# Package 'Giotto'

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```
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2 R topics documented:

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reticulate (>= 1.14),
  magrittr,
  limma,
  ggdendro,
  smfishHmrf,
  devtools,
  reshape2,
  ggraph,
  Rcpp,
  Rfast,
  Rtsne (>= 0.15),
  rlang (>= 0.4.3),
  R.utils,
  fitdistrplus,
  quadprog
Suggests knitr,
  rmarkdown,
  MAST,
  scran (>= 1.10.1),
  png,
  FactoMineR,
  tiff,
  biomaRt,
  trendsceek.
  multinet (>= 3.0.2),
  RTriangle (>= 1.6-0.10)
biocViews
VignetteBuilder knitr
LinkingTo Rcpp,
  RcppArmadillo
Remotes lambdamoses/smfishhmrf-r
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## Description

Creates an additional metadata column with information about interacting and non-interacting cell types of the selected cell-cell interaction.

## Usage

```
addCellIntMetadata(
  gobject,
  spatial_network = "spatial_network",
  cluster_column,
  cell_interaction,
  name = "select_int",
  return_gobject = TRUE
)
```

## Arguments

## **Details**

This function will create an additional metadata column which selects interacting cell types for a specific cell-cell interaction. For example, if you want to color interacting astrocytes and oligodendrocytes it will create a new metadata column with the values "select\_astrocytes", "select\_oligodendrocytes", "other\_astrocytes", "other\_oligodendrocytes" and "other". Where "other" is all other cell types found within the selected cell type column.

## Value

Giotto object

```
addCellIntMetadata(gobject)
```

8 addCellMetadata

addCellMetadata

addCellMetadata

## Description

adds cell metadata to the giotto object

#### Usage

```
addCellMetadata(
  gobject,
  new_metadata,
  vector_name = NULL,
  by_column = FALSE,
  column_cell_ID = NULL)
```

## **Arguments**

```
gobject giotto object

new_metadata new cell metadata to use (data.table, data.frame, ...)

vector_name (optional) custom name if you provide a single vector

by_column merge metadata based on cell_ID column in pDataDT (default = FALSE)

column_cell_ID column name of new metadata to use if by_column = TRUE
```

## Details

You can add additional cell metadata in two manners:

- 1. Provide a data.table or data.frame with cell annotations in the same order as the cell\_ID column in pDataDT(gobject)
- 2. Provide a data.table or data.frame with cell annotations and specificy which column contains the cell IDs, these cell IDs need to match with the cell\_ID column in pDataDT(gobject)

## Value

```
giotto object
```

```
addCellMetadata(gobject)
```

addCellStatistics 9

addCellStatistics

addCellStatistics

## **Description**

adds cells statistics to the giotto object

## Usage

```
addCellStatistics(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  detection_threshold = 0,
  return_gobject = TRUE
)
```

## **Arguments**

## **Details**

This function will add the following statistics to cell metadata:

- nr\_genes: Denotes in how many genes are detected per cell
- perc\_genes: Denotes what percentage of genes is detected per cell
- total\_expr: Shows the total sum of gene expression per cell

## Value

```
giotto object if return_gobject = TRUE
```

```
addCellStatistics(gobject)
```

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addGeneMetadata

addGeneMetadata

## **Description**

adds gene metadata to the giotto object

### Usage

```
addGeneMetadata(gobject, new_metadata, by_column = F, column_gene_ID = NULL)
```

#### **Arguments**

```
gobject giotto object

new_metadata new metadata to use

by_column merge metadata based on gene_ID column in fDataDT

column_gene_ID column name of new metadata to use if by_column = TRUE
```

#### **Details**

You can add additional gene metadata in two manners: 1. Provide a data.table or data.frame with gene annotations in the same order as the gene\_ID column in fDataDT(gobject) 2. Provide a data.table or data.frame with gene annotations and specificy which column contains the gene IDs, these gene IDs need to match with the gene\_ID column in fDataDT(gobject)

#### Value

giotto object

## **Examples**

```
addGeneMetadata(gobject)
```

addGenesPerc

addGenesPerc

## **Description**

calculates the total percentage of (normalized) counts for a subset of selected genes

## Usage

```
addGenesPerc(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  genes = NULL,
  vector_name = "gene_perc",
  return_gobject = TRUE
)
```

addGeneStatistics 11

#### **Arguments**

#### Value

```
giotto object if return_gobject = TRUE, else a vector with
```

## **Examples**

```
addGenesPerc(gobject)
```

addGeneStatistics

addGeneStatistics

## **Description**

adds gene statistics to the giotto object

## Usage

```
addGeneStatistics(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  detection_threshold = 0,
  return_gobject = TRUE
)
```

## **Arguments**

```
gobject giotto object
expression_values
expression values to use
detection_threshold
detection threshold to consider a gene detected
return_gobject boolean: return giotto object (default = TRUE)
```

## **Details**

This function will add the following statistics to gene metadata:

- nr\_cells: Denotes in how many cells the gene is detected
- per\_cells: Denotes in what percentage of cells the gene is detected
- total\_expr: Shows the total sum of gene expression in all cells
- mean\_expr: Average gene expression in all cells
- mean\_expr\_det: Average gene expression in cells with detectable levels of the gene

#### Value

```
giotto object if return_gobject = TRUE
```

## **Examples**

addGeneStatistics(gobject)

 ${\tt addGiottoImage}$ 

add Giot to Image

## **Description**

Adds giotto image objects to your giotto object

## Usage

```
addGiottoImage(gobject, images)
```

## **Arguments**

gobject giotto object

images list of giotto image objects, see createGiottoImage

## Value

an updated Giotto object with access to the list of images

## **Examples**

```
addGiottoImage(mg_object)
```

 $add {\tt GiottoImageToSpatPlot}$ 

addGiottoImageToSpatPlot

## **Description**

Add a giotto image to a spatial ggplot object post creation

## Usage

```
addGiottoImageToSpatPlot(spatpl = NULL, gimage = NULL)
```

## **Arguments**

spatpl a spatial ggplot object

gimage a giotto image, see createGiottoImage

addHMRF

#### Value

an updated spatial ggplot object

## **Examples**

```
addGiottoImageToSpatPlot(mg_object)
```

addHMRF

addHMRF

## Description

Add selected results from doHMRF to the giotto object

## Usage

```
addHMRF(gobject, HMRFoutput, k = NULL, betas_to_add = NULL, hmrf_name = NULL)
```

## **Arguments**

gobject giotto object

HMRF output from doHMRF()

k number of domains

betas\_to\_add results from different betas that you want to add

hmrf\_name specify a custom name

### Value

giotto object

## **Examples**

```
addHMRF(gobject)
```

 ${\it addNetworkLayout}\\$ 

addNetworkLayout

## Description

Add a network layout for a selected nearest neighbor network

## Usage

```
addNetworkLayout(
  gobject,
  nn_network_to_use = "sNN",
  network_name = "sNN.pca",
  layout_type = c("drl"),
  options_list = NULL,
  layout_name = "layout",
  return_gobject = TRUE
)
```

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#### **Arguments**

### **Details**

This function creates layout coordinates based on the provided kNN or sNN. Currently only the force-directed graph layout "drl", see layout\_with\_drl, is implemented. This provides an alternative to tSNE or UMAP based visualizations.

#### Value

giotto object with updated layout for selected NN network

## **Examples**

```
addNetworkLayout(gobject)
```

 ${\sf addStatistics}$ 

addStatistics

## **Description**

adds genes and cells statistics to the giotto object

## Usage

```
addStatistics(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  detection_threshold = 0,
  return_gobject = TRUE
)
```

## **Arguments**

```
gobject giotto object

expression_values

expression values to use

detection_threshold

detection threshold to consider a gene detected

return_gobject boolean: return giotto object (default = TRUE)
```

adjustGiottoMatrix 15

#### **Details**

See addGeneStatistics and addCellStatistics

#### Value

```
giotto object if return_gobject = TRUE, else a list with results
```

#### **Examples**

```
addStatistics(gobject)
```

adjustGiottoMatrix adjustGiottoMatrix

## **Description**

normalize and/or scale expresion values of Giotto object

## Usage

```
adjustGiottoMatrix(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  batch_columns = NULL,
  covariate_columns = NULL,
  return_gobject = TRUE,
  update_slot = c("custom")
)
```

## Arguments

```
gobject giotto object
expression_values
expression values to use
batch_columns metadata columns that represent different batch (max = 2)
covariate_columns
metadata columns that represent covariates to regress out
return_gobject boolean: return giotto object (default = TRUE)
update_slot expression slot that will be updated (default = custom)
```

### **Details**

This function implements the removeBatchEffect function to remove known batch effects and to adjust expression values according to provided covariates.

#### Value

giotto object

```
{\tt adjustGiottoMatrix(gobject)}
```

16 annotateGiotto

annotateGiotto

annotate Giotto

## **Description**

Converts cluster results into provided annotation.

## Usage

```
annotateGiotto(
  gobject,
  annotation_vector = NULL,
  cluster_column = NULL,
  name = "cell_types"
)
```

## Arguments

## Details

You need to specifify which (cluster) column you want to annotate and you need to provide an annotation vector like this:

- 1. identify the cell type of each cluster
- 2. create a vector of these cell types, e.g. cell\_types = c('T-cell', 'B-cell', 'Stromal')
- 3. provide original cluster names to previous vector, e.g. names(cell\_types) = c(2, 1, 3)

## Value

giotto object

```
annotateGiotto(gobject)
```

annotateSpatialGrid 17

```
annotate {\tt Spatial Grid} \quad \textit{annotate Spatial Grid}
```

## **Description**

annotate spatial grid with cell ID and cell metadata (optional)

## Usage

```
annotateSpatialGrid(
  gobject,
  spatial_grid_name = "spatial_grid",
  cluster_columns = NULL
)
```

## Arguments

## Value

annotated spatial grid data.table

## **Examples**

```
annotateSpatialGrid()
```

```
annotateSpatialNetwork
```

annotate Spatial Network

## Description

Annotate spatial network with cell metadata information.

## Usage

```
annotateSpatialNetwork(
  gobject,
  spatial_network_name = "Delaunay_network",
  cluster_column,
  create_full_network = F
)
```

## **Arguments**

#### Value

annotated network in data.table format

## **Examples**

```
annotateSpatialNetwork(gobject)
```

```
average_gene_gene_expression_in_groups

average_gene_gene_expression_in_groups
```

## **Description**

calculate average expression per cluster

### Usage

```
average_gene_gene_expression_in_groups(
  gobject,
  cluster_column = "cell_types",
  gene_set_1,
  gene_set_2
)
```

## **Arguments**

```
gobject giotto object to use
cluster_column cluster column with cell type information
gene_set_1 first specific gene set from gene pairs
gene_set_2 second specific gene set from gene pairs
```

#### **Details**

Details will follow soon.

## Value

data.table with average expression scores for each cluster

```
average_gene_gene_expression_in_groups(gobject)
```

binSpect 19

binSpect binSpect

## Description

Previously: binGetSpatialGenes. BinSpect (Binary Spatial Extraction of genes) is a fast computational method that identifies genes with a spatially coherent expression pattern.

