

Package ‘blisa’

June 2, 2026

Type Package

Title Infer Cell-Cell Communication from Spatial Transcriptomics

Version 0.2.0

Description Identifies cell-cell communication hotspots in spatial transcriptomics data using bivariate Local Moran's I statistics on hexagonally binned cells. Provides functions for spatial weighting, ligand-receptor pair filtering, hotspot detection, and visualisation of sender-receiver cell-type interactions.

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Encoding UTF-8

Imports sf, spdep, Matrix, SpatialExperiment, SummarizedExperiment, ComplexHeatmap, ggplot2, viridisLite, grid

RoxygenNote 7.3.3

VignetteBuilder knitr

Suggests knitr, rmarkdown

NeedsCompilation no

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Repository CRAN

Date/Publication 2026-06-02 07:50:36 UTC

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blisa-package	<i>blisa: Bivariate Local Indicator of Spatial Association for spatial transcriptomics</i>
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Description

Tools for spatial cell-cell communication analysis of spatial transcriptomics data.

blisa	<i>Run BLISA spatial cell-cell communication analysis</i>
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Description

Generic function for running BLISA (Bivariate Local Indicator of Spatial Association). Dispatches on the class of `x`:

- `blisa.default` accepts a pre-binned gene-by-bin count matrix and a matching bins polygon object.
- `blisa.SpatialExperiment` accepts a cell-level `SpatialExperiment` object and bins cells into hexagonal tiles internally via `hexBinCells` before running the analysis.

Usage

```
blisa(x, ...)

## Default S3 method:
blisa(
  x,
  bins,
  LR_df = NULL,
  bin_size = 50,
  dmax = 250,
  nsim = 999,
  p_cutoff = 0.05,
  min_ligand = 10,
  min_receptor = 10,
```

```

min_cells = 1,
n_cells_col = NA,
annotation_col = "annotation",
default_mode = "diffuse",
diffuse_category = c("Secreted Signaling", "Non-protein Signaling"),
species = c("human", "mouse"),
genes = NULL,
counts_by_group = NULL,
...
)

## S3 method for class 'SpatialExperiment'
blisa(x, bin_size = 50, LR_df = NULL, group = "cell_type", genes = NULL, ...)

```

Arguments

<code>x</code>	A gene-by-bin count matrix (for <code>blisa.default</code>) or a cell-level <code>SpatialExperiment</code> object (for <code>blisa.SpatialExperiment</code>).
<code>...</code>	Additional arguments passed to the relevant method.
<code>bins</code>	An <code>sf</code> object of bin polygons. Row order must match the column order of <code>x</code> .
<code>LR_df</code>	Data frame of ligand-receptor pairs with columns <code>ligand.symbol</code> and <code>receptor.symbol</code> . When <code>NULL</code> , <code>CellChatDB</code> for the chosen species is downloaded automatically.
<code>bin_size</code>	Numeric. Width of each hexagonal bin in coordinate units (e.g. microns). Passed to <code>hexBinCells</code> and <code>computeSpatialWeights</code> . Default 50.
<code>dmax</code>	Numeric. Maximum distance for diffuse-mode neighbours. Default 250.
<code>nsim</code>	Integer. Number of permutations for Moran's I significance. Default 999.
<code>p_cutoff</code>	Numeric. P-value threshold for High-High hotspots. Default 0.05.
<code>min_ligand</code>	Numeric. Minimum ligand count threshold. Default 10.
<code>min_receptor</code>	Numeric. Minimum receptor count threshold. Default 10.
<code>min_cells</code>	Integer. Bins with fewer cells are excluded from Moran's I and assigned neutral statistics ($p = 1$, $LISA = 0$). Ignored when <code>n_cells_col = NA</code> . Default 1.
<code>n_cells_col</code>	Character or <code>NA</code> . Column in <code>bins</code> holding per-bin cell counts used for <code>min_cells</code> filtering. Set to <code>NA</code> to skip (default).
<code>annotation_col</code>	Character. Column in <code>LR_df</code> specifying interaction category used for communication-mode assignment. Default "annotation".
<code>default_mode</code>	Character. CCC mode assigned to LR pairs whose annotation does not match <code>diffuse_category</code> . Default "diffuse".
<code>diffuse_category</code>	Character vector of annotation categories treated as diffuse signalling.
<code>species</code>	Character. Which <code>CellChatDB</code> to download when <code>LR_df = NULL</code> . One of "human" (default) or "mouse".
<code>genes</code>	Character vector of gene names to consider when matching ligand-receptor pairs. Defaults to <code>rownames(x)</code> (all genes in the <code>SpatialExperiment</code> object).

