

Package ‘SpaceMarkers’

November 7, 2025

Type Package

Title Spatial Interaction Markers

Version 2.1.0

BugReports <https://github.com/DeshpandeLab/SpaceMarkers/issues>

URL <https://github.com/DeshpandeLab/SpaceMarkers>

Description Spatial transcriptomic technologies have helped to resolve the connection between gene expression and the 2D orientation of tissues relative to each other. However, the limited single-cell resolution makes it difficult to highlight the most important molecular interactions in these tissues. SpaceMarkers, R/Bioconductor software, can help to find molecular interactions, by identifying genes associated with latent space interactions in spatial transcriptomics.

Depends R (>= 4.4.0)

biocViews SingleCell, GeneExpression, Software, Spatial,
Transcriptomics

Imports matrixStats, matrixTests, rstatix, spatstat.explore,
spatstat.geom, ape, hdf5r, nanoparquet, jsonlite, Matrix,
qvalue, stats, utils, methods, ggplot2, reshape2, RColorBrewer,
circlize, mixtools, dplyr, readbitmap, rlang, effsize, viridis

Suggests data.table, devtools, knitr, cowplot, rjson, rmarkdown,
BiocStyle, testthat (>= 3.0.0), CoGAPS, ComplexHeatmap

Enhances BiocParallel

VignetteBuilder knitr

Config/testthat/edition 3

Encoding UTF-8

LazyData false

Roxygen list(markdown = TRUE)

RoxygenNote 7.3.3

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

git_branch devel

git_last_commit 2b359d8

git_last_commit_date 2025-10-29

Repository Bioconductor 3.23

Date/Publication 2025-11-06

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<code>.calc_IM_scores</code>	<i>Calculate interaction scores for a specific pattern pair</i>
------------------------------	---

Description

This function calculates interaction scores for a specific pattern pair using the `.classify_spots` function to determine the region of each spot.

Usage

```
.calc_IM_scores(
  data,
  pat_hotspots,
  influence_hotspots,
  patternpair,
  avoid_confounders = FALSE,
  ...
)
```

Arguments

- `data` A numeric matrix with genes as rows and barcodes as columns.
- `pat_hotspots` A data frame with pattern hotspots, containing columns for x, y, and barcode.
- `influence_hotspots` A data frame with influence hotspots, containing columns for x, y, and barcode.
- `patternpair` A character vector of length 2 specifying the pattern pair to analyze.
- `avoid_confounders` Logical (default=FALSE) indicating whether to avoid confounding effects due to colocalization.
- `...` Additional parameters to pass to lower level functions.

Value

A data frame with interaction scores for the specified pattern pair.

<code>.calc_threshold</code>	<i>Compute the threshold for identifying outlier values or hotspots</i>
------------------------------	---

Description

This function computes the threshold for identifying outlier values or hotspots by fitting a normal mixture model to the data.

Usage

```
.calc_threshold(df, minval = 0.01, maxval = 0.99, method = c("abs", "pct"))
```

Arguments

<code>df</code>	A vector containing pattern values
<code>minval</code>	Minimum value for quantile threshold
<code>maxval</code>	Maximum value for quantile threshold
<code>method</code>	Method to use for threshold calculation. Options are "abs" for absolute (default) and "pct" for percentile.

Value

A list containing the computed thresholds

<code>.find_genes_of_interest</code>	<i>.find_genes_of_interest Identify genes associated with pattern interaction. This function identifies genes exhibiting significantly higher values of testMat in the Interaction region of the two patterns compared to regions with exclusive influence from either pattern. It uses Kruskal-Wallis test followed by posthoc analysis using Dunn's Test to identify the genes.</i>
--------------------------------------	---

Description

`.find_genes_of_interest` Identify genes associated with pattern interaction. This function identifies genes exhibiting significantly higher values of testMat in the Interaction region of the two patterns compared to regions with exclusive influence from either pattern. It uses Kruskal-Wallis test followed by posthoc analysis using Dunn's Test to identify the genes.

Usage

```
.find_genes_of_interest(testMat, goodGenes, region, fdr.level=0.05,
  analysis=c("enrichment", "overlap"),...)
```

Arguments

<code>testMat</code>	A matrix of counts with cells as columns and genes as rows
<code>goodGenes</code>	A vector of user specified genes expected to interact a priori. The default for this is NULL as the function can find these genes itself
<code>region</code>	A data frame of the reference pattern regions that overlap with the other patterns
<code>fdr.level</code>	False Discovery Rate. The default value is 0.05.
<code>analysis</code>	a character string that specifies the type of analysis to carry out, whether overlap or enrichment.
<code>...</code>	Additional arguments to be passed to lower level functions

Value

a list of genes exhibiting significantly higher values of testMat in the Interaction region of the two #' patterns compared to regions with exclusive influence from either pattern.

<code>.get_BTME_features</code>	<i>.get_BTME_features Load features BayesTME object</i>
---------------------------------	---

Description

`.get_BTME_features` Load features BayesTME object

Usage

`.get_BTME_features(hf)`

<code>.get_cogaps_features</code>	<i>.get_cogaps_features Load features CoGAPS object</i>
-----------------------------------	---

Description

`.get_cogaps_features` Load features CoGAPS object

Usage

`.get_cogaps_features(obj)`

<code>.get_csv_features</code>	<i>.get_csv_features Load features from dataframe</i>
--------------------------------	---

Description

`.get_csv_features` Load features from dataframe

Usage

`.get_csv_features(obj)`

<code>.get_seurat_features</code>	<i>.get_seurat_features Load features Seurat object</i>
-----------------------------------	---

Description

`.get_seurat_features` Load features Seurat object

Usage

`.get_seurat_features(obj)`

<code>.infer_method</code>	<i>inferMethod Infer the method used to obtain spatial features</i>
----------------------------	---

Description

`inferMethod` Infer the method used to obtain spatial features

Usage

`.infer_method(spObject, method)`

<code>.pick_image</code>	<i>.pick_image</i>
--------------------------	--------------------

Description

The function picks the appropriate histology image file from the spatial directory based on the specified resolution.

Usage

```
.pick_image(sp_dir, res)
```

Arguments

<code>sp_dir</code>	path to the spatial directory
<code>res</code>	a character string specifying the resolution of the image

Value

a character string of the image file name

<code>.read_format</code>	<i>readFormat Reads a format into an R object</i>
---------------------------	---

Description

readFormat Reads a format into an R object

Usage

```
.read_format(path)
```

<code>.row_t_test</code>	<i>Perform row-wise t-tests from scratch</i>
--------------------------	--

Description

This function iterates over the rows of a matrix and performs a t-test comparing two groups of columns. It calculates the t-statistic, p-value, and sample sizes without relying on `stats::t.test()` for the core logic.

Usage

```
.row_t_test(in.data, region, min_bins = 50, ...)
```

Arguments

<code>in.data</code>	A numeric matrix. Rows represent features, columns represent samples.
<code>region</code>	A factor or vector indicating the group membership for each column of <code>in.data</code> . Must have exactly two levels/unique values. Its length must equal <code>ncol(in.data)</code> .
<code>min_bins</code>	Minimum number of non-missing observations required in each group to perform the t-test.
<code>...</code>	Additional parameters to pass to the t-test function.

Value

A matrix with rows corresponding to the features and columns: - `statistic`: The calculated t-statistic. - `p.value`: The calculated two-sided p-value. - `n1`: Number of non-missing observations in group 1 for that row. - `n2`: Number of non-missing observations in group 2 for that row.

