Package 'CytoMDS'

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Title Low Dimensions projection of cytometry samples

Version 1.0.0

Description This package implements a low dimensional visualization of a set
of cytometry samples, in order to visually assess the 'distances' between them.
This, in turn, can greatly help the user to identify quality issues
like batch effects or outlier samples, and/or check the presence of potential
sample clusters that might align with the exeprimental design.
The CytoMDS algorithm combines, on the one hand, the concept of Earth Mover's
Distance (EMD), a.k.a. Wasserstein metric and, on the other hand,
the Multi Dimensional Scaling (MDS) algorithm for the low dimensional
projection.
Also, the package provides some diagnostic tools for both checking the quality
of the MDS projection, as well as tools to help with the interpretation of
the axes of the projection.

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Encoding UTF-8

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BugReports https://github.com/UCLouvain-CBIO/CytoMDS/issues

URL https://uclouvain-cbio.github.io/CytoMDS

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Contents

CytoMDS-package   *CytoMDS: Low Dimensions projection of cytometry samples*

Description

This package implements a low dimensional visualization of a set of cytometry samples, in order
to visually assess the 'distances' between them. This, in turn, can greatly help the user to identify
quality issues like batch effects or outlier samples, and/or check the presence of potential sample
clusters that might align with the exeprimental design. The CytoMDS algorithm combines, on the
one hand, the concept of Earth Mover's Distance (EMD), a.k.a. Wasserstein metric and, on the other
hand, the Multi Dimensional Scaling (MDS) algorithm for the low dimensional projection. Also,
the package provides some diagnostic tools for both checking the quality of the MDS projection, as
well as tools to help with the interpretation of the axes of the projection.

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See Also

Useful links:

- https://uclouvain-cbio.github.io/CytoMDS
- Report bugs at https://github.com/UCLouvain-CBIO/CytoMDS/issues

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channelSummaryStats   *Summary statistics per channel computation*

Description

Computation of summary statistic for selected channels, for all flowFrames of a flowSet. This method provides two different input modes:

- the user provides directly a flowSet loaded in memory (RAM).
- the user provides (1.) a number of samples nSamples; (2.) an ad-hoc function that takes as input an index between 1 and nSamples, and codes the method to load the corresponding flowFrame in memory; Optional row and column ranges can be provided to limit the calculation to a specific rectangle of the matrix. These i.e. can be specified as a way to split heavy calculations of large distance matrices on several computation nodes.

Usage

```
channelSummaryStats(
  x,
  loadFlowFrameFUN = NULL,
  loadFlowFrameFUNArgs = NULL,
  channels = NULL,
  statFUNs = stats::median,
  verbose = FALSE,
  BPPARAM = BiocParallel::SerialParam(),
  BPOPTIONS = BiocParallel::bpoptions(packages = c("flowCore"))
)
```

Arguments

x	either a flowCore::flowSet, or the number of samples (integer >=1)
loadFlowFrameFUN	
	the function used to translate a flowFrame index into a flowFrame. In other words, the function should code how to load a specific flowFrame into memory. Important: the flow Frame index should be the first function argument and should be named ffIndex.
loadFlowFrameFUNArgs	
	(optional) a named list containing additional input parameters of loadFlowFrameFUN()
channels	which channels (integer index(ices) or character(s)):
	• if it is a character vector, it can refer to either the channel names, or the marker names
	• if it is a numeric vector, it refers to the indexes of channels in fs
	• if NULL all scatter and fluorescent channels of fs will be selected
statFUNs	a list (possibly of length one) of functions to call to calculate the statistics, or a simple function This list can be named, in that case, these names will be transfered to the returned value.
verbose	if TRUE, output a message after each single distance calculation
BPPARAM	sets the BPPARAM back-end to be used for the computation. If not provided, will use BiocParallel::SerialParam() (no task parallelization)
BPOPTIONS	sets the BPOPTIONS to be passed to bplapply() function. Note that if you use a SnowParams back-end, you need to specify all the packages that need to be loaded for the different CytoProcessingStep to work properly (visibility of functions). As a minimum, the flowCore package needs to be loaded. (hence the default BPOPTIONS = bpoptions(packages = c("flowCore")))

Value

a list of named statistic matrices. In each stat matrix, the columns are the channel statistics for all flowFrames of the flowSet. Exception: if only one stat function (and not a list) is passed in statFUNs, the return value is simplified to the stat matrix itself.