counts_by_group	Named list of gene-by-bin count matrices, one per group level (e.g. cell type), as returned by hexBinCells when group is supplied. When provided, runCCI is called automatically after the BLISA loop and its output is included in the result as CCI_scores. Default NULL.
group	Character. Column name in colData(x) to use as the grouping variable (e.g. cell type) for per-group bin aggregation and downstream CCI analysis via runCCI . If the column is not found in colData(x), a message is issued and CCI is skipped. Default "cell_type".

Value

A list; see individual method documentation for details.

An object of class blisa with four components:

LR_results Data frame of BLISA results for each LR pair, including ccc_mode, sig_numbers, sig_index, sig_pval, all_pval, all_lisa, all_quadrant, and original columns from LR_df. all_quadrant is a character vector of spdep: :hotspot quadrant labels ("High-High", "Low-Low", etc.) for every bin; non-tested bins are NA.

bins Bin-level sf object of hexagonal polygons.

spatial_weights Spatial weights list from [computeSpatialWeights](#).

CCI_scores NULL unless counts_by_group is supplied, in which case a wide data frame of interaction scores from [runCCI](#): rows are "Sender->Receiver" group pairs, columns are LR pairs.

Methods (by class)

- blisa(default): Method for a gene-by-bin count matrix.
- blisa(SpatialExperiment): Method for a cell-level SpatialExperiment object. Bins cells into hexagonal tiles via [hexBinCells](#) then delegates to blisa.default.

computeSpatialWeights *Compute Spatial Weights for BLISA*

Description

Builds queen (nearby) and distance-decay (diffuse) spatial weight matrices from a bin-level sf object, excluding isolated bins and optionally excluding low-cell bins. A second-pass isolation check further removes bins that become isolated after the initial subset.

Usage

```
computeSpatialWeights(
  bins,
  bin_size = 50,
  dmax = 250,
  min_cells = 1,
  n_cells_col = NA
)
```

Arguments

<code>bins</code>	An sf object of spatial bins.
<code>bin_size</code>	Numeric. Bin spacing used to define queen adjacency ($1.2 * \text{bin_size}$ radius).
<code>dmax</code>	Numeric. Maximum distance for diffuse-mode neighbours.
<code>min_cells</code>	Integer. Minimum cell count for a bin to be included. Ignored when <code>n_cells_col = NA</code> .
<code>n_cells_col</code>	Character or NA. Column name in bins holding per-bin cell counts. Set to NA to skip cell-count filtering (default).

Value

A list with:

- queen_wt** Spatial weights list for nearby (queen) mode.
- dist_wt** Spatial weights list for diffuse (distance-decay) mode.
- keep_idx_queen** Integer indices of bins used in queen-mode Moran.
- keep_idx_dist** Integer indices of bins used in diffuse-mode Moran.
- isolate_idx_queen** Integer indices of original queen-mode isolates.
- isolate_idx_dist** Integer indices of original diffuse-mode isolates.
- low_cell_idx** Integer indices of bins excluded for low cell counts.
- queen_nb_full** Full (unsubset) neighbour list for nearby mode, indexed over all bins.
- dist_nb_full** Full (unsubset) neighbour list for diffuse mode, indexed over all bins.

 filterLRpairs

Filter ligand-receptor pairs by expression threshold

Description

Retains only LR pairs where at least one bin/spot has counts at or above `min_ligand` for every ligand subunit and `min_receptor` for every receptor subunit.

