

# Package ‘spicyR’

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**Type** Package

**Title** Spatial analysis of in situ cytometry data

**Version** 1.16.0

**Description** The spicyR package provides a framework for performing inference on changes in spatial relationships between pairs of cell types for cell-resolution spatial omics technologies. spicyR consists of three primary steps: (i) summarizing the degree of spatial localization between pairs of cell types for each image; (ii) modelling the variability in localization summary statistics as a function of cell counts and (iii) testing for changes in spatial localizations associated with a response variable.

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**URL** <https://ellispatrick.github.io/spicyR/>

<https://github.com/SydneyBioX/spicyR>

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## Contents

Accessors . . . . .	2
as.data.frame.SegmentedCells . . . . .	4
bind . . . . .	5
colTest . . . . .	6
convPairs . . . . .	6
diabetesData . . . . .	7
diabetesData_SCE . . . . .	8
getPairwise . . . . .	8
getProp . . . . .	10
plot,SegmentedCells,ANY-method . . . . .	10
SegmentedCells-class . . . . .	11
show-SegmentedCells . . . . .	13
signifPlot . . . . .	14
spicyBoxPlot . . . . .	15
SpicyResults-class . . . . .	16
spicyTest . . . . .	18
topPairs . . . . .	18
<b>Index</b>	<b>20</b>

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Accessors

*Accessors for SegmentedCells*

---

## Description

Methods to access various components of the ‘SegmentedCells’ object.

## Usage

```
cellSummary(x, imageID = NULL, bind = TRUE)
```

```
cellSummary(x, imageID = NULL) <- value
```

```
cellMarks(x, imageID = NULL, bind = TRUE)
```

```
cellMarks(x, imageID = NULL) <- value
```

```
cellMorph(x, imageID = NULL, bind = TRUE)
```

```
cellMorph(x, imageID = NULL) <- value  
imagePheno(x, imageID = NULL, bind = TRUE, expand = FALSE)  
imagePheno(x, imageID = NULL) <- value  
imageID(x, imageID = NULL)  
cellID(x, imageID = NULL)  
cellID(x) <- value  
imageCellID(x, imageID = NULL)  
imageCellID(x) <- value  
cellType(x, imageID = NULL)  
cellType(x, imageID = NULL) <- value  
filterCells(x, select)  
cellAnnotation(x, variable, imageID = NULL)  
cellAnnotation(x, variable, imageID = NULL) <- value
```

### Arguments

x	A 'SegmentedCells' object.
imageID	A vector of imageIDs to specifically extract.
bind	When false outputs a list of DataFrames split by imageID
expand	Used to expand the phenotype information from per image to per cell.
value	The relevant information used to replace.
select	A logical vector of the cells to be kept.
variable	A variable to add or retrieve from cellSummary.

### Value

DataFrame or a list of DataFrames

### Descriptions

**'cellSummary'**: Retrieves the DataFrame containing 'x' and 'y' coordinates of each cell as well as 'cellID', 'imageID' and 'cellType'. imageID can be used to select specific images and bind=FALSE outputs the information as a list split by imageID.

**'cellMorph'**: Retrieves the DataFrame containing morphology information.

**‘cellMarks‘:** Retrieves the DataFrame containing intensity of gene or protein markers.

**‘imagePheno‘:** Retrieves the DataFrame containing the phenotype information for each image. Using `expand = TRUE` will produce a DataFrame with the number of rows equal to the number of cells.

### Examples

```
### Something that resembles cellProfiler data

set.seed(51773)

n = 10

cells <- data.frame(row.names = seq_len(n))
cells$ObjectNumber <- seq_len(n)
cells$imageNumber <- rep(1:2,c(n/2,n/2))
cells$AreaShape_Center_X <- runif(n)
cells$AreaShape_Center_Y <- runif(n)
cells$AreaShape_round <- rexp(n)
cells$AreaShape_diameter <- rexp(n, 2)
cells$Intensity_Mean_CD8 <- rexp(n, 10)
cells$Intensity_Mean_CD4 <- rexp(n, 10)

cellExp <- SegmentedCells(cells, cellProfiler = TRUE)

### Cluster cell types
intensities <- cellMarks(cellExp)
kM <- kmeans(intensities,2)
cellType(cellExp) <- paste('cluster',kM$cluster, sep = '')

cellSummary(cellExp, imageID = 1)
```

---

```
as.data.frame.SegmentedCells
      as.data.frame
```

---

### Description

Function to coerce a `SegmentedCells` object to a data frame.