Examples

```
library(CytoPipeline)

data(OMIP021Samples)

# estimate scale transformations
# and transform the whole OMIP021Samples

transList <- estimateScaleTransforms(
    ff = OMIP021Samples[[1]],
    fluoMethod = "estimateLogicle",
    scatterMethod = "linearQuantile",
    scatterRefMarker = "BV785 - CD3")
```

```
OMIP021Trans <- CytoPipeline::applyScaleTransforms(
    OMIP021Samples,
    transList)

channelsOrMarkers <- c("FSC-A", "SSC-A", "BV785 - CD3")

# calculate mean for each 4 selected channels, for each 2 samples

channelMeans <- channelSummaryStats(
    OMIP021Trans,
    channels = channelsOrMarkers,
    statFUNs = mean)

# calculate median AND std deviation
# for each 4 selected channels, for each 2 samples

channelMedians <- channelSummaryStats(
    OMIP021Trans,
    channels = channelsOrMarkers,
    statFUNs = list("median" = stats::median,
                    "std.dev" = stats::sd))
```

---

computeMetricMDS   *metric MDS projection of sample*

Description

Multi-dimensional scaling projection of samples, using a distance matrix as an input. The MDS algorithm is not the classical MDS (cmdscale alike, aka Torgerson's algorithm), but is the SMA-COF algorithm for metric distances that are not necessarily euclidean. After having obtained the projections on the nDim dimensions, we always apply svd decomposition to visualize as first axes the ones that contain the most variance of the projected dataset in nDim dimensions. Instead of being provided directly by the user, the nDim parameter can otherwise be found iteratively by finding the minimum nDim parameter that allows the projection to reach a target pseudo RSquare. If this is the case, the maxDim parameter is used to avoid looking for too big projection spaces.

Usage

```
computeMetricMDS(
  pwDist,
  nDim = NULL,
  seed = NULL,
  targetPseudoRSq = 0.95,
  maxDim = 128,
  ...
)
```

Arguments

`pwDist`	(nSamples rows, nSamples columns), previously calculated pairwise distances between samples, must be provided as a full symmetric square matrix, with 0. diagonal
`nDim`	number of dimensions of projection, as input to SMACOF algorithm if not provided, will be found iteratively using `targetPseudoRSq`
`seed`	seed to be set when launching SMACOF algorithm (e.g. when `init` is set to `"random"` but not only)
`targetPseudoRSq`	
	target pseudo RSquare to be reached (only used when `nDim` is set to NULL)
`maxDim`	in case `nDim` is found iteratively, maximum number of dimensions the search procedure is allowed to explore
`...`	additional parameters passed to SMACOF algorithm

Value

a list with six elements:

- `$pwDist` the initial pair-wise distance (same as input)
- `$proj` the final configuration, i.e. the projected data matrix (nSamples rows, nDim columns) in nDim dimensions
- `$projDist` the distance matrix of projected data
- stress the global stress loss function final value obtained from the SMACOF algorithm
- spp the stress per point obtained from the SMACOF algorithm, i.e. the contribution of each point to the stress loss function
- `$RSq` R squares, for each d, from 1 to nDim: the (pseudo) R square when taking all dims from 1 to d.
- `$GoF` Goodness of fit, for each d, from 1 to nDim: the goodness of fit indicator (b/w 0 and 1) when taking all dims from 1 to d. Note pseudo R square and goodness of fit indicators are essentially the same indicator, only the definition of total sum of squares differ:
- for pseudo RSq: TSS is calculated using the mean pairwise distance as minimum
- for goodness of fit: TSS is calculated using 0 as minimum

Examples

```
library(CytoPipeline)

data(OMIP021Samples)

# estimate scale transformations
# and transform the whole OMIP021Samples

transList <- estimateScaleTransforms(
    ff = OMIP021Samples[[1]],
    fluoMethod = "estimateLogicle",
    scatterMethod = "linearQuantile",
    scatterRefMarker = "BV785 - CD3")
```

```
OMIP021Trans <- CytoPipeline::applyScaleTransforms(
    OMIP021Samples,
    transList)

# As there are only 2 samples in OMIP021Samples dataset,
# we create artificial samples that are random combinations of both samples

ffList <- c(
    flowCore::flowSet_to_list(OMIP021Trans),
    lapply(3:5,
           FUN = function(i) {
               aggregateAndSample(
                   OMIP021Trans,
                   seed = 10*i,
                   nTotalEvents = 5000)[,1:22]
           }))

fsNames <- c("Donor1", "Donor2", paste0("Agg",1:3))
names(ffList) <- fsNames

fsAll <- as(ffList,"flowSet")

flowCore::pData(fsAll)$type <- factor(c("real", "real", rep("synthetic", 3)))
flowCore::pData(fsAll)$grpId <- factor(c("D1", "D2", rep("Agg", 3)))

# calculate all pairwise distances

pwDist <- pairwiseEMDDist(fsAll,
                          channels = c("FSC-A", "SSC-A"),
                          verbose = FALSE)

# compute Metric MDS object with explicit number of dimensions
mdsObj <- computeMetricMDS(pwDist, nDim = 4, seed = 0)

dim <- nDim(mdsObj) # should be 4

#' # compute Metric MDS object by reaching a target pseudo RSquare
mdsObj2 <- computeMetricMDS(pwDist, seed = 0, targetPseudoRSq = 0.999)
```

