata for subsets:
fSOM$metaData

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

FMeasure

*F measure***Description**

Compute the F measure between two clustering results

Usage

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

Arguments

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

Value

F measure score

Examples

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

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

GetClusters

*Get cluster label for all individual cells***Description**

Get cluster label for all individual cells

Usage

```
GetClusters(fsom)
```

Arguments

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

Value

vector label for every cell

Examples

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

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 <- GetCVs(flowSOM.res)
cvs <- GetCVs(flowSOM.res$FlowSOM)
```

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, wsp_file, group = "All Samples",
cell_types = NULL)
```

Arguments

files	The fcs files of interest
wsp_file	The FlowJo wsp file to read
group	The FlowJo group to parse. Default "All Samples".
cell_types	Cell types to use for final labeling the cells. Should correspond with a subset of the gate names in FlowJo.

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")
wsp_file <- system.file("extdata", "gating.wsp", package = "FlowSOM")

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

# Parse the FlowJo workspace
```

GetMFIs	<i>Get MFI values for all clusters</i>
---------	--

Description

Get MFI values for all clusters

Usage

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

Arguments

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

Value

Matrix with median values for each marker

Examples

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

get_channels	<i>get_channels</i>
--------------	---------------------

Description

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

Usage

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

Arguments

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

Value

Corresponding channel names

See Also

[get_markers](#)

Examples

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

get_markers

get_markers

Description

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

Usage

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

Arguments

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

Value

Corresponding marker names

See Also

[get_channels](#)

Examples

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

Initialize_KWSP	<i>Select k well spread points from X</i>
-----------------	---

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	<i>Create a grid from first 2 PCA components</i>
----------------	--

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)
```

MapDataToCodes	<i>Assign nearest node to each datapoint</i>
----------------	--

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	<i>MetaclusterCVs</i>
----------------	-----------------------

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 <- MetaclusterCVs(flowSOM.res)

```

 MetaClustering

MetaClustering

Description

Cluster data with automatic number of cluster determination for several algorithms

Usage

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

Arguments

data	Matrix containing the data to cluster
method	Clustering method to use
max	Maximum number of clusters to try out
...	Extra parameters to pass along

Value

Numeric array indicating cluster for each datapoint

See Also

[metaClustering_consensus](#)

Examples

```

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

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

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

```

metaClustering_consensus
MetaClustering

Description

Cluster data using hierarchical consensus clustering with k clusters

Usage

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

Arguments

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

Value

Numeric array indicating cluster for each datapoint

See Also

[MetaClustering](#)

Examples

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

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

MetaclusterMFIs *MetaclusterMFIs*

Description

Compute the median fluorescence intensities for the metaclusters

Usage

```
MetaclusterMFIs(fsom)
```

Arguments

fson Result of calling the FlowSOM function

Value

Metacluster MFIs

Examples

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

NewData

Map new data to a FlowSOM grid

Description

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.

Usage

```
NewData(fson, input, mad_allowed = 4, compensate = NULL,
  spillover = NULL, transform = NULL, toTransform = NULL,
  transformFunction = NULL, scale = NULL, scaled.center = NULL,
  scaled.scale = NULL)
```

Arguments

fson FlowSOM object

input A flowFrame, a flowSet or an array of paths to files or directories

mad_allowed 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 + mad_allowed * MAD. Default is 4.

compensate logical, does the data need to be compensated. If NULL, the same value as in the original FlowSOM call will be used.

spillover spillover matrix to compensate with. If NULL, the same value as in the original FlowSOM call will be used.

transform logical, does the data need to be transformed. If NULL, the same value as in the original FlowSOM call will be used.

toTransform	column names or indices that need to be transformed. If NULL, the same value as in the original FlowSOM call will be used.
transformFunction	If NULL, the same value as in the original FlowSOM call will be used.
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.

Value

A new FlowSOM object

See Also

[FlowSOMSubset](#) if you want to get a subset of the current data instead of a new dataset

Examples

