Package ‘spicyR’

May 4, 2024

Type Package

Title Spatial analysis of in situ cytometry data

Version 1.16.0

Description The spicyR package provides a framework for performing inference on changes in spatial relationships between pairs of cell types for cell-resolution spatial omics technologies. spicyR consists of three primary steps: (i) summarizing the degree of spatial localization between pairs of cell types for each image; (ii) modelling the variability in localization summary statistics as a function of cell counts and (iii) testing for changes in spatial localizations associated with a response variable.

License GPL (>=2)

LazyData true

biocViews SingleCell, CellBasedAssays, Spatial

Encoding UTF-8

Depends R (>= 4.1)

VignetteBuilder knitr

BugReports https://github.com/SydneyBioX/spicyR/issues

URL https://ellispatrick.github.io/spicyR/

https://github.com/SydneyBioX/spicyR

Imports ggplot2, concaveman, BiocParallel, spatstat.explore, spatstat.geom, lmerTest, BiocGenerics, S4Vectors, methods, mgcv, pheatmap, rlang, grDevices, IRanges, stats, data.table, dplyr, tidyr, scam, SingleCellExperiment, SpatialExperiment, SummarizedExperiment, ggforce, ClassifyR, tibble

Suggests BiocStyle, knitr, rmarkdown, pkgdown, imcRtools

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Accessors

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<tbody>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Description

Methods to access various components of the ‘SegmentedCells’ object.

Usage

cellSummary(x, imageID = NULL, bind = TRUE)
cellSummary(x, imageID = NULL) <- value
cellMarks(x, imageID = NULL, bind = TRUE)
cellMarks(x, imageID = NULL) <- value
cellMorph(x, imageID = NULL, bind = TRUE)
Accessors

\[
\begin{align*}
\text{cellMorph}(x, \text{imageID} = \text{NULL}) & \leftarrow \text{value} \\
\text{imagePheno}(x, \text{imageID} = \text{NULL}, \text{bind} = \text{TRUE}, \text{expand} = \text{FALSE}) & \\
\text{imagePheno}(x, \text{imageID} = \text{NULL}) & \leftarrow \text{value} \\
\text{imageID}(x, \text{imageID} = \text{NULL}) & \\
\text{cellID}(x, \text{imageID} = \text{NULL}) & \\
\text{cellID}(x) & \leftarrow \text{value} \\
\text{imageCellID}(x, \text{imageID} = \text{NULL}) & \\
\text{imageCellID}(x) & \leftarrow \text{value} \\
\text{cellType}(x, \text{imageID} = \text{NULL}) & \\
\text{cellType}(x, \text{imageID} = \text{NULL}) & \leftarrow \text{value} \\
\text{filterCells}(x, \text{select}) & \\
\text{cellAnnotation}(x, \text{variable}, \text{imageID} = \text{NULL}) & \\
\text{cellAnnotation}(x, \text{variable}, \text{imageID} = \text{NULL}) & \leftarrow \text{value}
\end{align*}
\]

Arguments

- **x**: A ‘SegmentedCells’ object.
- **imageID**: A vector of imageIDs to specifically extract.
- **bind**: When false outputs a list of DataFrames split by imageID
- **expand**: Used to expand the phenotype information from per image to per cell.
- **value**: The relevant information used to replace.
- **select**: A logical vector of the cells to be kept.
- **variable**: A variable to add or retrieve from cellSummary.

Value

DataFrame or a list of DataFrames

Descriptions

- **‘cellSummary’**: Retrieves the DataFrame containing ‘x’ and ‘y’ coordinates of each cell as well as ‘cellID’, ‘imageID’ and ‘cellType’. imageID can be used to select specific images and bind=FALSE outputs the information as a list split by imageID.
- **‘cellMorph’**: Retrieves the DataFrame containing morphology information.
‘cellMarks‘: Retrieves the DataFrame containing intensity of gene or protein markers.
‘imagePheno‘: Retrieves the DataFrame containing the phenotype information for each image.
  Using expand = TRUE will produce a DataFrame with the number of rows equal to the number of cells.

