

# Package ‘scHOT’

July 24, 2025

**Type** Package

**Title** single-cell higher order testing

**Version** 1.20.1

**Description** Single cell Higher Order Testing (scHOT) is an R package that facilitates testing changes in higher order structure of gene expression along either a developmental trajectory or across space. scHOT is general and modular in nature, can be run in multiple data contexts such as along a continuous trajectory, between discrete groups, and over spatial orientations; as well as accommodate any higher order measurement such as variability or correlation. scHOT meaningfully adds to first order effect testing, such as differential expression, and provides a framework for interrogating higher order interactions from single cell data.

**License** GPL-3

**Encoding** UTF-8

**LazyData** true

**Depends** R (>= 4.0)

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|       |                                      |
|-------|--------------------------------------|
| liver | <i>Liver trajectory example data</i> |
|-------|--------------------------------------|

---

### Description

A liver data set contains two branches (hepatocyte and cholangiocyte).

### Usage

```
data(liver, package = 'scHOT')
```

**Format**

A ‘list’ object.

---

|            |                                        |
|------------|----------------------------------------|
| MOB_subset | <i>MOB_subset spatial example data</i> |
|------------|----------------------------------------|

---

**Description**

A MOB spatial data set.

**Usage**

```
data(MOB_subset, package = 'schOT')
```

**Format**

An object of class list of length 1.

---

|          |                                           |
|----------|-------------------------------------------|
| params<- | <i>Setter functions for schOT objects</i> |
|----------|-------------------------------------------|

---

**Description**

Setter functions for schOT objects

**Usage**

```
params(x) <- value
```

**Arguments**

- |       |                                |
|-------|--------------------------------|
| x     | A schOT object                 |
| value | The value the slot should take |

**Value**

A schOT object

**Examples**

```
data(MOB_subset)
sce_MOB_subset <- MOB_subset$sce_MOB_subset
scHOT_spatial <- scHOT_buildFromSCE(sce_MOB_subset,
  assayName = "logcounts",
  positionType = "spatial",
  positionColData = c("x", "y"))

params = list(higherOrderFunction = weightedSpearman,
  higherOrderFunctionType = "weighted")

params(scHOT_spatial) <- params
```

---

plotColouredExpression

*plotColouredExpression*


---

**Description**

the plotColouredExpression function plots an n-panel scatterplot of the gene pairs split by early, mid, and late in the sample ordering.

**Usage**

```
plotColouredExpression(
  scHOT,
  genes,
  genes_delimiter = "_",
  branches = NULL,
  ranked_by = NULL,
  subsetBranch = NULL,
  n = 3,
  fittedline = TRUE,
  assayName = NULL
)
```

**Arguments**

|                 |                                                                                          |
|-----------------|------------------------------------------------------------------------------------------|
| scHOT           | A scHOT object.                                                                          |
| genes           | is either a single character string with a delimiter, or a length two character vector   |
| genes_delimiter | is the delimiter to split into two gene names if genes is provided as a single character |
| branches        | A character indicates that the colnames stored the branch information in colData         |

|              |                                                                                                                                                                                            |
|--------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| ranked_by    | A character indicates that the colnames stored the ranking information of the cells in colData, such as trajectory time, if it is NULL, it will be ranked based on the branch information. |
| subsetBranch | subsetBranch is a character vector containing the names of the branches to be plotted. If NULL it will plot all branches                                                                   |
| n            | number of panels to split ranked samples into, default 3.                                                                                                                                  |
| fittedline   | logical default TRUE, add a lm straight line to the plot                                                                                                                                   |
| assayName    | the name of the assay that are used to plot.                                                                                                                                               |

**Value**

ggplot a ggplot object of scatterplots of expression split by sample ordering

**Examples**

```
data(liver)

scHOT_traj <- scHOT_buildFromMatrix(
  mat = liver$liver_branch_hep,
  cellData = list(pseudotime = liver$liver_pseudotime_hep),
  positionType = "trajectory",
  positionColData = "pseudotime")

scHOT_traj

plotColouredExpression(scHOT_traj, c("Cdt1", "Top2a"), n = 5)
```

---

plotEgoNetwork

*plotEgoNetwork*


---

**Description**

the plotEgoNetwork function plots network graphs with edges coloured by weights in the network

**Usage**

```
plotEgoNetwork(
  scHOT,
  hubnode,
  network,
  weight = "higherOrderStatistic",
  subset = FALSE,
  thresh = NULL
)
```

**Arguments**

|         |                                                                                                            |
|---------|------------------------------------------------------------------------------------------------------------|
| scHOT   | a scHOT object                                                                                             |
| hubnode | is a character vector of node(s) to include as hub nodes                                                   |
| network | is an igraph network                                                                                       |
| weight  | A string indicates the column name stored in scHOT_output slot that are used as the weights of the network |
| subset  | is a logical asking if you should subset based on the weight (default FALSE)                               |
| thresh  | is the subset weight threshold                                                                             |

**Value**

igraph object containing the network graphed. Produces an igraph plot

---

plotHigherOrderSequence  
*plotHigherOrderSequence*

---

**Description**

the plotHigherOrderSequence function plots weighted higher order statistic vectors (stored in higherOrderSequence) as line plots

**Usage**

```
plotHigherOrderSequence(
  scHOT,
  gene,
  positionType = NULL,
  branches = NULL,
  positionColData = NULL
)
```