EMDDist	*Calculate Earth Mover's distance between two flowFrames*

Description

Calculate Earth Mover's distance between two flowFrames

Usage

```
EMDDist(
  ff1,
  ff2,
  channels = NULL,
  checkChannels = TRUE,
  binSize = 0.05,
  minRange = -10,
  maxRange = 10,
  returnAll = FALSE
)
```

Arguments

`ff1`	a flowCore::flowFrame
`ff2`	a flowCore::flowFrame
`channels`	which channels (integer index(ices) or character(s)):
	• if it is a character vector, it can refer to either the channel names, or the marker names
	• if it is a numeric vector, it refers to the indexes of channels in `ff1`
	• if NULL all scatter and fluorescent channels of `ff1` will be selected
`checkChannels`	if `TRUE`, will explicitly check that all provided channels are present in both flowFrames
`binSize`	size of equal bins to approximate the marginal distributions.
`minRange`	minimum value taken when approximating the marginal distributions
`maxRange`	maximum value taken when approximating the marginal distributions
`returnAll`	If `TRUE`, distributions and marginal distribution distances are returned as well. Default = FALSE.

Value

the Earth Mover's distance between `ff1` and `ff2`, which is calculated by summing up all EMD approximates for the marginal distributions of each channel

Examples

```
library(CytoPipeline)

data(OMIP021Samples)

# estimate scale transformations
# and transform the whole OMIP021Samples

transList <- estimateScaleTransforms(
    ff = OMIP021Samples[[1]],
    fluoMethod = "estimateLogicle",
    scatterMethod = "linearQuantile",
```

```
      scatterRefMarker = "BV785 - CD3")

  OMIP021Trans <- CytoPipeline::applyScaleTransforms(
      OMIP021Samples,
      transList)

  # distance with itself (all channels at once)
  # => should return 0
  dist0 <- EMDDist(
      ff1 = OMIP021Trans[[1]],
      ff2 = OMIP021Trans[[1]])

  # returning only distance, 2 channels
  dist1 <- EMDDist(
      ff1 = OMIP021Trans[[1]],
      ff2 = OMIP021Trans[[2]],
      channels = c("FSC-A", "SSC-A"))

  # using only one channel, passed by marker name
  dist2 <- EMDDist(ff1 = OMIP021Trans[[1]],
                   ff2 = OMIP021Trans[[2]],
                   channels = c("BV785 - CD3"))

  # using only one channel, passed by index
  dist3 <- EMDDist(ff1 = OMIP021Trans[[1]],
                   ff2 = OMIP021Trans[[2]],
                   channels = 10)

  dist2 == dist3
```

---

ggplotMarginalDensities

Plot of channel intensity marginal densities

Description

ggplotMarginalDensities uses ggplot2 to draw plots of marginal densities of selected channels of a flowSet. If the flowSet contains several flowFrames, all events are concatenated together. By default, a pseudo Rsquare projection quality indicator, and the number of dimensions of the MDS projection are provided in sub-title

Usage

```
ggplotMarginalDensities(
  x,
  sampleSubset,
  channels,
  pDataForColour,
```

```
    pDataForGroup,
    nEventInSubsample = Inf,
    seed = NULL,
    transList
)
```

Arguments

x	a `flowCore::flowSet` (or a single `flowCore::flowFrame`)
sampleSubset	(optional) a logical vector, of size `nrow(pData)`, which is by construction the nb of samples, indicating which samples to keep in the plot. Typically it is obtained through the evaluation of a logical condition on `pData` rows.
channels	(optional)
pDataForColour	(optional) which `phenoData(fs)` variable will be used as colour aesthetic. Should be a character.
pDataForGroup	(optional) which `phenoData(fs)` variable will be used as group aesthetic. Should be a character.
nEventInSubsample	
	how many event to take (per flowFrame of the flowSet).
seed	if not null, used in subsampling.
transList	a `flowCore::transformList` that will be applied before plotting.

