

# Package ‘hoodscanR’

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**Title** Spatial cellular neighbourhood scanning in R

**Version** 1.5.2

**Description** hoodscanR is an user-friendly R package providing functions to assist cellular neighborhood analysis of any spatial transcriptomics data with single-cell resolution. All functions in the package are built based on the SpatialExperiment object, allowing integration into various spatial transcriptomics-related packages from Bioconductor. The package can result in cell-level neighborhood annotation output, along with functions to perform neighborhood colocalization analysis and neighborhood-based cell clustering.

**biocViews** Spatial, Transcriptomics, SingleCell, Clustering

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**URL** <https://github.com/DavisLaboratory/hoodscanR>,  
<https://davislaboratory.github.io/hoodscanR/>

**BugReports** <https://github.com/DavisLaboratory/hoodscanR/issues>

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**Author** Ning Liu [aut, cre] (ORCID: <<https://orcid.org/0000-0002-9487-9305>>),  
Jarryd Martin [aut]

**Maintainer** Ning Liu <ning.liu@adelaide.edu.au>

## Contents

hoodscanR-package . . . . .	2
calcMetrics . . . . .	3
clustByHood . . . . .	4
findNearCells . . . . .	5
mergeByGroup . . . . .	6
mergeHoodSpe . . . . .	7
perplexityPermute . . . . .	8
plotColocal . . . . .	9
plotHoodMat . . . . .	10
plotProbDist . . . . .	12
plotTissue . . . . .	13
readHoodData . . . . .	14
scanHoods . . . . .	15
spe_test . . . . .	16
<b>Index</b>	<b>17</b>

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hoodscanR-package	<i>Method to identify cellular spatial neighbourhood from single cell spatial transcriptomics data.</i>
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## Description

hoodscanR implements a novel method to scan for cell neighbourhood from spatial transcriptomics data at single cell level, such as CosMx and MERFISH etc. hoodscanR takes the cellular position and cell type annotations as inputs, allowing cellular spatial neighbourhood analysis.

## Details

Key neighborhood analysis functions include `findNearCells`, `scanHoods`, `mergeByGroup`, `calcMetrics`, `clustByHood`.  
Key visualisation functions include `plotTissue`, `plotHoodMat`, `plotColocal`, `plotProbDist`.

## Author(s)

Ning Liu <liu.n@wehi.edu.au>

**See Also**

Useful links:

- <https://github.com/DavisLaboratory/hoodscanR>
- <https://davislaboratory.github.io/hoodscanR/>
- Report bugs at <https://github.com/DavisLaboratory/hoodscanR/issues>

---

calcMetrics

*Calculate metrics for probability matrix*

---

**Description**

Calculate metrics for probability matrix

**Usage**

```
calcMetrics(spe, pm = NA, pm_cols = NA, val_names = c("entropy", "perplexity"))
```

**Arguments**

spe	A SpatialExperiment object.
pm	Optional. The probability matrix.
pm_cols	The colnames of probability matrix. This is requires for SpatialExperiment input. Assuming that the probability is stored in the colData.
val_names	Character vector with length of 2. Column names used to store calculated entropy and perplexity.

**Value**

A SpatialExperiment object. Calculated entropy and perplexity are saved as columns in the colData of the SpatialExperiment object. Entropy and perplexity are calculated based on information theory:

$P(x)$  is the probability calculated from the scanHoods function.

Entropy  $H(x) = -P(x)\log_2(P(x))$

Perplexity  $P(x) = 2^{H(x)}$

By default, the calculated entropy and perplexity will be stored in the colData of the input spe, with column name as entropy and perplexity.