**Arguments**

|                 |                                                                                                                |
|-----------------|----------------------------------------------------------------------------------------------------------------|
| scHOT           | A scHOT object with higherOrderSequence in scHOT_output slot                                                   |
| gene            | is either a logical vector matching rows of entries in wcorsList, or a character of a gene                     |
| positionType    | A string indicates the position type, either trajectory or spatial                                             |
| branches        | A character indicates that the colnames stored the branch information in colData (for trajectory type of data) |
| positionColData | A vector indicates column names of colData that stored the position information (for spatial type of data)     |

**Value**

ggplot object with line plots

**Examples**

```
data(liver)

schOT_traj <- schOT_buildFromMatrix(
  mat = liver$liver_branch_hep,
  cellData = list(pseudotime = liver$liver_pseudotime_hep),
  positionType = "trajectory",
  positionColData = "pseudotime")
schOT_traj
plotColouredExpression(schOT_traj, c("Cdt1", "Top2a"), n = 5)

schOT_traj <- schOT_addTestingScaffold(schOT_traj,
  t(as.matrix(c("Cdt1", "Top2a"))))
schOT_traj <- schOT_setWeightMatrix(schOT_traj,
  positionColData = c("pseudotime"),
  positionType = "trajectory",
  nrow.out = NULL,
  span = 0.25)
schOT_traj <- schOT_calculateGlobalHigherOrderFunction(schOT_traj,
  higherOrderFunction =
    weightedSpearman,
  higherOrderFunctionType =
    "weighted")

schOT_traj <- schOT_calculateHigherOrderTestStatistics(schOT_traj,
  higherOrderSummaryFunction =
    sd)

slot(schOT_traj, "schOT_output")
plotHigherOrderSequence(schOT_traj, c("Cdt1_Top2a"))
```

---

plotOrderedExpression *plotOrderedExpression*

---

**Description**

the plotOrderedExpression function plots expression vectors along branches and genes as ribbon plots

**Usage**

```
plotOrderedExpression(
  schOT,
  genes,
```

```

    positionType = NULL,
    branches = NULL,
    ranked_by = NULL,
    xvals = NULL,
    subsetBranch = NULL,
    facet = FALSE,
    positionColData = NULL,
    assayName = NULL
  )

```

### Arguments

|                              |                                                                                                                          |
|------------------------------|--------------------------------------------------------------------------------------------------------------------------|
| <code>scHOT</code>           | A scHOT object, where the expression data is stored in the assay slot, with assay name "expression".                     |
| <code>genes</code>           | is a character vector for gene names                                                                                     |
| <code>positionType</code>    | A string indicates the position type, either trajectory or spatial                                                       |
| <code>branches</code>        | A character indicates that the colnames stored the branch information in colData                                         |
| <code>ranked_by</code>       | A character indicates that the colnames stored the ranking information of the cells in colData, such as trajectory time  |
| <code>xvals</code>           | A character indicates that the colnames stored in colData of the x-values associated with the samples in branchData      |
| <code>subsetBranch</code>    | subsetBranch is a character vector containing the names of the branches to be plotted. If NULL it will plot all branches |
| <code>facet</code>           | can either be FALSE, "branch", "gene", or "both"                                                                         |
| <code>positionColData</code> | A vector indicates column names of colData that stored the position information (for spatial type of data)               |
| <code>assayName</code>       | the name of the assay that are used to plot.                                                                             |

### Value

ggplot a ggplot object for ribbon plot with points

---

`positionColData<-`      *Setter functions for scHOT objects*

---

### Description

Setter functions for scHOT objects

### Usage

```
positionColData(x) <- value
```

*positionType*<-

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### Arguments

|       |                                |
|-------|--------------------------------|
| x     | A scHOT object                 |
| value | The value the slot should take |

### Value

A scHOT object

### Examples

```
data(MOB_subset)
sce_MOB_subset <- MOB_subset$sce_MOB_subset
scHOT_spatial <- scHOT_buildFromSCE(sce_MOB_subset,
  assayName = "logcounts",
  positionType = "spatial",
  positionColData = c("x", "y"))

positionColData(scHOT_spatial) <- c("x", "y")
```

---

|                        |                                           |
|------------------------|-------------------------------------------|
| <i>positionType</i> <- | <i>Setter functions for scHOT objects</i> |
|------------------------|-------------------------------------------|

---

### Description

Setter functions for scHOT objects

### Usage

```
positionType(x) <- value
```

### Arguments

|       |                                |
|-------|--------------------------------|
| x     | A scHOT object                 |
| value | The value the slot should take |

### Value

A scHOT object

### Examples

```
data(MOB_subset)
sce_MOB_subset <- MOB_subset$sce_MOB_subset
scHOT_spatial <- scHOT_buildFromSCE(sce_MOB_subset,
  assayName = "logcounts",
  positionType = "spatial",
  positionColData = c("x", "y"))
```

```
positionType(scHOT_spatial) <- "spatial"
```