<code>calculate_gene_scores_directed</code>	<i>Calculate interaction scores for all pattern pairs</i>
---	---

Description

This function calculates interaction scores for all pattern pairs using the `.calc_IM_scores` function. It can run in parallel if `BiocParallel` is available.

Usage

```
calculate_gene_scores_directed(
  data,
  pat_hotspots,
  influence_hotspots,
  pattern_pairs = NULL,
  ...
)
```

Arguments

<code>data</code>	A numeric matrix with genes as rows and barcodes as columns.
<code>pat_hotspots</code>	A data frame with pattern hotspots, containing columns for x, y, and barcode.
<code>influence_hotspots</code>	A data frame with influence hotspots, containing columns for x, y, and barcode.
<code>pattern_pairs</code>	A data frame with pattern pairs to calculate interaction scores for. If NULL, all combinations of patterns in <code>pat_hotspots</code> will be used. If provided, it should have two columns with pattern names. Each row should represent a pair of patterns for which interaction scores will be calculated.
<code>...</code>	Additional parameters to pass to lower level functions.

Value

A data frame with interaction scores for all pattern pairs.

```
calculate_gene_set_score
      calculate_gene_set_score
```

Description

Calculate the mean interaction score for a set of genes

Usage

```
calculate_gene_set_score(
  IMscores,
  gene_sets,
  weighted = TRUE,
  method = c("geometric_mean", "arithmetic_mean")
)
```

Arguments

<code>IMscores</code>	A matrix of interaction scores
<code>gene_sets</code>	A list of gene sets, where each set is a vector of gene names
<code>weighted</code>	Logical; if TRUE, gene scores are weighted by their occurrence in multiple gene sets
<code>method</code>	Character; specifies the aggregation method for gene set scores. Options are "geometric_mean" or "arithmetic_mean"

Details

This function computes mean interaction scores for given gene sets across cell interactions. It supports both geometric and arithmetic means, and can weight gene contributions based on their presence in multiple gene sets.

Value

A matrix of mean interaction scores for genes in each gene set, with attributes for log p-value sums and number of genes for later fisher combination

```
calculate_gene_set_specificity
      calculate_gene_set_specificity
```

Description

This function computes specificity scores for given gene sets across cell types or spatial patterns. It uses fold-change scores and p-values to weight gene contributions, and supports both geometric and arithmetic means.

Usage

```
calculate_gene_set_specificity(
  data,
  spPatterns,
  gene_sets,
  weighted = TRUE,
  method = c("geometric_mean", "arithmetic_mean")
)
```

Arguments

data	A numeric matrix or data frame of gene expression values (genes x samples).
spPatterns	A data frame or matrix containing spatial pattern information, with columns for cell types and optionally "x", "y", "barcode".
gene_sets	A named list of character vectors, where each vector contains gene names for a gene set.
weighted	Logical; if TRUE, gene scores are weighted by their occurrence in multiple gene sets.
method	Character; specifies the aggregation method for gene set scores. Options are "geometric_mean" or "arithmetic_mean".

Details**Calculate Gene Set Specificity Scores**

- Genes not present in the data are excluded.
- Genes with all zero expression are removed.
- Fold-change scores and p-values are calculated using `.calculate_all_fc_scores`.
- Scores are normalized and weighted by p-value significance.
- For each gene set, scores are aggregated using the specified method and gene weights.

Value

A numeric matrix of gene set specificity scores (gene sets x cell types).

Examples

```
# Example usage:
# gene_set_scores <- calculate_gene_set_specificity(expr_matrix, spPatterns, gene_sets)
```

calculate_influence	<i>Compute the spatial influence of a spatial feature</i>
---------------------	---

Description

This function computes the spatial influence of a specified pattern

Usage

```
calculate_influence(spPatterns, optParams, ...)
```

Arguments

spPatterns	A data frame containing x, y coordinates and pattern name
optParams	A data frame with optimal parameters for the pattern
...	Additional parameters for the Smooth function

Value

A data frame with the spatial influence of the specified pattern

calculate_lr_scores	<i>calculate_lr_scores</i>
---------------------	----------------------------

Description

Calculate L-R pair scores using Fisher's method

Usage

```
calculate_lr_scores(
  ligand_scores,
  receptor_scores,
  lr_pairs,
  ligand_test = c("greater", "two.sided"),
  method = c("geometric_mean", "arithmetic_mean"),
  weighted = TRUE
)
```

Arguments

ligand_scores	Output from getGeneSetScore for ligands
receptor_scores	Output from getGeneSetScore for receptors
lr_pairs	Data frame with columns 'ligand' and 'receptor'
ligand_test	Character; specifies the type of test for ligand overexpression. Options are "greater" or "two.sided"
method	Character; specifies the aggregation method for L-R scores. Options are "geometric_mean" or "arithmetic_mean"
weighted	Logical; if TRUE, L-R scores are weighted by their occurrence in multiple L-R pairs

Details

This function computes L-R pair scores by combining ligand and receptor overexpression scores using either geometric or arithmetic mean. It can also weight L-R pairs based on their presence in multiple pairs to reduce bias from promiscuous ligands or receptors.

Value

Data frame with L-R scores and p-values

```
calculate_overlap_directed
      calculate_overlap_directed
```

Description

Calculate the overlap scores between patterns in hotspots

Usage

```
calculate_overlap_directed(
  pat_hotspots,
  influence_hotspots,
  patternList = NULL,
  method = c("relative-abundance", "differential-abundance", "absolute")
)
```

Arguments

pat_hotspots	A data frame with columns x, y, barcode and pattern names
influence_hotspots	A data frame with columns x, y, barcode and pattern names

patternList	A character vector of pattern names to calculate overlap scores for. If NULL, all patterns in pat_hotspots and influence_hotspots will be used.
method	The method to calculate overlapping abundance scores. Options are "relative-abundance", "differential-abundance" and "absolute"

Details

The function calculates the overlap scores between patterns hotspots using the specified method. The default method is "relative-abundance"

Value

A data frame with columns pattern, influence and overlapping abundance

Examples

```
hotspots <- data.frame(x = c(1,2,3,4,5),
  y = c(1,2,3,4,5),
  barcode = c("A","B","C","D","E"),
  pattern1 = c(1,0,1,0,1),
  pattern2 = c(1,1,0,0,1))
influence_hotspots <- data.frame(x = c(1,2,3,4,5),
  y = c(1,2,3,4,5),
  barcode = c("A","B","C","D","E"),
  pattern1 = c(0,1,1,0,0),
  pattern2 = c(0,1,0,1,1))
calculate_overlap_directed(pat_hotspots = hotspots, influence_hotspots = influence_hotspots)
```

```
calculate_overlap_undirected
      calculate_overlap_undirected
```

Description

Calculate the overlap scores between patterns in hotspots

Usage

```
calculate_overlap_undirected(
  hotspots,
  patternList = NULL,
  method = c("Szymkiewicz-Simpson", "Jaccard", "Sorensen-Dice", "Ochiai", "absolute")
)
```

Arguments

hotspots	A data frame with columns x, y, barcode and pattern names
patternList	A character vector of pattern names to calculate overlap scores for
method	The method to calculate overlap scores. Options are "Szymkiewicz-Simpson", "Jaccard", "Sorensen-Dice", "Ochiai" and "absolute"

Details

The function calculates the overlap scores between patterns hotspots using the specified method. The default method is "Szymkiewicz-Simpson" overlap coefficient.