Value

a ggplot object

Examples

```
library(CytoPipeline)

data(OMIP021Samples)

# estimate scale transformations
# and transform the whole OMIP021Samples

transList <- estimateScaleTransforms(
    ff = OMIP021Samples[[1]],
    fluoMethod = "estimateLogicle",
    scatterMethod = "linearQuantile",
    scatterRefMarker = "BV785 - CD3")

OMIP021Trans <- CytoPipeline::applyScaleTransforms(
    OMIP021Samples,
    transList)


# As there are only 2 samples in OMIP021Samples dataset,
# we create artificial samples that are random combinations of both samples
```

```
ffList <- c(
    flowCore::flowSet_to_list(OMIP021Trans),
    lapply(3:5,
            FUN = function(i) {
                aggregateAndSample(
                    OMIP021Trans,
                    seed = 10*i,
                    nTotalEvents = 5000)[,1:22]
            }))

fsNames <- c("Donor1", "Donor2", paste0("Agg",1:3))
names(ffList) <- fsNames

fsAll <- as(ffList,"flowSet")

flowCore::pData(fsAll)$grpId <- factor(c("D1", "D2", rep("Agg", 3)))
flowCore::pData(fsAll)$lbl <- paste0("S", 1:5)

# plot densities, all samples together
p <- ggplotMarginalDensities(fsAll)

# plot densities, per sample
p <- ggplotMarginalDensities(fsAll, pDataForGroup = "lbl")

# plot densities, per sample and coloured by group
p <- ggplotMarginalDensities(
    fsAll,
    pDataForGroup = "lbl",
    pDataForColour = "grpId")
```

---

ggplotSampleMDS *Plot of Metric MDS object*

Description

ggplotSampleMDS uses ggplot2 to provide plots of Metric MDS results. By default, a pseudo Rsquare projection quality indicator, and the number of dimensions of the MDS projection are provided in sub-title

Usage

```
ggplotSampleMDS(
  mdsObj,
  pData,
  sampleSubset,
  projectionAxes = c(1, 2),
  biplot = FALSE,
  biplotType = c("correlation", "regression"),
```

```
    extVariables,
    pDataForColour,
    pDataForShape,
    pDataForLabel,
    pDataForAdditionalLabelling,
    sizeReflectingStress = FALSE,
    title = "Multi Dimensional Scaling",
    displayPointLabels = TRUE,
    pointLabelSize = 3.88,
    repelPointLabels = TRUE,
    displayArrowLabels = TRUE,
    arrowLabelSize = 3.88,
    repelArrowLabels = FALSE,
    arrowThreshold = 0.8,
    flipXAxis = FALSE,
    flipYAxis = FALSE,
    displayPseudoRSq = TRUE,
    ...
)
```

Arguments

mdsObj   a MDS object, output of the `computeMetricMDS()` method.

pData   (optional) a data.frame providing user input sample data. These can be design of
                       experiment variables, phenotype data per sample,... and will be used to highlight
                       sample categories in the plot and/or for subsetting.

sampleSubset   (optional) a logical vector, of size `nrow(pData)`, which is by construction the nb
                       of samples, indicating which samples to keep in the plot. Typically it is obtained
                       through the evaluation of a logical condition on `pData` rows.

projectionAxes   which two axes should be plotted (should be a numeric vector of length 2)

biplot   if TRUE, adds projection of external variables

biplotType   type of biplot used:

                       • if "correlation", projection of external variables will be according to Pear-
                         son correlations w.r.t. projection axes (arrow x & y coordinates)

                       • if "regression", a linear regression of external variables using the 2 projec-
                         tion axes as explanatory variables is performed, and the projection of exter-
                         nal variables will be according to regression coefficients (arrow direction)
                         and R square of regression (arrow size)

extVariables   are used to generate a biplot these are the external variables that will be used
                       in the biplot. They should be provided as a matrix with named columns cor-
                       responding to the variables. The number of rows should be the same as the
                       number of samples. The matrix might contain some NA's, in that case only
                       complete rows will be used to calculate biplot arrows.

pDataForColour   (optional) which `pData` variable will be used as colour aesthetic. Should be a
                       character.