Usage

```
filterLRpairs(
  counts,
  min_ligand = 10,
  min_receptor = 10,
  LR_df = NULL,
  species = c("human", "mouse")
)
```

Arguments

counts	Gene-by-bin count matrix (dense or sparse). Row names must be gene symbols.
min_ligand	Numeric. Minimum count threshold for ligand genes. At least one bin must meet or exceed this value. Default 10.
min_receptor	Numeric. Minimum count threshold for receptor genes. At least one bin must meet or exceed this value. Default 10.
LR_df	Data frame of ligand-receptor pairs with columns <code>ligand.symbol</code> and <code>receptor.symbol</code> (comma-separated gene symbols for multi-subunit complexes). When NULL, the CellChatDB for the chosen species is downloaded automatically.
species	Character. Which CellChatDB to download when LR_df is NULL. One of "human" (default) or "mouse".

Value

A subset of LR_df containing only pairs that pass the expression thresholds for both ligand and receptor.

hexBinCells

Bin cells into hexagonal spatial bins

Description

Aggregates single-cell spatial data into hexagonal bins and returns a bin-level count matrix together with a matching `sf` polygon object, ready to pass directly to [blisa.default](#).

Usage

```
hexBinCells(
  coords_df,
  counts_matrix,
  bin_size = 50,
  min_cells = 1,
  group = NULL
)
```

Arguments

<code>coords_df</code>	Data frame or matrix with columns <code>x_centroid</code> and <code>y_centroid</code> (e.g. the output of <code>SpatialExperiment::spatialCoords()</code>). Row names must be cell IDs matching the column names of <code>counts_matrix</code> .
<code>counts_matrix</code>	Gene-by-cell count matrix (dense or sparse). Row names must be gene symbols; column names must be cell IDs present in <code>coords_df</code> .
<code>bin_size</code>	Numeric. Approximate width of each hexagonal bin in coordinate units (e.g. microns). Analogous to <code>grid.length.x</code> in <code>sciderHex::gridDensity</code> . Default 50.
<code>min_cells</code>	Integer. Bins containing fewer than <code>min_cells</code> cells are dropped from the output. Default 1.
<code>group</code>	Factor or character vector of length <code>ncol(counts_matrix)</code> giving the cell-type label of each cell. When supplied, a named list of per-cell-type gene-by-bin matrices is included in the output as <code>counts_by_group</code> . Default NULL (not computed).

Value

A list with:

counts_matrix Gene-by-bin sparse count matrix (all cells combined). Column *i* corresponds to row *i* of bins.

bins An sf object of hexagonal bin polygons with an `n_cells` column recording how many cells fall in each bin. Row order matches the columns of `counts_matrix`.

counts_by_group (Only when `group` is supplied.) A named list of gene-by-bin sparse matrices, one per cell-type level, with the same bin order as `counts_matrix`.

<code>is.blisa</code>	<i>Test if an object is a blisa object</i>
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Description

Test if an object is a blisa object

Usage

```
is.blisa(x)
```

Arguments

`x` Any R object.

Value

Logical.

plotCCI

Heatmap of CCI scores across all ligand-receptor pairs

Description

Generic function. Draws a clustered heatmap (via `ComplexHeatmap`) with rows as Sender \rightarrow Receiver cell-type pairs and columns as LR pairs. Row annotations colour-code the sender and receiver cell types.

Usage

```
plotCCI(x, ...)

## S3 method for class 'blisa'
plotCCI(
  x,
  top_lr = 20,
  top_pairs = 30,
  sender = NULL,
  receiver = NULL,
  colors = NULL,
  colours = NULL,
  ...
)

## Default S3 method:
plotCCI(
  x,
  top_lr = 20,
  top_pairs = 30,
  sender = NULL,
  receiver = NULL,
  colors = NULL,
  colours = NULL,
  ...
)
```

Arguments

<code>x</code>	A <code>blisa</code> object or a CCI scores data frame (the <code>CCI_scores</code> slot of a <code>blisa</code> object). The data frame must contain columns <code>Sender</code> , <code>Receiver</code> , and one column per LR pair.
<code>...</code>	Additional arguments passed to the relevant method.
<code>top_lr</code>	Integer or <code>NULL</code> . Number of top-ranked LR pairs (by <code>sig_numbers</code>) to display as columns. LR pairs in <code>CCI_scores</code> are already ordered by rank, so this simply takes the first <code>top_lr</code> columns. Default 20.