Value

A data frame with columns pattern1, pattern2 and overlapScore

Examples

```
hotspots <- data.frame(x = c(1,2,3,4,5),
                      y = c(1,2,3,4,5),
                      barcode = c("A","B","C","D","E"),
                      pattern1 = c("pattern1",NA,"pattern1",NA,"pattern1"),
                      pattern2 = c("pattern2","pattern2",NA,NA,"pattern2"))
calculate_overlap_undirected(hotspots)
calculate_overlap_undirected(hotspots, c("pattern1","pattern2"))
```

calculate_thresholds	<i>Compute the thresholds for all columns in a data frame</i>
----------------------	---

Description

This function computes the thresholds for all columns in a data frame. The data frame could be an spPatterns object or an spInfluence object.

Usage

```
calculate_thresholds(df, minvals = 0.01, maxvals = 0.99, ...)
```

Arguments

df	A data frame with pattern values (optionally with x, y, barcode columns)
minvals	Minimum value for quantile threshold
maxvals	Maximum value for quantile threshold
...	Additional parameters to pass to lower level functions

Value

A list containing the computed thresholds for each pattern

curated_genes	<i>Curated Genes for example purposes</i>
---------------	---

Description

A vector with genes selected based on previous runs of SpaceMarkers on the Visium 10x breast ductal carcinoma spatial transcriptomics dataset

Format

A vector with 114 pre-selected genes

Value

a vector of genes

find_all_hotspots	<i>Find hotSpots for all spatial patterns</i>
-------------------	---

Description

Convenience function to find hotspots for all spatial patterns

Usage

```
find_all_hotspots(
  spPatterns,
  params = NULL,
  outlier = "positive",
  nullSamples = 1000,
  includeSelf = TRUE,
  ...
)
```

Arguments

spPatterns	A data frame that contains the spatial coordinates and metrics for spatial features (cell types/cell processes). The column names must include 'x' and 'y' as well as the spatially varying features.
params	a named vector of the optimal sigma and threshold for a given spatial pattern. The names are should be 'sigmaOpt' and 'threshOpt'. The default value is NULL.
outlier	a character string specifying whether to apply the outlier threshold to the kernel density distribution in a one-sided manner (specify 'positive' the default) or in a two sided manner (specify 'two.sided').

nullSamples	a numeric values specifying the number of spatial patterns to randomly sample for a null distribution.
includeSelf	a logic value specifying whether to consider the spatial influence the pattern has on surrounding regions only (set to FALSE), or whether to also consider the influence of the pattern itself (set to TRUE , the default).
...	Arguments passed to methods

find_hotspots_gmm	<i>Find hotspots for all patterns or influences based on values</i>
-------------------	---

Description

Convenience function to find hotspots for all spatial patterns or influence dataframes based on provided thresholds

Usage

```
find_hotspots_gmm(df, threshold = 0.1, ...)
```

Arguments

df	A data frame with pattern values (optionally with x, y, barcode columns)
threshold	a scalar or vector of thresholds for each column in the data frame. Either user provided or the output of @calculate_thresholds
...	Additional parameters to pass to lower level functions

Value

a data frame with the same dimensions as the input data frame.

find_pattern_hotspots	<i>Identify hotspots of spatial pattern influence</i>
-----------------------	---

Description

This function calculates 'hotspots' which are regions of high spatial influence based on an outlier threshold from a null distribution.

Usage

```
find_pattern_hotspots(
  spPatterns,
  params = NULL,
  patternName = "Pattern_1",
  outlier = "positive",
  nullSamples = 1000,
  includeSelf = TRUE,
  ...
)
```

Arguments

spPatterns	A data frame that contains the spatial coordinates and metrics for spatial features (cell types/cell processes). The column names must include 'x' and 'y' as well as the spatially varying features.
params	a named vector of the optimal sigma and threshold for a given spatial pattern. The names should be 'sigmaOpt' and 'threshOpt'. The default value is NULL.
patternName	a character string that specifies the pattern of interest
outlier	a character string specifying whether to apply the outlier threshold to the kernel density distribution in a one-sided manner (specify 'positive' the default) or in a two sided manner (specify 'two.sided').
nullSamples	a numeric values specifying the number of spatial patterns to randomly sample for a null distribution.
includeSelf	a logic value specifying whether to consider the spatial influence the pattern has on surrounding regions only (set to FALSE), or whether to also consider the influence of the pattern itself (set to TRUE, the default).
...	Arguments passed to methods

Value

a character vector with the spatial feature name if the spatial influence exceeded the threshold for that spot/cell, and NA otherwise

See Also

Other getIntGenes: [get_interacting_genes\(\)](#), [get_pairwise_interacting_genes\(\)](#)

Examples

```
library(SpaceMarkers)
#Visium data links
urls <- read.csv(system.file("extdata","visium_data.txt",
                             package="SpaceMarkers",mustWork = TRUE))
sp_url <- urls[["visium_url"]][2]
#Remove present Directories if any
unlink(basename(sp_url))
```

```

unlink("spatial", recursive = TRUE)
#Obtaining CoGAPS Patterns i.e Spatial Features
cogaps_result <- readRDS(system.file("extdata", "CoGAPS_result.rds",
                                     package="SpaceMarkers", mustWork = TRUE))
spFeatures <- slot(cogaps_result, "sampleFactors")
#Obtaining Spatial Coordinates
download.file(sp_url, basename(sp_url), mode = "wb")
untar(basename(sp_url))
spCoords <- load10XCoords(visiumDir = ".", version = "1.0")
rownames(spCoords) <- spCoords$barcode
#Match Dimensions
barcodes <- intersect(rownames(spFeatures), spCoords$barcode)
spCoords <- spCoords[barcodes,]
spFeatures <- spFeatures[barcodes,]
spPatterns <- cbind(spCoords, spFeatures[barcodes,])
spPatterns <- spPatterns[c("barcode", "y", "x", "Pattern_1", "Pattern_5")]
data("optParams")
hotspots <- find_pattern_hotspots(
  spPatterns = spPatterns,
  patternName = "Pattern_1",
  params = optParams["Pattern_1"],
  outlier = "positive", nullSamples = 1000, includeSelf = TRUE)
#Remove present Directories if any
unlink(basename(sp_url))
unlink("spatial", recursive = TRUE)

```

get_im_scores

get_im_scores

Description

Get the interaction scores for SpaceMarkers

Usage

```
get_im_scores(SpaceMarkers)
```

Arguments

SpaceMarkers A list of SpaceMarkers objects

Value

A data frame with columns Gene and SpaceMarkersMetric

Examples

```

example(get_pairwise_interacting_genes)
get_im_scores(SpaceMarkers)

```

get_interacting_genes *Calculate Interaction Regions and Associated Genes*

Description

This function calculates statistically significant genes using a non-parametric Kruskal-Wallis test for genes in any one region of influence and a post hoc Dunn's test is used for analysis of genes between regions.

