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 experimental 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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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 experimental 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.
channelSummaryStats

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See Also

Useful links:

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

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 `flowFrame` 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 transferred 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

```r
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,
  ...
)
```
**computeMetricMDS**

**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**

```r
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", paste("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
EMDDist

Usage

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

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ff1</td>
<td>a flowCore::flowFrame</td>
</tr>
<tr>
<td>ff2</td>
<td>a flowCore::flowFrame</td>
</tr>
</tbody>
</table>
| 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`,
  `plot.title`,
  `plot.subtitle`,
  `plot.xlab`,
  `plot.ylab`,
  `plot.width`,
  `plot.height`,
  `plot.main`,
  `plot.main.args`,
  `plot.theme`,
  `plot.theme.args`,
  `plot.xlim`,
  `plot.ylim`,
  `plot.equal_aspect_ratio`,
  `plot.equal_heights`,
  `plot.equal_widths`,
  `plot.equal_scales`,
  `plot.equal_axes`,
  `plot.equal_legends`,
  `plot.equal_colors`,
  `plot.equal_shapes`,
  `plot.equal_text`,
  `plot.equal_titles`,
  `plot.equal_colors`,
  `plot.equal_shapes`,
  `plot.equal_text`,
  `plot.equal_titles`
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
```


```r
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 R-square projection quality indicator, and the number of dimensions of the MDS projection are provided in sub-title

**Usage**

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

```r
```
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 Pearson correlations w.r.t. projection axes (arrow x & y coordinates)
• if "regression", a linear regression of external variables using the 2 projection axes as explanatory variables is performed, and the projection of external 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 corresponding 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) whichpData 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 added to the ggplot mapping, as to make them available for plotly toolipping. 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

```r
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

```r
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

```r
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

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

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

```r
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
ggplotSampleMDSShepard

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
'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")
Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>mdsObj</td>
<td>a MDS object, output of the <code>computeMetricMDS()</code> method</td>
</tr>
<tr>
<td>extVariableList</td>
<td>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.</td>
</tr>
<tr>
<td>ncol</td>
<td>passed to <code>patchwork::wrap_plots()</code></td>
</tr>
<tr>
<td>nrow</td>
<td>passed to <code>patchwork::wrap_plots()</code></td>
</tr>
<tr>
<td>byrow</td>
<td>passed to <code>patchwork::wrap_plots()</code></td>
</tr>
<tr>
<td>displayLegend</td>
<td>if FALSE, will de-active the legend display</td>
</tr>
<tr>
<td>...</td>
<td>additional parameters passed to <code>ggplotSampleMDS()</code> (if used)</td>
</tr>
</tbody>
</table>

Value

a ggplot object

See Also

`ggplotSampleMDS`, `ggplotSampleMDSShepard`, `computeMetricMDS`

Examples

```r
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,
```
ggplotSampleMDSWrapBiplots

```r
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)

# 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)
```
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 min-
mimization 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**

```r
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 flowFrame 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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