---

scHOT

*scHOT*


---

## Description

A wrapper function to perform scHOT

## Usage

```
scHOT(
  scHOT,
  testingScaffold = NULL,
  weightMatrix = NULL,
  positionType = NULL,
  positionColData = NULL,
  nrow.out = NULL,
  averageAcrossTrajectoryTies = FALSE,
  higherOrderFunction = NULL,
  higherOrderFunctionType = NULL,
  numberPermutations = 1000,
  numberScaffold = 100,
  storePermutations = TRUE,
  higherOrderSummaryFunction = sd,
  parallel = FALSE,
  BPPARAM = BiocParallel::SerialParam(),
  useperm_estimate = FALSE,
  nperm_estimate = 10000,
  maxDist = 0.1,
  plot = FALSE,
  verbose = TRUE,
  ...
)
```

## Arguments

|                 |                                                                         |
|-----------------|-------------------------------------------------------------------------|
| scHOT           | A scHOT object                                                          |
| testingScaffold | A matrix with rows for each testing combination                         |
| weightMatrix    | A matrix indicates the weight matrix for scHOT analysis                 |
| positionType    | A string indicating the position type, either "trajectory" or "spatial" |

|                             |                                                                                                                                                                                                                                                                                                                                                                          |
|-----------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| positionColData             | Either trajectory or spatial information for each sample. If positionType is "trajectory" then positionColData should be a character or numeric indicating the subset of colData of the scHOT object. If positionType is "spatial" then positionColData should be a character or numeric vector indicating the subset of colData that give the full spatial coordinates. |
| nrow.out                    | The number of weightings to include for testing, a smaller value is faster for computation                                                                                                                                                                                                                                                                               |
| averageAcrossTrajectoryTies | Logical indicating whether ties in the trajectory should be given the same local weights                                                                                                                                                                                                                                                                                 |
| higherOrderFunction         | A function object indicates the higher order function                                                                                                                                                                                                                                                                                                                    |
| higherOrderFunctionType     | is "weighted" or "unweighted", determines if there is a weighting argument in the higher order function                                                                                                                                                                                                                                                                  |
| numberPermutations          | The number of permutations, set as 1000 by default                                                                                                                                                                                                                                                                                                                       |
| numberScaffold              | The number of testing scaffolds to perform permutations, set as 100 by default                                                                                                                                                                                                                                                                                           |
| storePermutations           | a logical flag on whether permutation values should be saved                                                                                                                                                                                                                                                                                                             |
| higherOrderSummaryFunction  | A function indicating the higher order summary function (default is standard deviation 'sd')                                                                                                                                                                                                                                                                             |
| parallel                    | A logical input indicating whether to run the permutation test using multiple cores in parallel.                                                                                                                                                                                                                                                                         |
| BPPARAM                     | A BiocParallelParam class object from the BiocParallel package is used. Default is SerialParam().                                                                                                                                                                                                                                                                        |
| usenperm_estimate           | Logical (default FALSE) if number of neighbouring permutations should be used to estimate P-values, or if difference of global higher order statistic should be used                                                                                                                                                                                                     |
| nperm_estimate              | Number of neighbouring permutations to use for p-value estimation                                                                                                                                                                                                                                                                                                        |
| maxDist                     | max difference of global higher order statistic to use for p-value estimation (default 0.1)                                                                                                                                                                                                                                                                              |
| plot                        | A logical input indicating whether the results are plotted                                                                                                                                                                                                                                                                                                               |
| verbose                     | A logical input indicating whether the intermediate steps will be printed                                                                                                                                                                                                                                                                                                |
| ...                         | parameters for function trajectoryWeightMatrix or spatialWeightMatrix                                                                                                                                                                                                                                                                                                    |

**Value**

A scHOT object

**Examples**

```

data(MOB_subset)
sce_MOB_subset <- MOB_subset$sce_MOB_subset
scHOT_spatial <- scHOT_buildFromSCE(sce_MOB_subset,
                                   assayName = "logcounts",
                                   positionType = "spatial",
                                   positionColData = c("x", "y"))
pairs <- matrix(c("Arrb1", "Mtor", "Dnm1l", "Gucy1b3"), ncol = 2, byrow = TRUE)
rownames(pairs) <- apply(pairs,1,paste0,collapse = "_")

scHOT_spatial <- scHOT(scHOT_spatial,
                      testingScaffold = pairs,
                      positionType = "spatial",
                      positionColData = c("x", "y"),
                      nrow.out = NULL,
                      higherOrderFunction = weightedSpearman,
                      higherOrderFunctionType = "weighted",
                      numberPermutations = 100,
                      higherOrderSummaryFunction = sd,
                      parallel = FALSE,
                      verbose = TRUE,
                      span = 0.05)

```

---

scHOT-class

*scHOT class*


---

**Description**

scHOT class

**Slots**

testingScaffold A matrix with rows for each testing combination

weightMatrix A matrix or dgCMatrix indicates the weight matrix

scHOT\_output A data.frame or DtatFrame to store output from scHOT

params A list of parameters

positionType A character indicates the type of the position, either trajectory or spatial

positionColData A vector indicates column names of colData that stored the position informaton

---

```
scHOT_addTestingScaffold
      scHOT_addTestingScaffold
```

---

**Description**

Add a testing scaffold to a scHOT object

**Usage**

```
scHOT_addTestingScaffold(scHOT, testingScaffold)
```

**Arguments**

|                 |                                                                                                                   |
|-----------------|-------------------------------------------------------------------------------------------------------------------|
| scHOT           | A scHOT object                                                                                                    |
| testingScaffold | A matrix with rows for each testing combination, and columns for level of dimensionality (1 for single gene etc.) |