```
# Build FlowSom result
fileName <- system.file("extdata", "68983.fcs", package="FlowSOM")
ff <- flowCore::read.FCS(fileName)
ff <- flowCore::compensate(ff, ff@description$SPILL)
ff <- flowCore::transform(ff,
  flowCore::transformList(colnames(ff@description$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,])
```

 PlotCenters

Plot cluster centers on a 2D plot

Description

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

Usage

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

Arguments

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

Value

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

See Also

[PlotStars](#), [PlotPies](#), [PlotMarker](#), [BuildMST](#)

Examples

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

# Plot centers
PlotCenters(flowSOM.res, "FSC-A", "SSC-A")
PlotCenters(flowSOM.res, 2, 5)
```

PlotClusters2D

Plot nodes on scatter plot

Description

Plot a 2D scatter plot. All cells of `fson$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.

Usage

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

Arguments

<code>fsom</code>	FlowSOM object, as generated by BuildMST
<code>marker1</code>	Marker to plot on the x-axis
<code>marker2</code>	Marker to plot on the y-axis
<code>nodes</code>	Nodes of which the cells should be plotted in red
<code>col</code>	Colors for all the cells in the selected nodes (ordered array)
<code>maxBgPoints</code>	Maximum number of background points to plot
<code>pchBackground</code>	Character to use for background cells
<code>pchCluster</code>	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

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

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

# Plot cells
PlotClusters2D(flowSOM.res, 1, 2, 91)
```

PlotGroups

Plot differences between groups

Description

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

Usage

```
PlotGroups(fsom, groups, tresh = NULL, p_tresh = 0.05,
           heatmap = FALSE, ...)
```

Arguments

fsom	FlowSOM object, as generated by BuildMST or the first list item of FlowSOM
groups	groups result as generated by CountGroups
tresh	Relative difference in groups before the node is coloured
p_tresh	Threshold on p-value from wilcox-test before the node is coloured. If this is not NULL, tresh will be ignored.
heatmap	If TRUE, the scores are plotted in a gradient instead of only the selection that passes the threshold
...	Other parameters to pass to PlotStars

Value

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

See Also

[PlotStars](#), [CountGroups](#)

Examples

```
## Use the wrapper function to build a flowSOM object (saved in fsom[[1]])
## and a metaclustering (saved in fsom[[2]])
# fsom <- FlowSOM(ff,compensate = FALSE, transform = FALSE,scale = TRUE,
#               colsToUse = colsToUse, nClus = 10, silent = FALSE,
#               xdim=7, ydim=7)

## Make a list with for each group a list of files
## The reference group should be the first
# groups <- list("C"=file.path(workDir,grep("C",files,value = TRUE)),
#               "D"=file.path(workDir,grep("D",files,value=TRUE)))

## Compute the percentages for all groups
# groups_res <- CountGroups(fsom[[1]],groups)

## Plot the groups. For all groups except the first, differences with the
## first group are indicated
# annotation <- PlotGroups(fsom[[1]],groups_res)
```

PlotLabels

Plot a label in each node

Description

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

Usage

```
PlotLabels(fsom, labels, view = "MST", main = NULL,
           nodeSize = fsom$MST$size, fontSize = 1, backgroundValues = NULL,
           backgroundColor = function(n) { grDevices::rainbow(n, alpha = 0.3)
           }, backgroundLim = NULL, backgroundBreaks = NULL)
```

Arguments

fsom	FlowSOM object, as generated by BuildMST
labels	A label for every node
view	Preferred view, options: "MST", "grid" or "tSNE" (if this option was selected while building the MST)
main	Title of the plot
nodeSize	Nodesize. The plot might be easier to read if this is a constant number, e.g. 10 or 15

fontSize	Fontsize, passed to label.cex
backgroundValues	Values to be used for background coloring, either numerical values or something that can be made into a factor (e.g. a clustering)
backgroundColor	Colorpalette 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.
backgroundBreaks	Breaks to pass on to <code>cut</code> , to split numerical background values. If NULL, the length of backgroundColor will be used (default 100).

Value

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

See Also

[PlotNumbers](#)

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, ff@description$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
PlotLabels( flowSOM.res$FlowSOM, flowSOM.res$metaclustering, nodeSize=15)
```