### Usage

```
## S3 method for class 'SegmentedCells'
as.data.frame(x, ...)
```

### Arguments

```
x          A SegmentedCells object.
...        Other arguments.
```

**Value**

A data.frame

```
## Generate toy data set.seed(51773) x <- round(c(runif(200),runif(200)+1,runif(200)+2,runif(200)+3,
runif(200)+3,runif(200)+2,runif(200)+1,runif(200)),4) y <- round(c(runif(200),runif(200)+1,runif(200)+2,runif(200)+3,
runif(200),runif(200)+1,runif(200)+2,runif(200)+3),4) cellType <- factor(paste('c',rep(rep(c(1:2),rep(200,2)),4),sep
= '')) imageID <- rep(c('s1', 's2'),c(800,800)) cells <- data.frame(x, y, cellType, imageID)

## Store data in SegmentedCells object cellExp <- SegmentedCells(cells, cellTypeString = 'cell-
Type')

## Generate LISA cellsDF <- as.data.frame(cellExp)

NULL
```

---

bind	<i>Produces a dataframe showing L-function metric for each imageID entry.</i>
------	---

---

**Description**

Produces a dataframe showing L-function metric for each imageID entry.

**Usage**

```
bind(results, pairName = NULL)
```

**Arguments**

results	Spicy test result obtained from spicy.
pairName	A string specifying the pairwise interaction of interest. If NULL, all pairwise interactions are shown.

**Value**

A data.frame containing the colData related to the results.

**Examples**

```
data(spicyTest)
df <- bind(spicyTest)
```

---

colTest	<i>Perform a simple wilcoxon-rank-sum test or t-test on the columns of a data frame</i>
---------	---

---

**Description**

Perform a simple wilcoxon-rank-sum test or t-test on the columns of a data frame

**Usage**

```
colTest(df, condition, type = NULL, feature = NULL, imageID = "imageID")
```

**Arguments**

df	A data.frame or SingleCellExperiment, SpatialExperiment
condition	The condition of interest
type	The type of test, "wilcox", "ttest" or "survival".
feature	Can be used to calculate the proportions of this feature for each image
imageID	The imageID's if presenting a SingleCellExperiment

**Value**

Proportions

**Examples**

```
# Test for an association with long-duration diabetes
# This is clearly ignoring the repeated measures...
data("diabetesData")
props <- getProp(diabetesData)
condition <- imagePheno(diabetesData)$stage
names(condition) <- imagePheno(diabetesData)$imageID
condition <- condition[condition %in% c("Long-duration", "Onset")]
test <- colTest(props[names(condition), ], condition)
```

---

convPairs	<i>Converts colPairs object into an abundance matrix based on number of nearby interactions for every cell type.</i>
-----------	--

---

**Description**

Converts colPairs object into an abundance matrix based on number of nearby interactions for every cell type.

**Usage**

```
convPairs(cells, colPair, cellType = "cellType", imageID = "imageID")
```

**Arguments**

cells	A SingleCellExperiment that contains objects in the colPairs slot.
colPair	The name of the object in the colPairs slot for which the dataframe is constructed from.
cellType	The cell type if using SingleCellExperiment.
imageID	The image ID if using SingleCellExperiment.

**Value**

Matrix of abundances

**Examples**

```
data("diabetesData_SCE")

diabetesData_SPE <- SpatialExperiment::SpatialExperiment(diabetesData_SCE,
  colData = SingleCellExperiment::colData(diabetesData_SCE))
SpatialExperiment::spatialCoords(diabetesData_SPE) <- data.frame(
  SingleCellExperiment::colData(diabetesData_SPE)$x,
  SingleCellExperiment::colData(diabetesData_SPE)$y) |>
  as.matrix()

SpatialExperiment::spatialCoordsNames(diabetesData_SPE) <- c("x", "y")

diabetesData_SPE <- imcRtools::buildSpatialGraph(diabetesData_SPE,
  img_id = "imageID",
  type = "knn",
  k = 20,
  coords = c("x", "y"))

pairAbundances <- convPairs(diabetesData_SPE,
  colPair = "knn_interaction_graph")
```

---

diabetesData

*Diabetes IMC data*

---

**Description**

This is a subset of the Damond et al 2019 imaging mass cytometry dataset. The data contains cells in the pancreatic islets of individuals with early onset diabetes and healthy controls. The object contains single-cell data of 160 images from 8 subjects, with 20 images per subject.