**Examples**

```

data("spe_test")

spe <- readHoodData(spe, anno_col = "celltypes")

fnc <- findNearCells(spe, k = 100)

pm <- scanHoods(fnc$distance)

pm2 <- mergeByGroup(pm, fnc$cells)

spe <- mergeHoodSpe(spe, pm2)

spe <- calcMetrics(spe, pm_cols = colnames(pm2))

```

---

clustByHood

---

*Cluster the probability matrix with K-means*


---

**Description**

Cluster the probability matrix with K-means

**Usage**

```

clustByHood(object, ...)

## S4 method for signature 'matrix'
clustByHood(object, k = 2^ncol(object) - 1, iter_max = 1000, nstart = 5)

## S4 method for signature 'SpatialExperiment'
clustByHood(
  object,
  pm_cols,
  k = 0,
  iter_max = 1000,
  nstart = 5,
  algo = "Hartigan-Wong",
  val_name = "clusters"
)

```

**Arguments**

object	A probability matrix or a SpatialExperiment.
...	Ignore parameter.
k	The number of clusters. By default is $2^{\text{ncol}(\text{object})}-1$ .
iter_max	the maximum number of iterations allowed.

nstart	how many random sets should be chosen.
pm_cols	The colnames of probability matrix. This is requires for SpatialExperiment input. Assuming that the probability is stored in the colData.
algo	Algorithm to be used. Options include Hartigan-Wong, Lloyd, and MacQueen.
val_name	Character. Column name used to store the clusters.

**Value**

A probability matrix or a SpatialExperiment object. For latter, the clustering results are saved in the colData of the SpatialExperiment object.

**Examples**

```
m <- matrix(abs(rnorm(1000 * 100)), 1000, 100)
clust <- clustByHood(m, k = 3)
```

---

findNearCells	<i>Find the k-th nearest cells for each cell</i>
---------------	--

---

**Description**

Find the k-th nearest cells for each cell

**Usage**

```
findNearCells(
  dat,
  k = 100,
  targetCell = FALSE,
  reportCellID = FALSE,
  reportDist = TRUE,
  anno_col = 0
)
```

**Arguments**

dat	A SpatialExperiment object, can be generated using function readHoodData.
k	The maximum number of nearest cells to compute.
targetCell	Specify the cells to be the target cell for finding nearest cells.
reportCellID	Logical. Set to TRUE to report cell id instead of cell types.
reportDist	Logical. Set to TRUE to report the distance matrix.
anno_col	Character vector. The name of annotation column to use.

**Details**

The `findNearCells` function uses the `nn2` function from the RANN package, which uses the Approximate Near Neighbor (ANN) C++ library. For more information on the ANN library please see <http://www.cs.umd.edu/~mount/ANN/>.

**Value**

A list includes a `data.frame` and a matrix, describing the cell types and distances of the `k`-th nearest cells of each cell.

**Examples**

```
data("spe_test")

spe <- readHoodData(spe, anno_col = "celltypes")

fnc <- findNearCells(spe, k = 100)
```

---

mergeByGroup

*Merge probability matrix based on annotations*

---

**Description**

Merge probability matrix based on annotations

**Usage**

```
mergeByGroup(pm, group_df)
```

**Arguments**

`pm` A numeric matrix. Probability matrix generated by the `soft_max` function.  
`group_df` A character matrix. Annotation of the neighboring cells to be used.

**Value**

A probability matrix, describing the probability of each cell being in each cellular neighborhood.

**Examples**

```
data("spe_test")

spe <- readHoodData(spe, anno_col = "celltypes")

fnc <- findNearCells(spe, k = 100)

pm <- scanHoods(fnc$distance)
```

```
pm2 <- mergeByGroup(pm, fnc$cells)
```

---

mergeHoodSpe	<i>Merge probability matrix into SpatialExperiment object.</i>
--------------	--

---

### Description

Merge probability matrix into SpatialExperiment object.

### Usage

```
mergeHoodSpe(spe, pm, val_names = NULL)
```

### Arguments

spe	A SpatialExperiment object.
pm	Probability matrix. Can be obtained by the function mergeByGroup.
val_names	Character vector with length of the ncol of pm.

