Package 'CytoSimplex'

Type Package

Title Simplex Visualization of Cell Fate Similarity in Single-Cell
Data

Version 0.1.1

Maintainer Yichen Wang <wayichen@umich.edu>

Description Create simplex plots to visualize the similarity between
single-cells and selected clusters in a 1-/2-/3-simplex space.
Velocity information can be added as an additional layer.
See Liu J, Wang Y et al (2023) <doi:10.1101/2023.12.07.570655> for more details.

URL https://welch-lab.github.io/CytoSimplex/,
https://github.com/welch-lab/CytoSimplex

License GPL-3

Encoding UTF-8

LazyData true

Depends stats, methods, R (>= 3.6)

LinkingTo Rcpp, RcppArmadillo

biocViews

Imports ggplot2, Matrix, plot3D, Rcpp, rlang,

Suggests knitr, magick, patchwork, rgl, rmarkdown, Seurat,
SeuratObject, SingleCellExperiment, SummarizedExperiment,
testthat (>= 3.0.0)

RoxygenNote 7.2.3

Config/testthat/edition 3

VignetteBuilder knitr

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R **topics documented:**

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colNormalize   *Normalize each column of the input matrix by the column sum*

Description

Normalize each column of the input matrix by the column sum

Usage

```
colNormalize(x, scaleFactor = NULL, log = FALSE, ...)

## Default S3 method:
colNormalize(x, scaleFactor = NULL, log = FALSE, ...)

## S3 method for class 'dgCMatrix'
colNormalize(x, scaleFactor = NULL, log = FALSE, ...)

## S3 method for class 'Seurat'
colNormalize(
  x,
  scaleFactor = NULL,
  log = FALSE,
  assay = NULL,
  layer = "counts",
  ...
)

## S3 method for class 'SingleCellExperiment'
colNormalize(x, scaleFactor = NULL, log = FALSE, assay.type = "counts", ...)
```

Arguments

x	Feature by observation matrix. Alternatively, `Seurat` object or `SingleCellExperiment` object with raw counts available are also supported.
scaleFactor	Multiplier on normalized data. Default `NULL`.
log	Logical. Whether to take log1p transformation after scaling. Default `FALSE`
...	Additional arguments passed to methods
assay	For "Seurat" method, the specific assay to get data from. Default `NULL` to the default assay.
layer	For "Seurat" method, which layer of the assay to be used. Default `"counts"`.
assay.type	For "SingleCellExperiment" method, the assay type to get data from. Default `"counts"`.

Value

Normalized matrix of the same size

A Seurat object with normalized data in the specified slot of the specified assay.

A SingleCellExperiment object with normalized data in the specified assay. `"normcounts"` if `log = FALSE` and `"logcounts"` if `log = TRUE`.

Examples

```
rnaNorm <- colNormalize(rnaRaw)

# Seurat example
library(Seurat)
srt <- CreateSeuratObject(rnaRaw)
srt <- colNormalize(srt)


# SingleCellExperiment example
library(SingleCellExperiment)
sce <- SingleCellExperiment(assays = list(counts = rnaRaw))
sce <- colNormalize(sce)
```

---

plotBinary *Create binary plots*

Description

Create binary plots that show similarity between single cells and two selected terminals in a barycentric coordinate. The two vertices are placed at the left and right of a 2D plot where x-axis measures the similarity. Y-axis is jittered for a clear view. A density (histogram) curve is added for indicating the distribution.

See plotTernary manual for more details.

Usage

```
plotBinary(x, ...)

## Default S3 method:
plotBinary(
  x,
  clusterVar,
  vertices,
  features = NULL,
  byCluster = NULL,
  processed = FALSE,
  method = c("euclidean", "cosine", "pearson", "spearman"),
  force = FALSE,
  sigma = 0.08,
  scale = TRUE,
  dotColor = "grey60",
  returnData = FALSE,
  ...
)

## S3 method for class 'Seurat'
plotBinary(
  x,
  layer = "counts",
  assay = NULL,
  clusterVar = NULL,
  processed = FALSE,
  ...
)

## S3 method for class 'SingleCellExperiment'
plotBinary(x, assay.type = "counts", clusterVar = NULL, processed = FALSE, ...)

## S3 method for class 'simMat'
plotBinary(
  x,
  dotSize = 0.6,
  dotColor = "grey60",
  densLinewidth = 0.8,
  labelColors = c("#3B4992FF", "#EE0000FF"),
  title = NULL,
  ...
)
```