**Value**

A scHOT object with slot testingScaffold saved

**Examples**

```
data(MOB_subset)
sce_MOB_subset <- MOB_subset$sce_MOB_subset
scHOT_spatial <- scHOT_buildFromSCE(sce_MOB_subset,
  assayName = "logcounts",
  positionType = "spatial",
  positionColData = c("x", "y"))
pairs <- matrix(c("Arrb1", "Mtor", "Dnm1l", "Gucy1b3"), ncol = 2, byrow = TRUE)
scHOT_spatial <- scHOT_addTestingScaffold(scHOT_spatial, pairs)
```

---

```
scHOT_buildFromMatrix  scHOT_buildFromMatrix
```

---

**Description**

Create scHOT object from a matrix

**Usage**

```
scHOT_buildFromMatrix(
  mat,
  cellData = NULL,
  positionType = NULL,
  positionColData = NULL
)
```

**Arguments**

|                              |                                                                                                                                                                                                                                                                                                   |
|------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>mat</code>             | A matrix with rows for genes and columns for cells                                                                                                                                                                                                                                                |
| <code>cellData</code>        | A dataframe or DataFrame object with rows for cells                                                                                                                                                                                                                                               |
| <code>positionType</code>    | A string indicating the position type, either "trajectory" or "spatial"                                                                                                                                                                                                                           |
| <code>positionColData</code> | Strings indicate the position information stored in <code>colData</code> . If <code>positionType</code> is "trajectory" then <code>positionColData</code> should be a sortable vector if <code>positionType</code> is "spatial" then <code>positionColData</code> should be a matrix type object. |

**Value**

A scHOT object

**Examples**

```
dat <- rbind(rnorm(50), rnorm(50), rnorm(50))
colnames(dat) <- paste0("cell_", 1:ncol(dat))
rownames(dat) <- c("gene_1", "gene_2", "gene_2")

scHOT <- scHOT_buildFromMatrix(dat, cellData = data.frame(1:ncol(dat)))
```

---

|                    |                           |
|--------------------|---------------------------|
| scHOT_buildFromSCE | <i>scHOT_buildFromSCE</i> |
|--------------------|---------------------------|

---

**Description**

Create scHOT object from a SingleCellExperiment object

**Usage**

```
scHOT_buildFromSCE(
  sce,
  assayName = "counts",
  positionType = NULL,
  positionColData = NULL
)
```

**Arguments**

**sce** A SingleCellExperiment object  
**assayName** is a single assay to pull out from sce as the expression matrix input of scHOT  
**positionType** A string indicates the position type, either trajectory or spatial  
**positionColData** Strings indicate the position information stored in colData. If positionType is "trajectory" then positionColData should be a sortable vector if positionType is "spatial" then positionColData should be a matrix type object.

**Value**

A scHOT object

**Examples**

```

library(SingleCellExperiment)
dat <- rbind(rnorm(50), rnorm(50), rnorm(50))
colnames(dat) <- paste0("cell_", 1:ncol(dat))
rownames(dat) <- c("gene_1", "gene_2", "gene_2")

sce <- SingleCellExperiment::SingleCellExperiment(assays =
S4Vectors::SimpleList(counts = dat))
scHOT <- scHOT_buildFromSCE(sce)

```

---

scHOT\_calculateGlobalHigherOrderFunction

*scHOT\_calculateGlobalHigherOrderFunction*

---

**Description**

this calculates the global higher order function and stores it in the output if these aren't found in the params slot then they need to be specified here

**Usage**

```

scHOT_calculateGlobalHigherOrderFunction(
  scHOT,
  higherOrderFunction = NULL,
  higherOrderFunctionType = NULL
)

```

**Arguments**

scHOT                    A scHOT object

higherOrderFunction                    A function object indicating the higher order function

higherOrderFunctionType                    is "weighted" or "unweighted", determines if there is a weighting argument in the higher order function

**Details**

Calculates the global higher order function

**Value**

A scHOT object with scHOT\_output\$globalHigherOrderFunction in slot scHOT\_output saved

**Examples**

```
data(MOB_subset)
sce_MOB_subset <- MOB_subset$sce_MOB_subset
scHOT_spatial <- scHOT_buildFromSCE(sce_MOB_subset,
                                     assayName = "logcounts",
                                     positionType = "spatial",
                                     positionColData = c("x", "y"))
pairs <- matrix(c("Arrb1", "Mtor", "Dnm1l", "Gucy1b3"), ncol = 2, byrow = TRUE)
rownames(pairs) <- apply(pairs, 1, paste0, collapse = "_")
scHOT_spatial <- scHOT_addTestingScaffold(scHOT_spatial, pairs)
scHOT_spatial <- scHOT_setWeightMatrix(scHOT_spatial,
                                       positionColData = c("x", "y"),
                                       positionType = "spatial",
                                       nrow.out = NULL,
                                       span = 0.05)
scHOT_spatial <- scHOT_calculateGlobalHigherOrderFunction(
  scHOT_spatial,
  higherOrderFunction = weightedSpearman,
  higherOrderFunctionType = "weighted")
```