### Usage

```
binSpect(
  gobject,
  bin_method = c("kmeans", "rank"),
  expression_values = c("normalized", "scaled", "custom"),
  subset_genes = NULL,
  spatial_network_name = "Delaunay_network",
  reduce_network = FALSE,
  kmeans_algo = c("kmeans", "kmeans_arma", "kmeans_arma_subset"),
  nstart = 3,
  iter_max = 10,
  extreme_nr = 50,
  sample_nr = 50,
  percentage_rank = 30,
  do_fisher_test = TRUE,
  adjust_method = "fdr",
  calc_hub = FALSE,
  hub_min_int = 3,
  get_av_expr = TRUE,
  get_high_expr = TRUE,
  implementation = c("data.table", "simple", "matrix"),
  group_size = "automatic",
  do_parallel = TRUE,
  cores = NA,
  verbose = T,
  set.seed = NULL
)
```

## **Arguments**

```
gobject giotto object

bin_method method to binarize gene expression

expression_values

expression values to use

subset_genes only select a subset of genes to test

spatial_network_name

name of spatial network to use (default = 'spatial_network')

reduce_network default uses the full network

kmeans_algo kmeans algorithm to use (kmeans, kmeans_arma, kmeans_arma_subset)

nstart kmeans: nstart parameter
```

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iter\_max kmeans: iter.max parameter

extreme\_nr number of top and bottom cells (see details)
sample\_nr total number of cells to sample (see details)

percentage\_rank

percentage of top cells for binarization

do\_fisher\_test perform fisher test

adjust\_method p-value adjusted method to use (see p.adjust)

calc\_hub calculate the number of hub cells

hub\_min\_int minimum number of cell-cell interactions for a hub cell

get\_av\_expr calculate the average expression per gene of the high expressing cells

get\_high\_expr calculate the number of high expressing cells per gene implementation enrichment implementation (data.table, simple, matrix)

group\_size number of genes to process together with data.table implementation (default =

automatic)

do\_parallel run calculations in parallel with mclapply cores number of cores to use if do\_parallel = TRUE

verbose be verbose

#### **Details**

We provide two ways to identify spatial genes based on gene expression binarization. Both methods are identicial except for how binarization is performed.

- 1. binarize: Each gene is binarized (0 or 1) in each cell with **kmeans** (k = 2) or based on **rank** percentile
- 2. network: Alll cells are connected through a spatial network based on the physical coordinates
- 3. contingency table: A contingency table is calculated based on all edges of neighboring cells and the binarized expression (0-0, 0-1, 1-0 or 1-1)
- 4. For each gene an odds-ratio (OR) and fisher.test (optional) is calculated

Three different kmeans algorithmes have been implemented:

- 1. kmeans: default, see kmeans
- 2. kmeans\_arma: from ClusterR, see KMeans\_arma
- 3. kmeans\_arma\_subst: from ClusterR, see KMeans\_arma, but random subsetting the vector for each gene to increase speed. Change extreme\_nr and sample\_nr for control.

Other statistics are provided (optional):

- Number of cells with high expression (binary = 1)
- Average expression of each gene within high expressing cells
- Number of hub cells, these are high expressing cells that have a user defined number of high expressing neighbors

By selecting a subset of likely spatial genes (e.g. soft thresholding highly variable genes) can accelerate the speed. The simple implementation is usually faster, but lacks the possibility to run in parallel and to calculate hub cells. The data.table implementation might be more appropriate for large datasets by setting the group\_size (number of genes) parameter to divide the workload.

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#### Value

```
data.table with results (see details)
```

#### **Examples**

```
binSpect(gobject)
```

calculateHVG

calculateHVG

## **Description**

compute highly variable genes

## Usage

```
calculateHVG(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  method = c("cov_groups", "cov_loess"),
  reverse_log_scale = FALSE,
  logbase = 2,
  expression_threshold = 0,
  nr_expression_groups = 20,
  zscore_threshold = 1.5,
  HVGname = "hvg",
  difference_in_cov = 0.1,
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "HVGplot",
  return_gobject = TRUE
)
```

## **Arguments**

```
gobject
                  giotto object
expression_values
                  expression values to use
method
                  method to calculate highly variable genes
reverse_log_scale
                  reverse log-scale of expression values (default = FALSE)
                  if reverse_log_scale is TRUE, which log base was used?
logbase
expression_threshold
                  expression threshold to consider a gene detected
nr_expression_groups
                  number of expression groups for cov_groups
zscore_threshold
                  zscore to select hvg for cov_groups
```

22 calculateMetaTable

#### **Details**

Currently we provide 2 ways to calculate highly variable genes: 1. high coeff of variance (COV) within groups:

First genes are binned (*nr\_expression\_groups*) into average expression groups and the COV for each gene is converted into a z-score within each bin. Genes with a z-score higher than the threshold (*zscore\_threshold*) are considered highly variable.

## 2. high COV based on loess regression prediction:

A predicted COV is calculated for each gene using loess regression (COV~log(mean expression)) Genes that show a higher than predicted COV (difference\_in\_cov) are considered highly variable.

#### Value

giotto object highly variable genes appended to gene metadata (fDataDT)

## **Examples**

```
# 1. create giotto object
expr_path = system.file("extdata", "seqfish_field_expr.txt", package = 'Giotto')
loc_path = system.file("extdata", "seqfish_field_locs.txt", package = 'Giotto')
VC_small <- createGiottoObject(raw_exprs = expr_path, spatial_locs = loc_path)
# 2. normalize giotto
VC_small <- normalizeGiotto(gobject = VC_small, scalefactor = 6000)
VC_small <- addStatistics(gobject = VC_small)
# 3. highly variable genes detection
VC_small <- calculateHVG(gobject = VC_small)</pre>
```

calculateMetaTable calculateMetaTable

#### **Description**

calculates the average gene expression for one or more (combined) annotation columns.

calculateMetaTableCells 23

### Usage

```
calculateMetaTable(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  metadata_cols = NULL,
  selected_genes = NULL
)
```

## **Arguments**

```
gobject giotto object
expression_values
expression values to use
metadata_cols annotation columns found in pDataDT(gobject)
selected_genes subset of genes to use
```

#### Value

data.table with average expression values for each gene per (combined) annotation

## **Examples**

```
calculateMetaTable(gobject)
```

```
calculateMetaTableCells
```

 $calculate {\it MetaTable Cells}$ 

## **Description**

calculates the average metadata values for one or more (combined) annotation columns.

## Usage

```
calculateMetaTableCells(
  gobject,
  value_cols = NULL,
  metadata_cols = NULL,
  spat_enr_names = NULL
)
```

## Arguments

```
gobject giotto object
value_cols metadata or enrichment value columns to use
metadata_cols annotation columns found in pDataDT(gobject)
spat_enr_names which spatial enrichment results to include
```

#### Value

data.table with average metadata values per (combined) annotation

24 cellProximityBarplot

### **Examples**

```
calculateMetaTableCells(gobject)
```

```
cellProximityBarplot cellProximityBarplot
```

#### **Description**

Create barplot from cell-cell proximity scores

### Usage

```
cellProximityBarplot(
  gobject,
  CPscore,
  min_orig_ints = 5,
  min_sim_ints = 5,
  p_val = 0.05,
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "cellProximityBarplot"
)
```

## **Arguments**

```
gobject
                  giotto object
CPscore
                  CPscore, output from cellProximityEnrichment()
                  filter on minimum original cell-cell interactions
min_orig_ints
                  filter on minimum simulated cell-cell interactions
min_sim_ints
                  p-value
p_val
show_plot
                  show plot
return_plot
                  return ggplot object
                  directly save the plot [boolean]
save_plot
                  list of saving parameters from all_plots_save_function
save_param
default_save_name
                  default save name for saving, don't change, change save_name in save_param
```

#### **Details**

This function creates a barplot that shows the spatial proximity enrichment or depletion of cell type pairs.

## Value

ggplot barplot

```
cellProximityBarplot(CPscore)
```

```
cell Proximity Enrichment \\ cell Proximity Enrichment
```

## **Description**

Compute cell-cell interaction enrichment (observed vs expected)

## Usage

## **Arguments**

## **Details**

Spatial proximity enrichment or depletion between pairs of cell types is calculated by calculating the observed over the expected frequency of cell-cell proximity interactions. The expected frequency is the average frequency calculated from a number of spatial network simulations. Each individual simulation is obtained by reshuffling the cell type labels of each node (cell) in the spatial network.

#### Value

List of cell Proximity scores (CPscores) in data.table format. The first data.table (raw\_sim\_table) shows the raw observations of both the original and simulated networks. The second data.table (enrichm\_res) shows the enrichment results.

```
cellProximityEnrichment(gobject)
```

```
cellProximityHeatmap cellProximityHeatmap
```

## **Description**

Create heatmap from cell-cell proximity scores

## Usage

```
cellProximityHeatmap(
  gobject,
  CPscore,
  scale = T,
  order_cell_types = T,
  color_breaks = NULL,
  color_names = NULL,
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "cellProximityHeatmap")
```

## Arguments

```
giotto object
gobject
                  CPscore, output from cellProximityEnrichment()
CPscore
scale
                  scale cell-cell proximity interaction scores
order_cell_types
                  order cell types based on enrichment correlation
color_breaks
                  numerical vector of length 3 to represent min, mean and maximum
                  character color vector of length 3
color_names
show_plot
                  show plot
return_plot
                  return ggplot object
save_plot
                  directly save the plot [boolean]
save_param
                  list of saving parameters from all_plots_save_function
default_save_name
                  default save name for saving, don't change, change save_name in save_param
```

#### **Details**

This function creates a heatmap that shows the spatial proximity enrichment or depletion of cell type pairs.

## Value

```
ggplot heatmap
```

cellProximityNetwork 27

#### **Examples**

```
cellProximityHeatmap(CPscore)
```

```
cell Proximity Network \qquad cell Proximity Network
```

## **Description**

Create network from cell-cell proximity scores

### Usage

```
cellProximityNetwork(
  gobject,
  CPscore,
  remove_self_edges = FALSE,
  self_loop_strength = 0.1,
  color_depletion = "lightgreen",
  color_enrichment = "red",
  rescale_edge_weights = TRUE,
  edge_weight_range_depletion = c(0.1, 1),
  edge_weight_range_enrichment = c(1, 5),
  layout = c("Fruchterman", "DrL", "Kamada-Kawai"),
  only_show_enrichment_edges = F,
  edge_width_range = c(0.1, 2),
  node_size = 4,
  node_text_size = 6,
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "cellProximityNetwork"
```

## **Arguments**

```
gobject
                  giotto object
CPscore
                  CPscore, output from cellProximityEnrichment()
remove_self_edges
                  remove enrichment/depletion edges with itself
self_loop_strength
                  size of self-loops
color_depletion
                  color for depleted cell-cell interactions
color_enrichment
                  color for enriched cell-cell interactions
rescale_edge_weights
                  rescale edge weights (boolean)
{\tt edge\_weight\_range\_depletion}
                  numerical vector of length 2 to rescale depleted edge weights
```

```
edge_weight_range_enrichment
```

numerical vector of length 2 to rescale enriched edge weights

layout algorithm to use to draw nodes and edges

only\_show\_enrichment\_edges

show only the enriched pairwise scores

edge\_width\_range

range of edge width

node\_size size of nodes
node\_text\_size size of node labels

show\_plot show plot

return\_plot return ggplot object

save\_plot directly save the plot [boolean]

save\_param list of saving parameters from all\_plots\_save\_function

default\_save\_name

default save name for saving, don't change, change save\_name in save\_param

#### **Details**

This function creates a network that shows the spatial proximity enrichment or depletion of cell type pairs.

#### Value

igraph plot

## **Examples**

cellProximityNetwork(CPscore)

 $cell Proximity SpatPlot \ \ \textit{cellProximitySpatPlot}$ 

## **Description**

Visualize 2D cell-cell interactions according to spatial coordinates in ggplot mode

## Usage

```
cellProximitySpatPlot(gobject, ...)
```

## **Arguments**

gobject giotto object

... Arguments passed on to cellProximitySpatPlot2D

interaction\_name cell-cell interaction name
cluster\_column cluster column with cell clusters
sdimx x-axis dimension name (default = 'sdimx')
sdimy y-axis dimension name (default = 'sdimy')

cellProximitySpatPlot

```
cell_color color for cells (see details)
cell_color_code named vector with colors
color_as_factor convert color column to factor
show_other_cells decide if show cells not in network
show_network show spatial network of selected cells
show_other_network show spatial network of not selected cells
network_color color of spatial network
spatial_network_name name of spatial network to use
show_grid show spatial grid
grid_color color of spatial grid
spatial_grid_name name of spatial grid to use
coord_fix_ratio fix ratio between x and y-axis
show_legend show legend
point_size_select size of selected points
point_select_border_col border color of selected points
point_select_border_stroke stroke size of selected points
point_size_other size of other points
point_alpha_other opacity of other points
point_other_border_col border color of other points
point_other_border_stroke stroke size of other points
show_plot show plots
return_plot return ggplot object
save_plot directly save the plot [boolean]
save_param list of saving parameters from all_plots_save_function
default_save_name default save name for saving, don't change, change save_name
    in save_param
```

#### **Details**

Description of parameters.