Arguments

x   Input data. Can be a `matrix` or `dgCMatrix` object with cells as columns, a
            `Seurat` or `SingleCellExperiment` object. "simMat" method takes intermedi-

	ate values.
`...`	Arguments passed to other methods.
`clusterVar`	A vector/factor assigning the cluster variable to each column of the matrix object. For "Seurat" method, NULL (default) for `Idents(x)`, or a variable name in `meta.data` slot. For "SingleCellExperiment" method, NULL (default) for `colLabels(x)`, or a variable name in `colData` slot.
`vertices`	Vector of three unique cluster names that will be used for plotting. Or a named list that groups clusters as three terminal vertices. There must not be any overlap between groups.
`features`	Valid matrix row subsetting index to select features for similarity calculation. Default NULL uses all available features.
`byCluster`	Default NULL to generate one plot with all cells. Set `"all"` to split cells in plot by cluster and returns a list of subplots for each cluster as well as the plot including all cells. Otherwise, a vector of cluster names to generate a list of subplots for the specified clusters.
`processed`	Logical. Whether the input matrix is already processed. TRUE will bypass internal preprocessing and input matrix will be directly used for similarity calculation. Default FALSE and raw count input is recommended. If missing in call, using `slot = "counts"` in "Seurat" method or using `assay.type = "counts"` in "SingleCellExperiment" method will force this argument to be FALSE and others for TRUE.
`method`	Similarity calculation method. Default `"euclidean"`. Choose from `"euclidean"`, `"cosine"`, `"pearson"`, `"spearman"`.
`force`	Whether to force calculate the similarity when more then 500 features are detected, which is generally not recommended. Default FALSE.
`sigma`	Gaussian kernel parameter that controls the effect of variance. Only effective when using a distance metric (i.e. `method` is `"euclidian"` or `"cosine"`). Larger value tighten the dot spreading on figure. Default `0.08`.
`scale`	Whether to min-max scale the distance matrix by clusters. Default TRUE.
`returnData`	Logical. Whether to return similarity data instead of generating plot. Default FALSE.
`layer`	For "Seurat" method, which layer of the assay to be used. Default `"counts"`.
`assay`	For "Seurat" method, the specific assay to get data from. Default NULL to the default assay.
`assay.type`	For "SingleCellExperiment" methods. Which assay to use for calculating the similarity. Default `"counts"`.
`dotSize, dotColor`	
	Dot aesthetics passed to [`geom_point`](). Default `0.6` and `"grey60"`.
`densLinewidth`	Density plot line aesthetic. Default `0.8`.
`labelColors`	Color of the axis lines and vertex labels. Default `c("#3B4992FF", "#EE0000FF")` (blue and red).
`title`	Title text of the plot. Default NULL.

Value

For 'simMat' method, a ggplot object. For other methods, a ggplot object when `splitCluster =` `FALSE`, or a list of ggplot objects when `splitCluster = TRUE`.

Examples

```
gene <- selectTopFeatures(rnaRaw, rnaCluster, c("RE", "OS"))
plotBinary(rnaRaw, rnaCluster, c("RE", "OS"), gene)

# Seurat example
library(Seurat)
srt <- CreateSeuratObject(rnaRaw)
Idents(srt) <- rnaCluster
gene <- selectTopFeatures(srt, vertices = c("OS", "RE"))
plotBinary(srt, features = gene, vertices = c("OS", "RE"))


# SingleCellExperiment example
library(SingleCellExperiment)
sce <- SingleCellExperiment(assays = list(counts = rnaRaw))
colLabels(sce) <- rnaCluster
gene <- selectTopFeatures(sce, vertices = c("OS", "RE"))
plotBinary(sce, features = gene, vertices = c("OS", "RE"))
```

---

 plotQuaternary   *Create quaternary simplex plots*

Description

Create quaternary plots that show similarity between single cells and selected four terminals in a baricentric coordinate.

See `plotTernary` for more details on methodologies.

A dynamic rotating view in a GIF image file can be created with `writeQuaternaryGIF`. Package `magick` must be installed in advance. Linux users may refer to this installation guide.