**Usage**

diabetesData

**Format**

diabetesData a SegmentedCells object

---

diabetesData\_SCE      *Diabetes IMC data in SCE format.*

---

**Description**

This is a subset of the Damond et al 2019 imaging mass cytometry dataset. The data contains cells in the pancreatic islets of individuals with early onset diabetes and healthy controls. The object contains single-cell data of 160 images from 8 subjects, with 20 images per subject.

**Usage**

diabetesData\_SCE

**Format**

diabetesData\_SCE a SingleCellExperiment object

**Details**

Converted into a SingleCellExperiment format.

---

getPairwise      *Get statistic from pairwise L curve of a single image.*

---

**Description**

Get statistic from pairwise L curve of a single image.

**Usage**

```
getPairwise(  
  cells,  
  from = NULL,  
  to = NULL,  
  window = "convex",  
  window.length = NULL,  
  Rs = c(20, 50, 100),  
  sigma = NULL,
```



```

minLambda = 0.05,
edgeCorrect = TRUE,
includeZeroCells = TRUE,
BPPARAM = BiocParallel::SerialParam(),
imageID = "imageID",
cellType = "cellType",
spatialCoords = c("x", "y")
)

```

### Arguments

cells	A SegmentedCells or data frame that contains at least the variables x and y, giving the location coordinates of each cell, and cellType.
from	The 'from' cellType for generating the L curve.
to	The 'to' cellType for generating the L curve.
window	Should the window around the regions be 'square', 'convex' or 'concave'.
window.length	A tuning parameter for controlling the level of concavity when estimating concave windows.
Rs	A vector of the radii that the measures of association should be calculated.
sigma	A numeric variable used for scaling when fitting inhomogeneous L-curves.
minLambda	Minimum value for density for scaling when fitting inhomogeneous L-curves.
edgeCorrect	A logical indicating whether to perform edge correction.
includeZeroCells	A logical indicating whether to include cells with zero counts in the pairwise association calculation.
BPPARAM	A BiocParallelParam object.
imageID	The imageID if using a SingleCellExperiment or SpatialExperiment.
cellType	The cellType if using a SingleCellExperiment or SpatialExperiment.
spatialCoords	The spatialCoords if using a SingleCellExperiment or SpatialExperiment.

### Value

Statistic from pairwise L curve of a single image.

### Examples

```

data("diabetesData")
pairAssoc <- getPairwise(diabetesData[1, ])

```

---

getProp	<i>Get proportions from a SegmentedCells, SingleCellExperiment, SpatialExperiment or data.frame.</i>
---------	--

---

**Description**

Get proportions from a SegmentedCells, SingleCellExperiment, SpatialExperiment or data.frame.

**Usage**

```
getProp(cells, feature = "cellType", imageID = "imageID")
```

**Arguments**

cells	SegmentedCells, SingleCellExperiment, SpatialExperiment or data.frame
feature	The feature of interest
imageID	The imageID's

**Value**

Proportions

**Examples**

```
data("diabetesData")
prop <- getProp(diabetesData)
```

---

plot, SegmentedCells, ANY-method	<i>A basic plot for SegmentedCells object</i>
----------------------------------	---

---

**Description**

This function generates a basic x-y plot of the location coordinates and cellType data.

**Usage**

```
## S4 method for signature 'SegmentedCells, ANY'
plot(x, imageID = NULL)
```

**Arguments**

x	A SegmentedCells object.
imageID	The image that should be plotted.

**Value**

A ggplot object.

**usage**

```
'plot(x, imageID = NULL)'
```

**Examples**

```
### Something that resembles cellProfiler data

set.seed(51773)

n = 10

cells <- data.frame(row.names = seq_len(n))
cells$ObjectNumber <- seq_len(n)
cells$ImageNumber <- rep(1:2,c(n/2,n/2))
cells$AreaShape_Center_X <- runif(n)
cells$AreaShape_Center_Y <- runif(n)
cells$AreaShape_round <- rexp(n)
cells$AreaShape_diameter <- rexp(n, 2)
cells$Intensity_Mean_CD8 <- rexp(n, 10)
cells$Intensity_Mean_CD4 <- rexp(n, 10)

cellExp <- SegmentedCells(cells, cellProfiler = TRUE)

### Cluster cell types
markers <- cellMarks(cellExp)
kM <- kmeans(markers,2)
cellType(cellExp) <- paste('cluster',kM$cluster, sep = '')

#plot(cellExp, imageID=1)
```