#### Value

ggplot

## See Also

cellProximitySpatPlot2D and cellProximitySpatPlot3D for 3D

## Examples

cellProximitySpatPlot(gobject)

```
cell Proximity Spat Plot 2D \\ cell Proximity Spat Plot 2D
```

## **Description**

Visualize 2D cell-cell interactions according to spatial coordinates in ggplot mode

#### Usage

```
cellProximitySpatPlot2D(
  gobject,
  interaction_name = NULL,
  cluster_column = NULL,
  sdimx = "sdimx",
  sdimy = "sdimy",
  cell_color = NULL,
  cell_color_code = NULL,
  color_as_factor = T,
  show_other_cells = F,
  show_network = F,
  show_other_network = F,
  network_color = NULL,
  spatial_network_name = "Delaunay_network",
  show\_grid = F,
  grid_color = NULL,
  spatial_grid_name = "spatial_grid",
  coord_fix_ratio = 1,
  show_legend = T,
  point_size_select = 2,
  point_select_border_col = "black",
  point_select_border_stroke = 0.05,
  point_size_other = 1,
  point_alpha_other = 0.3,
  point_other_border_col = "lightgrey",
  point_other_border_stroke = 0.01,
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "cellProximitySpatPlot2D"
)
```

## **Arguments**

sdimy y-axis dimension name (default = 'sdimy') cell\_color color for cells (see details) cell\_color\_code named vector with colors color\_as\_factor convert color column to factor show\_other\_cells decide if show cells not in network show\_network show spatial network of selected cells show\_other\_network show spatial network of not selected cells network\_color color of spatial network spatial\_network\_name name of spatial network to use show\_grid show spatial grid grid\_color color of spatial grid spatial\_grid\_name name of spatial grid to use coord\_fix\_ratio fix ratio between x and y-axis show\_legend show legend point\_size\_select size of selected points point\_select\_border\_col border color of selected points point\_select\_border\_stroke stroke size of selected points point\_size\_other size of other points point\_alpha\_other opacity of other points point\_other\_border\_col border color of other points point\_other\_border\_stroke stroke size of other points show\_plot show plots return\_plot return ggplot object save\_plot directly save the plot [boolean] list of saving parameters from all\_plots\_save\_function save\_param default\_save\_name default save name for saving, don't change, change save\_name in save\_param

## **Details**

Description of parameters.

#### Value

ggplot

#### **Examples**

```
cellProximitySpatPlot2D(gobject)
```

```
{\tt cellProximitySpatPlot3D}
```

cellProximitySpatPlot2D

## Description

Visualize 3D cell-cell interactions according to spatial coordinates in plotly mode

## Usage

```
cellProximitySpatPlot3D(
  gobject,
  interaction_name = NULL,
  cluster_column = NULL,
  sdimx = "sdimx",
  sdimy = "sdimy",
  sdimz = "sdimz",
  cell_color = NULL,
  cell_color_code = NULL,
  color_as_factor = T,
  show_other_cells = T,
  show_network = T,
  show\_other\_network = F,
  network_color = NULL,
  spatial_network_name = "Delaunay_network",
  show_grid = F,
  grid_color = NULL,
  spatial_grid_name = "spatial_grid",
  show_legend = T,
  point_size_select = 4,
  point_size_other = 2,
  point_alpha_other = 0.5,
  axis_scale = c("cube", "real", "custom"),
  custom_ratio = NULL,
  x_ticks = NULL,
  y_ticks = NULL,
  z_ticks = NULL,
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "cellProximitySpatPlot3D",
)
```

### **Arguments**

gobject giotto object interaction\_name cell-cell interaction name cluster\_column cluster column with cell clusters x-axis dimension name (default = 'sdimx') sdimx sdimy y-axis dimension name (default = 'sdimy') sdimz z-axis dimension name (default = 'sdimz') cell\_color color for cells (see details) cell\_color\_code named vector with colors color\_as\_factor convert color column to factor show\_other\_cells decide if show cells not in network show\_network show spatial network of selected cells show\_other\_network show spatial network of not selected cells network\_color color of spatial network spatial\_network\_name name of spatial network to use show\_grid show spatial grid grid\_color color of spatial grid spatial\_grid\_name name of spatial grid to use show\_legend show legend point\_size\_select size of selected points point\_size\_other size of other points point\_alpha\_other opacity of other points axis\_scale scale of axis custom\_ratio custom ratio of axes x\_ticks ticks on x-axis ticks on y-axis y\_ticks ticks on z-axis z\_ticks show\_plot show plots return\_plot return plotly object save\_plot directly save the plot [boolean] list of saving parameters from all\_plots\_save\_function save\_param default\_save\_name default save name for saving, don't change, change save\_name in save\_param additional parameters

cellProximityVisPlot

#### **Details**

Description of parameters.

#### Value

plotly

#### **Examples**

```
cellProximitySpatPlot3D(gobject)
```

```
cellProximityVisPlot cellProximityVisPlot
```

#### **Description**

Visualize cell-cell interactions according to spatial coordinates

### Usage

```
cellProximityVisPlot(
 gobject,
 interaction_name = NULL,
 cluster_column = NULL,
  sdimx = NULL,
 sdimy = NULL,
 sdimz = NULL,
 cell_color = NULL,
 cell_color_code = NULL,
  color_as_factor = T,
  show_other_cells = F,
  show_network = F,
  show_other_network = F,
 network_color = NULL,
  spatial_network_name = "Delaunay_network",
  show\_grid = F,
 grid_color = NULL,
 spatial_grid_name = "spatial_grid",
 coord_fix_ratio = 1,
  show_legend = T,
 point_size_select = 2,
 point_select_border_col = "black",
 point_select_border_stroke = 0.05,
 point_size_other = 1,
 point_alpha_other = 0.3,
 point_other_border_col = "lightgrey",
 point_other_border_stroke = 0.01,
 axis_scale = c("cube", "real", "custom"),
 custom_ratio = NULL,
 x_ticks = NULL,
 y_ticks = NULL,
```

cellProximityVisPlot 35

```
z_ticks = NULL,
 plot_method = c("ggplot", "plotly"),
)
```

## **Arguments**

```
gobject
                  giotto object
interaction_name
                  cell-cell interaction name
cluster_column cluster column with cell clusters
                  x-axis dimension name (default = 'sdimx')
sdimx
                  y-axis dimension name (default = 'sdimy')
sdimy
sdimz
                  z-axis dimension name (default = 'sdimz')
                  color for cells (see details)
cell_color
cell_color_code
                  named vector with colors
color_as_factor
                  convert color column to factor
show_other_cells
                  show not selected cells
show_network
                  show underlying spatial network
show_other_network
                  show underlying spatial network of other cells
                 color of spatial network
network_color
spatial_network_name
                  name of spatial network to use
show_grid
                  show spatial grid
grid_color
                  color of spatial grid
spatial_grid_name
                  name of spatial grid to use
coord_fix_ratio
                  fix ratio between x and y-axis
show_legend
                  show legend
point_size_select
                  size of selected points
point_select_border_col
                  border color of selected points
point_select_border_stroke
                  stroke size of selected points
point_size_other
                  size of other points
point_alpha_other
                  alpha of other points
point_other_border_col
```

border color of other points

```
point_other_border_stroke
stroke size of other points

axis_scale scale of axis

custom_ratio custom ratio of scales

x_ticks x ticks

y_ticks y ticks

z_ticks z ticks

plot_method method to plot

... additional parameters
```

## **Details**

Description of parameters.

## Value

```
ggplot or plotly
```

## **Examples**

```
cellProximityVisPlot(gobject)
```

```
change Giot to Instructions
```

change Giot to Instructions

## Description

Function to change one or more instructions from giotto object

## Usage

```
changeGiottoInstructions(
  gobject,
  params = NULL,
  new_values = NULL,
  return_gobject = TRUE
)
```

## **Arguments**

```
gobject giotto object
params parameter(s) to change
new_values new value(s) for parameter(s)
return_gobject (boolean) return giotto object
```

#### Value

giotto object with one or more changed instructions

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## **Examples**

changeGiottoInstructions()

changeImageBg

change Image Bg

# Description

Function to change the background color of a magick image plot to another color

## Usage

```
changeImageBg(
  mg_object,
  bg_color,
  perc_range = 10,
  new_color = "#FFFFFF",
  new_name = NULL
)
```

## Arguments

mg\_object magick image or giotto image object
bg\_color estimated current background color

perc\_range range around estimated background color to include (percentage)

new\_color new background color

new\_name change name of Giotto image

## Value

magick image or giotto image object with updated background color

## **Examples**

```
changeImageBg(mg_object)
```

clusterCells

clusterCells

# Description

cluster cells using a variety of different methods

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#### Usage

```
clusterCells(
  gobject,
 cluster_method = c("leiden", "louvain_community", "louvain_multinet", "randomwalk",
    "sNNclust", "kmeans", "hierarchical"),
  name = "cluster_name"
  nn_network_to_use = "sNN";
  network_name = "sNN.pca",
  pyth_leid_resolution = 1,
  pyth_leid_weight_col = "weight",
  pyth_leid_part_type = c("RBConfigurationVertexPartition",
    "ModularityVertexPartition"),
  pyth_leid_init_memb = NULL,
  pyth_leid_iterations = 1000,
  pyth_louv_resolution = 1,
  pyth_louv_weight_col = NULL,
  python_louv_random = F,
  python_path = NULL,
  louvain_gamma = 1,
  louvain_omega = 1,
  walk\_steps = 4,
  walk\_clusters = 10,
  walk_weights = NA,
  sNNclust_k = 20,
  sNNclust_eps = 4,
  sNNclust_minPts = 16,
  borderPoints = TRUE,
  expression_values = c("normalized", "scaled", "custom"),
  genes_to_use = NULL,
  dim_reduction_to_use = c("cells", "pca", "umap", "tsne"),
  dim_reduction_name = "pca",
  dimensions_to_use = 1:10,
  distance_method = c("original", "pearson", "spearman", "euclidean", "maximum",
    "manhattan", "canberra", "binary", "minkowski"),
  km_centers = 10,
  km_iter_max = 100,
  km_nstart = 1000,
  km_algorithm = "Hartigan-Wong",
 hc_agglomeration_method = c("ward.D2", "ward.D", "single", "complete", "average",
    "mcquitty", "median", "centroid"),
  hc_k = 10,
  hc_h = NULL
  return_gobject = TRUE,
  set_seed = T,
  seed_number = 1234
```

#### **Arguments**

```
gobject giotto object
cluster_method community cluster method to use
name name for new clustering result
```

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nn\_network\_to\_use

type of NN network to use (kNN vs sNN)

network\_name name of NN network to use

pyth\_leid\_resolution

resolution for leiden

pyth\_leid\_weight\_col

column to use for weights

pyth\_leid\_part\_type

partition type to use

pyth\_leid\_init\_memb

initial membership

 $pyth\_leid\_iterations$ 

number of iterations

pyth\_louv\_resolution

resolution for louvain

pyth\_louv\_weight\_col

python louvain param: weight column

python\_louv\_random

python louvain param: random

python\_path specify specific path to python if required

louvain\_gamma louvain param: gamma or resolution

louvain\_omega louvain param: omega

 $walk\_steps \qquad \quad random walk: number \ of \ steps$ 

 ${\tt walk\_clusters} \quad random walk: number of clusters$ 

walk\_weights randomwalk: weight column

sNNclust\_k SNNclust: k neighbors to use

sNNclust\_eps SNNclust: epsilon

sNNclust\_minPts

SNNclust: min points

borderPoints SNNclust: border points

expression\_values

expression values to use

 $genes\_to\_use = NULL,$ 

dim\_reduction\_to\_use

dimension reduction to use

dim\_reduction\_name

name of reduction 'pca',

dimensions\_to\_use

dimensions to use

distance\_method

distance method

km\_centers kmeans centers km\_iter\_max kmeans iterations

km\_nstart kmeans random starting points

km\_algorithm kmeans algorithm

hc\_agglomeration\_method

hierarchical clustering method

hc\_k hierachical number of clusters

hc\_h hierarchical tree cutoff

return\_gobject boolean: return giotto object (default = TRUE)

set\_seed set seed

seed\_number number for seed

#### **Details**

Wrapper for the different clustering methods.