PlotMarker

Plot marker values

Description

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

Usage

```
PlotMarker(fsom, marker = NULL, view = "MST", main = NULL,
           colorPalette = grDevices::colorRampPalette(c("#00007F", "blue",
           "#007FFF", "cyan", "#7FFF7F", "yellow", "#FF7F00", "red", "#7F0000")),
           backgroundValues = NULL, backgroundColor = function(n) {
           grDevices::rainbow(n, alpha = 0.3) }, backgroundBreaks = NULL,
           backgroundLim = NULL)
```

Arguments

fsom	FlowSOM object, as generated by BuildMST
marker	Name or index of marker to plot
view	Preferred view, options: "MST" (default), "grid" or "tSNE" (if this option was selected while building the MST)
main	Title of the plot
colorPalette	Color palette to use
backgroundValues	Values to be used for background coloring, either numerical values or something that can be made into a factor (e.g. a clustering)
backgroundColor	Colorpalette to be used for the background coloring . Can be either a function or an array specifying colors
backgroundBreaks	Breaks to pass on to cut , to split numerical background values. If NULL, the length of backgroundColor will be used (default 100).
backgroundLim	Only used when backgroundValues are numerical. Defaults to min and max of the backgroundValues.

Value

Nothing is returned. A plot is drawn in which each node is coloured depending on its median value for the given marker

References

This visualization technique resembles SPADE results. M. Linderman, P. Qiu, E. Simonds and Z. Bjornson (). [spade](#): SPADE – An analysis and visualization tool for Flow Cytometry. R package version 1.12.2. <http://cytospade.org>

See Also

[PlotStars](#), [PlotPies](#), [PlotCenters](#), [BuildMST](#)

Examples

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

# Plot one marker
PlotMarker(flowSOM.res, "FSC-A")
```

PlotNode	<i>Plot star chart</i>
----------	------------------------

Description

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

Usage

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

Arguments

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

Value

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

See Also

[PlotStars](#), [PlotNumbers](#), [FlowSOM](#)

Examples

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

# Plot stars indicating the MFI of the cells present in the nodes
PlotNode(flowSOM.res$FlowSOM,1)
```

PlotNumbers

*Plot the index of each node***Description**

Plot FlowSOM grid or tree, with in each node a number indicating its index

Usage

```
PlotNumbers(fsom, view = "MST", main = NULL,
            nodeSize = fsom$MST$size, fontSize = 1, backgroundValues = NULL,
            backgroundColor = function(n) { grDevices::rainbow(n, alpha = 0.3)
            }, backgroundLim = NULL, backgroundBreaks = NULL)
```

Arguments

fsom	FlowSOM object, as generated by BuildMST
view	Preferred view, options: "MST", "grid" or "tSNE" (if this option was selected while building the MST)
main	Title of the plot
nodeSize	Nodesize. The plot might be easier to read if this is a constant number, e.g. 10 or 15
fontSize	Fontsize, passed to label.cex
backgroundValues	Values to be used for background coloring, either numerical values or something that can be made into a factor (e.g. a clustering)
backgroundColor	Colorpalette 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.
backgroundBreaks	Breaks to pass on to cut , to split numerical background values. If NULL, the length of backgroundColor will be used (default 100).