---

scHOT\_calculateHigherOrderTestStatistics

*scHOT\_calculateHigherOrderTestStatistics*

---

**Description**

Calculate and store the higherOrderSequence and higherOrderTestStatistic

**Usage**

```
scHOT_calculateHigherOrderTestStatistics(
  scHOT,
  higherOrderSummaryFunction = stats::sd,
  ...
)
```

**Arguments**

|                            |                                                                                       |
|----------------------------|---------------------------------------------------------------------------------------|
| scHOT                      | A scHOT object                                                                        |
| higherOrderSummaryFunction | A function which indicates how the higher order sequence is summarised, default is sd |
| ...                        | parameters for higherOrderSummaryFunction                                             |

**Value**

scHOT A scHOT object with results stored in scHOT\_output slot

**Examples**

```
data(MOB_subset)
sce_MOB_subset <- MOB_subset$sce_MOB_subset
scHOT_spatial <- scHOT_buildFromSCE(sce_MOB_subset,
                                   assayName = "logcounts",
                                   positionType = "spatial",
                                   positionColData = c("x", "y"))
pairs <- matrix(c("Arrb1", "Mtor", "Dnm1l", "Gucy1b3"), ncol = 2, byrow = TRUE)

rownames(pairs) <- apply(pairs, 1, paste0, collapse = "_")
scHOT_spatial <- scHOT_addTestingScaffold(scHOT_spatial, pairs)

scHOT_spatial <- scHOT_setWeightMatrix(scHOT_spatial,
                                       positionColData = c("x", "y"),
                                       positionType = "spatial",
                                       nrow.out = NULL,
                                       span = 0.05)

scHOT_spatial <- scHOT_calculateGlobalHigherOrderFunction(
  scHOT_spatial,
  higherOrderFunction = weightedSpearman,
  higherOrderFunctionType = "weighted")
scHOT_spatial <- scHOT_setPermutationScaffold(scHOT_spatial,
                                             numberPermutations = 100)

scHOT_spatial <- scHOT_calculateHigherOrderTestStatistics(
  scHOT_spatial,
  higherOrderSummaryFunction = sd)
```

---

scHOT\_estimatePvalues *scHOT\_estimatePvalues*


---

### Description

Estimate p-values based on already run permutation tests

### Usage

```
scHOT_estimatePvalues(
  scHOT,
  usenperm_estimate = FALSE,
  nperm_estimate = 10000,
  maxDist = 0.1,
  plot = FALSE,
  verbose = FALSE
)
```

### Arguments

|                   |                                                                                                                                                 |
|-------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|
| scHOT             | A scHOT object                                                                                                                                  |
| usenperm_estimate | Logical (default FALSE) if number of neighbouring permutations should be used, or if difference of global higher order statistic should be used |
| nperm_estimate    | Number of neighbouring permutations to use for p-value estimation                                                                               |
| maxDist           | max difference of global higher order statistic to use for p-value estimation (default 0.1)                                                     |
| plot              | A logical input indicating whether the results are plotted                                                                                      |
| verbose           | A logical input indicating whether the intermediate steps will be printed                                                                       |

### Value

scHOT A scHOT object with results stored in scHOT\_output slot

### Examples

```
data(MOB_subset)
sce_MOB_subset <- MOB_subset$sce_MOB_subset
scHOT_spatial <- scHOT_buildFromSCE(sce_MOB_subset,
                                   assayName = "logcounts",
                                   positionType = "spatial",
                                   positionColData = c("x", "y"))
pairs <- matrix(c("Arrb1", "Mtor", "Dnm1l", "Gucylb3"), ncol = 2, byrow = TRUE)
rownames(pairs) <- apply(pairs, 1, paste0, collapse = "_")

scHOT_spatial <- scHOT_addTestingScaffold(scHOT_spatial, pairs)
```

```

scHOT_spatial <- scHOT_setWeightMatrix(scHOT_spatial,
                                       positionColData = c("x","y"),
                                       positionType = "spatial",
                                       nrow.out = NULL,
                                       span = 0.05)
scHOT_spatial <- scHOT_calculateGlobalHigherOrderFunction(
  scHOT_spatial,
  higherOrderFunction = weightedSpearman,
  higherOrderFunctionType = "weighted")
scHOT_spatial <- scHOT_setPermutationScaffold(scHOT_spatial,
                                              numberPermutations = 100)
scHOT_spatial <- scHOT_calculateHigherOrderTestStatistics(
  scHOT_spatial,
  higherOrderSummaryFunction = sd)

scHOT_spatial <- scHOT_performPermutationTest(
  scHOT_spatial,
  verbose = TRUE,
  parallel = FALSE)

scHOT_spatial <- scHOT_estimatePvalues(scHOT_spatial)

```

---

scHOT\_output<-

*Setter functions for scHOT objects*


---

## Description

Setter functions for scHOT objects

## Usage

```
scHOT_output(x) <- value
```

## Arguments

|       |                                |
|-------|--------------------------------|
| x     | A scHOT object                 |
| value | The value the slot should take |

## Value

A scHOT object

## Examples

```

data(MOB_subset)
sce_MOB_subset <- MOB_subset$sce_MOB_subset
scHOT_spatial <- scHOT_buildFromSCE(sce_MOB_subset,
  assayName = "logcounts",

```

```

positionType = "spatial",
positionColData = c("x", "y"))

scHOT_output(scHOT_spatial) <- data.frame()

```

---

```

scHOT_performPermutationTest
      scHOT_performPermutationTest

```

---

## Description

Perform permutation test

## Usage

```

scHOT_performPermutationTest(
  scHOT,
  verbose = FALSE,
  parallel = FALSE,
  BPPARAM = BiocParallel::SerialParam()
)