pDataForShape	(optional) which pData variable will be used as shape aesthetic. Should be a character.
pDataForLabel	(optional) which pData variable will be used as point labels in the plot. Should be a character. If missing, point labels will be set equal to point names defined in MDS object (if not NULL, otherwise no labels will be set).
pDataForAdditionalLabelling	
	(optional) which pData variable(s) will be add to the ggplot mapping, as to make them available for *plotly* tooltipping. Should be an array of character of maximum length 3. Note this works only if biplot=FALSE, as biplots contain circle and arrows that are currently not supported under ggplotly.
sizeReflectingStress	
	if TRUE, size of points will appear proportional to stress by point, i.e. the bigger the sample point appears, the less accurate its representation is (in terms of distances w.r.t. other points)
title	title to give to the plot
displayPointLabels	
	if TRUE, displays labels attached to points (see pDataForLabels for the setting of the label values)
pointLabelSize	size of point labels (default: 3.88 as in geom_text())
repelPointLabels	
	if TRUE, uses ggrepel::geom_text_repel() instead of ggplot2::geom_text() (try to split the labels such that they do not overlap) for the points
displayArrowLabels	
	if TRUE, displays arrows labels (only with biplot)
arrowLabelSize	size of arrow labels (default: 3.88 as in geom_text())
repelArrowLabels	
	if TRUE, uses ggrepel::geom_text_repel() instead of ggplot2::geom_text() for the arrows (only with biplot)
arrowThreshold	(only with biplot), arrows will be made barely visible if their length is (in absolute value) less than this threshold.
flipXAxis	if TRUE, take the opposite of x values (provided as it might ease low dimensional projection comparisons)
flipYAxis	if TRUE, take the opposite of y values (provided as it might ease low dimensional projection comparisons)
displayPseudoRSq	
	if TRUE, display pseudo RSquare in subtitle, on top of nb of dimensions
...	additional parameters passed to ggrepel::geom_text_repel() (if used)

Value

a ggplot object

See Also

[ggplotSampleMDSWrapBiplots](#), [ggplotSampleMDSShepard](#), [computeMetricMDS](#)

Examples

```
library(CytoPipeline)

data(OMIP021Samples)

# estimate scale transformations
# and transform the whole OMIP021Samples

transList <- estimateScaleTransforms(
    ff = OMIP021Samples[[1]],
    fluoMethod = "estimateLogicle",
    scatterMethod = "linearQuantile",
    scatterRefMarker = "BV785 - CD3")

OMIP021Trans <- CytoPipeline::applyScaleTransforms(
    OMIP021Samples,
    transList)

# As there are only 2 samples in OMIP021Samples dataset,
# we create artificial samples that are random combinations of both samples

ffList <- c(
    flowCore::flowSet_to_list(OMIP021Trans),
    lapply(3:5,
           FUN = function(i) {
               aggregateAndSample(
                   OMIP021Trans,
                   seed = 10*i,
                   nTotalEvents = 5000)[,1:22]
           }))

fsNames <- c("Donor1", "Donor2", paste0("Agg",1:3))
names(ffList) <- fsNames

fsAll <- as(ffList,"flowSet")

flowCore::pData(fsAll)$type <- factor(c("real", "real", rep("synthetic", 3)))
flowCore::pData(fsAll)$grpId <- factor(c("D1", "D2", rep("Agg", 3)))

# calculate all pairwise distances

pwDist <- pairwiseEMDDist(fsAll,
                          channels = c("FSC-A", "SSC-A"),
                          verbose = FALSE)

# compute Metric MDS object with explicit number of dimensions
mdsObj <- computeMetricMDS(pwDist, nDim = 4, seed = 0)

dim <- nDim(mdsObj) # should be 4

#' # compute Metric MDS object by reaching a target pseudo RSquare
mdsObj2 <- computeMetricMDS(pwDist, seed = 0, targetPseudoRSq = 0.999)
```

```
# plot mds projection on axes 1 and 2,
# use 'grpId' for colour, 'type' for shape, and no label

p_12 <- ggplotSampleMDS(
    mdsObj = mdsObj,
    pData = flowCore::pData(fsAll),
    projectionAxes = c(1,2),
    pDataForColour = "grpId",
    pDataForShape = "type")

# plot mds projection on axes 3 and 4,
# use 'grpId' for colour, and 'name' as point label

p_34 <- ggplotSampleMDS(
    mdsObj = mdsObj,
    pData = flowCore::pData(fsAll),
    projectionAxes = c(3,4),
    pDataForColour = "grpId",
    pDataForLabel = "name")

# plot mds projection on axes 1 and 2,
# use 'group' for colour, 'type' for shape, and 'name' as point label
# have sample point size reflecting 'stress'
# i.e. quality of projection w.r.t. distances to other points

p12_Stress <- ggplotSampleMDS(
    mdsObj = mdsObj,
    pData = flowCore::pData(fsAll),
    projectionAxes = c(1,2),
    pDataForColour = "grpId",
    pDataForLabel = "name",
    pDataForShape = "type",
    sizeReflectingStress = TRUE)

# try to associate axes with median of each channel
# => use bi-plot

extVars <- channelSummaryStats(
    fsAll,
    channels = c("FSC-A", "SSC-A"),
    statFUNs = stats::median)


bp_12 <- ggplotSampleMDS(
    mdsObj = mdsObj,
    pData = flowCore::pData(fsAll),
    projectionAxes = c(1,2),
    biplot = TRUE,
    extVariables = extVars,
    pDataForColour = "grpId",
```

```
    pDataForShape = "type",
    seed = 0)

bp_34 <- ggplotSampleMDS(
    mdsObj = mdsObj,
    pData = flowCore::pData(fsAll),
    projectionAxes = c(3,4),
    biplot = TRUE,
    extVariables = extVars,
    pDataForColour = "grpId",
    pDataForLabel = "name",
    seed = 0)
```