Usage

```
get_interacting_genes(
  data,
  spPatterns,
  refPattern = "Pattern_1",
  mode = c("DE", "residual"),
  optParams = NULL,
  reconstruction = NULL,
  hotspots = NULL,
  analysis = c("enrichment", "overlap"),
  minOverlap = 50,
  ...
)
```

Arguments

data	original spatial data matrix.
spPatterns	A data frame that contains the spatial coordinates and metrics for spatial features (cell types/cell processes). The column names must include 'x' and 'y' as well as the spatially varying features.
refPattern	a character string that specifies the pattern whose "interaction" with every other pattern we want to study. The default value is "Pattern_1".
mode	SpaceMarkers mode of operation. Possible values are "DE" (the default) or "residual".
optParams	a matrix with dimensions 2 X N, where N is the number of spatial patterns with optimal parameters. The first row contains the kernel width 'sigmaOpt' for each pattern, and the second row is the threshOpt (outlier threshold) for each pattern. Users can also input their preferred param values. The default value is NULL.
reconstruction	reconstruction of the data matrix from latent spaces. Required for "residual" mode.
hotspots	a vector that specifies the patterns to compare to the 'refPattern'. The default is NULL which indicates that all patterns would be compared to the 'refPattern'.
analysis	a character string that specifies the type of downstream analysis to be performed. Possible values are "enrichment" (default) and "overlap". In enrichment mode, all genes are returned, ranked by the SpaceMarkers metric. In overlap mode,

only the genes which are significantly overexpressed in the interaction region are returned.

`minOverlap` a number that specifies the minimum overlap between genes in two patterns to be considered for the statistical tests. The default is 50.

... Arguments passed to methods

Value

a list of data frames with information about the interacting genes of the `refPattern` and each latent feature pattern matrix (`interacting_genes` object). There is also a data frame with all of the regions of influence for any two of patterns (the `hotspots` object).

See Also

Other `getIntGenes`: [find_pattern_hotspots\(\)](#), [get_pairwise_interacting_genes\(\)](#)

Examples

```
library(SpaceMarkers)
#Visium data links
urls <- read.csv(system.file("extdata", "visium_data.txt",
package="SpaceMarkers", mustWork = TRUE))
counts_url <- urls[["visium_url"]][1]
sp_url <- urls[["visium_url"]][2]
#Remove present Directories if any
unlink(basename(sp_url))
unlink("spatial", recursive = TRUE)
files <- list.files(".")[grepl(basename(counts_url), list.files("."))]
unlink(files)
download.file(counts_url, basename(counts_url), mode = "wb")
counts_matrix <- load10XExpr(visiumDir = ".", h5filename = basename(counts_url))
#Obtaining CoGAPS Patterns
cogaps_result <- readRDS(system.file("extdata", "CoGAPS_result.rds",
package="SpaceMarkers", mustWork = TRUE))
features <- intersect(rownames(counts_matrix), rownames(
  slot(cogaps_result, "featureLoadings")))
barcodes <- intersect(colnames(counts_matrix), rownames(
  slot(cogaps_result, "sampleFactors")))
counts_matrix <- counts_matrix[features, barcodes]
cogaps_matrix <- slot(cogaps_result, "featureLoadings")[features, ] %*%
  t(slot(cogaps_result, "sampleFactors")[barcodes, ])
#Obtaining Spatial Coordinates
download.file(sp_url, basename(sp_url), mode = "wb")
untar(basename(sp_url))
spCoords <- load10XCoords(visiumDir = ".", version = "1.0")
rownames(spCoords) <- spCoords$barcode
spCoords <- spCoords[barcodes, ]
spPatterns <- cbind(spCoords, slot(cogaps_result,
"sampleFactors")[barcodes, ])
data("curated_genes")
spPatterns <- spPatterns[c("barcode", "y", "x", "Pattern_1", "Pattern_5")]
```

```

counts_matrix <- counts_matrix[curated_genes,]
cogaps_matrix <- cogaps_matrix[curated_genes, ]
data("optParams")
SpaceMarkersMode <- "DE"
ref_Pattern <- "Pattern_1"
SpaceMarkers_test <- get_interacting_genes(
  data=counts_matrix,reconstruction=NULL,
  optParams = optParams,
  spPatterns = spPatterns,
  refPattern = "Pattern_1",
  mode="DE",analysis="overlap")
#Remove present Directories if any
unlink(basename(sp_url))
unlink("spatial", recursive = TRUE)
files <- list.files(".")[grepl(basename(counts_url),list.files("."))]
unlink(files)

```

```

get_pairwise_interacting_genes
      get_pairwise_interacting_genes

```

Description

Performs pairwise analysis to find genes associated with spatial interaction between pairs of spatially varying patterns.

Usage

```

get_pairwise_interacting_genes(
  data,
  spPatterns,
  mode = c("DE", "residual"),
  optParams = NULL,
  reconstruction = NULL,
  hotspots = NULL,
  minOverlap = 50,
  analysis = c("enrichment", "overlap"),
  pattern_pairs = NULL,
  ...,
  workers = NULL
)

```

Arguments

data	original spatial data matrix.
spPatterns	A data frame that contains the spatial coordinates and metrics for spatial features (cell types/cell processes). The column names must include 'x' and 'y' as well as the spatially varying features.

mode	SpaceMarkers mode of operation. Possible values are "DE" (the default) or "residual".
optParams	a matrix with dimensions 2 X N, where N is the number of spatial patterns with optimal parameters. The first row contains the kernel width 'sigmaOpt' for each pattern, and the second row is the threshOpt (outlier threshold) for each pattern. Users can also input their preferred param values. The default value is NULL.
reconstruction	reconstruction of the data matrix from latent spaces. Required for "residual" mode.
hotspots	a vector that specifies the patterns to compare to the 'refPattern'. The default is NULL which indicates that all patterns would be compared to the 'refPattern'.
minOverlap	a number that specifies the minimum overlap between genes in two patterns to be considered for the statistical tests. The default is 50.
analysis	a character string that specifies the type of downstream analysis to be performed. Possible values are "enrichment" (default) and "overlap". In enrichment mode, all genes are returned, ranked by the SpaceMarkers metric. In overlap mode, only the genes which are significantly overexpressed in the interaction region are returned.
pattern_pairs	A matrix of pattern pairs to be analyzed. Default is
...	Arguments passed to methods
workers	(optional) Number of workers to be used for parallel processing.

Details

=====

Value

a list of data frames for each pattern with 1) names of the patterns (patterns object) 2) data frame with the hotspots of influence for the two patterns (the hotspots object). 3) data frame with the genes associated with the interaction between the two patterns (interacting genes object, empty if insufficient interaction).