---

SegmentedCells-class    *The SegmentedCells class*

---

**Description**

The SegmentedCells S4 class is for storing data from segmented imaging cytometry and spatial omics data. It extends DataFrame and defines methods that take advantage of DataFrame nesting to represent elements of cell-based experiments with spatial orientation that are commonly encountered. This object is able to store information on a cell's spatial location, cellType, morphology, intensity of gene/protein markers as well as image level phenotype information.

**Usage**

```
SegmentedCells(
  cellData,
  cellProfiler = FALSE,
  spatialCoords = c("x", "y"),
  cellTypeString = "cellType",
  intensityString = "intensity_",
  morphologyString = "morphology_",
  phenotypeString = "phenotype_",
  cellIDString = "cellID",
  cellAnnotations = NULL,
  imageCellIDString = "imageCellID",
  imageIDString = "imageID",
  verbose = TRUE
)
```

**Arguments**

<code>cellData</code>	A data frame that contains at least the columns <code>x</code> and <code>y</code> giving the location coordinates of each cell.
<code>cellProfiler</code>	A logical indicating that <code>cellData</code> is in a format similar to what <code>cellProfiler</code> outputs.
<code>spatialCoords</code>	The column names corresponding to spatial coordinates. eg. <code>x</code> , <code>y</code> , <code>z</code> ...
<code>cellTypeString</code>	The name of the column that contains cell type calls.
<code>intensityString</code>	A string which can be used to identify the columns which contain marker intensities. (This needs to be extended to take the column names themselves.)
<code>morphologyString</code>	A string which can be used to identify the columns which contains morphology information.
<code>phenotypeString</code>	A string which can be used to identify the columns which contains phenotype information.
<code>cellIDString</code>	The column name for <code>cellID</code> .
<code>cellAnnotations</code>	A vector of variables that provide additional annotation of a cell.
<code>imageCellIDString</code>	The column name for <code>imageCellID</code> .
<code>imageIDString</code>	The column name for <code>imageIDString</code> .
<code>verbose</code>	logical indicating whether to output messages.

**Value**

A `SegmentedCells` object

**Examples**

```
### Something that resembles cellProfiler data

set.seed(51773)

n = 10

cells <- data.frame(row.names = seq_len(n))
cells$ObjectNumber <- seq_len(n)
cells$ImageNumber <- rep(seq_len(2),c(n/2,n/2))
cells$AreaShape_Center_X <- runif(n)
cells$AreaShape_Center_Y <- runif(n)
cells$AreaShape_round <- rexp(n)
cells$AreaShape_diameter <- rexp(n, 2)
cells$Intensity_Mean_CD8 <- rexp(n, 10)
cells$Intensity_Mean_CD4 <- rexp(n, 10)

cellExp <- SegmentedCells(cells, cellProfiler = TRUE)

### Cluster cell types
intensities <- cellMarks(cellExp)
kM <- kmeans(intensities,2)
cellType(cellExp) <- paste('cluster',kM$cluster, sep = '')
cellSummary(cellExp)
```

---

show-SegmentedCells    *Show SegmentedCells*

---

**Description**

This outputs critical information about a SegmentedCells.

**Arguments**

object            A SegmentedCells.

**Value**

Information of the number of images, cells, intensities, morphologies and phenotypes.

**usage**

‘show(object)’

## Examples

```
### Something that resembles cellProfiler data

set.seed(51773)

n = 10

cells <- data.frame(row.names = seq_len(n))
cells$ObjectNumber <- seq_len(n)
cells$ImageNumber <- rep(1:2,c(n/2,n/2))
cells$AreaShape_Center_X <- runif(n)
cells$AreaShape_Center_Y <- runif(n)
cells$AreaShape_round <- rexp(n)
cells$AreaShape_diameter <- rexp(n, 2)
cells$Intensity_Mean_CD8 <- rexp(n, 10)
cells$Intensity_Mean_CD4 <- rexp(n, 10)

cellExp <- SegmentedCells(cells, cellProfiler = TRUE)

### Cluster cell types
markers <- cellMarks(cellExp)
kM <- kmeans(markers,2)
cellType(cellExp) <- paste('cluster',kM$cluster, sep = '')

cellExp
```

---

signifPlot

*Plots result of signifPlot.*

---

## Description

Plots result of signifPlot.