### Value

A SpatialExperiment object. Cell-level neighborhood information are saved in the colData of the SpatialExperiment object.

### Examples

```
data("spe_test")  
  
spe <- readHoodData(spe, anno_col = "celltypes")  
  
fnc <- findNearCells(spe, k = 100)  
  
pm <- scanHoods(fnc$distance)  
  
pm2 <- mergeByGroup(pm, fnc$cells)  
  
spe <- mergeHoodSpe(spe, pm2)
```

---

perplexityPermute      *Compute p-value for perplexity via permutation*

---

### Description

Compute p-value for perplexity via permutation

### Usage

```
perplexityPermute(spe, pm = NA, pm_cols = NA, n_perm = 1000)
```

### Arguments

spe	A SpatialExperiment object.
pm	Optional. The probability matrix.
pm_cols	The colnames of probability matrix. This is requires for SpatialExperiment input. Assuming that the probability is stored in the colData.
n_perm	Integer number. The number of permutation. 1000 by default.

### Value

A SpatialExperiment object. Calculated P-value and adjusted P-value are saved as columns in the colData of the SpatialExperiment object. P-value and adjusted P-value are calculated based on permutation test and Benjamini Hochberg correction.

### Examples

```
data("spe_test")  
  
spe <- readHoodData(spe, anno_col = "celltypes")  
  
fnc <- findNearCells(spe, k = 100)  
  
pm <- scanHoods(fnc$distance)  
  
pm2 <- mergeByGroup(pm, fnc$cells)  
  
spe <- mergeHoodSpe(spe, pm2)  
  
spe <- perplexityPermute(spe, pm_cols = colnames(pm2))
```



---

plotColocal

*Plot heatmap for neighbourhood analysis*


---

### Description

Plot heatmap for neighbourhood analysis

### Usage

```
plotColocal(object, ...)

## S4 method for signature 'matrix'
plotColocal(object, hm_width = 5, hm_height = 5)

## S4 method for signature 'SpatialExperiment'
plotColocal(
  object,
  pm_cols,
  self_cor = TRUE,
  by_group = NULL,
  hm_width = 5,
  hm_height = 5,
  cluster_row = TRUE,
  cluster_col = TRUE,
  return_matrix = FALSE
)
```

### Arguments

object	A probability matrix or SpatialExperiment.
...	Ignore parameter.
hm_width	Integer. The width of heatmap.
hm_height	Integer. The height of heatmap.
pm_cols	The colnames of probability matrix. This is requires for SpatialExperiment input. Assuming that the probability is stored in the colData.
self_cor	Logical. By default is TRUE, indicating running a correlation between neighbourhoods to perform a simple co-localization analysis. When this set to FALSE, it will plot the average probability of each neighbourhood by group using the by_group parameter.
by_group	Character. This is required when self_cor is set to FALSE.
cluster_row	Logical. Cluster rows.
cluster_col	Logical. Cluster columns.
return_matrix	Logical. Export a numeric matrix .

**Value**

A ComplexHeatmap plot. When return\_matrix is set to TRUE, return a matrix Object.

**Examples**

```
data("spe_test")

spe <- readHoodData(spe, anno_col = "celltypes")

fnc <- findNearCells(spe, k = 100)

pm <- scanHoods(fnc$distance)

pm2 <- mergeByGroup(pm, fnc$cells)

spe <- mergeHoodSpe(spe, pm2)

plotColocal(spe, pm_cols = colnames(pm2))

plotColocal(spe, pm_cols = colnames(pm2), self_cor = FALSE, by_group = "cell_annotation")
```