See Also

Other getIntGenes: [find_pattern_hotspots\(\)](#), [get_interacting_genes\(\)](#)

Examples

```
library(SpaceMarkers)
#Visium data links
urls <- read.csv(system.file("extdata", "visium_data.txt",
package="SpaceMarkers", mustWork = TRUE))
counts_url <- urls[["visium_url"]][1]
sp_url <- urls[["visium_url"]][2]
#Remove present Directories if any
unlink(basename(sp_url))
unlink("spatial", recursive = TRUE)
files <- list.files(".") [grepl(basename(counts_url), list.files("."))]
```

```

unlink(files)
download.file(counts_url,basename(counts_url), mode = "wb")
counts_matrix<-load10XExpr(visiumDir=".",
h5filename = basename(counts_url))
#Obtaining CoGAPS Patterns
cogaps_result <- readRDS(system.file("extdata","CoGAPS_result.rds",
package="SpaceMarkers",mustWork = TRUE))
features <- intersect(rownames(counts_matrix),rownames(
  slot(cogaps_result,"featureLoadings")))
barcodes <- intersect(colnames(counts_matrix),rownames(
  slot(cogaps_result,"sampleFactors")))
counts_matrix <- counts_matrix[features,barcodes]
cogaps_matrix <- slot(cogaps_result,"featureLoadings")[features,]%*%
  t(slot(cogaps_result,"sampleFactors")[barcodes,])
#Obtaining Spatial Coordinates
download.file(sp_url, basename(sp_url), mode = "wb")
untar(basename(sp_url))
spCoords <- load10XCoords(visiumDir = ".", version = "1.0")
rownames(spCoords) <- spCoords$barcode
spCoords <- spCoords[barcodes,]
spPatterns <- cbind(spCoords,
slot(cogaps_result,"sampleFactors")[barcodes,])
data("curated_genes")
spPatterns<-spPatterns[c("barcode","y","x","Pattern_1",
"Pattern_3","Pattern_5")]
counts_matrix <- counts_matrix[curated_genes,]
cogaps_matrix <- cogaps_matrix[curated_genes, ]
optParams <- matrix(c(6, 2, 6, 2, 6, 2), nrow = 2)
rownames(optParams) <- c("sigmaOpt","threshOpt")
colnames(optParams) <- c("Pattern_1","Pattern_3","Pattern_5")
SpaceMarkersMode <- "DE"
pattern_pairs <- matrix(c("Pattern_1", "Pattern_1",
"Pattern_3", "Pattern_5"), nrow=2)
SpaceMarkers <- get_pairwise_interacting_genes(
  data=counts_matrix,reconstruction=NULL,
  optParams = optParams,
  spPatterns = spPatterns,
  mode="DE",analysis="enrichment", pattern_pairs=pattern_pairs)
#Remove present Directories if any
unlink(basename(sp_url))
unlink("spatial", recursive = TRUE)
files <- list.files(".")[grepl(basename(counts_url),list.files("."))]
unlink(files)

```

get_spatial_features *Load spatial features*

Description

This function loads spatial features from a file containing spatial features

Usage

```
get_spatial_features(filePath, method = NULL, featureNames = ".")
```

Arguments

filePath	A string path to the location of the file containing the spatial features.
method	A string specifying the type of object to obtain spatial feature from. Default NULL, where the method is inferred based on object type. Other methods are: "CoGAPS", "Seurat", or "BayesTME".
featureNames	An array of strings specifying the column names corresponding to the feature names or a regex string. In the case of Seurat, all metadata columns with "_Feature" suffix are selected.

Value

a matrix of spatial features with barcodes associated with individual coordinates

Examples

```
library(SpaceMarkers)
#CoGAPS data filePath
filePath <- system.file("extdata", "CoGAPS_result.rds",
package = "SpaceMarkers", mustWork = TRUE)
spFeatures <- get_spatial_features(filePath, method = "CoGAPS")
head(spFeatures)
```

```
get_spatial_parameters
```

Read optimal parameters for spatial kernel density from user input or .json file

Description

This function obtains the width of a spatial kernel density (sigma) from either the user input or from a scale factors .json file. The outlier threshold around the set of spots (threshold) for each pattern is specified by the user (default is 4).

Usage

```
get_spatial_parameters(
  spatialPatterns,
  visiumDir = ".",
  spatialDir = "spatial",
  pattern = "scalefactors_json.json",
  sigma = NULL,
  threshold = 4,
```

```

    resolution = c("fullres", "lowres", "hires"),
    ...
)

```

Arguments

spatialPatterns	A data frame that contains the spatial coordinates for each cell type. The column names must include 'x' and 'y' as well as a set of numbered columns named 'Pattern_1.....N'.
visiumDir	A string path specifying the location of the 10xVisium directory
spatialDir	A string path specifying the location of the spatial folder containing the .json file of the spot characteristics
pattern	A string specifying the name of the .json file
sigma	A numeric value specifying the width of the kernel density estimate to be used for smoothing
threshold	A numeric value specifying how many standard deviations above the mean of a null distribution to use an outlier threshold for identifying 'hotspots'
resolution	A string specifying image resolution to be used for spot diameter. Can take values of "fullres" (default), "lowres" or "hires".
...	Arguments passed to methods

Value

a numeric matrix of sigmaOpts - the optimal width of the gaussian distribution, and the threshOpt - outlier threshold around the set of spots for each pattern

Examples

```

library(SpaceMarkers)
# Create test data
cells <- c()
test_num <- 500
for(i in 1:test_num){
  cells[length(cells)+1] <- paste0("cell_",i)
}
spPatterns <- data.frame(barcode = cells,
y = runif(test_num, min=0, max=test_num),
x = runif(test_num, min=0, max=test_num),
Pattern_1 = runif(test_num, min=0, max=1),
Pattern_2 = runif(test_num, min=0, max=1) )
# Call the get_spatial_parameters function with the test data
optParams <- get_spatial_parameters(spPatterns, sigma = 10)

```

```
get_spatial_params_morans_i
```

Calculate the optimal parameters from spatial kernel density for cell-cell interactions

Description

This function uses Morans.I to calculate the optimal width of the kernel density (sigmaOpt) as well as the outlier threshold around the set of spots (threshOpt) for a null distribution.

Usage

```
get_spatial_params_morans_i(spatialPatterns, ...)
```

Arguments

spatialPatterns

A data frame that contains the spatial coordinates for each cell type. The column names must include 'x' and 'y' as well as a set of numbered columns named 'Pattern_1.....N'.

...

Arguments passed to methods

Value

a numeric matrix of sigmaOpts - the optimal width of the gaussian distribution, and the threshOpt - outlier threshold around the set of spots for each pattern

Examples

```
library(SpaceMarkers)
# Create test data
cells <- c()
test_num <- 500
for(i in 1:test_num){
  cells[length(cells)+1] <- paste0("cell_",i)
}
spPatterns <- data.frame(barcode = cells,
y = runif(test_num, min=0, max=test_num),
x = runif(test_num, min=0, max=test_num),
Pattern_1 = runif(test_num, min=0, max=1),
Pattern_2 = runif(test_num, min=0, max=1) )
# Call the get_spatial_params_morans_i function with the test data
optParams <- get_spatial_params_morans_i(spPatterns)
```

load10XCoords

*Load 10x Visium Spatial Coordinates***Description**

This function loads spatial coordinates for each cell from a 10X Visium spatial folder.

Usage

```
load10XCoords(
  visiumDir,
  resolution = c("fullres", "lowres", " hires"),
  version = NULL
)
```

Arguments

visiumDir	A string path to the location of the folder containing the spatial coordinates. The folder in your visiumDir must be named 'spatial' and must contain files 'scalefactors_json.json' and 'tissue_positions_list.csv.'
resolution	A string specifying which values to look for in the .json object. Can be either fullres (default), lowres or hires.
version	A string specifying the version of the spaceranger data.

Value

a data frame of the spatial coordinates (x and y) for each spot/cell

Examples

```
library(SpaceMarkers)
#Visium data links
urls <- read.csv(system.file("extdata","visium_data.txt",
  package = "SpaceMarkers",mustWork = TRUE))
sp_url <- urls[["visium_url"]][2]
# Spatial Coordinates
download.file(sp_url, basename(sp_url), mode = "wb")
untar(basename(sp_url))
spCoords <- load10XCoords(visiumDir = ".", version = "1.0")
unlink("spatial", recursive = TRUE)
unlink("Visium_Human_Breast_Cancer_spatial.tar.gz")
```

load10XExpr	<i>Load 10X Visium Expression Data</i>
-------------	--

Description

This loads log-transformed 10X Visium expression data from standard 10X Visium folder.