```

## Arguments

|          |                                                                                                   |
|----------|---------------------------------------------------------------------------------------------------|
| scHOT    | A scHOT object                                                                                    |
| verbose  | A logical input indicates whether the intermediate steps will be printed                          |
| parallel | A logical input indicates whether run the permutation test using multiple cores in parallel.      |
| BPPARAM  | A BiocParallelParam class object from the BiocParallel package is used. Default is SerialParam(). |

## Value

scHOT A scHOT object with results stored in scHOT\_output slot

## Examples

```

data(MOB_subset)
sce_MOB_subset <- MOB_subset$sce_MOB_subset
scHOT_spatial <- scHOT_buildFromSCE(sce_MOB_subset,
                                     assayName = "logcounts",
                                     positionType = "spatial",
                                     positionColData = c("x", "y"))
pairs <- matrix(c("Arrb1", "Mtor", "Dnm1l", "Gucylb3"), ncol = 2, byrow = TRUE)
rownames(pairs) <- apply(pairs,1,paste0,collapse = "_")
scHOT_spatial <- scHOT_addTestingScaffold(scHOT_spatial, pairs)
scHOT_spatial <- scHOT_setWeightMatrix(scHOT_spatial,

```

```

                                positionColData = c("x","y"),
                                positionType = "spatial",
                                nrow.out = NULL,
                                span = 0.05)
scHOT_spatial <- scHOT_calculateGlobalHigherOrderFunction(
  scHOT_spatial,
  higherOrderFunction = weightedSpearman,
  higherOrderFunctionType = "weighted")
scHOT_spatial <- scHOT_setPermutationScaffold(scHOT_spatial,
                                              numberPermutations = 100)
scHOT_spatial <- scHOT_calculateHigherOrderTestStatistics(
  scHOT_spatial,
  higherOrderSummaryFunction = sd)

scHOT_spatial <- scHOT_performPermutationTest(
  scHOT_spatial,
  verbose = TRUE,
  parallel = FALSE)

```

---

scHOT\_plotPermutationDistributions

*scHOT\_plotPermutationDistributions*


---

## Description

the scHOT\_plotPermutationDistributions function plots the permutation test statistics as a diagnostic plot for estimating p-values

## Usage

```
scHOT_plotPermutationDistributions(scHOT)
```

## Arguments

scHOT                      a scHOT object

## Value

ggplot graph of global higher order function and the permutation scHOT test statistics. This should have a continuous pattern to be reliably used for p-value estimation

---

```
scHOT_setPermutationScaffold
      scHOT_setPermutationScaffold
```

---

## Description

Set permutation scaffold

## Usage

```
scHOT_setPermutationScaffold(
  scHOT,
  numberPermutations = 1000,
  numberScaffold = 100,
  storePermutations = TRUE
)
```

## Arguments

|                    |                                                                                                                                                                         |
|--------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| scHOT              | A scHOT object                                                                                                                                                          |
| numberPermutations | The number of permutations, set as 1000 by default                                                                                                                      |
| numberScaffold     | The number of scaffold, set as 100 by default, minimum 6. if you want all combinations to do permutations then set, numberScaffold much higher than the testingScaffold |
| storePermutations  | a logical flag on whether Permutations should be stored, or discarded once used                                                                                         |

## Value

A scHOT object with storePermutations in slot scHOT\_output saved

## Examples

```
data(MOB_subset)
sce_MOB_subset <- MOB_subset$sce_MOB_subset
scHOT_spatial <- scHOT_buildFromSCE(sce_MOB_subset,
                                   assayName = "logcounts",
                                   positionType = "spatial",
                                   positionColData = c("x", "y"))
pairs <- matrix(c("Arrb1", "Mtor", "Dnm1l", "Gucy1b3"), ncol = 2, byrow = TRUE)
rownames(pairs) <- apply(pairs, 1, paste0, collapse = "_")
scHOT_spatial <- scHOT_addTestingScaffold(scHOT_spatial, pairs)
scHOT_spatial <- scHOT_setWeightMatrix(scHOT_spatial,
                                       positionColData = c("x", "y"),
                                       positionType = "spatial",
                                       nrow.out = NULL,
                                       span = 0.05)
```

```

scHOT_spatial <- scHOT_calculateGlobalHigherOrderFunction(
  scHOT_spatial,
  higherOrderFunction = weightedSpearman,
  higherOrderFunctionType = "weighted")
scHOT_spatial <- scHOT_setPermutationScaffold(scHOT_spatial,
                                              numberPermutations = 100)

```

---

scHOT\_setWeightMatrix *scHOT\_setWeightMatrix*

---

## Description

Create scHOT object from a SingleCellExperiment object

## Usage

```

scHOT_setWeightMatrix(
  scHOT,
  weightMatrix = NULL,
  positionType = NULL,
  positionColData = NULL,
  nrow.out = NULL,
  averageAcrossTrajectoryTies = FALSE,
  ...
)