top_pairs	Integer or NULL. Number of top sender-receiver pairs to display as rows, ranked by their maximum interaction score across the displayed LR pairs (after top_lr is applied). When NULL all rows are shown. Default 30.
sender	Character vector or NULL. If provided, only rows where Sender is in this vector are kept. Applied independently of receiver (AND logic when both are supplied). Default NULL (all senders).
receiver	Character vector or NULL. If provided, only rows where Receiver is in this vector are kept. Applied independently of sender (AND logic when both are supplied). Default NULL (all receivers).
colors	Named character vector mapping cell-type names to colours, used for the sender/receiver row annotations. When NULL (default), colours are assigned automatically from the package palette. The British spelling colours is accepted as an alias.
colours	Alias for colors (British spelling). Ignored when colors is supplied.

Value

Invisibly returns the Heatmap object.

Methods (by class)

- `plotCCI(blisa)`: Method for a `blisa` object. Extracts `CCI_scores` and delegates to `plotCCI.default`. Stops with an informative error if `CCI_scores` is NULL.
- `plotCCI(default)`: Method for a CCI scores data frame (e.g. the `CCI_scores` slot of a `blisa` object).

See Also

[plotCCILR](#) for a sender-by-receiver heatmap of a single LR pair; [plotCCIsummary](#) for an aggregated sender-by-receiver heatmap across all LR pairs.

plotCCILR	<i>Sender-by-receiver heatmap of CCI scores for one ligand-receptor pair</i>
-----------	--

Description

Generic function. Reshapes the CCI data frame into a receiver-by-sender cell-type matrix for one selected LR pair and draws a clustered heatmap.

Usage

```
plotCCILR(x, ...)

## S3 method for class 'blisa'
plotCCILR(x, index = 1, ligand = NULL, receptor = NULL, ...)

## Default S3 method:
plotCCILR(x, lr_pair, ...)
```

Arguments

x	A blisa object or a CCI scores data frame (the CCI_scores slot of a blisa object).
...	Additional arguments passed to the relevant method.
index	Integer. Row index into LR_results selecting the ligand-receptor pair to visualise. Ignored when both ligand and receptor are supplied. Default 1 (top-ranked pair).
ligand	Character. Ligand gene symbol. When both ligand and receptor are provided the matching LR pair is located automatically and index is ignored. Must be supplied together with receptor.
receptor	Character. Receptor gene symbol. Must be supplied together with ligand.
lr_pair	Character. Column name in the CCI scores data frame corresponding to the ligand-receptor pair to visualise (e.g. "CXCL12_CXCR4").

Value

A Heatmap object.

Methods (by class)

- plotCCILR(blisa): Method for a blisa object. The LR pair is selected by index (default 1, the top-ranked pair) unless both ligand and receptor are supplied, in which case the matching row is located automatically and index is ignored. Stops with an informative error if CCI_scores is NULL or the selected LR pair has no significant hotspots.
- plotCCILR(default): Method for a CCI scores data frame (e.g. the CCI_scores slot of a blisa object). The LR pair is selected by column name via lr_pair.

See Also

[plotCCI](#) for an overview heatmap across all LR pairs; [plotCCIsummary](#) for an aggregated sender-by-receiver heatmap.

plotCCIspatial	<i>Spatial map of dominant sender-receiver cell-type pairs at BLISA hotspots</i>
----------------	--

Description

For a selected ligand-receptor pair, identifies the dominant interacting cell-type pair at each significant hotspot bin and draws a spatial map of the tissue coloured by those pairs. Receiver cells are those inside hotspot bins; sender cells are drawn from the immediate neighbourhood.