Usage

```
plotQuaternary(x, ...)

## Default S3 method:
plotQuaternary(
  x,
  clusterVar,
  vertices,
  features = NULL,
  veloGraph = NULL,
```

```
    byCluster = NULL,
    processed = FALSE,
    method = c("euclidean", "cosine", "pearson", "spearman"),
    force = FALSE,
    sigma = 0.05,
    scale = TRUE,
    dotColor = "grey60",
    returnData = FALSE,
    ...
)

## S3 method for class 'Seurat'
plotQuaternary(
    x,
    layer = "counts",
    assay = NULL,
    clusterVar = NULL,
    processed = FALSE,
    ...
)

## S3 method for class 'SingleCellExperiment'
plotQuaternary(
    x,
    assay.type = "counts",
    clusterVar = NULL,
    processed = FALSE,
    ...
)

## S3 method for class 'simMat'
plotQuaternary(
    x,
    veloMat = NULL,
    nGrid = 10,
    radius = 0.2,
    dotSize = 0.6,
    dotColor = "grey60",
    labelColors = c("#3B4992FF", "#EE0000FF", "#008B45FF", "#631879FF"),
    arrowLinewidth = 0.6,
    arrowAngle = 20,
    arrowLen = 0.1,
    vertexLabelSize = 1,
    edgeLinewidth = 1,
    title = NULL,
    titleSize = 1,
    titleColor = "black",
    theta = 20,
```

```
    phi = 0,
    interactive = FALSE,
    ...
)
```

Arguments

x	Input data. Can be a `matrix` or `dgCMatrix` object with cells as columns, a `Seurat` or `SingleCellExperiment` object. "simMat" method takes intermediate values.
...	Arguments passed to other methods.
clusterVar	A vector/factor assigning the cluster variable to each column of the matrix object. For "Seurat" method, `NULL` (default) for `Idents(x)`, or a variable name in `meta.data` slot. For "SingleCellExperiment" method, `NULL` (default) for `colLabels(x)`, or a variable name in `colData` slot.
vertices	Vector of three unique cluster names that will be used for plotting. Or a named list that groups clusters as three terminal vertices. There must not be any overlap between groups.
features	Valid matrix row subsetting index to select features for similarity calculation. Default `NULL` uses all available features.
veloGraph	Cell x cell `dgCMatrix` object containing velocity information. Shows velocity grid-arrow layer when specified. Default `NULL` does not show velocity.
byCluster	Default `NULL` to generate one plot with all cells. Set `"all"` to split cells in plot by cluster and returns a list of subplots for each cluster as well as the plot including all cells. Otherwise, a vector of cluster names to generate a list of subplots for the specified clusters.
processed	Logical. Whether the input matrix is already processed. `TRUE` will bypass internal preprocessing and input matrix will be directly used for similarity calculation. Default `FALSE` and raw count input is recommended. If missing in call, using `slot = "counts"` in "Seurat" method or using `assay.type = "counts"` in "SingleCellExperiment" method will force this argument to be `FALSE` and others for `TRUE`.
method	Similarity calculation method. Default `"euclidean"`. Choose from `"euclidean"`, `"cosine"`, `"pearson"`, `"spearman"`.
force	Whether to force calculate the similarity when more then 500 features are detected, which is generally not recommended. Default `FALSE`.
sigma	Gaussian kernel parameter that controls the effect of variance. Only effective when using a distance metric (i.e. `method` is `"euclidian"` or `"cosine"`). Larger values tighten the dot spreading on figure. Default `0.05`.
scale	Whether to min-max scale the distance matrix by clusters. Default `TRUE`.
returnData	Logical. Whether to return similarity and aggregated velocity data if applicable instead of generating plot. Default `FALSE`.
layer	For "Seurat" method, which layer of the assay to be used. Default `"counts"`.
assay	For "Seurat" method, the specific assay to get data from. Default `NULL` to the default assay.