```

## Arguments

|                             |                                                                                                                                                                                                                                                                                                                                                                          |
|-----------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| scHOT                       | A scHOT object                                                                                                                                                                                                                                                                                                                                                           |
| weightMatrix                | A matrix indicating the weight matrix for scHOT analysis, such as the output from 'trajectoryWeightMatrix' or 'spatialWeightMatrix'. If this is not NULL then other parameters are ignored.                                                                                                                                                                              |
| positionType                | A string indicating the position type, either "trajectory" or "spatial"                                                                                                                                                                                                                                                                                                  |
| positionColData             | Either trajectory or spatial information for each sample. If positionType is "trajectory" then positionColData should be a character or numeric indicating the subset of colData of the scHOT object. If positionType is "spatial" then positionColData should be a character or numeric vector indicating the subset of colData that give the full spatial coordinates. |
| nrow.out                    | The number of weightings to include for testing, a smaller value is faster for computation                                                                                                                                                                                                                                                                               |
| averageAcrossTrajectoryTies | Logical indicating whether ties in the trajectory should be given the same local weights                                                                                                                                                                                                                                                                                 |
| ...                         | parameters for function trajectoryWeightMatrix or spatialWeightMatrix                                                                                                                                                                                                                                                                                                    |

**Value**

A scHOT object with slot weightMatrix saved

**Examples**

```
data(MOB_subset)
sce_MOB_subset <- MOB_subset$sce_MOB_subset
scHOT_spatial <- scHOT_buildFromSCE(sce_MOB_subset,
                                   assayName = "logcounts",
                                   positionType = "spatial",
                                   positionColData = c("x", "y"))
pairs <- matrix(c("Arrb1", "Mtor", "Dnm1l", "Gucy1b3"), ncol = 2, byrow = TRUE)
rownames(pairs) <- apply(pairs,1,paste0,collapse = "_")
scHOT_spatial <- scHOT_addTestingScaffold(scHOT_spatial, pairs)
scHOT_spatial <- scHOT_setWeightMatrix(scHOT_spatial,
                                       positionColData = c("x","y"),
                                       positionType = "spatial",
                                       nrow.out = NULL,
                                       span = 0.05)
```

---

|                   |                          |
|-------------------|--------------------------|
| scHOT_stripOutput | <i>scHOT_stripOutput</i> |
|-------------------|--------------------------|

---

**Description**

Strip the scHOT output

**Usage**

```
scHOT_stripOutput(scHOT, force = TRUE, store = FALSE, file_name = NULL)
```

**Arguments**

|           |                                                                   |
|-----------|-------------------------------------------------------------------|
| scHOT     | A scHOT object                                                    |
| force     | A logical indicates whther forcing stripping the scHOT output     |
| store     | A logical flag on whether the scHOT should be stored as .rds file |
| file_name | A string indicates the file name of the scHOT will be stored      |

**Value**

A scHOT object with scHOT\_output striped

**Examples**

```
data(MOB_subset)
sce_MOB_subset <- MOB_subset$sce_MOB_subset
schOT_spatial <- schOT_buildFromSCE(sce_MOB_subset,
                                     assayName = "logcounts",
                                     positionType = "spatial",
                                     positionColData = c("x", "y"))

schOT_spatial <- schOT_stripOutput(schOT_spatial)
```

---

|                     |                            |
|---------------------|----------------------------|
| spatialWeightMatrix | <i>spatialWeightMatrix</i> |
|---------------------|----------------------------|

---

**Description**

Create weight matrix for spatial data

**Usage**

```
spatialWeightMatrix(x, span = NULL)
```

**Arguments**

|      |                                                                                                                                                              |
|------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|
| x    | a matrix with rows corresponding to cells and columns corresponding to dimensions to calculate Euclidean distance                                            |
| span | proportion of samples to include on either side, default is 13/(number of rows in 'x'), corresponding roughly to points within a diamond shape distance away |

**Value**

A weighted matrix

**Examples**

```
spat_x <- rnorm(50)
spat_y <- rnorm(50)
spat_coord <- cbind(spat_x, spat_y)
W <- spatialWeightMatrix(spat_coord)
```

---

```
testingScaffold<-      Setter functions for scHOT objects
```

---

**Description**

Setter functions for scHOT objects

**Usage**

```
testingScaffold(x) <- value
```

**Arguments**

|       |                                |
|-------|--------------------------------|
| x     | A scHOT object                 |
| value | The value the slot should take |

**Value**

A scHOT object

**Examples**

```
data(MOB_subset)
sce_MOB_subset <- MOB_subset$sce_MOB_subset
scHOT_spatial <- scHOT_buildFromSCE(sce_MOB_subset,
  assayName = "logcounts",
  positionType = "spatial",
  positionColData = c("x", "y"))

pairs <- t(combn(rownames(sce_MOB_subset),2))
rownames(pairs) <- apply(pairs,1,paste0,collapse = "_")

testingScaffold(scHOT_spatial) <- pairs
```

---

```
thin
```

```
thin
```

---

**Description**

The thin function extracts the rows of a matrix evenly so that roughly n number of rows remain. Used for thinning down the weight matrix to speed up overall computation.

**Usage**

```
thin(W, n = 100)
```

**Arguments**

|   |                              |
|---|------------------------------|
| W | matrix                       |
| n | rough number of rows to keep |

**Value**

matrix of thinned matrix keeping only roughly n rows.