Usage

```
load10XExpr(visiumDir = NULL, h5filename = "filtered_feature_bc_matrix.h5")
```

Arguments

- visiumDir A string path to the h5 file with expression information.
- h5filename A string of the name of the h5 file in the directory.

Value

A matrix of class dgeMatrix or Matrix that contains the expression info for each sample (cells) across multiple features (genes)

Examples

```
library(SpaceMarkers)
#Visium data links
urls <- read.csv(system.file("extdata","visium_data.txt",
package = "SpaceMarkers",mustWork = TRUE))
counts_url <- urls[["visium_url"]][1]
#Remove present Directories if any
files <- list.files(".")[grepl(basename(counts_url),list.files("."))]
unlink(files)
download.file(counts_url,basename(counts_url), mode = "wb")
counts_matrix<-load10XExpr(visiumDir=".",h5filename = basename(counts_url))
files <- list.files(".")[grepl(basename(counts_url),list.files("."))]
unlink(files)
```

lrd	<i>Curated Ligand-receptor interaction genes A list of vectors with genes associated with ligand-receptor interactions from CellChat database</i>
-----	---

Description

Curated Ligand-receptor interaction genes A list of vectors with genes associated with ligand-receptor interactions from CellChat database

Format

A data frame with LR interaction genes

Value

A list of vectors of LR genes

optParams	<i>Optimal paramters of 5 patterns from CoGAPS.</i>
-----------	---

Description

A dataset with the optimal width of the gaussian distribution (sigmaOpt) and the outlier threshold around the set of spots (thresOpt) for each pattern obtained from CoGAPS. CoGAPS was ran on spatial transcriptomic data from a breast cancer sample.

Format

A data frame with 2 rows and 5 columns:

- Pattern_1** immune cell pattern paramters
- Pattern_2** Disp.1 parameters
- Pattern_3** intraductal carcinoma (DCIS) parameters
- Pattern_4** Disp.2 parameters
- Pattern_5** invasive carcinoma lesion pattern paramters

Value

A matrix of optimal parameters for patterns identified by CoGAPS

plot_cell_interaction_circos	<i>Plot Ligand-Receptor Interactions between Cell Types</i>
------------------------------	---

Description

Visualizes ligand-receptor interactions as a circular plot, allowing for selection of cell types, customization of segment and link appearance, and different modes of representing interactions.

Usage

```
plot_cell_interaction_circos(
  lr_interactions_df,
  selected_cell_types = NULL,
  cell_order = NULL,
  gap_degree_after_sector = 5,
  track_height_molecules = 0.1,
  molecule_label_cex = 0.6,
  cell_label_cex = 0.9,
  link_transparency = 0.5,
  link_connection_rou = 0.7,
  link_arrowhead_type = "big.arrow",
  link_arrowhead_width = NULL,
  link_arrowhead_length = NULL,
  score_color_palette_fun = NULL,
  split_segments_for_links = TRUE,
  link_buffer_fraction = 0.1,
  scale_link_width_by_score = FALSE,
  score_transform_for_width = function(s) s,
  use_individual_molecule_colors = TRUE,
  default_ligand_color = "lightgreen",
  default_receptor_color = "lightblue",
  individual_ligand_palette_generator = NULL,
  individual_receptor_palette_generator = NULL,
  molecule_segment_border_col = NA,
  inter_molecule_segment_gap = 0.1
)
```

Arguments

lr_interactions_df
A data.frame with columns: ligand, receptor, source_cell_type, target_cell_type, score.

selected_cell_types
Optional character vector. If provided, only these cell types and interactions *between* them will be shown.

cell_order
Optional character vector for specific cell type ordering. If NULL, alphabetical order is used for the (selected) cell types.

gap_degree_after_sector
Numeric, gap in degrees after each sector. Default is 5.

track_height_molecules
Numeric, height of the track for molecule segments. Default is 0.1.

molecule_label_cex
Numeric, cex for molecule labels. Default is 0.6.

cell_label_cex
Numeric, cex for cell type labels. Default is 0.9.

link_transparency
Numeric, alpha for links (0 to 1). Default is 0.5.

link_connection_rou	Numeric (0-1) or vector of two for rou in <code>circos.link</code> . Default is 0.7.
link_arrowhead_type	Character. Type of arrowhead (e.g., "triangle", "big.arrow"). Default is "big.arrow".
link_arrowhead_width	Numeric. Width of the arrowhead. Default uses <code>circlize</code> default.
link_arrowhead_length	Numeric. Length of the arrowhead. Default uses <code>circlize</code> default.
score_color_palette_fun	A function (e.g., from <code>circlize::colorRamp2</code>) to map scores to colors.
split_segments_for_links	Logical. If TRUE, molecule segments are sized by interaction count/score and links connect to unique sub-segments. Default is TRUE.
link_buffer_fraction	Numeric (0 to <0.5). Buffer around each link within its sub-segment. Applies if <code>split_segments_for_links</code> is TRUE. Default is 0.1.
scale_link_width_by_score	Logical. If TRUE (and <code>split_segments_for_links</code> is TRUE), link/sub-segment width is proportional to score. Default is FALSE.
score_transform_for_width	Function to transform scores for width scaling. Default is <code>function(s) s</code> .
use_individual_molecule_colors	Logical. If TRUE, each unique ligand/receptor name gets a distinct color. Default is TRUE.
default_ligand_color	Character, color for all ligand segments if <code>use_individual_molecule_colors</code> is FALSE. Default is "lightgreen".
default_receptor_color	Character, color for all receptor segments if <code>use_individual_molecule_colors</code> is FALSE. Default is "lightblue".
individual_ligand_palette_generator	Function (takes n, returns n colors) for unique ligands. Default generates a green palette.
individual_receptor_palette_generator	Function (takes n, returns n colors) for unique receptors. Default generates a blue palette.
molecule_segment_border_col	Color for the border of L/R segments. Default NA (no border).
inter_molecule_segment_gap	Numeric, gap between L/R segments on the same track. Default is 0.1.

Value

Invisibly returns NULL. The function is called for its side effect of creating a plot.