Examples

```r
### Something that resembles cellProfiler data

set.seed(51773)

n = 10

cells <- data.frame(row.names = seq_len(n))
cells$ObjectNumber <- seq_len(n)
cells$ImageNumber <- rep(1:2,c(n/2,n/2))
cells$AreaShape_Center_X <- runif(n)
cells$AreaShape_Center_Y <- runif(n)
cells$AreaShape_round <- rexp(n)
cells$AreaShape_diameter <- rexp(n, 2)
cells$Intensity_Mean_CD8 <- rexp(n, 10)
cells$Intensity_Mean_CD4 <- rexp(n, 10)

cellExp <- SegmentedCells(cells, cellProfiler = TRUE)

### Cluster cell types

intensities <- cellMarks(cellExp)
km <- kmeans(intensities, 2)
cellType(cellExp) <- paste('cluster', km$cluster, sep = '')

cellSummary(cellExp, imageID = 1)
```

as.data.frame.SegmentedCells

as.data.frame

Description

Function to coerce a SegmentedCells object to a data frame.

Usage

```
## S3 method for class 'SegmentedCells'
as.data.frame(x, ...)
```

Arguments

- **x**: A SegmentedCells object.
- **...**: Other arguments.
## Value

A data.frame

```r
## Generate toy data set.seed(51773) x <- round(c(runif(200),runif(200)+1,runif(200)+2,runif(200)+3,
runif(200)+3,runif(200)+2,runif(200)+1,runif(200)),4) y <- round(c(runif(200),runif(200)+1,runif(200)+2,runif(200)+3,runif(200),runif(200)+1,runif(200)+2,runif(200)+3,4) cellType <- factor(paste('c',rep(rep(c(1:2),rep(200,2)),4),sep = "")) imageID <- rep(c('s1', 's2'),c(800,800)) cells <- data.frame(x, y, cellType, imageID)

## Store data in SegmentedCells object cellExp <- SegmentedCells(cells, cellTypeString = 'cell-Type')

## Generate LISA cellsDF <- as.data.frame(cellExp)

NULL
```

## Description

Produces a dataframe showing L-function metric for each imageID entry.

## Usage

```r
bind(results, pairName = NULL)
```

## Arguments

- **results**: Spicy test result obtained from spicy.
- **pairName**: A string specifying the pairwise interaction of interest. If NULL, all pairwise interactions are shown.

## Value

A data.frame containing the colData related to the results.

## Examples

```r
data(spicyTest)
df <- bind(spicyTest)
```
### colTest

Perform a simple wilcoxon-rank-sum test or t-test on the columns of a data frame

#### Description

Perform a simple wilcoxon-rank-sum test or t-test on the columns of a data frame

#### Usage

```
colTest(df, condition, type = NULL, feature = NULL, imageID = "imageID")
```

#### Arguments

- **df**: A data.frame or SingleCellExperiment, SpatialExperiment
- **condition**: The condition of interest
- **type**: The type of test, "wilcox", "ttest" or "survival".
- **feature**: Can be used to calculate the proportions of this feature for each image
- **imageID**: The imageID’s if presenting a SingleCellExperiment

#### Value

Proportions

#### Examples

```
# Test for an association with long-duration diabetes
# This is clearly ignoring the repeated measures...
data("diabetesData")
props <- getProp(diabetesData)
condition <- imagePheno(diabetesData)$stage
names(condition) <- imagePheno(diabetesData)$imageID
condition <- condition[condition %in% c("Long-duration", "Onset")]
test <- colTest(props[names(condition), ], condition)
```

### convPairs

Converts colPairs object into an abundance matrix based on number of nearby interactions for every cell type.

#### Description

Converts colPairs object into an abundance matrix based on number of nearby interactions for every cell type.
**Usage**

```r
convPairs(cells, colPair, cellType = "cellType", imageID = "imageID")
```

**Arguments**

- `cells` A `SingleCellExperiment` that contains objects in the `colPairs` slot.
- `colPair` The name of the object in the `colPairs` slot for which the dataframe is constructed from.
- `cellType` The cell type if using `SingleCellExperiment`.
- `imageID` The image ID if using `SingleCellExperiment`.

**Value**

Matrix of abundances

**Examples**

```r
data("diabetesData_SCE")
diabetesData_SPE <- SpatialExperiment::SpatialExperiment(diabetesData_SCE, colData = SingleCellExperiment::colData(diabetesData_SCE))
SpatialExperiment::spatialCoords(diabetesData_SPE) <- data.frame(
  SingleCellExperiment::colData(diabetesData_SPE)$x, SingleCellExperiment::colData(diabetesData_SPE)$y) |> as.matrix()
SpatialExperiment::spatialCoordsNames(diabetesData_SPE) <- c("x", "y")
diabetesData_SPE <- imcRtools::buildSpatialGraph(diabetesData_SPE, img_id = "imageID", type = "knn", k = 20, coords = c("x", "y"))
pairAbundances <- convPairs(diabetesData_SPE, colPair = "knn_interaction_graph")
```

---

**diabetesData**

*Diabetes IMC data*

**Description**

This is a subset of the Damond et al 2019 imaging mass cytometry dataset. The data contains cells in the pancreatic islets of individuals with early onset diabetes and healthy controls. The object contains single-cell data of 160 images from 8 subjects, with 20 images per subject.
getPairwise

Usage
diabetesData

Format
diabetesData a SegmentedCells object

---
diabetesData_SCE Diabetes IMC data in SCE format.