#### Value

giotto object with new clusters appended to cell metadata

#### See Also

 $\label{local-community} do Louvain Cluster\_community, do Louvain Cluster\_multinet, do Louvain Cluster, do Random Walk Cluster, do SNN Cluster, do Kmeans, do H clust \\$ 

#### **Examples**

```
clusterCells(gobject)
```

clusterSpatialCorGenes

clusterSpatialCorGenes

### **Description**

Cluster based on spatially correlated genes

## Usage

```
clusterSpatialCorGenes(
   spatCorObject,
   name = "spat_clus",
   hclust_method = "ward.D",
   k = 10,
   return_obj = TRUE
)
```

#### **Arguments**

spatCorObject spatial correlation object

name name for spatial clustering results
hclust\_method method for hierarchical clustering
k number of clusters to extract

return\_obj return spatial correlation object (spatCorObject)

combCCcom 41

#### Value

spatCorObject or cluster results

### **Examples**

```
clusterSpatialCorGenes(gobject)
```

combCCcom

combCCcom

## **Description**

Combine spatial and expression based cell-cell communication data.tables

## Usage

```
combCCcom(
  spatialCC,
  exprCC,
  min_lig_nr = 3,
  min_rec_nr = 3,
  min_padj_value = 1,
  min_log2fc = 0,
  min_av_diff = 0,
  detailed = FALSE
)
```

### **Arguments**

```
spatialCC spatial cell-cell communication scores
exprCC expression cell-cell communication scores
min_lig_nr minimum number of ligand cells
min_rec_nr minimum number of receptor cells
min_padj_value minimum adjusted p-value
min_log2fc minimum log2 fold-change
min_av_diff minimum average expression difference
detailed detailed option used with spatCellCellCom (default = FALSE)
```

### Value

combined data.table with spatial and expression communication data

## **Examples**

```
combCCcom(gobject)
```

```
combineCellProximityGenes
```

combineCellProximityGenes

## Description

Combine CPG scores in a pairwise manner.

#### Usage

```
combineCellProximityGenes(
  cpgObject,
  selected_ints = NULL,
  selected_genes = NULL,
  specific_genes_1 = NULL,
  specific_genes_2 = NULL,
  min_cells = 5,
  min_int_cells = 3,
  min_fdr = 0.05,
  min_spat_diff = 0,
  min_log2_fc = 0.5,
  do_parallel = TRUE,
  cores = NA,
  verbose = T
)
```

## **Arguments**

```
cpgObject
                  cell proximity gene score object
selected_ints
                  subset of selected cell-cell interactions (optional)
selected_genes subset of selected genes (optional)
specific_genes_1
                  specific geneset combo (need to position match specific_genes_2)
specific_genes_2
                  specific geneset combo (need to position match specific_genes_1)
min_cells
                  minimum number of target cell type
min_int_cells
                  minimum number of interacting cell type
min_fdr
                  minimum adjusted p-value
                  minimum absolute spatial expression difference
min_spat_diff
min_log2_fc
                  minimum absolute log2 fold-change
do_parallel
                  run calculations in parallel with mclapply
                  number of cores to use if do_parallel = TRUE
cores
                  verbose
verbose
```

#### Value

cpgObject that contains the filtered differential gene scores

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## **Examples**

combineCellProximityGenes(gobject)

combineCPG combineCPG

### Description

Combine CPG scores in a pairwise manner.

## Usage

```
combineCPG(
  cpgObject,
  selected_ints = NULL,
  selected_genes = NULL,
  specific_genes_1 = NULL,
  specific_genes_2 = NULL,
  min_cells = 5,
  min_int_cells = 3,
  min_fdr = 0.05,
  min_spat_diff = 0,
  min_log2_fc = 0.5,
  do_parallel = TRUE,
  cores = NA,
  verbose = T
)
```

### **Arguments**

```
cell proximity gene score object
cpg0bject
                  subset of selected cell-cell interactions (optional)
selected_ints
selected_genes subset of selected genes (optional)
specific_genes_1
                  specific geneset combo (need to position match specific_genes_2)
specific_genes_2
                  specific geneset combo (need to position match specific_genes_1)
min_cells
                  minimum number of target cell type
min_int_cells
                  minimum number of interacting cell type
min_fdr
                  minimum adjusted p-value
min_spat_diff
                  minimum absolute spatial expression difference
                  minimum absolute log2 fold-change
min_log2_fc
do_parallel
                  run calculations in parallel with mclapply
                  number of cores to use if do_parallel = TRUE
cores
verbose
                  verbose
```

#### Value

cpgObject that contains the filtered differential gene scores

## **Examples**

```
combineCPG(gobject)
```

combineMetadata

combineMetadata

## **Description**

This function combines the cell metadata with spatial locations and enrichment results from createSpatialEnrich

#### Usage

```
combineMetadata(gobject, spat_enr_names = NULL)
```

## Arguments

```
gobject Giotto object
spat_enr_names names of spatial enrichment results to include
```

### Value

Extended cell metadata in data.table format.

### **Examples**

```
combineMetadata(gobject)
```

```
convertEnsemblToGeneSymbol
```

convertEnsemblToGeneSymbol

### **Description**

This function convert ensembl gene IDs from a matrix to official gene symbols

#### Usage

```
convertEnsemblToGeneSymbol(matrix, species = c("mouse", "human"))
```

### **Arguments**

matrix an expression matrix with ensembl gene IDs as rownames

species species to use for gene symbol conversion

createCrossSection 45

#### **Details**

This function requires that the biomaRt library is installed

#### Value

expression matrix with gene symbols as rownames

## Description

Create a virtual 2D cross section.

## Usage

```
createCrossSection(
  gobject,
  name = "cross_section",
  spatial_network_name = "Delaunay_network",
  thickness_unit = c("cell", "natural"),
  slice_thickness = 2,
  cell_distance_estimate_method = "mean",
  extend_ratio = 0.2,
  method = c("equation", "3 points", "point and norm vector",
    "point and two plane vectors"),
  equation = NULL,
  point1 = NULL,
  point2 = NULL,
  point3 = NULL,
  normVector = NULL,
  planeVector1 = NULL,
  planeVector2 = NULL,
  mesh\_grid\_n = 20,
  return_gobject = TRUE
)
```

## **Arguments**

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extend_ratio	deciding the span of the cross section meshgrid, as a ratio of extension compared to the borders of the vitural tissue section. (default = $0.2$ )
method	method to define the cross section plane. If equation, the plane is defined by a four element numerical vector (equation) in the form of $c(A,B,C,D)$ , corresponding to a plane with equation $Ax+By+Cz=D$ . If 3 points, the plane is define by the coordinates of 3 points, as given by point1, point2, and point3. If point and norm vector, the plane is defined by the coordinates of one point (point1) in the plane and the coordinates of one norm vector (normVector) to the plane. If point and two plane vector, the plane is defined by the coordinates of one point (point1) in the plane and the coordinates of two vectors (planeVector1, planeVector2) in the plane. (default = equation)
equation	equation required by method "equation".equations needs to be a numerical vector of length 4, in the form of $c(A,B,C,D)$ , which defines plane $Ax+By+Cz=D$ .
point1	coordinates of the first point required by method "3 points", "point and norm vector", and "point and two plane vectors".
point2	coordinates of the second point required by method "3 points"
point3	coordinates of the third point required by method "3 points"
normVector	coordinates of the norm vector required by method "point and norm vector"
planeVector1	coordinates of the first plane vector required by method "point and two plane vectors"
planeVector2	coordinates of the second plane vector required by method "point and two plane vectors"
mesh_grid_n	numer of meshgrid lines to generate along both directions for the cross section plane.
return_gobject	boolean: return giotto object (default = TRUE)

### **Details**

Creates a virtual 2D cross section object for a given spatial network object. The users need to provide the definition of the cross section plane (see method).

## Value

giotto object with updated spatial network slot

createGiottoImage	createGiottoImage	

# Description

Creates a giotto image that can be added to a Giotto object and/or used to add an image to the spatial plotting functions

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#### Usage

```
createGiottoImage(
  gobject = NULL,
  spatial_locs = NULL,
  mg_object,
  name = "image",
  xmax_adj = 0,
  xmin_adj = 0,
  ymax_adj = 0,
  ymin_adj = 0
)
```

## **Arguments**

```
gobject
                  giotto object
                  spatial locations (alternative if giobject = NULL)
spatial_locs
                  magick image object
mg\_object
                  name for the image
name
                  adjustment of the maximum x-value to align the image
xmax_adj
                  adjustment of the minimum x-value to align the image
xmin_adj
                  adjustment of the maximum y-value to align the image
ymax_adj
                  adjustment of the minimum y-value to align the image
ymin_adj
```

#### Value

a giotto image object

### **Examples**

```
createGiottoImage(mg_object)
```

createGiottoInstructions

create Giot to Instructions

## Description

Function to set global instructions for giotto functions

## Usage

```
createGiottoInstructions(
  python_path = NULL,
  show_plot = NULL,
  return_plot = NULL,
  save_plot = NULL,
  save_dir = NULL,
  plot_format = NULL,
  dpi = NULL,
```

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```
units = NULL,
height = NULL,
width = NULL
)
```

### **Arguments**

python\_path path to python binary to use show\_plot print plot to console, default = TRUE return plot as object, default = TRUE return\_plot automatically save plot, dafault = FALSE save\_plot save\_dir path to directory where to save plots plot\_format format of plots (defaults to png) resolution for raster images dpi units of format (defaults to in) units height height of plots width width of plots

#### Value

named vector with giotto instructions

#### See Also

More online information can be found here https://rubd.github.io/Giotto\_site/articles/instructions\_and\_plotting.html

### **Examples**

createGiottoInstructions()

### **Description**

Function to create a giotto object

### Usage

```
createGiottoObject(
  raw_exprs,
  spatial_locs = NULL,
  norm_expr = NULL,
  norm_scaled_expr = NULL,
  custom_expr = NULL,
  cell_metadata = NULL,
  gene_metadata = NULL,
  spatial_network = NULL,
```

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```
spatial_network_name = NULL,
spatial_grid = NULL,
spatial_grid_name = NULL,
spatial_enrichment = NULL,
spatial_enrichment_name = NULL,
dimension_reduction = NULL,
nn_network = NULL,
images = NULL,
offset_file = NULL,
instructions = NULL,
cores = NA
```

#### Arguments

```
raw_exprs
                  matrix with raw expression counts [required]
spatial_locs
                  data.table or data.frame with coordinates for cell centroids
norm_expr
                  normalized expression values
norm_scaled_expr
                  scaled expression values
                  custom expression values
custom_expr
cell_metadata
                  cell annotation metadata
gene_metadata
                  gene annotation metadata
spatial_network
                  list of spatial network(s)
spatial_network_name
                  list of spatial network name(s)
                  list of spatial grid(s)
spatial_grid
spatial_grid_name
                  list of spatial grid name(s)
spatial_enrichment
                  list of spatial enrichment score(s) for each spatial region
spatial_enrichment_name
                  list of spatial enrichment name(s)
dimension_reduction
                  list of dimension reduction(s)
                  list of nearest neighbor network(s)
nn_network
                  list of images
images
offset_file
                  file used to stitch fields together (optional)
instructions
                  list of instructions or output result from createGiottoInstructions
                  how many cores or threads to use to read data if paths are provided
cores
```

#### **Details**

[Requirements] To create a giotto object you need to provide at least a matrix with genes as row names and cells as column names. This matrix can be provided as a base matrix, sparse Matrix, data.frame, data.table or as a path to any of those. To include spatial information about cells (or regions) you need to provide a matrix, data.table or data.frame (or path to them) with coordinates

for all spatial dimensions. This can be 2D (x and y) or 3D (x, y, x). The row order for the cell coordinates should be the same as the column order for the provided expression data.