Examples

```
## Not run:
if (requireNamespace("circlize", quietly = TRUE) &&
    requireNamespace("RColorBrewer", quietly = TRUE)) {
  lr_data <- data.frame(
    ligand = c("LGF1", "LGF1", "LGF2", "LGF3", "LGF4", "LGF1", "LGF5", "LGF6"),
    receptor = c("REC1", "REC2A", "REC1", "REC3B", "REC1", "REC4", "REC4", "REC2A"),
    source_cell_type = c("CellA", "CellA", "CellB", "CellC",
                        "CellA", "CellD", "CellD", "CellB"),
    target_cell_type = c("CellB", "CellC", "CellB", "CellA",
                        "CellD", "CellA", "CellD", "CellC"),
    score = c(4, 0.9, 0.7, 0.95, 0.6, 0.1, 0.88, 2.5),
    stringsAsFactors = FALSE
  )
  # Basic plot with defaults
  # plot_cell_interaction_circos(lr_data)

  # Plot with selected cell types and custom order
  # plot_cell_interaction_circos(lr_data,
  #                             selected_cell_types = c("CellA", "CellB", "CellD"),
  #                             cell_order = c("CellD", "CellA", "CellB"))
}

## End(Not run)
```

plot_im_scores

plot_im_scores

Description

Plot the top SpaceMarkers IMScores

Usage

```
plot_im_scores(
  df,
  interaction,
  cutOff = 0,
  nGenes = 20,
  geneText = 12,
  metricText = 12,
  increments = 1,
  out = NULL
)
```

Arguments

df A data frame with columns Gene and SpaceMarkersMetric

interaction	The interaction to plot
cutOff	The cut off value for the plot
nGenes	The number of genes to plot
geneText	The font size for the gene text
metricText	The font size for the metric text
increments	The increments for the y-axis
out	The output path for the plot

Examples

```
example(get_pairwise_interacting_genes)
plot_im_scores(get_im_scores(SpaceMarkers), "Pattern_1_Pattern_3")
```

plot_overlap_scores	<i>plot_overlap_scores</i>
---------------------	----------------------------

Description

Plot the overlap scores between patterns in hotspots

Usage

```
plot_overlap_scores(
  df,
  title = "Spatial Overlap Scores",
  out = NULL,
  fontsize = 15
)
```

Arguments

df	A data frame with columns pattern1, pattern2 and overlapScore
title	The title of the plot
out	The output path for the plot
fontsize	The font size of the plot

Value

A ggplot object

Examples

```
df <- data.frame(pattern1 = c("pattern1", "pattern1", "pattern2", "pattern2"),
                  pattern2 = c("pattern1", "pattern2", "pattern1", "pattern2"),
                  overlapScore = c(0.5, 0.7, 0.3, 0.9))
plot_overlap_scores(df)
plot_overlap_scores(df, "Overlap Scores", "overlapScores.png", 15)
```

`plot_source_to_target_circos`*Plot Ligand-Receptor Interactions from a Single Source to Target Cell Types*

Description

Visualizes ligand-receptor interactions focusing on a single source cell type and its outgoing interactions to a specified set of target cell types. Only ligands from the source and relevant receptors on the targets are shown.

Usage

```
plot_source_to_target_circos(  
  lr_interactions_df,  
  source_cell_name,  
  target_cell_names,  
  cell_order = NULL,  
  gap_degree_after_sector = 5,  
  track_height_molecules = 0.1,  
  molecule_label_cex = 0.6,  
  cell_label_cex = 0.9,  
  link_transparency = 0.5,  
  link_connection_rou = 0.7,  
  link_arrowhead_type = "triangle",  
  link_arrowhead_width = NULL,  
  link_arrowhead_length = NULL,  
  score_color_palette_fun = NULL,  
  split_segments_for_links = TRUE,  
  link_buffer_fraction = 0.1,  
  scale_link_width_by_score = FALSE,  
  score_transform_for_width = function(s) s,  
  use_individual_molecule_colors = TRUE,  
  default_ligand_color = "lightgreen",  
  default_receptor_color = "lightblue",  
  individual_ligand_palette_generator = NULL,  
  individual_receptor_palette_generator = NULL,  
  molecule_segment_border_col = NA,  
  inter_molecule_segment_gap = 0.1  
)
```

Arguments

`lr_interactions_df`

A data.frame with columns: ligand, receptor, source_cell_type, target_cell_type, score.

source_cell_name	Character, name of the source cell type.
target_cell_names	Character vector, names of target cell types to show.
cell_order	Optional character vector for specific cell type ordering. If NULL, source cell is first, then targets alphabetically.
gap_degree_after_sector	Numeric, gap in degrees after each sector. Default is 5.
track_height_molecules	Numeric, height of the track for molecule segments. Default is 0.1.
molecule_label_cex	Numeric, cex for molecule labels. Default is 0.6.
cell_label_cex	Numeric, cex for cell type labels. Default is 0.9.
link_transparency	Numeric, alpha for links (0 to 1). Default is 0.5.
link_connection_rou	Numeric (0-1) or vector of two for rou in <code>circos.link</code> . Default is 0.7.
link_arrowhead_type	Character. Type of arrowhead (e.g., "triangle", "big.arrow"). Default is "big.arrow".
link_arrowhead_width	Numeric. Width of the arrowhead. Default uses <code>circlize</code> default.
link_arrowhead_length	Numeric. Length of the arrowhead. Default uses <code>circlize</code> default.
score_color_palette_fun	A function (e.g., from <code>circlize::colorRamp2</code>) to map scores to colors.
split_segments_for_links	Logical. If TRUE, molecule segments are sized by interaction count/score and links connect to unique sub-segments. Default is TRUE.
link_buffer_fraction	Numeric (0 to <0.5). Buffer around each link within its sub-segment. Applies if <code>split_segments_for_links</code> is TRUE. Default is 0.1.
scale_link_width_by_score	Logical. If TRUE (and <code>split_segments_for_links</code> is TRUE), link/sub-segment width is proportional to score. Default is FALSE.
score_transform_for_width	Function to transform scores for width scaling. Default is <code>function(s) s</code> .
use_individual_molecule_colors	Logical. If TRUE, each unique ligand/receptor name gets a distinct color. Default is TRUE.
default_ligand_color	Character, color for all ligand segments if <code>use_individual_molecule_colors</code> is FALSE. Default is "lightgreen".
default_receptor_color	Character, color for all receptor segments if <code>use_individual_molecule_colors</code> is FALSE. Default is "lightblue".

individual_ligand_palette_generator
 Function (takes n, returns n colors) for unique ligands. Default generates a green palette.

individual_receptor_palette_generator
 Function (takes n, returns n colors) for unique receptors. Default generates a blue palette.

molecule_segment_border_col
 Color for the border of L/R segments. Default NA (no border).

inter_molecule_segment_gap
 Numeric, gap between L/R segments on the same track. Default is 0.1.

Value

Invisibly returns NULL. The function is called for its side effect of creating a plot.

Examples

```
## Not run:
if (requireNamespace("circlize", quietly = TRUE) &&
    requireNamespace("RColorBrewer", quietly = TRUE)) {
  lr_data <- data.frame(
    ligand = c("LGF1", "LGF1", "LGF2", "LGF3", "LGF4", "LGF1", "LGF5", "LGF6", "LGF7"),
    receptor = c("REC1", "REC2A", "REC1", "REC3B", "REC1", "REC4", "REC4", "REC2A", "REC5"),
    source_cell_type = c("CellA", "CellA", "CellB", "CellC",
                        "CellA", "CellD", "CellD", "CellB", "CellA"),
    target_cell_type = c("CellB", "CellC", "CellB", "CellA",
                        "CellD", "CellA", "CellD", "CellC", "CellE"),
    score = c(4,0.9,0.7,0.95,0.6,0.1,0.88,2.5,3.0),
    stringsAsFactors = FALSE
  )
  # Plot interactions from CellA to CellB and CellC
  # plot_source_to_target_circos(lr_data,
  #                             source_cell_name = "CellA",
  #                             target_cell_names = c("CellB", "CellC"))

  # Custom order and score-based link widths
  # score_pal <- circlize::colorRamp2(c(0, 4), c("white", "blue"))
  # plot_source_to_target_circos(lr_data,
  #                             source_cell_name = "CellD",
  #                             target_cell_names = c("CellA", "CellD"), # Include autocrine
  #                             cell_order = c("CellD", "CellA"),
  #                             scale_link_width_by_score = TRUE,
  #                             score_color_palette_fun = score_pal)
}

## End(Not run)
```

```
plot_spatial_data_over_image
      plotSpatialDataOverImage
```