## Usage

```
signifPlot(
  results,
  fdr = FALSE,
  type = "bubble",
  breaks = NULL,
  comparisonGroup = NULL,
  colours = c("#4575B4", "white", "#D73027"),
  marksToPlot = NULL,
  cutoff = 0.05
)
```

**Arguments**

results	Data frame obtained from spicy.
fdr	TRUE if FDR correction is used.
type	Where to make a bubble plot or heatmap.
breaks	Vector of 3 numbers giving breaks used in pheatmap. The first number is the minimum, the second is the maximum, the third is the number of breaks.
comparisonGroup	A string specifying the name of the outcome group to compare with the base group.
colours	Vector of colours to use in pheatmap.
marksToPlot	Vector of marks to include in pheatmap.
cutoff	significance threshold for circles in bubble plot

**Value**

a pheatmap object

**Examples**

```
data(spicyTest)
signifPlot(spicyTest, breaks = c(-3, 3, 0.5))
```

---

spicyBoxPlot	<i>Plots boxplot for a specified cell-cell relationship</i>
--------------	---

---

**Description**

Plots boxplot for a specified cell-cell relationship

**Usage**

```
spicyBoxPlot(results, from = NULL, to = NULL, rank = NULL)
```

**Arguments**

results	Data frame obtained from spicy.
from	Cell type which you would like to compare to the to cell type.
to	Cell type which you would like to compare to the from cell type.
rank	Ranking of cell type in terms of p-value, the smaller the p-value the higher the rank.

**Value**

a ggplot2 boxplot

**Examples**

```
data(spicyTest)

spicyBoxPlot(spicyTest,
             rank = 1)
```

---

SpicyResults-class      *Performs spatial tests on spatial cytometry data.*

---

**Description**

Performs spatial tests on spatial cytometry data.

**Usage**

```
spicy(
  cells,
  condition = NULL,
  subject = NULL,
  covariates = NULL,
  from = NULL,
  to = NULL,
  alternateResult = NULL,
  verbose = TRUE,
  weights = TRUE,
  weightsByPair = FALSE,
  weightFactor = 1,
  window = "convex",
  window.length = NULL,
  BPPARAM = BiocParallel::SerialParam(),
  sigma = NULL,
  Rs = NULL,
  minLambda = 0.05,
  edgeCorrect = TRUE,
  includeZeroCells = FALSE,
  imageID = "imageID",
  cellType = "cellType",
  spatialCoords = c("x", "y"),
  ...
)
```

**Arguments**

**cells**      A SegmentedCells or data frame that contains at least the variables x and y, giving the location coordinates of each cell, and cellType.



condition	Vector of conditions to be tested corresponding to each image if cells is a data frame.
subject	Vector of subject IDs corresponding to each image if cells is a data frame.
covariates	Vector of covariate names that should be included in the mixed effects model as fixed effects.
from	vector of cell types which you would like to compare to the to vector
to	vector of cell types which you would like to compare to the from vector
alternateResult	An pairwise association statistic between each combination of celltypes in each image.
verbose	logical indicating whether to output messages.
weights	logical indicating whether to include weights based on cell counts.
weightsByPair	logical indicating whether weights should be calculated for each cell type pair.
weightFactor	numeric that controls the convexity of the weight function.
window	Should the window around the regions be 'square', 'convex' or 'concave'.
window.length	A tuning parameter for controlling the level of concavity when estimating concave windows.
BPPARAM	A BiocParallelParam object.
sigma	A numeric variable used for scaling when fitting inhomogeneous L-curves.
Rs	A vector of radii that the measures of association should be calculated.
minLambda	Minimum value for density for scaling when fitting inhomogeneous L-curves.
edgeCorrect	A logical indicating whether to perform edge correction.
includeZeroCells	A logical indicating whether to include cells with zero counts in the pairwise association calculation.
imageID	The image ID if using SingleCellExperiment.
cellType	The cell type if using SingleCellExperiment.
spatialCoords	The spatial coordinates if using a SingleCellExperiment.
...	Other options.

**Value**

Data frame of p-values.