`assay.type`	For "SingleCellExperiment" methods. Which assay to use for calculating the similarity. Default `"counts"`.
`veloMat`	Aggregated velocity matrix. Output of `aggrVeloGraph`.
`nGrid`	Number of grids along the x-axis of the tetrahedron triangle. Default `10`.
`radius`	Arrow length of unit velocity. Lower this when arrows point outside of the tetrahedron. Default `0.2`.
`dotSize, dotColor`	
	Dot aesthetics. Default `0.6` and `"grey60"`.
`labelColors`	Colors of the vertex labels. Default `c("#3B4992FF", "#EE0000FF", "#008B45FF", "#631879FF")` (blue, red, green and purple).
`arrowLinewidth`	Arrow aesthetics. Default `0.6`.
`arrowAngle, arrowLen`	
	Arrow aesthetics passed to `grid::`[`arrow`](#). The length of the arrow will be internally converted to unit onject in inches. Default `20` and `0.1`.
`vertexLabelSize`	
	Numeric, size of vertex text label relative to default size. Default `1`.
`edgeLinewidth`	Controls the linewidth of the edges of the tetrahedron. Default `1`.
`title`	Title text of the plot. Default `NULL`.
`titleSize, titleColor`	
	Setting on the main title text. Default `1`, and `"black"`.
`theta, phi`	Numeric scalar. The angles defining the viewing direction. `theta` gives the azimuthal direction and `phi` the colatitude. Default `20` and `0`.
`interactive`	Logical. Whether to use "rgl" library to create interactive device. Default `FALSE`.

Value

For "simMat" method, a "plist" (plot3D package product) object. For other methods, a "plist" object when splitCluster = FALSE, or a list of "plist" objects when splitCluster = TRUE. A "plist" object can be viewed with `print()`, `show()` or a direct run of the object variable name in interactive console.

Examples

```
gene <- selectTopFeatures(rnaRaw, rnaCluster, c("RE", "OS", "CH", "ORT"))
plotQuaternary(rnaRaw, rnaCluster, c("RE", "OS", "CH", "ORT"), gene)

# Seurat example
library(Seurat)
srt <- CreateSeuratObject(rnaRaw)
Idents(srt) <- rnaCluster
gene <- selectTopFeatures(srt, vertices = c("OS", "RE", "CH", "ORT"))
plotQuaternary(srt, features = gene,
               vertices = c("OS", "RE", "CH", "ORT"))


# SingleCellExperiment example
library(SingleCellExperiment)
```

```
sce <- SingleCellExperiment(assays = list(counts = rnaRaw))
colLabels(sce) <- rnaCluster
gene <- selectTopFeatures(sce, vertices = c("OS", "RE", "CH", "ORT"))
plotQuaternary(sce, features = gene,
                vertices = c("OS", "RE", "CH", "ORT"))
```

---

plotTernary   *Create ternary plots*

Description

Create ternary plots that show similarity between single cells and selected three terminals in a
ternary baricentric coordinate.

Usage

```
plotTernary(x, ...)

## Default S3 method:
plotTernary(
  x,
  clusterVar,
  vertices,
  features = NULL,
  veloGraph = NULL,
  byCluster = NULL,
  processed = FALSE,
  method = c("euclidean", "cosine", "pearson", "spearman"),
  force = FALSE,
  sigma = 0.08,
  scale = TRUE,
  dotColor = "grey60",
  returnData = FALSE,
  ...
)

## S3 method for class 'Seurat'
plotTernary(
  x,
  layer = "counts",
  assay = NULL,
  clusterVar = NULL,
  processed = FALSE,
  ...
)
```

```
## S3 method for class 'SingleCellExperiment'
plotTernary(
  x,
  assay.type = "counts",
  clusterVar = NULL,
  processed = FALSE,
  ...
)

## S3 method for class 'simMat'
plotTernary(
  x,
  title = NULL,
  veloMat = NULL,
  nGrid = 10,
  radius = 0.1,
  dotSize = 0.6,
  dotColor = "grey60",
  labelColors = c("#3B4992FF", "#EE0000FF", "#008B45FF"),
  vertexLabelSize = 5,
  vertexLabelDrift = 0.03,
  axisBreak = 5,
  axisTextShow = TRUE,
  axisTextSize = 4,
  axisTextDrift = 0.02,
  gridLineAlpha = 0.6,
  arrowLinewidth = 0.25,
  arrowAngle = 20,
  arrowLen = 0.2,
  titleSize = 14,
  equilateral = TRUE,
  margin = 0.1,
  ...
)
```