Value

Nothing is returned. A plot is drawn in which each node is assigned a number

See Also

[PlotMarker](#), [PlotStars](#), [PlotPies](#), [PlotCenters](#), [BuildMST](#)

Examples

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

```

flowSOM.res <- BuildMST(flowSOM.res)

# Plot the node IDs
PlotNumbers(flowSOM.res)

# Adapt node size for easier readability
PlotNumbers(flowSOM.res, nodeSize=14)

```

PlotOverview2D

Plot metaclusters on scatter plots

Description

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

Usage

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

Arguments

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

Value

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

See Also

[PlotClusters2D](#)

Examples

```

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

```

```

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

# Recommended to write to png

png("Markeroverview.png",
    width = 500 * length(markers_of_interest),
    height = 500 * length(metaclusters_of_interest))
PlotOverview2D(flowSOM.res,
               markerlist = markers_of_interest,
               metaclusters = metaclusters_of_interest,
               pchCluster = 19,
               ff = flowCore::read.FCS(fileName))

dev.off()

```

PlotPies

Plot comparison with other clustering

Description

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

Usage

```

PlotPies(fsom, cellTypes, view = "MST",
         colorPalette = grDevices::colorRampPalette(c("white", "#00007F",
             "blue", "#007FFF", "cyan", "#7FFF7F", "yellow", "#FF7F00", "red")),
         backgroundValues = NULL, backgroundColor = function(n) {
             grDevices::rainbow(n, alpha = 0.3) }, backgroundLim = NULL,
         backgroundBreaks = NULL, legend = TRUE, main = "")

```

Arguments

fsom	FlowSOM object, as generated by BuildMST
cellTypes	Array of factors indicating the celltypes
view	Preferred view, options: "MST", "grid" or "tSNE" (if this option was selected while building the MST)
colorPalette	Colorpalette to be used for the markers
backgroundValues	Values to be used for background coloring, either numerical values or something that can be made into a factor (e.g. a clustering)
backgroundColor	Colorpalette 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.

backgroundBreaks	Breaks to pass on to <code>cut</code> , to split numerical background values. If NULL, the length of <code>backgroundColor</code> will be used (default 100).
legend	Logicle, if T add a legend
main	Title of the plot

Value

Nothing is returned. A plot is drawn in which each node is represented by a pie chart indicating the percentage of cells present of each cell type. At the end, the layout is set to 1 figure again.

See Also

[PlotStars](#), [PlotMarker](#), [PlotCenters](#), [BuildMST](#)

Examples

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

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

# Parse the FlowJo workspace
library(flowWorkspace)
gatingResult <- GetFlowJoLabels(fcs_file, wsp_file,
                               cell_types = cell_types)

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

# Build a FlowSOM tree
flowSOM.res <- FlowSOM(fcs_file,
                      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$FlowSOM,
         gatingResult$manual,
         backgroundValues = flowSOM.res$metaclustering)
```

Description

— Function in development, use with caution — Plot FlowSOM grid or tree, coloured by standard deviation

Usage

```
PlotSD(fsom, marker = NULL, view = "MST", main = NULL,
       colorPalette = grDevices::colorRampPalette(c("#00007F", "blue",
"#007FFF", "cyan", "#7FFF7F", "yellow", "#FF7F00", "red", "#7F0000")),
       symmetric = FALSE, lim = NULL, backgroundValues = NULL,
       backgroundColor = function(n) { grDevices::rainbow(n, alpha = 0.3)
}, backgroundLim = NULL, backgroundBreaks = NULL)
```

Arguments

fsom	FlowSOM object, as generated by BuildMST
marker	If a marker is given, the sd for this marker is shown. Otherwise, the maximum ratio is used.
view	Preferred view, options: "MST", "grid" or "tSNE" (if this option was selected while building the MST)
main	Title of the plot
colorPalette	Color palette to use
symmetric	Plot colours symmetric around zero
lim	Variable limits
backgroundValues	Values to be used for background coloring, either numerical values or something that can be made into a factor (e.g. a clustering)
backgroundColor	Colorpalette 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.
backgroundBreaks	Breaks to pass on to cut , to split numerical background values. If NULL, the length of backgroundColor will be used (default 100).

Details

From suggestion in email: I am currently considering a way to summarize for each node all the SD as one value. After computing the SD matrix (nrow = # nodes, ncol = # markers), I compute the median value per column, then divide the SD matrix by it, and finally take the maximum ratio of each line (aka node). Doing so I got a unique dispersion score per node.

Value

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

See Also

[PlotMarker](#), [PlotStars](#), [PlotPies](#), [PlotCenters](#), [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)

PlotSD(flowSOM.res)
```

plotStarLegend	<i>Plot legend for star plot</i>
----------------	----------------------------------