Description

This function plots spatial data over the complementary histology image of varying resolutions

Usage

```
plot_spatial_data_over_image(
  visiumDir,
  df,
  feature_col,
  barcode_col = "barcode",
  resolution = c("lowres", "hires", "fullres"),
  version = NULL,
  colors = NULL,
  point_size = 2.5,
  stroke = 0.05,
  alpha = 0.5,
  title = "Spatial Heatmap",
  bg_color = NULL,
  crop = TRUE,
  text_size = 15
)
```

Arguments

visiumDir	directory with a spatial folder containing scalefactors_json.json, images (lowres, or hires), and coordinates (tissue_positons_(list).csv or probe.csv)
df	a dataframe with the features of interest. For example, can behotspots (character), and/or influence (numeric))
feature_col	feature to plot over spots, Default: NULL
barcode_col	barcode column name to match with coordinates, Default: 'barcode'
resolution	Image resoulution to scale coordinates too, Default: c("lowres", "hires", "fullres")
version	Visium version. Automatically infers from load10XCoords if NULL, Default: NULL
colors	colors to be displayed over spots. If set to NULL, it automatically colors the spots red for character values and uses viridis for numeric values. Default: NULL
point_size	size of spots displayed on the plot, Default: 2.5
stroke	thickness of spot outline, Default: 0.05
alpha	Transparency of the spots, Default: 0.5

title	Title displayed on the plot, Default: 'Spatial Heatmap'
bg_color	background color of ggplot box, Default: NULL
crop	crop spatial plot to a zoomed in window, Default: TRUE
text_size	size of text on the plot, Default: 15

Value

a ggplot object

plot_target_from_sources_circos

Plot Ligand-Receptor Interactions from Multiple Source to a Single Target Cell Type

Description

Visualizes ligand-receptor interactions focusing on a single target cell type and its incoming interactions from a specified set of source cell types. Only relevant ligands from the source cells and receptors on the target cell are shown.

Usage

```
plot_target_from_sources_circos(
  lr_interactions_df,
  source_cell_names,
  target_cell_name,
  cell_order = NULL,
  gap_degree_after_sector = 5,
  track_height_molecules = 0.1,
  molecule_label_cex = 0.6,
  cell_label_cex = 0.9,
  link_transparency = 0.5,
  link_connection_rou = 0.7,
  link_arrowhead_type = "triangle",
  link_arrowhead_width = 0.1,
  link_arrowhead_length = 0.1,
  score_color_palette_fun = NULL,
  split_segments_for_links = TRUE,
  link_buffer_fraction = 0.1,
  scale_link_width_by_score = FALSE,
  score_transform_for_width = function(s) s,
  use_individual_molecule_colors = TRUE,
  default_ligand_color = "lightgreen",
  default_receptor_color = "lightblue",
  individual_ligand_palette_generator = NULL,
  individual_receptor_palette_generator = NULL,
```

```

    molecule_segment_border_col = NA,
    inter_molecule_segment_gap = 0.1
)

```

Arguments

lr_interactions_df
A data.frame with columns: ligand, receptor, source_cell_type, target_cell_type, score.

source_cell_names
Character vector, names of source cell types to show.

target_cell_name
Character, name of the single target cell type.

cell_order
Optional character vector for specific cell type ordering. If NULL, target cell is first, then sources alphabetically.

gap_degree_after_sector
Numeric, gap in degrees after each sector. Default is 5.

track_height_molecules
Numeric, height of the track for molecule segments. Default is 0.1.

molecule_label_cex
Numeric, cex for molecule labels. Default is 0.6.

cell_label_cex
Numeric, cex for cell type labels. Default is 0.9.

link_transparency
Numeric, alpha for links (0 to 1). Default is 0.5.

link_connection_rou
Numeric (0-1) or vector of two for rou in `circos.link`. Default is 0.7.

link_arrowhead_type
Character. Type of arrowhead (e.g., "triangle", "big.arrow"). Default is "big.arrow".

link_arrowhead_width
Numeric. Width of the arrowhead. Default uses `circlize` default.

link_arrowhead_length
Numeric. Length of the arrowhead. Default uses `circlize` default.

score_color_palette_fun
A function (e.g., from `circlize::colorRamp2`) to map scores to colors.

split_segments_for_links
Logical. If TRUE, molecule segments are sized by interaction count/score and links connect to unique sub-segments. Default is TRUE.

link_buffer_fraction
Numeric (0 to <0.5). Buffer around each link within its sub-segment. Applies if `split_segments_for_links` is TRUE. Default is 0.1.

scale_link_width_by_score
Logical. If TRUE (and `split_segments_for_links` is TRUE), link/sub-segment width is proportional to score. Default is FALSE.

score_transform_for_width
Function to transform scores for width scaling. Default is `function(s) s`.

`use_individual_molecule_colors`
 Logical. If TRUE, each unique ligand/receptor name gets a distinct color. Default is TRUE.

`default_ligand_color`
 Character, color for all ligand segments if `use_individual_molecule_colors` is FALSE. Default is "lightgreen".

`default_receptor_color`
 Character, color for all receptor segments if `use_individual_molecule_colors` is FALSE. Default is "lightblue".

`individual_ligand_palette_generator`
 Function (takes n, returns n colors) for unique ligands. Default generates a green palette.

`individual_receptor_palette_generator`
 Function (takes n, returns n colors) for unique receptors. Default generates a blue palette.

`molecule_segment_border_col`
 Color for the border of L/R segments. Default NA (no border).

`inter_molecule_segment_gap`
 Numeric, gap between L/R segments on the same track. Default is 0.1.

Value

Invisibly returns NULL. The function is called for its side effect of creating a plot.

Examples

```
## Not run:
if (requireNamespace("circlize", quietly = TRUE) &&
    requireNamespace("RColorBrewer", quietly = TRUE)) {
  # Use the same test data from previous examples
  test_lr_data <- data.frame(
    ligand = c("LGF1", "LGF1", "LGF2", "LGF3", "LGF4", "LGF1", "LGF5", "LGF6", "LGF5"),
    receptor = c("REC1", "REC2", "REC1", "REC1", "REC5", "REC4", "REC4", "REC6", "REC2"),
    source_cell_type = c("CellA", "CellA", "CellB", "CellC", "CellA", "CellD", "CellD", "CellE", "CellD"),
    target_cell_type = c("CellB", "CellC", "CellA", "CellD", "CellD", "CellA", "CellD", "CellF", "CellA"),
    score = c(4.0, 2.5, 3.0, 2.2, 1.5, 1.0, 3.5, 0.5, 2.8),
    stringsAsFactors = FALSE
  )
  # Plot interactions from CellB and CellD converging on CellA
  # plot_target_from_sources_circos(test_lr_data,
  #                               source_cell_names = c("CellB", "CellD"),
  #                               target_cell_name = "CellA")

  # Another example: Interactions from CellA and CellC targeting CellD
  # plot_target_from_sources_circos(test_lr_data,
  #                               source_cell_names = c("CellA", "CellC"),
  #                               target_cell_name = "CellD",
  #                               scale_link_width_by_score = TRUE)
}

## End(Not run)
```

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