ggplotSampleMDSShepard

Plot of Metric MDS object - Shepard diagram

Description

ggplotSampleMDSShepard uses ggplot2 to provide plot of Metric MDS results. Shepard diagram provides a scatter plot of :

- on the x axis, the high dimensional pairwise distances between each sample pairs
- on the y axis, the corresponding pairwise distances in the obtained low dimensional projection

Usage

```
ggplotSampleMDSShepard(
  mdsObj,
  nDim,
  title = "Multi Dimensional Scaling - Shepard's diagram",
  pointSize = 0.5,
  lineWidth = 0.5,
  displayPseudoRSq = TRUE
)
```

Arguments

mdsObj	a MDS object, output of the computeMetricMDS() method.
nDim	(optional) number of dimensions to use when calculating Shepard's diagram and pseudoRSquare. If missing, it will be set equal to the number of projection dimensions as calculated in mdsObj
title	title to give to the plot
pointSize	point size in plot
lineWidth	line width in plot
displayPseudoRSq	
	if TRUE, display pseudo RSquare in subtitle, on top of nb of dimensions

Value

a ggplot object

See Also

ggplotSampleMDS, computeMetricMDS

Examples

```
library(CytoPipeline)

data(OMIP021Samples)

# estimate scale transformations
# and transform the whole OMIP021Samples

transList <- estimateScaleTransforms(
    ff = OMIP021Samples[[1]],
    fluoMethod = "estimateLogicle",
    scatterMethod = "linearQuantile",
    scatterRefMarker = "BV785 - CD3")

OMIP021Trans <- CytoPipeline::applyScaleTransforms(
    OMIP021Samples,
    transList)

ffList <- c(
    flowCore::flowSet_to_list(OMIP021Trans),
    lapply(3:5,
           FUN = function(i) {
               aggregateAndSample(
                   OMIP021Trans,
                   seed = 10*i,
                   nTotalEvents = 5000)[,1:22]
           }))

fsNames <- c("Donor1", "Donor2", paste0("Agg",1:3))
names(ffList) <- fsNames

fsAll <- as(ffList,"flowSet")

flowCore::pData(fsAll)$type <- factor(c("real", "real", rep("synthetic", 3)))
flowCore::pData(fsAll)$grpId <- factor(c("D1", "D2", rep("Agg", 3)))

# calculate all pairwise distances

pwDist <- pairwiseEMDDist(fsAll,
                          channels = c("FSC-A", "SSC-A"),
                          verbose = FALSE)

# compute Metric MDS object with explicit number of dimensions
```

```
mdsObj <- computeMetricMDS(pwDist, nDim = 4, seed = 0)

dim <- nDim(mdsObj) # should be 4

#' # compute Metric MDS object by reaching a target pseudo RSquare
mdsObj2 <- computeMetricMDS(pwDist, seed = 0, targetPseudoRSq = 0.999)

# Shepard diagrams

p2D <- ggplotSampleMDSShepard(
    mdsObj,
    nDim = 2,
    pointSize = 1,
    title = "Shepard with 2 dimensions")

p3D <- ggplotSampleMDSShepard(
    mdsObj,
    nDim = 3,
    title = "Shepard with 3 dimensions")
    #'
pDefD <- ggplotSampleMDSShepard(
    mdsObj,
    title = "Shepard with default nb of dimensions")
```

---

ggplotSampleMDSWrapBiplots

SampleMDS biplot wrapping

Description

ggplotSampleMDSWrapBiplots calls ggplotSampleMDS repeatly to generate biplots with different sets of external variables and align them in a grid using the patchwork package, in a similar fashion as ggplot2::facet_wrap() does.