**Examples**

```
data("diabetesData")

# Test with random effect for patient on a pairwise combination of cell
# types.
spicy(diabetesData,
      condition = "stage", subject = "case",
      from = "Tc", to = "Th")
```

```

)

# Test all pairwise combinations of cell types without random effect of
# patient.
# spicityTest <- spicity(diabetesData, condition = "stage", subject = "case")

# Test all pairwise combination of cell types with random effect of patient.
# spicityTest <- spicity(diabetesData, condition = "condition", subject = "subject")

```

---

spicityTest

*Results from spicity for diabetesData*

---

### Description

Results from the call: `spicityTest <- spicity(diabetesData, condition = "condition", subject = "subject")`

### Usage

spicityTest

### Format

spicityTest a spicity object

---

topPairs

*A table of the significant results from spicity tests*

---

### Description

A table of the significant results from spicity tests

### Usage

`topPairs(x, coef = NULL, n = 10, adj = "fdr", cutoff = NULL, figures = NULL)`

### Arguments

x	The output from spicity.
coef	Which coefficient to list.
n	Extract the top n most significant pairs.
adj	Which p-value adjustment method to use, argument for <code>p.adjust()</code> .
cutoff	A p-value threshold to extract significant pairs.
figures	Round to 'figures' significant figures.

**Value**

A data.frame

**Examples**

```
data(spicyTest)  
topPairs(spicyTest)
```

# Index

## \* datasets

diabetesData, 7  
diabetesData\_SCE, 8  
spicyTest, 18

Accessors, 2

as.data.frame.SegmentedCells, 4

bind, 5

cellAnnotation (Accessors), 2

cellAnnotation, SegmentedCells-method  
(Accessors), 2

cellAnnotation<- (Accessors), 2

cellAnnotation<- , SegmentedCells-method  
(Accessors), 2

cellID (Accessors), 2

cellID, SegmentedCells-method  
(Accessors), 2

cellID<- (Accessors), 2

cellID<- , SegmentedCells-method  
(Accessors), 2

cellMarks (Accessors), 2

cellMarks, SegmentedCells-method  
(Accessors), 2

cellMarks<- (Accessors), 2

cellMarks<- , SegmentedCells-method  
(Accessors), 2

cellMorph (Accessors), 2

cellMorph, SegmentedCells-method  
(Accessors), 2

cellMorph<- (Accessors), 2

cellMorph<- , SegmentedCells-method  
(Accessors), 2

cellSummary (Accessors), 2

cellSummary, SegmentedCells-method  
(Accessors), 2

cellSummary<- (Accessors), 2

cellSummary<- , SegmentedCells-method  
(Accessors), 2

cellType (Accessors), 2

cellType, SegmentedCells-method  
(Accessors), 2

cellType<- (Accessors), 2

cellType<- , SegmentedCells-method  
(Accessors), 2

colTest, 6

convPairs, 6

diabetesData, 7

diabetesData\_SCE, 8

filterCells (Accessors), 2

filterCells, SegmentedCells-method  
(Accessors), 2

getPairwise, 8

getProp, 10

imageCellID (Accessors), 2

imageCellID, SegmentedCells-method  
(Accessors), 2

imageCellID<- (Accessors), 2

imageCellID<- , SegmentedCells-method  
(Accessors), 2

imageID (Accessors), 2

imageID, SegmentedCells-method  
(Accessors), 2

imagePheno (Accessors), 2

imagePheno, SegmentedCells-method  
(Accessors), 2

imagePheno<- (Accessors), 2

imagePheno<- , SegmentedCells-method  
(Accessors), 2

plot (plot, SegmentedCells, ANY-method),  
10

plot, SegmentedCells

(plot, SegmentedCells, ANY-method),  
10

plot, SegmentedCells, ANY-method, 10

SegmentedCells (SegmentedCells-class),  
11

SegmentedCells, SegmentedCells-method  
(SegmentedCells-class), 11

SegmentedCells-class, 11

show (show-SegmentedCells), 13

show-SegmentedCells, 13

signifPlot, 14

spicy (SpicyResults-class), 16

spicy, spicy-method  
(SpicyResults-class), 16

spicyBoxPlot, 15

SpicyResults, list, ANY-method  
(SpicyResults-class), 16

SpicyResults-class, 16

spicyTest, 18

  

topPairs, 18

topPairs, SpicyResults-method  
(topPairs), 18