

Package ‘lisaClust’

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Type Package

Title lisaClust: Clustering of Local Indicators of Spatial Association

Version 1.19.0

Description lisaClust provides a series of functions to identify and visualise regions of tissue where spatial associations between cell-types is similar. This package can be used to provide a high-level summary of cell-type colocalization in multiplexed imaging data that has been segmented at a single-cell resolution.

License GPL (>=2)

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Depends R (>= 4.0)

VignetteBuilder knitr

BugReports <https://github.com/ellispatrick/lisaClust/issues>

URL <https://ellispatrick.github.io/lisaClust/>,
<https://github.com/ellispatrick/lisaClust>

Imports ggplot2, class, concaveman, grid, BiocParallel, spatstat.explore, spatstat.geom, BiocGenerics, S4Vectors, methods, spicyR, purrr, stats, data.table, dplyr, tidyr, SingleCellExperiment, SpatialExperiment, SummarizedExperiment, pheatmap, spatstat.random, lifecycle, simpleSeg, rlang,

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hatchingPlot	<i>hatchingPlot</i>
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Description

The hatchingPlot() function is used to create hatching patterns for representating spatial regions and cell-types.

The hatching geom is used to create hatching patterns for representation of spatial regions.

Usage

```
hatchingPlot(
  cells,
  useImages = NULL,
  region = "region",
  imageID = "imageID",
  cellType = "cellType",
  spatialCoords = c("x", "y"),
  window = "concave",
  line.spacing = 21,
  hatching.colour = 1,
  nbp = 50,
  window.length = NULL
)

geom_hatching(
```

```

mapping = NULL,
data = NULL,
stat = "identity",
position = "identity",
na.rm = FALSE,
show.legend = NA,
inherit.aes = TRUE,
line.spacing = 21,
hatching.colour = 1,
window = "concave",
window.length = NULL,
nbp = 250,
line.width = 1,
...
)

```

Arguments

cells	A data.frame or SingleCellExperiment.
useImages	A vector of images to plot.
region	The region column to plot.
imageID	The imageIDs column if using data.frame or SingleCellExperiment.
cellType	The cellType column if using data.frame or SingleCellExperiment.
spatialCoords	The spatial coordinates columns if using data.frame or SingleCellExperiment.
window	Should the window around the regions be 'square', 'convex' or 'concave'.
line.spacing	A integer indicating the spacing between hatching lines.
hatching.colour	A colour for the hatching.
nbp	An integer tuning the granularity of the grid used when defining regions
window.length	A tuning parameter for controlling the level of concavity when estimating concave windows.
mapping	Set of aesthetic mappings created by aes() or aes_(). If specified and inherit.aes = TRUE (the default), it is combined with the default mapping at the top level of the plot. You must supply mapping if there is no plot mapping.
data	The data to be displayed in this layer. There are three options: If NULL, the default, the data is inherited from the plot data as specified in the call to ggplot(). A data.frame, or other object, will override the plot data. All objects will be fortified to produce a data frame. See fortify() for which variables will be created. A function will be called with a single argument, the plot data. The return value must be a data.frame, and will be used as the layer data. A function can be created from a formula (e.g. ~ head(x, 10)).
stat	The statistical transformation to use on the data for this layer as a string.
position	adjustment, either as a string, or the result of a call to a position adjustment function.

<code>na.rm</code>	If FALSE, the default, missing values are removed with a warning. If TRUE, missing values are silently removed.
<code>show.legend</code>	logical. Should this layer be included in the legends? NA, the default, includes if any aesthetics are mapped. FALSE never includes, and TRUE always includes. It can also be a named logical vector to finely select the aesthetics to display.
<code>inherit.aes</code>	If FALSE, overrides the default aesthetics, rather than combining with them. This is most useful for helper functions that define both data and aesthetics and shouldn't inherit behaviour from the default plot specification, e.g. <code>borders()</code> .
<code>line.width</code>	A numeric controlling the width of the hatching lines
<code>...</code>	Other arguments passed on to <code>layer()</code> . These are often aesthetics, used to set an aesthetic to a fixed value, like <code>colour = "red"</code> or <code>size = 3</code> . They may also be parameters to the paired <code>geom/stat</code> .