Description

Plot a single star chart, annotated with labels

Usage

```
plotStarLegend(labels, colors = grDevices::rainbow(length(labels)),
              main = "")
```

Arguments

labels	Names to show in the legend
colors	Corresponding colors
main	Title of the legend

Value

Nothing is returned. A plot is drawn with 1 star chart, which is filled completely and annotated with the given labels.

See Also

[PlotStars](#)

PlotStars	<i>Plot star charts</i>
-----------	-------------------------

Description

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

Usage

```
PlotStars(fsom, markers = fsom$map$colsUsed, view = "MST",
  colorPalette = grDevices::colorRampPalette(c("#00007F", "blue",
    "#007FFF", "cyan", "#7FFF7F", "yellow", "#FF7F00", "red", "#7F0000")),
  starBg = "white", backgroundValues = NULL,
  backgroundColor = function(n) { grDevices::rainbow(n, alpha = 0.3)
  }, backgroundLim = NULL, backgroundBreaks = NULL,
  backgroundSize = NULL, thresholds = NULL, legend = TRUE,
  query = NULL, main = "")
```

Arguments

fsom	FlowSOM object, as generated by BuildMST
markers	Array of markers to use. Default: the markers used to build the tree
view	Preferred view, options: "MST", "grid" or "tSNE" (if this option was selected while building the MST)
colorPalette	Colorpalette to be used for the markers
starBg	Background color inside the star circle. Default is "white". Can also be put to "transparent" (as was the case for older versions).
backgroundValues	Values to be used for background coloring, either numerical values or something that can be made into a factor (e.g. a clustering)
backgroundColor	Colorpalette 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.
backgroundBreaks	Breaks to pass on to cut , to split numerical background values. If NULL, the length of backgroundColor will be used (default 100).
backgroundSize	Size of the background circles. Default 15.
thresholds	Optional. Array containing a number for each of the markers to be used as the split between high/low. If provided, the percentage of positive cells is indicated instead of the MFI
legend	Logical, if TRUE add a legend
query	Show a low/high profile for certain markers in the legend. See also QueryStarPlot
main	Title of the plot

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

[PlotPies](#), [PlotMarker](#), [PlotCenters](#), [BuildMST](#)

Examples

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

# Plot stars indicating the MFI of the cells present in the nodes
PlotStars(flowSOM.res)
```

PlotVariable	<i>Plot a variable for all nodes</i>
--------------	--------------------------------------

Description

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

Usage

```
PlotVariable(fsom, variable, view = "MST", main = NULL,
            colorPalette = grDevices::colorRampPalette(c("#00007F", "blue",
            "#007FFF", "cyan", "#7FFF7F", "yellow", "#FF7F00", "red", "#7F0000")),
            symmetric = FALSE, lim = NULL, backgroundValues = NULL,
            backgroundColor = function(n) { grDevices::rainbow(n, alpha = 0.3)
            }, backgroundLim = NULL, backgroundBreaks = NULL)
```

Arguments

fsom	FlowSOM object, as generated by BuildMST
variable	Vector containing a value for each node
view	Preferred view, options: "MST", "grid" or "tSNE" (if this option was selected while building the MST)
main	Title of the plot
colorPalette	Color palette to use
symmetric	Plot colours symmetric around zero
lim	Variable limits
backgroundValues	Values to be used for background coloring, either numerical values or something that can be made into a factor (e.g. a clustering)
backgroundColor	Colorpalette 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.
backgroundBreaks	Breaks to pass on to cut , to split numerical background values. If NULL, the length of backgroundColor will be used (default 100).

Value

Nothing is returned. A plot is drawn in which each node is coloured depending on its value for the given variable

See Also

[PlotMarker](#), [PlotStars](#), [PlotPies](#), [PlotCenters](#), [BuildMST](#)

Examples

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

# Plot some random values
rand <- runif(flowSOM.res$map$nNodes)
PlotVariable(flowSOM.res, rand)
```