Usage

```
ggplotSampleMDSWrapBiplots(
  mdsObj,
  extVariableList,
  ncol = NULL,
  nrow = NULL,
  byrow = NULL,
  displayLegend = TRUE,
  ...
)
```

Arguments

`mdsObj`	a MDS object, output of the `computeMetricMDS()` method
`extVariableList`	
	should be a named list of external variable matrices Each element of the list should be a matrix with named columns corresponding to the variables. The number of rows should be the same as the number of samples.
`ncol`	passed to `patchwork::wrap_plots()`
`nrow`	passed to `patchwork::wrap_plots()`
`byrow`	passed to `patchwork::wrap_plots()`
`displayLegend`	if FALSE, will de-active the legend display
`...`	additional parameters passed to `ggplotSampleMDS()` (if used)

Value

a ggplot object

See Also

ggplotSampleMDS, ggplotSampleMDSShepard, computeMetricMDS

Examples

```
library(CytoPipeline)

data(OMIP021Samples)

# estimate scale transformations
# and transform the whole OMIP021Samples

transList <- estimateScaleTransforms(
    ff = OMIP021Samples[[1]],
    fluoMethod = "estimateLogicle",
    scatterMethod = "linearQuantile",
    scatterRefMarker = "BV785 - CD3")

OMIP021Trans <- CytoPipeline::applyScaleTransforms(
    OMIP021Samples,
    transList)

# As there are only 2 samples in OMIP021Samples dataset,
# we create artificial samples that are random combinations of both samples

ffList <- c(
    flowCore::flowSet_to_list(OMIP021Trans),
    lapply(3:5,
           FUN = function(i) {
               aggregateAndSample(
                   OMIP021Trans,
```

```
                           seed = 10*i,
                           nTotalEvents = 5000)[,1:22]
             }))

fsNames <- c("Donor1", "Donor2", paste0("Agg",1:3))
names(ffList) <- fsNames

fsAll <- as(ffList,"flowSet")

flowCore::pData(fsAll)$type <- factor(c("real", "real", rep("synthetic", 3)))
flowCore::pData(fsAll)$grpId <- factor(c("D1", "D2", rep("Agg", 3)))

# calculate all pairwise distances

pwDist <- pairwiseEMDDist(fsAll,
                          channels = c("FSC-A", "SSC-A"),
                          verbose = FALSE)

# compute Metric MDS object with explicit number of dimensions
mdsObj <- computeMetricMDS(pwDist, nDim = 4, seed = 0)

dim <- nDim(mdsObj) # should be 4

#' # compute Metric MDS object by reaching a target pseudo RSquare
mdsObj2 <- computeMetricMDS(pwDist, seed = 0, targetPseudoRSq = 0.999)

# plot mds projection on axes 1 and 2,
# use 'group' for colour, 'type' for shape, and no label

p_12 <- ggplotSampleMDS(
    mdsObj = mdsObj,
    pData = flowCore::pData(fsAll),
    projectionAxes = c(1,2),
    pDataForColour = "grpId",
    pDataForShape = "type")

# try to associate axes with median or std deviation of each channel
# => use bi-plots

extVarList <- channelSummaryStats(
    fsAll,
    channels = c("FSC-A", "SSC-A"),
    statFUNs = c("median" = stats::median,
                 "std.dev" = stats::sd))

bpFull <- ggplotSampleMDSWrapBiplots(
    mdsObj = mdsObj,
    extVariableList = extVarList,
    pData = flowCore::pData(fsAll),
    projectionAxes = c(1,2),
    pDataForColour = "group",
    pDataForShape = "type",
    seed = 0)
```

MDS-class   *MDS class*

Description

Class representing Multi Dimensional Scaling (MDS) projection.

returns the value of the stress criterion, minimized by the SMACOF algorithm.

returns a vector of nPoints dimension, containing the stress indicator per point. The `stress` minimization criterion can indeed be allocated per represented point. The more the stress of a particular point, the less accurate its distances w.r.t. the other points.

Usage

```
## S4 method for signature 'MDS'
show(object)

nDim(x)

nPoints(x)

pwDist(x)

projections(x)

projDist(x)

stress(x)

spp(x)

eigenVals(x)

pctvar(x)

RSq(x)

RSqVec(x)

GoF(x)

smacofRes(x)
```

Arguments

object	a MDS object
x	a MDS object

Value

nothing

Slots

nDim numeric, nb of dimensions of the projection

pwDist An object of class dist storing the triangular relevant part of the symmetric, zero diagonal pairwise distance matrix (nPoints * nPoints), BEFORE projection.

proj The projection matrix, resulting from MDS

projDist An object of class dist storing the triangular relevant part of the symmetric, zero diagonal pairwise distance matrix (nPoints * nPoints), AFTER projection.

eigen numeric, vector of nDim length, containing the eigen values of the PCA that is applied after the Smacof algorithm.

pctvar numeric, vector of nDim length, containing the percentage of explained variance per axis.