Arguments

x	Input data. Can be a matrix or dgCMatrix object with cells as columns, a Seurat or SingleCellExperiment object. "simMat" method takes intermediate values.
...	Arguments passed to other methods.
clusterVar	A vector/factor assigning the cluster variable to each column of the matrix object. For "Seurat" method, NULL (default) for Idents(x), or a variable name in meta.data slot. For "SingleCellExperiment" method, NULL (default) for colLabels(x), or a variable name in colData slot.
vertices	Vector of three unique cluster names that will be used for plotting. Or a named list that groups clusters as three terminal vertices. There must not be any overlap between groups.

features	Valid matrix row subsetting index to select features for similarity calculation. Default NULL uses all available features.
veloGraph	Cell x cell dgCMatrix object containing velocity information. Shows velocity grid-arrow layer when specified. Default NULL does not show velocity.
byCluster	Default NULL to generate one plot with all cells. Set "all" to split cells in plot by cluster and returns a list of subplots for each cluster as well as the plot including all cells. Otherwise, a vector of cluster names to generate a list of subplots for the specified clusters.
processed	Logical. Whether the input matrix is already processed. TRUE will bypass internal preprocessing and input matrix will be directly used for similarity calculation. Default FALSE and raw count input is recommended. If missing in call, using slot = "counts" in "Seurat" method or using assay.type = "counts" in "SingleCellExperiment" method will force this argument to be FALSE and others for TRUE.
method	Similarity calculation method. Default "euclidean". Choose from "euclidean", "cosine", "pearson", "spearman".
force	Whether to force calculate the similarity when more then 500 features are detected, which is generally not recommended. Default FALSE.
sigma	Gaussian kernel parameter that controls the effect of variance. Only effective when using a distance metric (i.e. method is "euclidian" or "cosine"). Larger values tighten the dot spreading on figure. Default 0.08.
scale	Whether to min-max scale the distance matrix by clusters. Default TRUE.
returnData	Logical. Whether to return similarity and aggregated velocity data if applicable instead of generating plot. Default FALSE.
layer	For "Seurat" method, which layer of the assay to be used. Default "counts".
assay	For "Seurat" method, the specific assay to get data from. Default NULL to the default assay.
assay.type	For "SingleCellExperiment" methods. Which assay to use for calculating the similarity. Default "counts".
title	Title text of the plot. Default NULL.
veloMat	Aggregated velocity matrix. Output of aggrVeloGraph.
nGrid	Number of grids along the bottom side of the equilateral triangle. Default 10.
radius	Arrow length of unit velocity. Lower this when arrows point outside of the coordinate. Default 0.1.
dotSize, dotColor	
	Dot aesthetics passed to [geom_point](). Default 0.6 and "grey60".
labelColors	Colors of the axis lines and vertex labels. Default c("#3B4992FF", "#EE0000FF", "#008B45FF") (blue, red and green)
vertexLabelSize, vertexLabelDrift	
	Adjustment on the three vertex text labels. Drift means the distance that the labels should be moved against the center of the plot. Default size 5, drifted distance 0.03.
axisBreak	Number of breaks to be labeled along axis. Default 5.

axisTextShow	Logical, whether to show axis text. Default TRUE.
axisTextSize, axisTextDrift	
	Similar to the vertex adjustment applied to the text label along the axis breaks. Default size 4, drifted distance 0.02.
gridLineAlpha	Transparency of background grid lines. Default 0.6.
arrowLinewidth, arrowAngle, arrowLen	
	Arrow aesthetics, see Details.
titleSize	Size of title text. Default 14.
equilateral	Logical, whether to always display the triangle as equilateral. Default TRUE.
margin	Margin allowed around of the triangle plotting region when equilateral = TRUE

Details

Argument inheritance - For matrix/dgCMatrix ("default" method), we first calculate the similarity matrix and obtain a "simMat" object. Then the "simMat" method is internally called. For data container objects (e.g. Seurat), we obtain the correct data matrix first and then call the "default" method. The arguments inherits as the flow described above.

The calculation of similarity matrix - The similarity is calculated either by converting a distance metric ("euclidean" or "cosine") with Gaussian kernel, or directly computed with correlation metrics ("pearson" or "spearman"). The centroid of each terminal is obtained first, and the specified metric from each cell to each terminal is calculated. The similarity matrix (n cells by v terminals) is lastly normalized to sum to 1 for each cell, so it becomes a baricentric coordinate.