[Instructions] Additionally an instruction file, generated manually or with createGiottoInstructions can be provided to instructions, if not a default instruction file will be created for the Giotto object.

[Multiple fields] In case a dataset consists of multiple fields, like seqFISH+ for example, an offset file can be provided to stitch the different fields together. stitchFieldCoordinates can be used to generate such an offset file.

[**Processed data**] Processed count data, such as normalized data, can be provided using one of the different expression slots (norm\_expr, norm\_scaled\_expr, custom\_expr).

[Metadata] Cell and gene metadata can be provided using the cell and gene metadata slots. This data can also be added afterwards using the addGeneMetadata or addCellMetadata functions.

[Other information] Additional information can be provided through the appropriate slots:

- · spatial networks
- · spatial girds
- · spatial enrichments
- · dimensions reductions
- nearest neighbours networks
- images

#### Value

giotto object

#### **Examples**

```
createGiottoObject(raw_exprs, spatial_locs)
```

createGiottoVisiumObject

 $create Giot to {\it Visium Object}$ 

# Description

creates Giotto object directly from a 10X visium folder

#### Usage

```
createGiottoVisiumObject(
  visium_dir = NULL,
  expr_data = c("raw", "filter"),
  gene_column_index = 1,
  png_name = NULL,
  xmax_adj = 0,
  xmin_adj = 0,
  ymax_adj = 0,
  ymin_adj = 0,
  instructions = NULL,
  cores = NA
)
```

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#### **Arguments**

```
visium_dir
                  path to the 10X visium directory [required]
                  raw or filtered data (see details)
expr_data
gene_column_index
                  which column index to select (see details)
                  select name of png to use (see details)
png_name
                  adjustment of the maximum x-value to align the image
xmax_adj
                  adjustment of the minimum x-value to align the image
xmin_adj
                  adjustment of the maximum y-value to align the image
ymax_adj
                  adjustment of the minimum y-value to align the image
ymin_adj
                  list of instructions or output result from createGiottoInstructions
instructions
cores
                  how many cores or threads to use to read data if paths are provided
```

#### Details

- expr\_data: raw will take expression data from raw\_feature\_bc\_matrix and filter from filtered\_feature\_bc\_matrix
- gene\_column\_index: which gene identifiers (names) to use if there are multiple columns (e.g. ensemble and gene symbol)
- png\_name: by default the first png will be selected, provide the png name to override this (e.g. myimage.png)

#### Value

giotto object

### **Examples**

```
createGiottoVisiumObject(visium_dir)
```

createHeatmap\_DT

createHeatmap\_DT

#### **Description**

creates order for clusters

### Usage

```
createHeatmap_DT(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  genes,
  cluster_column = NULL,
  cluster_order = c("size", "correlation", "custom"),
  cluster_custom_order = NULL,
  cluster_cor_method = "pearson",
  cluster_hclust_method = "ward.D",
```

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```
gene_order = c("correlation", "custom"),
gene_custom_order = NULL,
gene_cor_method = "pearson",
gene_hclust_method = "complete"
)
```

## **Arguments**

```
gobject
                 giotto object
expression_values
                 expression values to use
genes
                 genes to use
cluster_column name of column to use for clusters
cluster_order
                 method to determine cluster order
cluster_custom_order
                 custom order for clusters
cluster_cor_method
                 method for cluster correlation
cluster_hclust_method
                 method for hierarchical clustering of clusters
gene_order
                 method to determine gene order
gene_custom_order
                 custom order for genes
gene_cor_method
                 method for gene correlation
gene_hclust_method
                 method for hierarchical clustering of genes
```

#### **Details**

Creates input data.tables for plotHeatmap function.

#### Value

list

### **Examples**

```
createHeatmap_DT(gobject)
```

createMetagenes createMetagenes

## Description

This function creates an average metagene for gene clusters.

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#### Usage

```
createMetagenes(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  gene_clusters,
  name = "metagene",
  return_gobject = TRUE
)
```

### **Arguments**

```
gobject Giotto object
expression_values
expression values to use
gene_clusters numerical vector with genes as names
name name of the metagene results
return_gobject return giotto object
```

### **Details**

```
An example for the 'gene_clusters' could be like this: cluster_vector = c(1, 1, 2, 2); names(cluster_vector) = c('geneA', 'geneB', 'geneC', 'geneD')
```

#### Value

giotto object

### **Examples**

```
createMetagenes(gobject)
```

createNearestNetwork createNearestNetwork

### **Description**

create a nearest neighbour (NN) network

### Usage

```
createNearestNetwork(
  gobject,
  type = c("sNN", "kNN"),
  dim_reduction_to_use = "pca",
  dim_reduction_name = "pca",
  dimensions_to_use = 1:10,
  genes_to_use = NULL,
  expression_values = c("normalized", "scaled", "custom"),
  name = "sNN.pca",
  return_gobject = TRUE,
```

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```
k = 30,
minimum_shared = 5,
top_shared = 3,
verbose = T,
...
)
```

### **Arguments**

```
gobject
                 giotto object
type
                 sNN or kNN
dim_reduction_to_use
                 dimension reduction method to use
dim_reduction_name
                 name of dimension reduction set to use
dimensions_to_use
                 number of dimensions to use as input
                 if dim_reduction_to_use = NULL, which genes to use
genes_to_use
expression_values
                 expression values to use
                 arbitrary name for NN network
name
return_gobject boolean: return giotto object (default = TRUE)
                 number of k neighbors to use
minimum_shared minimum shared neighbors
top_shared
                 keep at ...
verbose
                 be verbose
                 additional parameters for kNN and sNN functions from dbscan
. . .
```

#### **Details**

This function creates a k-nearest neighbour (kNN) or shared nearest neighbour (sNN) network based on the provided dimension reduction space. To run it directly on the gene expression matrix set  $dim\_reduction\_to\_use = NULL$ .

See also kNN and sNN for more information about how the networks are created.

Output for kNN:

• from: cell\_ID for source cell

• to: cell\_ID for target cell

• distance: distance between cells

• weight: weight = 1/(1 + distance)

## Output for sNN:

• from: cell\_ID for source cell

• to: cell\_ID for target cell

• distance: distance between cells

• weight: 1/(1 + distance)

• shared: number of shared neighbours

• rank: ranking of pairwise cell neighbours

For sNN networks two additional parameters can be set:

- minimum\_shared: minimum number of shared neighbours needed
- top\_shared: keep this number of the top shared neighbours, irrespective of minimum\_shared setting

#### Value

giotto object with updated NN network

### **Examples**

```
createNearestNetwork(gobject)
```

```
createSpatialDefaultGrid
```

createSpatialDefaultGrid

### **Description**

Create a spatial grid using the default method

### Usage

```
createSpatialDefaultGrid(
  gobject,
  sdimx_stepsize = NULL,
  sdimy_stepsize = NULL,
  sdimz_stepsize = NULL,
  minimum_padding = 1,
  name = NULL,
  return_gobject = TRUE
)
```

# Arguments

```
gobject giotto object

sdimx_stepsize stepsize along the x-axis

sdimy_stepsize stepsize along the y-axis

sdimz_stepsize stepsize along the z-axis

minimum_padding

minimum padding on the edges

name name for spatial grid (default = 'spatial_grid')

return_gobject boolean: return giotto object (default = TRUE)
```

### **Details**

Creates a spatial grid with defined x, y (and z) dimensions. The dimension units are based on the provided spatial location units.

#### Value

giotto object with updated spatial grid slot

```
createSpatialDelaunayNetwork
```

createSpatialDelaunayNetwork

#### **Description**

Create a spatial Delaunay network based on cell centroid physical distances.

#### Usage

```
createSpatialDelaunayNetwork(
  gobject,
  method = c("deldir", "delaunayn_geometry", "RTriangle"),
  dimensions = "all",
  name = "Delaunay_network",
  maximum_distance = "auto",
  minimum_k = 0,
  options = "Pp",
  Y = TRUE,
  j = TRUE,
  S = 0,
  verbose = T,
  return_gobject = TRUE,
  ...
)
```

## Arguments

Υ

gobject	giotto object

method package to use to create a Delaunay network

dimensions which spatial dimensions to use. Use "sdimx" (spatial dimension x), "sdimy",

"sdimz" respectively to refer to X (or the 1st), Y (or the 2nd) and Z(or the 3rd)

dimension, see details. (default = all)

name name for spatial network (default = 'delaunay\_network')

maximum\_distance

distance cuttof for Delaunay neighbors to consider. If "auto", "upper wisker" value of the distance vector between neighbors is used; see the boxplotgraphics

documentation for more details.(default = "auto")

minimum\_k minimum number of neighbours if maximum\_distance != NULL

options (geometry) String containing extra control options for the underlying Qhull

command; see the Qhull documentation (../doc/qhull/html/qdelaun.html) for the available options. (default = 'Pp', do not report precision problems)

(RTriangle) If TRUE prohibits the insertion of Steiner points on the mesh bound-

ary.

j (RTriangle) If TRUE jettisons vertices that are not part of the final triangulation

from the output.

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S (RTriangle) Specifies the maximum number of added Steiner points.

verbose verbose

return\_gobject boolean: return giotto object (default = TRUE)

... Other additional parameters

#### **Details**

Creates a spatial Delaunay network as explained in delaunayn (default), deldir, or triangulate.

#### Value

giotto object with updated spatial network slot

### **Examples**

createSpatialDelaunayNetwork(gobject)

createSpatialEnrich createSpatialEnrich

#### **Description**

Function to calculate gene signature enrichment scores per spatial position using an enrichment test.

#### Usage

```
createSpatialEnrich(...)
```

### **Arguments**

... Arguments passed on to runSpatialEnrich

gobject Giotto object

enrich\_method method for gene signature enrichment calculation
sign\_matrix Matrix of signature genes for each cell type / process

expression\_values expression values to use

reverse\_log\_scale reverse expression values from log scale

logbase log base to use if reverse\_log\_scale = TRUE

p\_value calculate p-value (default = FALSE)

n\_times (page/rank) number of permutation iterations to calculate p-value

top\_percentage (hyper) percentage of cells that will be considered to have gene expression with matrix binarization

output\_enrichment how to return enrichment output

name to give to spatial enrichment results, default = PAGE

return\_gobject return giotto object

#### See Also

runSpatialEnrich

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# Description

Create a spatial grid using the default method

## Usage

```
createSpatialGrid(
  gobject,
  name = NULL,
  method = c("default"),
  sdimx_stepsize = NULL,
  sdimy_stepsize = NULL,
  sdimz_stepsize = NULL,
  minimum_padding = 1,
  return_gobject = TRUE
)
```

### **Arguments**

```
gobject giotto object

name name for spatial grid

method method to create a spatial grid

sdimx_stepsize stepsize along the x-axis

sdimy_stepsize stepsize along the y-axis

sdimz_stepsize stepsize along the z-axis

minimum_padding

minimum padding on the edges

return_gobject boolean: return giotto object (default = TRUE)
```

#### **Details**

Creates a spatial grid with defined x, y (and z) dimensions. The dimension units are based on the provided spatial location units.