Value

A `ggplot` object

A `ggplot` geom

Examples

```
## 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) * 100
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) * 100
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)
cells <- SingleCellExperiment::SingleCellExperiment(colData = cells)

## Generate regions
cells <- lisaClust(cells, k = 2)

## Plot regions
hatchingPlot(cells)

## Generate toy data
set.seed(51773)
library(ggplot2)
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) * 100
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) * 100
  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)
  ## Generate regions
  cells <- lisaClust(cells, k = 2)

# Plot the regions with geom_hatching()
ggplot(
  cells, aes(x = x, y = y, colour = cellType, region = region)
) +
  geom_point() +
  facet_wrap(~imageID) +
  geom_hatching()

```

 inhomLocalK

Calculate the inhomogenous local K function.

Description

Calculate the inhomogenous local K function.

Usage

```

inhomLocalK(
  data,
  Rs = c(20, 50, 100, 200),
  sigma = 10000,
  window = "convex",
  window.length = NULL,
  minLambda = 0.05,
  lisaFunc = "K"
)

```

Arguments

data	The data.
Rs	A vector of the radii that the measures of association should be calculated.
sigma	A numeric variable used for scaling when filtering inhomogeneous L-curves.
window	Should the window around the regions be 'square', 'convex' or 'concave'.
window.length	A tuning parameter for controlling the level of concavity.
minLambda	Minimum value for density for scaling when fitting inhomogeneous L-curves.
lisaFunc	Either "K" or "L" curve.

Value

A matrix of LISA curves

Examples

```
library(spicyR)
# Read in data
isletFile <- system.file("extdata", "isletCells.txt.gz", package = "spicyR")
cells <- read.table(isletFile, header = TRUE)
cells$x <- cells$AreaShape_Center_X
cells$y <- cells$AreaShape_Center_Y
cells$cellType <- as.factor(sample(
  c("big", "medium", "small"),
  length(cells$AreaShape_Center_Y),
  replace = TRUE
))
cells$cellID <- as.factor(cells$ObjectNumber)

inhom <- inhomLocalK(cells[1:100, ])
```

lisa

Generate local indicators of spatial association

Description

Generate local indicators of spatial association

Usage

```
lisa(
  cells,
  Rs = NULL,
  imageID = "imageID",
  cellType = "cellType",
  spatialCoords = c("x", "y"),
  cores = 1,
  window = "convex",
  window.length = NULL,
  whichParallel = "imageID",
  sigma = NULL,
  lisaFunc = "K",
  minLambda = 0.05,
  BPPARAM = BiocParallel::SerialParam()
)
```

Arguments

cells	A SingleCellExperiment, SpatialExperiment or data frame that contains at least the variables x and y, giving the coordinates of each cell, imageID and cellType.
Rs	A vector of the radii that the measures of association should be calculated.
imageID	The column which contains image identifiers.
cellType	The column which contains the cell types.
spatialCoords	The columns which contain the x and y spatial coordinates.
cores	Number of cores to use for parallel processing, or a BiocParallel MulticoreParam or SerialParam object.
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.
whichParallel	Should the function use parallization on the imageID or the cellType.
sigma	A numeric variable used for scaling when filtering inhomogeneous L-curves.
lisaFunc	Either "K" or "L" curve.
minLambda	Minimum value for density for scaling when fitting inhomogeneous L-curves.
BPPARAM	{DEPRECATED} A BiocParallel MulticoreParam or SerialParam object.

Value

A matrix of LISA curves

Examples

```
library(spicyR)
library(SingleCellExperiment)
# Read in data
isletFile <- system.file("extdata", "isletCells.txt.gz", package = "spicyR")
cells <- read.table(isletFile, header = TRUE)
cellExp <- SingleCellExperiment(
  assay = list(intensities = t(cells[, grepl(names(cells), pattern = "Intensity_")])),
  colData = cells[, !grepl(names(cells), pattern = "Intensity_")]
)

# Cluster cell types
markers <- t(assay(cellExp, "intensities"))
kM <- kmeans(markers, 8)
colData(cellExp)$cluster <- paste("cluster", kM$cluster, sep = "")

# Generate LISA
lisaCurves <- lisa(
  cellExp,
  spatialCoords = c("Location_Center_X", "Location_Center_Y"),
  cellType = "cluster", imageID = "ImageNumber"
)

# Cluster the LISA curves
```

```
kM <- kmeans(lisaCurves, 2)
```

lisaClust	<i>Use k-means clustering to cluster local indicators of spatial association. For other clustering use lisa.</i>
-----------	--

Description

Use k-means clustering to cluster local indicators of spatial association. For other clustering use lisa.