Description
This is a subset of the Damond et al 2019 imaging mass cytometry dataset. The data contains cells in the pancreatic islets of individuals with early onset diabetes and healthy controls. The object contains single-cell data of 160 images from 8 subjects, with 20 images per subject.

Usage
diabetesData_SCE

Format
diabetesData_SCE a SingleCellExperiment object

Details
Converted into a SingleCellExperiment format.

---
getPairwise Get statistic from pairwise L curve of a single image.

Description
Get statistic from pairwise L curve of a single image.

Usage
getPairwise(
cells,
from = NULL,
to = NULL,
window = "convex",
window.length = NULL,
Rs = c(20, 50, 100),
sigma = NULL,
minLambda = 0.05,
edgeCorrect = TRUE,
includeZeroCells = TRUE,
BPPARAM = BiocParallel::SerialParam(),
imageID = "imageID",
cellType = "cellType",
spatialCoords = c("x", "y")
)

Arguments

cells A SegmentedCells or data frame that contains at least the variables x and y, giving the location coordinates of each cell, and cellType.
from The 'from' cellType for generating the L curve.
to The 'to' cellType for generating the L curve.
window Should the window around the regions be 'square', 'convex' or 'concave'.
window.length A tuning parameter for controlling the level of concavity when estimating concave windows.
Rs A vector of the radii that the measures of association should be calculated.
sigma A numeric variable used for scaling when fitting inhomogeneous L-curves.
minLambda Minimum value for density for scaling when fitting inhomogeneous L-curves.
edgeCorrect A logical indicating whether to perform edge correction.
includeZeroCells A logical indicating whether to include cells with zero counts in the pairwise association calculation.
BPPARAM A BiocParallelParam object.
imageID The imageID if using a SingleCellExperiment or SpatialExperiment.
cellType The cellType if using a SingleCellExperiment or SpatialExperiment.
spatialCoords The spatialCoords if using a SingleCellExperiment or SpatialExperiment.

Value

Statistic from pairwise L curve of a single image.

Examples

data("diabetesData")
pairAssoc <- getPairwise(diabetesData[1, ])

getProp

Get proportions from a SegmentedCells, SingleCellExperiment, SpatialExperiment or data.frame.

Description

Get proportions from a SegmentedCells, SingleCellExperiment, SpatialExperiment or data.frame.

Usage

getProp(cells, feature = "cellType", imageID = "imageID")

Arguments

cells: SegmentedCells, SingleCellExperiment, SpatialExperiment or data.frame
feature: The feature of interest
imageID: The imageID’s

Value

Proportions

Examples

data("diabetesData")
prop <- getProp(diabetesData)

plot,SegmentedCells,ANY-method

A basic plot for SegmentedCells object

Description

This function generates a basic x-y plot of the location coordinates and cellType data.

Usage

## S4 method for signature 'SegmentedCells,ANY'
plot(x, imageID = NULL)

Arguments

x: A SegmentedCells object.
imageID: The image that should be plotted.
The SegmentedCells S4 class is for storing data from segmented imaging cytometry and spatial omics data. It extends DataFrame and defines methods that take advantage of DataFrame nesting to represent elements of cell-based experiments with spatial orientation that are commonly encountered. This object is able to store information on a cell’s spatial location, cellType, morphology, intensity of gene/protein markers as well as image level phenotype information.
Usage

SegmentedCells(
  cellData,
  cellProfiler = FALSE,
  spatialCoords = c("x", "y"),
  cellTypeString = "cellType",
  intensityString = "intensity_",
  morphologyString = "morphology_",
  phenotypeString = "phenotype_",
  cellIDString = "cellID",
  cellAnnotations = NULL,
  imageCellIDString = "imageCellID",
  imageIDString = "imageID",
  verbose = TRUE
)

Arguments

cellData A data frame that contains at least the columns x and y giving the location coordinates of each cell.
cellProfiler A logical indicating that cellData is in a format similar to what cellProfiler outputs.
spatialCoords The column names corresponding to spatial coordinates. eg. x, y, z...
cellTypeString The name of the column that contains cell type calls.
intensityString A string which can be used to identify the columns which contain marker intensities. (This needs to be extended to take the column names themselves.)
morphologyString A string which can be used to identify the columns which contains morphology information.
phenotypeString A string which can be used to identify the columns which contains phenotype information.
cellIDString The column name for cellID.
cellAnnotations A vector of variables that provide additional annotation of a cell.
imageCellIDString The column name for imageCellID.
imageIDString The column name for imageIDString.
verbose logical indicating whether to output messages.