Arrow aesthetics parameters - The shape of arrows is controlled by 3 arguments. Considering an arrow as the combination of a line segment and a triangle, arrowLinewidth controls the width of the line as well as the edge line of the triangle; arrowAngle equals to angle of the arrow-tip vertex of the triangle devided by 2 (e.g. the triangle is equilateral when arrowAngle = 20); arrowLen controls the absolute length from the arrow-tip vertex to its opposite edge.

Value

For "simMat" method, a ggplot object. For other methods, a ggplot object when splitCluster = FALSE, or a list of ggplot objects when splitCluster = TRUE.

Examples

```
gene <- selectTopFeatures(rnaRaw, rnaCluster, c("OS", "RE", "CH"))
plotTernary(rnaRaw, rnaCluster, c("OS", "RE", "CH"), gene)

# Seurat example
library(Seurat)
srt <- CreateSeuratObject(rnaRaw)
Idents(srt) <- rnaCluster
gene <- selectTopFeatures(srt, vertices = c("OS", "RE", "CH"))
plotTernary(srt, features = gene, vertices = c("OS", "RE", "CH"))


# SingleCellExperiment example
```

```
library(SingleCellExperiment)
sce <- SingleCellExperiment(assays = list(counts = rnaRaw))
colLabels(sce) <- rnaCluster
gene <- selectTopFeatures(sce, vertices = c("OS", "RE", "CH"))
plotTernary(sce, features = gene, vertices = c("OS", "RE", "CH"))
```

---

rnaCluster   *Major cell type annotation of the example mouse bone marrow data*

Description

Major cell type annotation of the example mouse bone marrow data

Usage

```
rnaCluster
```

Format

`factor` object

Source

https://www.nature.com/articles/s41467-023-38034-2

References

Matsushita, Y., Liu, J., Chu, A.K.Y. et al. Bone marrow endosteal stem cells dictate active osteogenesis and aggressive tumorigenesis. Nat Commun 14, 2383 (2023).

---

rnaRaw   *Mouse bone marrow scRNAseq example data*

Description

Mouse bone marrow scRNAseq example data

Usage

```
rnaRaw
```

Format

[dgCMatrix](#) object

Source

https://www.nature.com/articles/s41467-023-38034-2

References

Matsushita, Y., Liu, J., Chu, A.K.Y. et al. Bone marrow endosteal stem cells dictate active osteogenesis and aggressive tumorigenesis. Nat Commun 14, 2383 (2023).

---

rnaVelo   *Velocity graph of the example mouse bone marrow data*

Description

Velocity graph of the example mouse bone marrow data

Usage

```
rnaVelo
```

Format

[dgCMatrix](#) object

Source

https://www.nature.com/articles/s41467-023-38034-2

References

Matsushita, Y., Liu, J., Chu, A.K.Y. et al. Bone marrow endosteal stem cells dictate active osteogenesis and aggressive tumorigenesis. Nat Commun 14, 2383 (2023).

---

selectTopFeatures   *Pick top differentially presented features for similarity calculation*

Description

Performs wilcoxon rank-sum test on input matrix. While `clusterVar` and `vertices` together defines the groups of cells to be set as terminals of the simplex, this function will test each of these groups against the rest of the cells. The U-Statistics (`statistic`), p-value (`pval`) and adjusted p-value (`padj`), together with average presence in group (`avgExpr`), log fold-change (`logFC`), AUC (`auc`), percentage in group (`pct_in`) and percentage out of group (`pct_out`) will be calculated. Set `returnStats = TRUE` to return the full statistics table.

Top features are selected by sorting primarily on adjusted p-value, and secondarily on log fold-change, after filtering for up-regulated features.

Usage

```
selectTopFeatures(x, clusterVar, vertices, ...)

## Default S3 method:
selectTopFeatures(
  x,
  clusterVar,
  vertices,
  nTop = 30,
  processed = FALSE,
  lfcThresh = 0.1,
  returnStats = FALSE,
  ...
)

## S3 method for class 'Seurat'
selectTopFeatures(
  x,
  clusterVar = NULL,
  vertices,
  assay = NULL,
  layer = "counts",
  processed = FALSE,
  ...
)

## S3 method for class 'SingleCellExperiment'
selectTopFeatures(
  x,
  clusterVar = NULL,
  vertices,
  assay.type = "counts",
  processed = FALSE,
  ...
)
```