Usage

```
lisaClust(
  cells,
  k = 2,
  Rs = NULL,
  imageID = "imageID",
  cellType = "cellType",
  spatialCoords = c("x", "y"),
  regionName = "region",
  cores = 1,
  window = "convex",
  window.length = NULL,
  whichParallel = "imimageID",
  sigma = NULL,
  lisaFunc = "K",
  minLambda = 0.05,
  BPPARAM = BiocParallel::SerialParam()
)
```

Arguments

cells	A SingleCellExperiment, SpatialExperiment or data frame that contains at least the variables x and y, giving the coordinates of each cell, imageID and cellType.
k	The number of regions to cluster.
Rs	A vector of the radii that the measures of association should be calculated.
imageID	The column which contains image identifiers.
cellType	The column which contains the cell types.
spatialCoords	The columns which contain the x and y spatial coordinates.
regionName	The output column for the lisaClust regions.
cores	Number of cores to use for parallel processing, or a BiocParallel MulticoreParam or SerialParam object.
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.
whichParallel	Should the function use parallelization on the imageID or the cellType.
sigma	A numeric variable used for scaling when fitting inhomogeneous L-curves.
lisaFunc	Either "K" or "L" curve.
minLambda	Minimum value for density for scaling when fitting inhomogeneous L-curves.
BPPARAM	{DEPRECATED} A BiocParallel MulticoreParam or SerialParam object.

Value

A matrix of LISA curves

Examples

```
library(spicyR)
library(SingleCellExperiment)
# Read in data
isletFile <- system.file("extdata", "isletCells.txt.gz", package = "spicyR")
cells <- read.table(isletFile, header = TRUE)
cellExp <- SingleCellExperiment(
  assay = list(intensities = t(cells[, grepl(names(cells), pattern = "Intensity_")])),
  colData = cells[, !grepl(names(cells), pattern = "Intensity_")]
)

# Cluster cell types
markers <- t(assay(cellExp, "intensities"))
kM <- kmeans(markers, 8)
colData(cellExp)$cluster <- paste("cluster", kM$cluster, sep = "")

# Generate LISA
cellExp <- lisaClust(cellExp,
  k = 2,
  imageID = "ImageNumber",
  cellType = "cluster",
  spatialCoords = c("Location_Center_X", "Location_Center_Y")
)
```

regionMap

Plot heatmap of cell type enrichment for lisaClust regions

Description

Plot heatmap of cell type enrichment for lisaClust regions

Usage

```
regionMap(
  cells,
  type = "bubble",
  cellType = "cellType",
  region = "region",
  limit = c(0.33, 3),
  ...
)
```

Arguments

cells	SingleCellExperiment, SpatialExperiment or data.frame
type	Make a "bubble" or "heatmap" plot.
cellType	The column storing the cell types
region	The column storing the regions
limit	limits to the lower and upper relative frequencies
...	Any arguments to be passed to the pheatmap package

Value

A bubble plot or heatmap

Examples

```
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) * 100
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) * 100
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)

cells <- lisaClust(cells, k = 2)

regionMap(cells)
```

scale_region	<i>Scale constructor for regions</i>
--------------	--------------------------------------

Description

Region scale constructor.

Usage

```
scale_region(aesthetics = "region", ..., guide = "legend")
scale_region_manual(..., values)
```

Arguments

aesthetics	The names of the aesthetics that this scale works with
...	Arguments passed on to <code>discrete_scale</code>
guide	A function used to create a guide or its name. See <code>guides()</code> for more info.
values	a set of aesthetic values to map data values to. If this is a named vector, then the values will be matched based on the names. If unnamed, values will be matched in order (usually alphabetical) with the limits of the scale. Any data values that don't match will be given <code>na.value</code> .

Value

a ggplot guide

Examples

```
library(spicyR)
## 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) * 100
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) * 100
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)
cells <- SingleCellExperiment::SingleCellExperiment(colData = cells)

## Generate regions
cells <- lisaClust(cells, k = 2)
```

```
# Plot the regions with hatchingPlot()
hatchingPlot(cells) +
  scale_region_manual(
    values = c(1, 4), labels = c("Region A", "Region B"),
    name = "Regions"
  )
```

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