**Examples**

```
W = trajectoryWeightMatrix(500)
W_small = thin(W, n = 100)
```

---

|                               |
|-------------------------------|
| trajectoryWeightMatrix        |
| <i>trajectoryWeightMatrix</i> |

---

**Description**

Create weight matrix for trajectory data

**Usage**

```
trajectoryWeightMatrix(n, type = NULL, span = NULL)
```

**Arguments**

|      |                                                                               |
|------|-------------------------------------------------------------------------------|
| n    | indicates the number of cels                                                  |
| type | Type of weight matrix, one of "triangular" (default), "block", and "harmonic" |
| span | proportion of samples to include on either side, default is 0.25              |

**Value**

A weighted matrix

**Examples**

```
W <- trajectoryWeightMatrix(100)
W <- trajectoryWeightMatrix(100, type = "triangular")
W <- trajectoryWeightMatrix(100, type = "block")
W <- trajectoryWeightMatrix(100, type = "harmonic")
```

---

|                 |                        |
|-----------------|------------------------|
| weightedPearson | <i>weightedPearson</i> |
|-----------------|------------------------|

---

**Description**

the weightedPearson function

**Usage**

```
weightedPearson(x, y, w = 1)
```

**Arguments**

|   |                                                 |
|---|-------------------------------------------------|
| x | x and y are data vectors                        |
| y | x and y are data vectors                        |
| w | weight vector, values should be between 0 and 1 |

**Value**

numeric weighted correlation value between x and y

**Examples**

```
x = rnorm(100)
y = rnorm(100)
w = runif(100)
weightedPearson(x,y,w)
```

---

|                  |                         |
|------------------|-------------------------|
| weightedSpearman | <i>weightedSpearman</i> |
|------------------|-------------------------|

---

**Description**

the weightedSpearman function

**Usage**

```
weightedSpearman(x, y, w = 1)
```

**Arguments**

|   |                                                 |
|---|-------------------------------------------------|
| x | x and y are data vectors                        |
| y | x and y are data vectors                        |
| w | weight vector, values should be between 0 and 1 |

**Value**

numeric weighted correlation value between x and y

**Examples**

```
x = rnorm(100)
y = rnorm(100)
w = runif(100)
weightedSpearman(x,y,w)
```

---

|                  |                         |
|------------------|-------------------------|
| weightedVariance | <i>weightedVariance</i> |
|------------------|-------------------------|

---

**Description**

the weightedVariance function

**Usage**

```
weightedVariance(x, y = NULL, w)
```

**Arguments**

|   |                                                 |
|---|-------------------------------------------------|
| x | x is a data vector                              |
| y | default to NULL, if given it is ignored         |
| w | weight vector, values should be between 0 and 1 |

**Value**

numeric weighted variance value for x

**Examples**

```
x = rnorm(100)
w = runif(100)
weightedVariance(x,w = w)
```

---

|                   |                          |
|-------------------|--------------------------|
| weightedZIKendall | <i>weightedZIKendall</i> |
|-------------------|--------------------------|

---

**Description**

the weightedZIKendall function calculates weighted  $\tau^*$ , where  $\tau^*$  is described in Pimentel et al (2015) doi:10.1016/j.spl.2014.09.002. This association measure is defined for zero-inflated, non-negative random variables.

**Usage**

```
weightedZIKendall(x, y, w = 1)
```

**Arguments**

|   |                                                 |
|---|-------------------------------------------------|
| x | x and y are non-negative data vectors           |
| y | x and y are non-negative data vectors           |
| w | weight vector, values should be between 0 and 1 |

**Value**

numeric weighted  $\tau^*$  association value between x and y

**Examples**

```
x = pmax(0, rnorm(100))
y = pmax(0, rnorm(100))
w = runif(100)
weightedZIKendall(x, y, w)
```

---

|                    |                           |
|--------------------|---------------------------|
| weightedZISpearman | <i>weightedZISpearman</i> |
|--------------------|---------------------------|

---

**Description**

the weightedZISpearman function calculates weighted  $\rho^*$ , where  $\rho^*$  is described in Pimentel et al (2009). This association measure is defined for zero-inflated, non-negative random variables.

**Usage**

```
weightedZISpearman(x, y, w = 1)
```

**Arguments**

|   |                                                 |
|---|-------------------------------------------------|
| x | x and y are non-negative data vectors           |
| y | x and y are non-negative data vectors           |
| w | weight vector, values should be between 0 and 1 |

**Value**

numeric weighted rho\* association value between x and y

Pimentel, Ronald Silva, "Kendall's Tau and Spearman's Rho for Zero-Inflated Data" (2009). Dissertations. 721. <https://scholarworks.wmich.edu/dissertations/721>

**Examples**

```
x = pmax(0, rnorm(100))
y = pmax(0, rnorm(100))
w = runif(100)
weightedZISpearman(x,y,w)
```

---

weightMatrix<-                      *Setter functions for scHOT objects*

---

**Description**

Setter functions for scHOT objects

**Usage**

```
weightMatrix(x) <- value
```

**Arguments**

|       |                                |
|-------|--------------------------------|
| x     | A scHOT object                 |
| value | The value the slot should take |

**Value**

A scHOT object

**Examples**

```
library(SingleCellExperiment)

data(MOB_subset)
sce_MOB_subset <- MOB_subset$sce_MOB_subset
schOT_spatial <- schOT_buildFromSCE(sce_MOB_subset,
  assayName = "logcounts",
  positionType = "spatial",
  positionColData = c("x", "y"))

W <- spatialWeightMatrix(colData(schOT_spatial)[,slot(schOT_spatial, "positionColData")])

weightMatrix(schOT_spatial) <- W
```

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