Value

A SegmentedCells object
Examples

```r
### Something that resembles cellProfiler data

set.seed(51773)

n = 10

cells <- data.frame(row.names = seq_len(n))
cells$ObjectNumber <- seq_len(n)
cells$ImageNumber <- rep(seq_len(2),c(n/2,n/2))
cells$AreaShape_Center_X <- runif(n)
cells$AreaShape_Center_Y <- runif(n)
cells$AreaShape_round <- rexp(n)
cells$AreaShape_diameter <- rexp(n, 2)
cells$Intensity_Mean_CD8 <- rexp(n, 10)
cells$Intensity_Mean_CD4 <- rexp(n, 10)

cellExp <- SegmentedCells(cells, cellProfiler = TRUE)

### Cluster cell types
intensities <- cellMarks(cellExp)
kM <- kmeans(intensities,2)
cellType(cellExp) <- paste('cluster',kM$cluster, sep = '')
cellSummary(cellExp)
```

show-SegmentedCells  

**Description**

This outputs critical information about a `SegmentedCells`.

**Arguments**

- **object**: A `SegmentedCells`.

**Value**

Information of the number of images, cells, intensities, morphologies and phenotypes.

**usage**

`'show(object)'`
Examples

### Something that resembles cellProfiler data

```r
set.seed(51773)

n = 10

cells <- data.frame(row.names = seq_len(n))
cells$ObjectNumber <- seq_len(n)
cells$ImageNumber <- rep(1:2, c(n/2, n/2))
cells$AreaShape_Center_X <- runif(n)
cells$AreaShape_Center_Y <- runif(n)
cells$AreaShape_round <- rexp(n)
cells$AreaShape_diameter <- rexp(n, 2)
cells$Intensity_Mean_CD8 <- rexp(n, 10)
cells$Intensity_Mean_CD4 <- rexp(n, 10)

cellExp <- SegmentedCells(cells, cellProfiler = TRUE)

### Cluster cell types
markers <- cellMarks(cellExp)
kM <- kmeans(markers, 2)
cellType(cellExp) <- paste('cluster', kM$cluster, sep = '')

cellExp
```

```r
signifPlot
Plots result of signifPlot.
```

### Description

Plots result of signifPlot.

### Usage

```r
signifPlot(
  results,
  fdr = FALSE,
  type = "bubble",
  breaks = NULL,
  comparisonGroup = NULL,
  colours = c("#4575B4", "white", "#D73027"),
  marksToPlot = NULL,
  cutoff = 0.05
)
```
spicyBoxPlot

**Arguments**

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>results</td>
<td>Data frame obtained from spicy.</td>
</tr>
<tr>
<td>fdr</td>
<td>TRUE if FDR correction is used.</td>
</tr>
<tr>
<td>type</td>
<td>Where to make a bubble plot or heatmap.</td>
</tr>
<tr>
<td>breaks</td>
<td>Vector of 3 numbers giving breaks used in heatmap. The first number is the minimum, the second is the maximum, the third is the number of breaks.</td>
</tr>
<tr>
<td>comparisonGroup</td>
<td>A string specifying the name of the outcome group to compare with the base group.</td>
</tr>
<tr>
<td>colours</td>
<td>Vector of colours to use in heatmap.</td>
</tr>
<tr>
<td>marksToPlot</td>
<td>Vector of marks to include in heatmap.</td>
</tr>
<tr>
<td>cutoff</td>
<td>significance threshold for circles in bubble plot</td>
</tr>
</tbody>
</table>

**Value**

a heatmap object

**Examples**

```r
data(spicyTest)
signifPlot(spicyTest, breaks = c(-3, 3, 0.5))
```

---

**SpicyBoxPlot**

*Plots boxplot for a specified cell-cell relationship*

**Description**

Plots boxplot for a specified cell-cell relationship

**Usage**

```
spicyBoxPlot(results, from = NULL, to = NULL, rank = NULL)
```

**Arguments**

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>results</td>
<td>Data frame obtained from spicy.</td>
</tr>
<tr>
<td>from</td>
<td>Cell type which you would like to compare to the to cell type.</td>
</tr>
<tr>
<td>to</td>
<td>Cell type which you would like to compare to the from cell type.</td>
</tr>
<tr>
<td>rank</td>
<td>Ranking of cell type in terms of p-value, the smaller the p-value the higher the rank.</td>
</tr>
</tbody>
</table>

**Value**

a ggplot2 boxplot
SpicyResults-class

Performs spatial tests on spatial cytometry data.