---

plotHoodMat

*Plot probability matrix as a heatmap*


---

**Description**

Plot probability matrix as a heatmap

**Usage**

```
plotHoodMat(object, ...)

## S4 method for signature 'matrix'
plotHoodMat(
  object,
  targetCells = NA,
  n = 30,
  hm_width = 4,
  hm_height = 15,
  clusterRows = TRUE,
  clusterCols = TRUE,
  title = "Probability of neighborhoods"
)

## S4 method for signature 'SpatialExperiment'
plotHoodMat(
  object,
```

```

    pm_cols,
    targetCells = NA,
    n = 30,
    hm_width = 4,
    hm_height = 15,
    clusterRows = TRUE,
    clusterCols = TRUE,
    title = "Probability of neighborhoods"
  )

```

### Arguments

object	A probability matrix or SpatialExperiment.
...	Ignore parameter.
targetCells	Character. Optional. Can specify one or more cells to be plotted.
n	Integer. The number of randomly selected cells to be plotted. This parameter will be used when targetCells is not specify.
hm_width	Integer. The width of heatmap.
hm_height	Integer. The height of heatmap.
clusterRows	Logical. Cluster rows or not.
clusterCols	Logical. Cluster columns or not.
title	Title of the heatmap.
pm_cols	The colnames of probability matrix. This is requires for SpatialExperiment input. Assuming that the probability is stored in the colData.

### Value

A ComplexHeatmap plot.

### Examples

```

data("spe_test")

spe <- readHoodData(spe, anno_col = "celltypes")

fnc <- findNearCells(spe, k = 100)

pm <- scanHoods(fnc$distance)

pm2 <- mergeByGroup(pm, fnc$cells)

spe <- mergeHoodSpe(spe, pm2)

plotHoodMat(spe, pm_cols = colnames(pm2))

```

---

plotProbDist	<i>Plot probability distribution</i>
--------------	--------------------------------------

---

**Description**

Plot probability distribution

**Usage**

```
plotProbDist(object, ...)

## S4 method for signature 'matrix'
plotProbDist(object, targetCells = NA, ...)

## S4 method for signature 'SpatialExperiment'
plotProbDist(
  object,
  pm_cols,
  targetCells = NA,
  by_cluster = FALSE,
  show_clusters = as.character(seq(6)),
  plot_all = FALSE,
  sample_size = 2,
  val_name = "clusters",
  ...
)
```

**Arguments**

object	A probability matrix or SpatialExperiment.
...	aesthetic mappings to pass to <code>ggplot2::aes_string()</code> .
targetCells	Character. Optional. Can specify one or more cells to be plotted.
pm_cols	The colnames of probability matrix. This is required for SpatialExperiment input. Assuming that the probability is stored in the colData.
by_cluster	Logical. By default is TRUE, to plot distribution by each cluster.
show_clusters	Character. The cluster to be plotted, by default is 1 to 6.
plot_all	Logical. By default is FALSE, set this to true to plot box plot instead of bar plot to show all cells in each cluster.
sample_size	Integer. By default is 2, sampling two cell from each cluster to be plotted.
val_name	Character. Column name used to store the clusters.

**Value**

A ggplot object.

**Examples**

```
data("spe_test")

spe <- readHoodData(spe, anno_col = "celltypes")

fnc <- findNearCells(spe, k = 100)

pm <- scanHoods(fnc$distance)

pm2 <- mergeByGroup(pm, fnc$cells)

spe <- mergeHoodSpe(spe, pm2)

plotProbDist(spe, pm_cols = colnames(pm2))
```

---

plotTissue	<i>Plot cells based on cell position on tissue.</i>
------------	---

---

**Description**

Plot cells based on cell position on tissue.

**Usage**

```
plotTissue(  
  spe,  
  targetcell = FALSE,  
  k_near = 100,  
  targetsize = 3,  
  targetshape = 1,  
  targetcolor = "red",  
  scaleFactor = 1,  
  reverseY = TRUE,  
  ...  
)
```

**Arguments**

spe	SpatialExperiment object.
targetcell	Optional. Can input ONE specific cell id to zoom-in on the region of a specific cell.
k_near	Optional. If targetcell is specified, the k_near cells around the targetcell will be plotted.
targetsize	Dot size of the targetcell.
targetshape	Shape of the targetcell.

targetcolor	Colour of the targetcell.
scaleFactor	Scale factor to align with the image.
reverseY	Reverse y coordinates.
...	aesthetic mappings to pass to <code>ggplot2::aes_string()</code> .