Purity

Calculate mean weighted cluster purity

Description

Calculate mean weighted cluster purity

Usage

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

Arguments

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

Value

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

Examples

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

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

QueryStarPlot

Query a certain cell type

Description

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

Usage

```
QueryStarPlot(fsom, query, plot = TRUE, color = "#ca0020",
  debug = FALSE, ...)
```

Arguments

fsom	FlowSOM object, as generated by BuildMST or the first list item of FlowSOM
query	Array containing "high" or "low" for the specified column names of the FlowSOM data
plot	If true, a plot with a gradient of scores for the nodes is shown
color	Color to use for nodes with a high score in the plot
debug	If TRUE, some extra output will be printed
...	Other parameters to pass to PlotStars

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

Examples

```
file <- system.file("extdata", "68983.fcs", package="FlowSOM")
# Use the wrapper function to build a flowSOM object (saved in fsom[[1]])
# and a metaclustering (saved in fsom[[2]])
fsom <- FlowSOM(file, compensate = TRUE, transform = TRUE, scale = TRUE,
  colsToUse = c(9,12,14:18), nClus = 10, silent = FALSE,
  xdim=7, ydim=7)
query <- c("PE-Cy7-A" = "high", #CD3
  "APC-Cy7-A" = "high", #TCRb
  "Pacific Blue-A" = "high") #CD8
query_res <- QueryStarPlot(UpdateNodeSize(fsom[[1]], reset=TRUE), query)

cellTypes <- factor(rep("Unknown",49), levels=c("Unknown", "CD8 T cells"))
cellTypes[query_res$selected] <- "CD8 T cells"
PlotStars(fsom[[1]],
  backgroundValues=cellTypes,
  backgroundColor=c("#FFFFFF00", "#ca0020aa"))
```

ReadInput

*Read fcs-files or flowframes***Description**

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

Usage

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

Arguments

input	a flowFrame, a flowSet 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. If NULL and transform=TRUE, column names of \$SPILL description in fcs file will be used.
transformFunction	Defaults to logicleTransform()
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

Value

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

See Also

[scale](#), [BuildSOM](#)

Examples

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

# Or read from flowFrame object
ff <- flowCore::read.FCS(fileName)
```

```

ff <- flowCore::compensate(ff,ff@description$SPILL)
ff <- flowCore::transform(ff,
                          flowCore::transformList(colnames(ff@description$SPILL),
                                                    flowCore::logicleTransform()))
flowSOM.res <- ReadInput(ff,scale=TRUE)

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

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

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

```

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

Description

Write FlowSOM clustering results to the original FCS files

Usage

```
SaveClustersToFCS(fsom, original_files, pp_files = original_files,
                 selection_files = NULL, silent = FALSE)
```

Arguments

<code>fsom</code>	FlowSOM object as generated by BuildSOM
<code>original_files</code>	FCS files that should be extended
<code>pp_files</code>	FCS files that correspond to the input of FlowSOM
<code>selection_files</code>	Files indicating which cells of the original files correspond to the input files
<code>silent</code>	If FALSE (default), print some extra output

Value

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

SOM

Build a self-organizing map

Description

Build a self-organizing map

Usage