• default method: createSpatialDefaultGrid

### Value

giotto object with updated spatial grid slot

```
createSpatialKNNnetwork
```

createSpatialKNN network

## Description

Create a spatial knn network.

### Usage

```
createSpatialKNNnetwork(
  gobject,
  method = "dbscan",
  dimensions = "all",
  name = "knn_network",
  k = 4,
  maximum_distance = NULL,
  minimum_k = 0,
  verbose = F,
  return_gobject = TRUE,
  ...
)
```

#### **Arguments**

gobject giotto object

method method to create kNN network

dimensions which spatial dimensions to use (default = all)

maximum\_distance

distance cuttof for nearest neighbors to consider for kNN network

minimum\_k minimum nearest neighbours if maximum\_distance != NULL

verbose verbose

return\_gobject boolean: return giotto object (default = TRUE)
... additional arguments to the selected method function

### Value

giotto object with updated spatial network slot

**dimensions:** default = 'all' which takes all possible dimensions. Alternatively you can provide a character vector that specififies the spatial dimensions to use, e.g. c("sdimx', "sdimy") or a numerical vector, e.g. 2:3

**maximum\_distance:** to create a network based on maximum distance only, you also need to set k to a very high value, e.g. k = 100

#### **Examples**

```
createSpatialKNNnetwork(gobject)
```

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```
create Spatial Network \\ create Spatial Network
```

## Description

Create a spatial network based on cell centroid physical distances.

## Usage

```
createSpatialNetwork(
  gobject,
  name = NULL,
  dimensions = "all",
  method = c("Delaunay", "kNN"),
delaunay_method = c("deldir", "delaunayn_geometry", "RTriangle"),
maximum_distance_delaunay = "auto",
  options = "Pp",
  Y = TRUE,
  j = TRUE,
  S = 0,
  minimum_k = 0,
  knn_method = "dbscan",
  k = 4,
  maximum_distance_knn = NULL,
  verbose = F,
  return_gobject = TRUE,
)
```

# Arguments

gobject	giotto object	
name	<pre>name for spatial network (default = 'spatial_network')</pre>	
dimensions	which spatial dimensions to use (default = all)	
method	which method to use to create a spatial network. (default = Delaunay)	
delaunay_method		
	Delaunay method to use	
maximum_distance_delaunay		
	distance cuttof for nearest neighbors to consider for Delaunay network	
options	(geometry) String containing extra control options for the underlying Qhull command; see the Qhull documentation (/doc/qhull/html/qdelaun.html) for the available options. (default = 'Pp', do not report precision problems)	
Υ	(RTriangle) If TRUE prohibits the insertion of Steiner points on the mesh boundary.	
j	(RTriangle) If TRUE jettisons vertices that are not part of the final triangulation from the output.	
S	(RTriangle) Specifies the maximum number of added Steiner points.	
minimum_k	minimum nearest neigbhours if maximum_distance != NULL	

#### **Details**

Creates a spatial network connecting single-cells based on their physical distance to each other. For Delaunay method, neighbors will be decided by delaunay triangulation and a maximum distance criteria. For kNN method, number of neighbors can be determined by k, or maximum distance from each cell with or without setting a minimum k for each cell.

**dimensions:** default = 'all' which takes all possible dimensions. Alternatively you can provide a character vector that specififies the spatial dimensions to use, e.g. c("sdimx', "sdimy") or a numerical vector, e.g. 2:3

#### Value

giotto object with updated spatial network slot

#### **Examples**

```
createSpatialNetwork(gobject)
```

```
create\_average\_detection\_DT \\ create\_average\_detection\_DT
```

#### **Description**

calculates average gene detection for a cell metadata factor (e.g. cluster)

## Usage

```
create_average_detection_DT(
  gobject,
  meta_data_name,
  expression_values = c("normalized", "scaled", "custom"),
  detection_threshold = 0
)
```

#### **Arguments**

#### Value

data.table with average gene epression values for each factor

## Description

calculates average gene expression for a cell metadata factor (e.g. cluster)

### Usage

```
create_average_DT(
  gobject,
  meta_data_name,
  expression_values = c("normalized", "scaled", "custom")
)
```

## **Arguments**

```
gobject giotto object

meta_data_name name of metadata column to use
expression_values

which expression values to use
```

#### Value

data.table with average gene epression values for each factor

### **Description**

creates randomized cell ids within a selection of cell types

## Usage

```
create_cell_type_random_cell_IDs(
  gobject,
  cluster_column = "cell_types",
  needed_cell_types
)
```

### **Arguments**

```
gobject giotto object to use

cluster_column cluster column with cell type information

needed_cell_types

vector of cell type names for which a random id will be found
```

#### **Details**

Details will follow.

#### Value

list of randomly sampled cell ids with same cell type composition

### **Examples**

```
create_cell_type_random_cell_IDs(gobject)
```

### **Description**

create a crossSection object

## Usage

```
create_crossSection_object(
  name = NULL,
  method = NULL,
  thickness_unit = NULL,
  slice_thickness = NULL,
  cell_distance_estimate_method = NULL,
  extend_ratio = NULL,
  plane_equation = NULL,
  mesh_grid_n = NULL,
  mesh_obj = NULL,
  cell_subset = NULL,
  cell_subset_spatial_locations = NULL,
  cell_subset_projection_locations = NULL,
  cell_subset_projection_PCA = NULL,
  cell_subset_projection_coords = NULL
)
```

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#### **Arguments**

name of cress section object. (default = cross\_sectino)

method method to define the cross section plane.

thickness\_unit unit of the virtual section thickness. If "cell", average size of the observed

cells is used as length unit. If "natural", the unit of cell location coordinates

is used.(default = cell)

slice\_thickness

thickness of slice

cell\_distance\_estimate\_method

method to estimate average distance between neighboring cells. (default = mean)

extend\_ratio deciding the span of the cross section meshgrid, as a ratio of extension compared

to the borders of the vitural tissue section. (default = 0.2)

plane\_equation a numerical vector of length 4, in the form of c(A,B,C,D), which defines plane

Ax+By+Cz=D.

mesh\_grid\_n numer of meshgrid lines to generate along both directions for the cross section

plane.

mesh\_obj object that stores the cross section meshgrid information.

cell\_subset cells selected by the cross section

cell\_subset\_spatial\_locations

locations of cells selected by the cross section

cell\_subset\_projection\_locations

3D projection coordinates of selected cells onto the cross section plane

cell\_subset\_projection\_PCA

pca of projection coordinates

cell\_subset\_projection\_coords

2D PCA coordinates of selected cells in the cross section plane

create\_screeplot

### **Description**

create screeplot with ggplot

#### Usage

```
create_screeplot(pca_obj, ncp = 20, ylim = c(0, 20))
```

### **Arguments**

pca\_obj pca dimension reduction object

ncp number of principal components to calculate

ylim y-axis limits on scree plot

#### Value

ggplot

crossSectionGenePlot 65

```
{\tt crossSectionGenePlot} \quad {\it crossSectionGenePlot}
```

## Description

Visualize cells and gene expression in a virtual cross section according to spatial coordinates

## Usage

```
crossSectionGenePlot(
  gobject = NULL,
  crossSection_obj = NULL,
  name = NULL,
  spatial_network_name = "Delaunay_network",
  default_save_name = "crossSectionGenePlot",
  ...
)
```

### **Arguments**

```
gobject giotto object

crossSection_obj

crossSection object

name name of virtual cross section to use

spatial_network_name

name of spatial network to use

default_save_name

default save name for saving, don't change, change save_name in save_param

parameters for spatGenePlot2D
```

### **Details**

Description of parameters.

#### Value

ggplot

#### See Also

```
spatGenePlot3D and spatGenePlot2D
```

66 crossSectionGenePlot3D

```
crossSectionGenePlot3D
```

crossSectionGenePlot3D

### **Description**

Visualize cells and gene expression in a virtual cross section according to spatial coordinates

## Usage

```
crossSectionGenePlot3D(
  gobject,
  crossSection_obj = NULL,
  name = NULL,
  spatial_network_name = "Delaunay_network",
  other_cell_color = alpha("lightgrey", 0),
  default_save_name = "crossSectionGenePlot3D",
   ...
)
```

### **Arguments**

#### **Details**

Description of parameters.

#### Value

ggplot

# Examples

```
{\tt crossSectionGenePlot3D(gobject)}
```

crossSectionPlot 67

crossSectionPlot

crossSectionPlot

# Description

Visualize cells in a virtual cross section according to spatial coordinates

### Usage

```
crossSectionPlot(
  gobject,
  crossSection_obj = NULL,
  name = NULL,
  spatial_network_name = "Delaunay_network",
  default_save_name = "crossSectionPlot",
  ...
)
```

## **Arguments**

### **Details**

Description of parameters.

### Value

ggplot

### See Also

crossSectionPlot

68 crossSectionPlot3D

crossSectionPlot3D

crossSectionPlot3D

### **Description**

Visualize cells in a virtual cross section according to spatial coordinates

### Usage

```
crossSectionPlot3D(
  gobject,
  crossSection_obj = NULL,
  name = NULL,
  spatial_network_name = "Delaunay_network",
  show_other_cells = T,
  other_cell_color = alpha("lightgrey", 0),
  default_save_name = "crossSection3D",
  ...
)
```

### **Arguments**

#### **Details**

Description of parameters.

#### Value

ggplot

### **Examples**

```
crossSectionPlot3D(gobject)
```

decide\_cluster\_order 69

```
decide_cluster_order
```

## Description

creates order for clusters

## Usage

```
decide_cluster_order(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  genes,
  cluster_column = NULL,
  cluster_order = c("size", "correlation", "custom"),
  cluster_custom_order = NULL,
  cor_method = "pearson",
  hclust_method = "ward.D"
)
```

## **Arguments**

```
gobject giotto object
expression_values
expression values to use
genes genes to use
cluster_column name of column to use for clusters
cluster_order method to determine cluster order
cluster_custom_order
custom order for clusters

cor_method method for correlation
hclust_method method for hierarchical clustering
```

### **Details**

Calculates order for clusters.

#### Value

custom

### **Examples**

```
decide_cluster_order(gobject)
```

detectSpatialCorGenes detectSpatialCorGenes

### **Description**

Detect genes that are spatially correlated

#### Usage

```
detectSpatialCorGenes(
  gobject,
  method = c("grid", "network"),
  expression_values = c("normalized", "scaled", "custom"),
  subset_genes = NULL,
  spatial_network_name = "Delaunay_network",
  network_smoothing = NULL,
  spatial_grid_name = "spatial_grid",
  min_cells_per_grid = 4,
  cor_method = c("pearson", "kendall", "spearman")
)
```

### **Arguments**

```
gobject
                  giotto object
method
                  method to use for spatial averaging
expression_values
                  gene expression values to use
subset_genes
                  subset of genes to use
spatial_network_name
                  name of spatial network to use
network_smoothing
                  smoothing factor beteen 0 and 1 (default: automatic)
spatial_grid_name
                  name of spatial grid to use
min_cells_per_grid
                  minimum number of cells to consider a grid
cor_method
                  correlation method
```

### **Details**

For method = network, it expects a fully connected spatial network. You can make sure to create a fully connected network by setting minimal\_k > 0 in the createSpatialNetwork function.

- 1. grid-averaging: average gene expression values within a predefined spatial grid
- 2. network-averaging: smoothens the gene expression matrix by averaging the expression within one cell by using the neighbours within the predefined spatial network. b is a smoothening factor that defaults to 1 1/k, where k is the median number of k-neighbors in the selected spatial network. Setting b = 0 means no smoothing and b = 1 means no contribution from its own expression.

The spatCorObject can be further explored with showSpatialCorGenes()

detectSpatialPatterns 71

#### Value

```
returns a spatial correlation object: "spatCorObject"
```

#### See Also

```
showSpatialCorGenes
```

### **Examples**

```
detectSpatialCorGenes(gobject)
```

```
detectSpatialPatterns detectSpatialPatterns
```

### **Description**

Identify spatial patterns through PCA on average expression in a spatial grid.

# Usage