Arguments

x	Dense or sparse matrix, observation per column. Preferrably a raw count matrix. Alternatively, a Seurat object or a SingleCellExperiment object.
clusterVar	A vector/factor assigning the cluster variable to each column of the matrix object. For "Seurat" method, NULL (default) for Idents(x), or a variable name in meta.data slot. For "SingleCellExperiment" method, NULL (default) for colLabels(x), or a variable name in colData slot.
vertices	Vector of cluster names that will be used for plotting. Or a named list that groups clusters as a terminal vertex. There must not be any overlap between groups.
...	Arguments passed to methods.

nTop	Number of top differentially presented features per terminal. Default 30.
processed	Logical. Whether the input matrix is already processed. TRUE will bypass internal preprocessing and input matrix will be directly used for rank-sum calculation. Default FALSE and raw count input is recommended.
lfcThresh	Threshold on log fold-change to identify up-regulated features. Default 0.1.
returnStats	Logical. Whether to return the full statistics table rather then returning the selected genes. Default FALSE
assay	Assay name of the Seurat object to be used. Default NULL.
layer	For "Seurat" method, which layer of the assay to be used. Default "counts".
assay.type	Assay name of the SingleCellExperiment object to be used. Default "counts".

Value

When returnStats = FALSE (default), a character vector of at most length(unique(vertices))*nTop feature names. When returnStats = TRUE, a data.frame of wilcoxon rank sum test statistics.

Examples

```
selectTopFeatures(rnaRaw, rnaCluster, c("OS", "RE"))

# Seurat example
library(Seurat)
srt <- CreateSeuratObject(rnaRaw)
Idents(srt) <- rnaCluster
gene <- selectTopFeatures(srt, vertices = c("OS", "RE"))


# SingleCellExperiment example
library(SingleCellExperiment)
sce <- SingleCellExperiment(assays = list(counts = rnaRaw))
colLabels(sce) <- rnaCluster
gene <- selectTopFeatures(sce, vertices = c("OS", "RE"))
```

---

show,plist-method   *Show plist object produced with plot3D package*

Description

Show plist object produced with plot3D package

Usage

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

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

Arguments

`object, x`	plist object
`...`	Graphic parameters passed to [plot](). mar is pre-specified.

Value

No return value. It displays the plot described in a 'plist' object returned by [plotQuaternary](), internally created by package 'plot3D'.

Examples

```
gene <- selectTopFeatures(rnaRaw, rnaCluster, c("RE", "OS", "CH", "ORT"))
plistObj <- plotQuaternary(rnaRaw, rnaCluster, c("RE", "OS", "CH", "ORT"), gene)
print(plistObj)
# equivalent to
show(plistObj)
```

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writeQuaternaryGIF	*Create GIF image for dynamic rotating view of 3D quaternary simplex plot*

Description

Create GIF image for dynamic rotating view of 3D quaternary simplex plot

Usage

```
writeQuaternaryGIF(
  x,
  ...,
  cluster = NULL,
  gifPath = "quaternary.gif",
  tmpDir = tempdir(),
  fps = 10,
  degreePerFrame = 10
)
```

Arguments

`x`	Input object that [plotQuaternary]() accepts.
`...`	All other arguments needed for [plotQuaternary](). Must be specified with exact argument names instead of a positional manner.
`cluster`	One cluster that exists in `clusterVar`, if users need to view the plot for specific group. Default `NULL` plot all cells.
`gifPath`	Output GIF image file path. Default `"quaternary.gif"`

tmpDir	A temprorary directory to store all PNG files for all perspectives created. Default `tempdir()`.
fps	Number of frame per second, must be a factor of 100. Default `10`.
degreePerFrame	Number of degree that the tetrahedron is rotated per frame. Default `10`.

Value

No object is returned. The `tmpDir` folder will be created with `360 / degreePerFrame` PNG image files in it. A GIF image file will be created at `gifPath`.

Examples

```
gene <- selectTopFeatures(rnaRaw, rnaCluster, c("RE", "OS", "CH", "ORT"))

writeQuaternaryGIF(rnaRaw, clusterVar = rnaCluster, features = gene,
                   vertices = c("RE", "OS", "CH", "ORT"),
                   gifPath = paste0(tempfile(), ".gif"))
```

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