```
SOM(data, xdim = 10, ydim = 10, rlen = 10, mst = 1,  
     alpha = c(0.05, 0.01), radius = stats::quantile(nhbrdist, 0.67) *  
     c(1, 0), init = FALSE, initf = Initialize_KWSP, distf = 2,  
     silent = FALSE, codes = NULL, importance = NULL)
```

Arguments

<code>data</code>	Matrix containing the training data
<code>xdim</code>	Width of the grid
<code>ydim</code>	Hight of the grid
<code>rlen</code>	Number of times to loop over the training data for each MST
<code>mst</code>	Number of times to build an MST
<code>alpha</code>	Start and end learning rate
<code>radius</code>	Start and end radius
<code>init</code>	Initialize cluster centers in a non-random way
<code>initf</code>	Use the given initialization function if <code>init==T</code> (default: <code>Initialize_KWSP</code>)
<code>distf</code>	Distance function (1=manhattan, 2=euclidean, 3=chebyshev, 4=cosine)
<code>silent</code>	If <code>FALSE</code> , print status updates
<code>codes</code>	Cluster centers to start with
<code>importance</code>	array with numeric values. Parameters will be scaled according to importance

Value

A list containing all parameter settings and results

References

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

See Also

[BuildSOM](#)

TestOutliers

Test if any cells are too far from their cluster centers

Description

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 + `mad_allowed` times the median absolute deviation of the distances.

Usage

```
TestOutliers(fsom, mad_allowed = 4, fsom_reference = NULL,  
             plot = FALSE, img_file = "testOutliers.pdf")
```

Arguments

<code>fsom</code>	FlowSOM object
<code>mad_allowed</code>	Number of median absolute deviations allowed. Default = 4.
<code>fsom_reference</code>	FlowSOM object to use as reference. If NULL (default), the original <code>fsom</code> object is used.
<code>plot</code>	Should a plot be generated showing the distribution of the distances. Default is FALSE.
<code>img_file</code>	If plot is TRUE, the output will be written to this file. Default is "testOutliers.pdf"

Value

A new FlowSOM object

See Also

[FlowSOMSubset](#) if you want to get a subset of the current data instead of a new dataset

Examples

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

UpdateNodeSize *Update nodesize of FlowSOM object*

Description

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

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

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
flowSOM.res <- UpdateNodeSize(flowSOM.res, reset=TRUE)
PlotStars(flowSOM.res)

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

Index

AddFlowFrame, 2
AggregateFlowFrames, 3
BuildMST, 4, 5, 9, 10, 21–24, 26–32, 34–37, 42
BuildSOM, 4, 5, 9, 38, 40
ceiling, 4
computeBackgroundColor, 6
CountGroups, 6, 23, 24
cut, 6, 25, 26, 28, 31, 32, 34, 35
Dist.MST, 7
FlowSOM, 8, 23, 27, 29, 37
FlowSOMSubset, 9, 21, 41
flowWorkspace, 12
FMeasure, 10
get_channels, 14, 15
get_markers, 15, 15
GetClusters, 11
GetCVs, 11
GetFlowJoLabels, 12
GetMetaclusters, 13
GetMFIs, 14
Initialize_KWSP, 16
Initialize_PCA, 16
MapDataToCodes, 17
MetaclusterCVs, 17
MetaClustering, 9, 18, 19
metaClustering_consensus, 18, 19
MetaclusterMFIs, 19
NewData, 20
PlotCenters, 21, 23, 26, 28, 31, 32, 34, 36
PlotClusters2D, 22, 29
PlotGroups, 23
PlotLabels, 24
PlotMarker, 22, 25, 28, 31, 32, 34, 36
PlotNode, 27
PlotNumbers, 23, 25, 27, 28
PlotOverview2D, 29
PlotPies, 12, 22, 26, 28, 30, 32, 34, 36
PlotSD, 31
plotStarLegend, 33
PlotStars, 4, 22–24, 26–28, 31–33, 33, 36, 37
PlotVariable, 35
Purity, 36
QueryStarPlot, 34, 37
ReadInput, 3, 5, 9, 38
SaveClustersToFCS, 39
scale, 8, 9, 21, 38
SOM, 40
TestOutliers, 41
UpdateNodeSize, 42