Description

Performs spatial tests on spatial cytometry data.

Usage

spicy(
cells,  
condition = NULL,  
subject = NULL,  
covariates = NULL,  
from = NULL,  
to = NULL,  
alternateResult = NULL,  
verbose = TRUE,  
weights = TRUE,  
weightsByPair = FALSE,  
weightFactor = 1,  
window = "convex",  
window.length = NULL,  
BPPARAM = BiocParallel::SerialParam(),  
sigma = NULL,  
Rs = NULL,  
minLambda = 0.05,  
edgeCorrect = TRUE,  
includeZeroCells = FALSE,  
imageID = "imageID",  
cellType = "cellType",  
spatialCoords = c("x", "y"),  
...  
)

Arguments

cells A SegmentedCells or data frame that contains at least the variables x and y, giving the location coordinates of each cell, and cellType.
SpicyResults-class

- **condition**: Vector of conditions to be tested corresponding to each image if cells is a data frame.
- **subject**: Vector of subject IDs corresponding to each image if cells is a data frame.
- **covariates**: Vector of covariate names that should be included in the mixed effects model as fixed effects.
- **from**: Vector of cell types which you would like to compare to the to vector.
- **to**: Vector of cell types which you would like to compare to the from vector.
- **alternateResult**: An pairwise association statistic between each combination of cell types in each image.
- **verbose**: Logical indicating whether to output messages.
- **weights**: Logical indicating whether to include weights based on cell counts.
- **weightsByPair**: Logical indicating whether weights should be calculated for each cell type pair.
- **weightFactor**: Numeric that controls the convexity of the weight function.
- **window**: Should the window around the regions be 'square', 'convex' or 'concave'.
- **window.length**: A tuning parameter for controlling the level of concavity when estimating concave windows.
- **BPPARAM**: A BiocParallelParam object.
- **sigma**: A numeric variable used for scaling when fitting inhomogeneous L-curves.
- **Rs**: A vector of radii that the measures of association should be calculated.
- **minLambda**: Minimum value for density for scaling when fitting inhomogeneous L-curves.
- **edgeCorrect**: A logical indicating whether to perform edge correction.
- **includeZeroCells**: A logical indicating whether to include cells with zero counts in the pairwise association calculation.
- **imageID**: The image ID if using SingleCellExperiment.
- **cellType**: The cell type if using SingleCellExperiment.
- **spatialCoords**: The spatial coordinates if using a SingleCellExperiment.
- **...**: Other options.

**Value**

Data frame of p-values.

**Examples**

```r
data("diabetesData")

# Test with random effect for patient on a pairwise combination of cell types.
spicy(diabetesData, 
  condition = "stage", subject = "case", 
  from = "Tc", to = "Th"
)```


# Test all pairwise combinations of cell types without random effect of patient.
# spicyTest <- spicy(diabetesData, condition = "stage", subject = "case")

# Test all pairwise combination of cell types with random effect of patient.
# spicy(diabetesData, condition = "condition", subject = "subject")

---

### spicyTest

**Results from spicy for diabetesData**

<table>
<thead>
<tr>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Results from the call: spicyTest &lt;- spicy(diabetesData, condition = &quot;condition&quot;, subject = &quot;subject&quot;)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Usage</th>
</tr>
</thead>
<tbody>
<tr>
<td>spicyTest</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Format</th>
</tr>
</thead>
<tbody>
<tr>
<td>spicyTest a spicy object</td>
</tr>
</tbody>
</table>

---

### topPairs

**A table of the significant results from spicy tests**

<table>
<thead>
<tr>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A table of the significant results from spicy tests</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Usage</th>
</tr>
</thead>
<tbody>
<tr>
<td>topPairs(x, coef = NULL, n = 10, adj = &quot;fdr&quot;, cutoff = NULL, figures = NULL)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Arguments</th>
</tr>
</thead>
<tbody>
<tr>
<td>x</td>
</tr>
<tr>
<td>coef</td>
</tr>
<tr>
<td>n</td>
</tr>
<tr>
<td>adj</td>
</tr>
<tr>
<td>cutoff</td>
</tr>
<tr>
<td>figures</td>
</tr>
</tbody>
</table>
**topPairs**

**Value**

A data.frame

**Examples**

```r
data(spicyTest)
topPairs(spicyTest)
```
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