```
detectSpatialPatterns(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  spatial_grid_name = "spatial_grid",
  min_cells_per_grid = 4,
  scale_unit = F,
  ncp = 100,
  show_plot = T,
  PC_zscore = 1.5
)
```

### Arguments

```
gobject
                  giotto object
expression_values
                  expression values to use
spatial_grid_name
                  name of spatial grid to use (default = 'spatial_grid')
min_cells_per_grid
                  minimum number of cells in a grid to be considered
                  scale features
scale_unit
                  number of principal components to calculate
ncp
show_plot
                  show plots
PC_zscore
                  minimum z-score of variance explained by a PC
```

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#### **Details**

Steps to identify spatial patterns:

- 1. average gene expression for cells within a grid, see createSpatialGrid
- 2. perform PCA on the average grid expression profiles
- 3. convert variance of principlal components (PCs) to z-scores and select PCs based on a z-score threshold

#### Value

```
spatial pattern object 'spatPatObj'
```

#### **Examples**

```
detectSpatialPatterns(gobject)
```

dimCellPlot

dimCellPlot

#### **Description**

Visualize cells according to dimension reduction coordinates

### Usage

```
dimCellPlot(gobject, ...)
```

#### **Arguments**

```
gobject
                 giotto object
                 Arguments passed on to dimCellPlot2D
                 dim_reduction_to_use dimension reduction to use
                 dim_reduction_name dimension reduction name
                 dim1_to_use dimension to use on x-axis
                 dim2_to_use dimension to use on y-axis
                 spat_enr_names names of spatial enrichment results to include
                 cell_annotation_values numeric cell annotation columns
                 show_NN_network show underlying NN network
                 nn_network_to_use type of NN network to use (kNN vs sNN)
                 network_name name of NN network to use, if show_NN_network = TRUE
                 cell_color_code named vector with colors for cell annotation values
                 cell_color_gradient vector with 3 colors for numeric data
                 gradient_midpoint midpoint for color gradient
                 gradient_limits vector with lower and upper limits
                 select_cell_groups select subset of cells/clusters based on cell_color param-
                 select_cells select subset of cells based on cell IDs
                 show_other_cells display not selected cells
```

```
other_cell_color color of not selected cells
other_point_size size of not selected cells
show_cluster_center plot center of selected clusters
show_center_label plot label of selected clusters
center_point_size size of center points
center_point_border_col border color of center points
center_point_border_stroke border stroke size of center points
label_size size of labels
label_fontface font of labels
edge_alpha column to use for alpha of the edges
point_shape point with border or not (border or no_border)
point_size size of point (cell)
point_alpha transparancy of dim. reduction points
point_border_col color of border around points
point_border_stroke stroke size of border around points
show_legend show legend
legend_text size of legend text
legend_symbol_size size of legend symbols
background_color color of plot background
axis_text size of axis text
axis_title size of axis title
cow_n_col cowplot param: how many columns
cow_rel_h cowplot param: relative height
cow_rel_w cowplot param: relative width
cow_align cowplot param: how to align
show_plot show plot
return_plot return ggplot object
save_plot directly save the plot [boolean]
save_param list of saving parameters, see showSaveParameters
default_save_name default save name for saving, don't change, change save_name
    in save_param
```

# Details

Description of parameters. For 3D plots see dimCellPlot2D

### Value

ggplot

# See Also

Other dimension reduction cell annotation visualizations: dimCellPlot2D()

### **Examples**

```
dimCellPlot(gobject)
```

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dimCellPlot2D

dimCellPlot2D

### **Description**

Visualize cells according to dimension reduction coordinates

```
dimCellPlot2D(
  gobject,
  dim_reduction_to_use = "umap",
  dim_reduction_name = "umap",
  dim1_to_use = 1,
  dim2\_to\_use = 2,
  spat_enr_names = NULL,
  cell_annotation_values = NULL,
  show_NN_network = F,
  nn_network_to_use = "sNN",
  network_name = "sNN.pca",
  cell_color_code = NULL,
  cell_color_gradient = c("blue", "white", "red"),
  gradient_midpoint = NULL,
  gradient_limits = NULL,
  select_cell_groups = NULL,
  select_cells = NULL,
  show_other_cells = T,
  other_cell_color = "lightgrey",
  other_point_size = 0.5,
  show_cluster_center = F,
  show_center_label = T,
  center_point_size = 4,
  center_point_border_col = "black",
  center_point_border_stroke = 0.1,
  label_size = 4,
  label_fontface = "bold",
  edge_alpha = NULL,
  point_shape = c("border", "no_border"),
  point_size = 1,
  point_alpha = 1,
  point_border_col = "black",
  point_border_stroke = 0.1,
  show_legend = T,
  legend_text = 8,
  legend_symbol_size = 1,
  background_color = "white",
  axis_text = 8,
  axis_title = 8,
  cow_n_col = 2,
  cow_rel_h = 1,
  cow_rel_w = 1,
```

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```
cow_align = "h",
      show_plot = NA,
      return_plot = NA,
      save_plot = NA,
      save_param = list(),
      default_save_name = "dimCellPlot2D"
    )
Arguments
    gobject
                     giotto object
    dim_reduction_to_use
                     dimension reduction to use
    dim_reduction_name
                     dimension reduction name
                     dimension to use on x-axis
    dim1_to_use
                     dimension to use on y-axis
    dim2_to_use
    spat_enr_names names of spatial enrichment results to include
    cell_annotation_values
                     numeric cell annotation columns
    show_NN_network
                     show underlying NN network
    nn_network_to_use
                     type of NN network to use (kNN vs sNN)
    network_name
                     name of NN network to use, if show_NN_network = TRUE
    cell_color_code
                     named vector with colors for cell annotation values
    cell_color_gradient
                     vector with 3 colors for numeric data
    gradient_midpoint
                     midpoint for color gradient
    gradient_limits
                     vector with lower and upper limits
    select_cell_groups
                     select subset of cells/clusters based on cell_color parameter
    select_cells
                     select subset of cells based on cell IDs
    show_other_cells
                     display not selected cells
    other_cell_color
                     color of not selected cells
    other_point_size
                     size of not selected cells
    show_cluster_center
                     plot center of selected clusters
    show_center_label
                     plot label of selected clusters
    center_point_size
```

size of center points

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```
center_point_border_col
                  border color of center points
center_point_border_stroke
                  border stroke size of center points
                  size of labels
label_size
label_fontface font of labels
edge_alpha
                  column to use for alpha of the edges
point_shape
                  point with border or not (border or no_border)
point_size
                  size of point (cell)
point_alpha
                  transparancy of dim. reduction points
point_border_col
                  color of border around points
point_border_stroke
                  stroke size of border around points
show_legend
                  show legend
legend_text
                  size of legend text
legend_symbol_size
                  size of legend symbols
background_color
                  color of plot background
axis_text
                  size of axis text
axis_title
                  size of axis title
cow_n_col
                  cowplot param: how many columns
cow_rel_h
                  cowplot param: relative height
cow_rel_w
                  cowplot param: relative width
cow_align
                  cowplot param: how to align
show_plot
                  show plot
return_plot
                  return ggplot object
                  directly save the plot [boolean]
save_plot
save_param
                  list of saving parameters, see showSaveParameters
default_save_name
                  default save name for saving, don't change, change save_name in save_param
```

### Details

Description of parameters. For 3D plots see dimPlot3D

### Value

ggplot

# See Also

Other dimension reduction cell annotation visualizations: dimCellPlot()

# **Examples**

```
dimCellPlot2D(gobject)
```

dimGenePlot 77

dimGenePlot

dimGenePlot

show\_plot show plots

return\_plot return ggplot object

### **Description**

Visualize gene expression according to dimension reduction coordinates

#### **Usage**

```
dimGenePlot(...)
```

#### **Arguments**

Arguments passed on to dimGenePlot2D gobject giotto object expression\_values gene expression values to use genes genes to show dim\_reduction\_to\_use dimension reduction to use dim\_reduction\_name dimension reduction name dim1\_to\_use dimension to use on x-axis dim2\_to\_use dimension to use on y-axis show\_NN\_network show underlying NN network nn\_network\_to\_use type of NN network to use (kNN vs sNN) network\_name name of NN network to use, if show\_NN\_network = TRUE network\_color color of NN network edge\_alpha column to use for alpha of the edges scale\_alpha\_with\_expression scale expression with ggplot alpha parameter point\_shape point with border or not (border or no\_border) point\_size size of point (cell) point\_alpha transparancy of points cell\_color\_gradient vector with 3 colors for numeric data gradient\_midpoint midpoint for color gradient gradient\_limits vector with lower and upper limits point\_border\_col color of border around points point\_border\_stroke stroke size of border around points show\_legend show legend legend\_text size of legend text background\_color color of plot background axis\_text size of axis text axis\_title size of axis title cow\_n\_col cowplot param: how many columns cow\_rel\_h cowplot param: relative height cow\_rel\_w cowplot param: relative width cow\_align cowplot param: how to align

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```
save_plot directly save the plot [boolean]
save_param list of saving parameters, see showSaveParameters
default_save_name default save name for saving, don't change, change save_name
in save_param
```

### **Details**

Description of parameters.

#### Value

ggplot

### See Also

```
dimGenePlot3D
```

Other dimension reduction gene expression visualizations: dimGenePlot2D(), dimGenePlot3D()

### **Examples**

```
dimGenePlot(gobject)
```

dimGenePlot2D

dimGenePlot2D

# **Description**

Visualize gene expression according to dimension reduction coordinates

```
dimGenePlot2D(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  genes = NULL,
  dim_reduction_to_use = "umap",
  dim_reduction_name = "umap",
  dim1_to_use = 1,
  dim2\_to\_use = 2,
  show_NN_network = F,
  nn_network_to_use = "sNN",
  network_name = "sNN.pca",
  network_color = "lightgray",
  edge_alpha = NULL,
  scale_alpha_with_expression = FALSE,
  point_shape = c("border", "no_border"),
  point_size = 1,
  point_alpha = 1,
  cell_color_gradient = c("blue", "white", "red"),
  gradient_midpoint = NULL,
  gradient_limits = NULL,
```

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```
point_border_col = "black",
      point_border_stroke = 0.1,
      show_legend = T,
      legend_text = 8,
      background_color = "white",
      axis_text = 8,
      axis_title = 8,
      cow_n_col = 2,
      cow_rel_h = 1,
      cow_rel_w = 1,
      cow_align = "h",
      show_plot = NA,
      return_plot = NA,
      save_plot = NA,
      save_param = list(),
      default_save_name = "dimGenePlot2D"
Arguments
   gobject
                     giotto object
    expression_values
                     gene expression values to use
    genes
                     genes to show
    dim_reduction_to_use
                     dimension reduction to use
   dim_reduction_name
                     dimension reduction name
                     dimension to use on x-axis
    dim1_to_use
    dim2_to_use
                     dimension to use on y-axis
    show_NN_network
                     show underlying NN network
   nn_network_to_use
                     type of NN network to use (kNN vs sNN)
   network_name
                     name of NN network to use, if show_NN_network = TRUE
   network_color
                     color of NN network
    edge_alpha
                     column to use for alpha of the edges
    scale_alpha_with_expression
                     scale expression with ggplot alpha parameter
                     point with border or not (border or no_border)
   point_shape
   point_size
                     size of point (cell)
   point_alpha
                     transparancy of points
    cell_color_gradient
                     vector with 3 colors for numeric data
    gradient_midpoint
                     midpoint for color gradient
    gradient_limits
                     vector with lower and upper limits
```

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```
point_border_col
                  color of border around points
point_border_stroke
                  stroke size of border around points
show_legend
                  show legend
legend_text
                  size of legend text
background_color
                  color of plot background
                  size of axis text
axis_text
                  size of axis title
axis_title
cow_n_col
                  cowplot param: how many columns
                  cowplot param: relative height
cow_rel_h
cow_rel_w
                  cowplot param: relative width
                  cowplot param: how to align
cow_align
show_plot
                  show plots
return_plot
                  return ggplot object
save_plot
                  directly save the plot [boolean]
save_param
                  list of saving parameters, see showSaveParameters
default_save_name
                  default save name for saving, don't change, change save_name in save_param
```

### **Details**

Description of parameters.