RSq numeric, vector of pseudo R square indicators, as a function of number of dimensions. RSq[nDim] is the global pseudo R square, as displayed on plots.

GoF numeric, vector of goodness of fit indicators, as a function of number of dimensions. GoF[nDim] is the global goodness of fit.

smacofRes an object of class 'smacofB' containing the algorithmic optimization results, for example stress and stress per point, as returned by smacof::smacofSym() method.

Examples

```
nHD <- 10
nLD <- 2
nPoints <- 20

# generate uniformly distributed points in 10 dimensions
points <- matrix(
    data = runif(n = nPoints * nHD),
    nrow = nPoints)

# calculate euclidian distances
pwDist  <- dist(points)

# compute Metric MDS object by reaching a target pseudo RSquare
mdsObj <- computeMetricMDS(pwDist, targetPseudoRSq = 0.95)

show(mdsObj)
```

---

pairwiseEMDDist   *Pairwise Earth Mover's Distance calculation*

Description

Computation of all EMD between pairs of flowFrames belonging to a flowSet. This method provides two different input modes:

- the user provides directly a flowSet loaded in memory (RAM).

- the user provides (1.) a number of samples nSamples; (2.) an ad-hoc function that takes as input an index between 1 and nSamples, and codes the method to load the corresponding flowFrame in memory; Optional row and column ranges can be provided to limit the calculation to a specific rectangle of the matrix. These i.e. can be specified as a way to split heavy calculations of large distance matrices on several computation nodes.

Usage

```
pairwiseEMDDist(
  x,
  rowRange = c(1, nSamples),
  colRange = c(min(rowRange), nSamples),
  loadFlowFrameFUN = NULL,
  loadFlowFrameFUNArgs = NULL,
  channels = NULL,
  verbose = FALSE,
  BPPARAM = BiocParallel::SerialParam(),
  BPOPTIONS = BiocParallel::bpoptions(packages = c("flowCore")),
  binSize = 0.05,
  minRange = -10,
  maxRange = 10
)
```

Arguments

x	either a flowCore::flowSet, or the number of samples (integer >=1)
rowRange	the range of rows of the distance matrix to be calculated
colRange	the range of columns of the distance matrix to be calculated
loadFlowFrameFUN	
	the function used to translate a flowFrame index into a flowFrame. In other words, the function should code how to load a specific flowFrame into memory. Important: the flow Frame index should be the first function argument and should be named ffIndex.
loadFlowFrameFUNArgs	
	(optional) a named list containing additional input parameters of loadFlowFrameFUN()
channels	which channels (integer index(ices) or character(s)):

- if it is a character vector, it can refer to either the channel names, or the marker names
- if it is a numeric vector, it refers to the indexes of channels in `fs`
- if NULL all scatter and fluorescent channels of `fs` #' will be selected

verbose	if TRUE, output a message after each single distance calculation
BPPARAM	sets the BPPARAM back-end to be used for the computation. If not provided, will use `BiocParallel::SerialParam()` (no task parallelization)
BPOPTIONS	sets the BPOPTIONS to be passed to bplapply() function. Note that if you use a `SnowParams` back-end, you need to specify all the packages that need to be loaded for the different CytoProcessingStep to work properly (visibility of functions). As a minimum, the `flowCore` package needs to be loaded. (hence the default BPOPTIONS = bpoptions(packages = c("flowCore")))
binSize	size of equal bins to approximate the marginal distributions.
minRange	minimum value taken when approximating the marginal distributions
maxRange	maximum value taken when approximating the marginal distributions

Value

a distance matrix of pairwise distances (full symmetric with 0. diagonal)

Examples

```
library(CytoPipeline)

data(OMIP021Samples)

# estimate scale transformations
# and transform the whole OMIP021Samples

transList <- estimateScaleTransforms(
    ff = OMIP021Samples[[1]],
    fluoMethod = "estimateLogicle",
    scatterMethod = "linearQuantile",
    scatterRefMarker = "BV785 - CD3")

OMIP021Trans <- CytoPipeline::applyScaleTransforms(
    OMIP021Samples,
    transList)

# calculate pairwise distances using only FSC-A & SSC-A channels
pwDist <- pairwiseEMDDist(
    x = OMIP021Trans,
    channels = c("FSC-A", "SSC-A"))
```

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