Usage

```
plotCCIspatial(
  x,
  counts_by_group,
  index = 1,
  ligand = NULL,
  receptor = NULL,
  top_pairs = 30
)
```

Arguments

x	A blisa object as returned by blisa .
counts_by_group	Named list of gene-by-bin count matrices, one per cell type. Typically the counts_by_group element returned by hexBinCells . Names must match the cell-type levels.
index	Integer. Row index into x\$LR_results selecting the ligand-receptor pair to visualise. Ignored when both ligand and receptor are supplied. Default 1 (top-ranked pair).
ligand	Character. Ligand gene symbol. When both ligand and receptor are provided the matching LR pair is located automatically and index is ignored. Must be supplied together with receptor.
receptor	Character. Receptor gene symbol. Must be supplied together with ligand.
top_pairs	Integer. Maximum number of distinct cell-type pairs to show in the legend; remaining pairs are grouped as "rare pairs". Default 30.

Value

A ggplot object.

See Also

[plotHotspots](#) for a significance-based spatial map of hotspot bins.

plotCCIsummary	<i>Sender-by-receiver heatmap of aggregated CCI scores across LR pairs</i>
----------------	--

Description

Generic function. Aggregates CCI scores across all (or the top-ranked) ligand-receptor pairs and draws a clustered receiver-by-sender heatmap, one cell per Sender → Receiver combination.

Usage

```
plotCCIsummary(x, ...)

## S3 method for class 'blisa'
plotCCIsummary(
  x,
  top_lr = NULL,
  sender = NULL,
  receiver = NULL,
  agg_fun = sum,
  ...
)

## Default S3 method:
plotCCIsummary(
  x,
  top_lr = NULL,
  sender = NULL,
  receiver = NULL,
  agg_fun = sum,
  ...
)
```

Arguments

x	A blisa object or a CCI scores data frame (the CCI_scores slot of a blisa object).
...	Additional arguments passed to the relevant method.
top_lr	Integer or NULL. Number of top-ranked LR pairs (by sig_numbers) to include before aggregating. LR pairs in CCI_scores are already ordered by rank, so this takes the first top_lr columns. NULL (default) uses all pairs.
sender	Character vector or NULL. If provided, only rows where Sender is in this vector are kept (AND logic with receiver). Default NULL (all senders).
receiver	Character vector or NULL. If provided, only rows where Receiver is in this vector are kept (AND logic with sender). Default NULL (all receivers).
agg_fun	Function used to aggregate scores across LR pairs for each Sender → Receiver combination. Receives a numeric vector with NAs already removed. Default sum.

Value

A Heatmap object.

Methods (by class)

- plotCCIsummary(blisa): Method for a blisa object. Stops with an informative error if CCI_scores is NULL.
- plotCCIsummary(default): Method for a CCI scores data frame (e.g. the CCI_scores slot of a blisa object).

See Also

[plotCCILR](#) for a per-LR-pair version of this plot; [plotCCI](#) for a heatmap with LR pairs as columns.

 plotHotspots

Spatial hotspot map for one ligand-receptor pair

Description

Generic function. Plots each bin coloured by significance status: empty, non-significant, or significant hotspot (continuous gradient of $-\log_{10}$ p-value or $1 - p$ -value).

Usage

```
plotHotspots(x, ...)

## S3 method for class 'blisa'
plotHotspots(
  x,
  index = 1,
  ligand = NULL,
  receptor = NULL,
  log_pval = TRUE,
  p_cutoff = NULL,
  ...
)
```

Arguments

x	A blisa object.
...	Additional arguments passed to the method.
index	Integer. Row index into LR_results selecting the ligand-receptor pair to visualise. Ignored when both ligand and receptor are supplied. Default 1 (top-ranked pair).
ligand	Character. Ligand gene symbol. When both ligand and receptor are provided the matching LR pair is located automatically and index is ignored. Must be supplied together with receptor.
receptor	Character. Receptor gene symbol. Must be supplied together with ligand.
log_pval	Logical. If TRUE (default), colour significant bins by $-\log_{10}$ (p-value). If FALSE, use $1 - p$ -value.
p_cutoff	Numeric or NULL. When NULL (default), the pre-computed hotspot bins stored in the blisa object are used, reflecting the p_cutoff and High-High quadrant classification applied during blisa. When a numeric value is supplied, bins are re-defined on the fly as those with $\text{all_pval} \leq p_cutoff$ and quadrant label "High-High" (from the stored all_quadrant), giving an exact re-threshold consistent with the original classification.

Value

A ggplot object.