**Value**

A ggplot object.

**Examples**

```
data("spe_test")

plotTissue(spe, color = celltypes)
```

---

readHoodData	<i>Read cellular position and annotation data into a list object.</i>
--------------	---

---

**Description**

Read cellular position and annotation data into a list object.

**Usage**

```
readHoodData(
  spe = NA,
  anno_col = NA,
  cell_pos_dat = NA,
  cell_anno_dat = NA,
  pos_col = NA
)
```

**Arguments**

spe	SpatialExperiment object.
anno_col	Character. The column name of the annotation to be used in the following neighbourhood analysis.
cell_pos_dat	data.frame object contains the cellular positions.
cell_anno_dat	data.frame object contains the cell annotations.
pos_col	Character. If the x and y are in the colData instead of in the SpatialCoords of spe, can specify this parameter.

**Value**

A SpatialExperiment object.

**Examples**

```
data("spe_test")  
  
spe <- readHoodData(spe, anno_col = "celltypes")
```

---

scanHoods	<i>Scan cellular neighbourhoods.</i>
-----------	--------------------------------------

---

**Description**

Scan cellular neighbourhoods.

**Usage**

```
scanHoods(  
  m,  
  mode = c("proximityFocused", "smoothFadeout"),  
  tau = NA,  
  t_init = NA  
)
```

**Arguments**

m	Distance matrix. Can be obtained from function findNearCells.
mode	Character. Either proximityFocused or smoothFadeout. By default is proximityFocused.
tau	The hyperparameter tau, by default is $\text{median}(m^{**2})/5$
t_init	An initial tau. In the smoothFadeout mode, user can provide an initial tau for optimization.

**Value**

A probability matrix.

**Examples**

```
m <- matrix(abs(rnorm(1000 * 100))), 1000, 100)  
  
pm <- scanHoods(m)
```

---

`spe_test`*Example test spatial transcriptomics data*

---

**Description**

hoodscanR-package has 1 datasets:

- `spe_test` Example test spatial transcriptomics data in `SpatialExperiment` format. This test data is randomly subsetting from the publicly available CosMx non-small cell lung cancer data. Source data: <https://nanosttring.com/products/cosmx-spatial-molecular-imager/nsclc-ffpe-dataset/>.

**Usage**

```
data("spe_test")
```

**Format**

A `SpatialExperiment` object

**Value**

A `SpatialExperiment` object

**Examples**

```
data(spe_test)
```



# Index

## \* **internal**

- hoodscanR-package, 2
- spe\_test, 16

calcMetrics, 2, 3

clustByHood, 2, 4

clustByHood, matrix-method  
(clustByHood), 4

clustByHood, SpatialExperiment-method  
(clustByHood), 4

findNearCells, 2, 5

hoodscanR (hoodscanR-package), 2

hoodscanR-package, 2

mergeByGroup, 2, 6

mergeHoodSpe, 7

perplexityPermute, 8

plotColocal, 2, 9

plotColocal, matrix-method  
(plotColocal), 9

plotColocal, SpatialExperiment-method  
(plotColocal), 9

plotHoodMat, 2, 10

plotHoodMat, matrix-method  
(plotHoodMat), 10

plotHoodMat, SpatialExperiment-method  
(plotHoodMat), 10

plotProbDist, 2, 12

plotProbDist, matrix-method  
(plotProbDist), 12

plotProbDist, SpatialExperiment-method  
(plotProbDist), 12

plotTissue, 2, 13

readHoodData, 14

scanHoods, 2, 15

spe (spe\_test), 16

spe\_test, 16