Methods (by class)

- plotHotspots(blisa): Method for a blisa object.

plotLRrank

Dot plot ranking LR pairs by number of significant hotspot bins

Description

Generic function for ranking LR pairs. Dispatches on the class of x:

- plotLRrank.blisa accepts a blisa object and uses its LR_results slot directly.
- plotLRrank.data.frame accepts the LR_results data frame directly.

Usage

```
plotLRrank(x, ...)
```

```
## S3 method for class 'blisa'
```

```
plotLRrank(x, top = 30, pt_size = 4, flip = FALSE, ...)
```

```
## S3 method for class 'data.frame'
```

```
plotLRrank(x, top = 30, pt_size = 4, flip = FALSE, ...)
```

Arguments

x	A blisa object or a data frame of LR results. The data frame must contain columns sig_numbers and annotation.
...	Additional arguments passed to the relevant method.
top	Integer or NULL. Number of top LR pairs (by sig_numbers) to display. Default 30.
pt_size	Numeric. Point size passed to geom_point. Default 4.
flip	Logical. When TRUE, LR pairs are placed on the x-axis and the hotspot count on the y-axis (vertical orientation). Default FALSE (LR pairs on y-axis, horizontal orientation).

Value

A ggplot object.

Methods (by class)

- `plotLRrank(blisa)`: Method for a `blisa` object. Extracts `LR_results` and delegates to `plotLRrank.data.frame`.
- `plotLRrank(data.frame)`: Method for a data frame of LR results (e.g. the `LR_results` slot of a `blisa` object).

runCCI

*Score cell-cell interactions from BLISA hotspots***Description**

Generic function for scoring cell-cell interactions. Dispatches on the class of `x`:

- `runCCI.blisa` accepts a `blisa` object. If `CCI_scores` are already present and `overwrite = FALSE` (the default), the object is returned unchanged. Set `overwrite = TRUE` with a `counts_by_group` to recompute and replace existing scores. If no scores exist, `counts_by_group` must be supplied and scores are computed and attached.
- `runCCI.default` performs the raw computation given a `blisa` object and a `counts_by_group` list, returning only the scores data frame. Used internally by `runCCI.blisa` and [blisa.default](#).

Usage

```
runCCI(x, ...)

## S3 method for class 'blisa'
runCCI(x, counts_by_group = NULL, overwrite = FALSE, ...)

## Default S3 method:
runCCI(x, counts_by_group, ...)
```

Arguments

<code>x</code>	A <code>blisa</code> object.
<code>...</code>	Additional arguments passed to the relevant method.
<code>counts_by_group</code>	Named list of gene-by-bin sparse count matrices, one per group level (e.g. cell type). Typically the <code>counts_by_group</code> element of the list returned by hexBinCells when <code>group</code> is supplied. Names must match the group levels. Required when <code>x\$CCI_scores</code> is <code>NULL</code> or when <code>overwrite = TRUE</code> .
<code>overwrite</code>	Logical. If <code>FALSE</code> (default) and <code>x\$CCI_scores</code> is already populated, the object is returned unchanged. If <code>TRUE</code> and <code>counts_by_group</code> is supplied, existing scores are recomputed and replaced.

Value

See individual method documentation.

`runCCI.blisa`: the input `blisa` object with `CCI_scores` populated (a wide data frame – rows are "Sender->Receiver" group pairs, columns are LR pairs).

`runCCI.default`: a data frame with "Sender->Receiver" row names and one column per significant LR pair containing the interaction score $0.5 * \log_2(\text{receiver} * \text{sender} + 1)$.

Methods (by class)

- `runCCI(blisa)`: Method for a `blisa` object. If `CCI_scores` are already present and `overwrite = FALSE` (the default), the object is returned unchanged. Set `overwrite = TRUE` with a `counts_by_group` to recompute and replace existing scores. If no scores exist, `counts_by_group` must be supplied and scores are computed and attached to `x$CCI_scores`.
- `runCCI(default)`: Default method. Performs the raw CCI computation and returns only the scores data frame. Typically called internally; use `runCCI.blisa` to compute and attach scores to a `blisa` object in one step.

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