# Value

ggplot

#### See Also

```
dimGenePlot3D
```

Other dimension reduction gene expression visualizations: dimGenePlot3D(), dimGenePlot()

# **Examples**

```
dimGenePlot2D(gobject)
```

dimGenePlot3D 81

dimGenePlot3D

dimGenePlot3D

### **Description**

Visualize cells and gene expression according to dimension reduction coordinates

# Usage

```
dimGenePlot3D(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  genes = NULL,
  dim_reduction_to_use = "umap",
  dim_reduction_name = "umap",
  dim1_to_use = 1,
  dim2_to_use = 2,
  dim3\_to\_use = 3,
  show_NN_network = F,
  nn_network_to_use = "sNN",
  network_name = "sNN.pca",
  network_color = "lightgray",
  cluster_column = NULL,
  select_cell_groups = NULL,
  select_cells = NULL,
  show_other_cells = T,
  other_cell_color = "lightgrey",
  other_point_size = 1,
  edge_alpha = NULL,
  point_size = 2,
  genes_high_color = NULL,
  genes_mid_color = "white",
  genes_low_color = "blue",
  show_legend = T,
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "dimGenePlot3D"
)
```

```
gobject giotto object
expression_values
gene expression values to use
genes genes to show
dim_reduction_to_use
dimension reduction to use
dim_reduction_name
dimension reduction name
```

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```
dim1_to_use
                  dimension to use on x-axis
dim2_to_use
                  dimension to use on y-axis
                  dimension to use on z-axis
dim3_to_use
show_NN_network
                  show underlying NN network
nn_network_to_use
                  type of NN network to use (kNN vs sNN)
                  name of NN network to use, if show_NN_network = TRUE
network_name
                  color of NN network
network_color
cluster_column cluster column to select groups
select_cell_groups
                  select subset of cells/clusters based on cell_color parameter
select_cells
                  select subset of cells based on cell IDs
show_other_cells
                  display not selected cells
other_cell_color
                  color of not selected cells
other_point_size
                  size of not selected cells
edge_alpha
                  column to use for alpha of the edges
point_size
                  size of point (cell)
genes_high_color
                  color for high expression levels
genes_mid_color
                  color for medium expression levels
genes_low_color
                  color for low expression levels
show_legend
                  show legend
show_plot
                  show plots
return_plot
                  return ggplot object
                  directly save the plot [boolean]
save_plot
                  list of saving parameters, see showSaveParameters
save_param
default_save_name
                  default save name for saving, don't change, change save_name in save_param
```

### **Details**

Description of parameters.

### Value

ggplot

### See Also

Other dimension reduction gene expression visualizations: dimGenePlot2D(), dimGenePlot()

### **Examples**

```
dimGenePlot3D(gobject)
```

dimPlot 83

dimPlot dimPlot

#### **Description**

Visualize cells according to dimension reduction coordinates

### Usage

```
dimPlot(...)
```

#### **Arguments**

Arguments passed on to dimPlot2D . . . gobject giotto object group\_by create multiple plots based on cell annotation column group\_by\_subset subset the group\_by factor column dim\_reduction\_to\_use dimension reduction to use dim\_reduction\_name dimension reduction name dim1\_to\_use dimension to use on x-axis dim2\_to\_use dimension to use on y-axis spat\_enr\_names names of spatial enrichment results to include show\_NN\_network show underlying NN network nn\_network\_to\_use type of NN network to use (kNN vs sNN) network\_name name of NN network to use, if show NN network = TRUE cell\_color color for cells (see details) color\_as\_factor convert color column to factor cell\_color\_code named vector with colors cell\_color\_gradient vector with 3 colors for numeric data gradient\_midpoint midpoint for color gradient gradient\_limits vector with lower and upper limits select\_cell\_groups select subset of cells/clusters based on cell color parameter select\_cells select subset of cells based on cell IDs show\_other\_cells display not selected cells other\_cell\_color color of not selected cells other\_point\_size size of not selected cells show\_cluster\_center plot center of selected clusters show\_center\_label plot label of selected clusters center\_point\_size size of center points center\_point\_border\_col border color of center points center\_point\_border\_stroke border stroke size of center points label\_size size of labels label\_fontface font of labels

edge\_alpha column to use for alpha of the edges

point\_shape point with border or not (border or no\_border)

```
point_size size of point (cell)
point_alpha transparancy of point
point_border_col color of border around points
point_border_stroke stroke size of border around points
title title for plot, defaults to cell_color parameter
show_legend show legend
legend_text size of legend text
legend_symbol_size size of legend symbols
background_color color of plot background
axis_text size of axis text
axis_title size of axis title
cow_n_col cowplot param: how many columns
cow_rel_h cowplot param: relative height
cow_rel_w cowplot param: relative width
cow_align cowplot param: how to align
show_plot show plot
return_plot return ggplot object
save_plot directly save the plot [boolean]
save_param list of saving parameters, see showSaveParameters
default_save_name default save name for saving, don't change, change save_name
    in save_param
```

### **Details**

Description of parameters, see dimPlot2D. For 3D plots see dimPlot3D

### Value

ggplot

### See Also

Other reduced dimension visualizations: dimPlot2D(), dimPlot3D(), plotPCA\_2D(), plotPCA\_3D(), plotPCA(), plotTSNE\_2D(), plotTSNE\_3D(), plotUMAP\_2D(), plotUMAP\_3D(), plotUMAP()

# **Examples**

dimPlot(gobject)

dimPlot2D

dimPlot2D

# Description

Visualize cells according to dimension reduction coordinates

```
dimPlot2D(
  gobject,
  group_by = NULL,
  group_by_subset = NULL,
  dim_reduction_to_use = "umap",
  dim_reduction_name = "umap",
  dim1_to_use = 1,
  dim2\_to\_use = 2,
  spat_enr_names = NULL,
  show_NN_network = F,
  nn_network_to_use = "sNN",
  network_name = "sNN.pca",
  cell_color = NULL,
  color_as_factor = T,
  cell_color_code = NULL,
  cell_color_gradient = c("blue", "white", "red"),
  gradient_midpoint = NULL,
  gradient_limits = NULL,
  select_cell_groups = NULL,
  select_cells = NULL,
  show_other_cells = T,
  other_cell_color = "lightgrey",
  other_point_size = 0.5,
  show_cluster_center = F,
  show_center_label = T,
  center_point_size = 4,
  center_point_border_col = "black",
  center_point_border_stroke = 0.1,
  label_size = 4,
  label_fontface = "bold",
  edge_alpha = NULL,
  point_shape = c("border", "no_border"),
  point_size = 1,
  point_alpha = 1,
  point_border_col = "black",
  point_border_stroke = 0.1,
  title = NULL,
  show_legend = T,
  legend_text = 8,
  legend_symbol_size = 1,
  background_color = "white",
  axis_text = 8,
  axis_title = 8,
  cow_n_col = 2,
  cow_rel_h = 1,
  cow_rel_w = 1,
  cow_align = "h",
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
```

```
default_save_name = "dimPlot2D"
)
```

### Arguments

gobject giotto object group\_by create multiple plots based on cell annotation column group\_by\_subset subset the group\_by factor column dim\_reduction\_to\_use dimension reduction to use dim\_reduction\_name dimension reduction name dim1\_to\_use dimension to use on x-axis dimension to use on y-axis dim2\_to\_use spat\_enr\_names names of spatial enrichment results to include show\_NN\_network show underlying NN network nn\_network\_to\_use type of NN network to use (kNN vs sNN) name of NN network to use, if show\_NN\_network = TRUE network\_name cell\_color color for cells (see details) color\_as\_factor convert color column to factor cell\_color\_code named vector with colors cell\_color\_gradient vector with 3 colors for numeric data gradient\_midpoint midpoint for color gradient gradient\_limits vector with lower and upper limits select\_cell\_groups select subset of cells/clusters based on cell\_color parameter select\_cells select subset of cells based on cell IDs show\_other\_cells display not selected cells other\_cell\_color color of not selected cells other\_point\_size size of not selected cells show\_cluster\_center plot center of selected clusters show\_center\_label plot label of selected clusters center\_point\_size size of center points

```
center_point_border_col
                  border color of center points
center_point_border_stroke
                  border stroke size of center points
                  size of labels
label_size
label_fontface font of labels
edge_alpha
                  column to use for alpha of the edges
point_shape
                  point with border or not (border or no_border)
point_size
                  size of point (cell)
point_alpha
                  transparancy of point
point_border_col
                  color of border around points
point_border_stroke
                  stroke size of border around points
title
                  title for plot, defaults to cell_color parameter
                  show legend
show_legend
legend_text
                  size of legend text
legend_symbol_size
                  size of legend symbols
background_color
                  color of plot background
axis_text
                  size of axis text
                  size of axis title
axis_title
cow_n_col
                  cowplot param: how many columns
                  cowplot param: relative height
cow_rel_h
                  cowplot param: relative width
cow_rel_w
cow_align
                  cowplot param: how to align
show_plot
                  show plot
return_plot
                  return ggplot object
                  directly save the plot [boolean]
save_plot
                  list of saving parameters, see showSaveParameters
save_param
default_save_name
                  default save name for saving, don't change, change save_name in save_param
```

### **Details**

Description of parameters. For 3D plots see dimPlot3D

### Value

ggplot

#### See Also

```
Other reduced dimension visualizations: dimPlot3D(), dimPlot(), plotPCA_2D(), plotPCA_3D(), plotPCA(), plotTSNE_2D(), plotTSNE_3D(), plotTSNE(), plotUMAP_2D(), plotUMAP_3D(), plotUMAP()
```

### **Examples**

```
dimPlot2D(gobject)
```

88 dimPlot3D

dimPlot3D

dimPlot3D

# **Description**

Visualize cells according to dimension reduction coordinates

# Usage

```
dimPlot3D(
  gobject,
  dim_reduction_to_use = "umap",
  dim_reduction_name = "umap",
  dim1_to_use = 1,
  dim2\_to\_use = 2,
  dim3_to_use = 3,
  spat_enr_names = NULL,
  select_cell_groups = NULL,
  select_cells = NULL,
  show_other_cells = T,
  other_cell_color = "lightgrey",
  other_point_size = 2,
  show_NN_network = F,
  nn_network_to_use = "sNN",
  network_name = "sNN.pca",
  color_as_factor = T,
  cell_color = NULL,
  cell_color_code = NULL,
  show_cluster_center = F,
  show_center_label = T,
  center_point_size = 4,
  label_size = 4,
  edge_alpha = NULL,
  point_size = 3,
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "dim3D"
)
```

dimPlot3D 89

```
dim3_to_use
                  dimension to use on z-axis
spat_enr_names names of spatial enrichment results to include
select_cell_groups
                  select subset of cells/clusters based on cell_color parameter
select_cells
                  select subset of cells based on cell IDs
show_other_cells
                  display not selected cells
other_cell_color
                  color of not selected cells
other_point_size
                  size of not selected cells
show_NN_network
                  show underlying NN network
nn_network_to_use
                  type of NN network to use (kNN vs sNN)
                  name of NN network to use, if show_NN_network = TRUE
network_name
color_as_factor
                  convert color column to factor
cell_color
                  color for cells (see details)
cell_color_code
                  named vector with colors
show_cluster_center
                  plot center of selected clusters
show_center_label
                  plot label of selected clusters
center_point_size
                  size of center points
label_size
                  size of labels
edge_alpha
                  column to use for alpha of the edges
point_size
                  size of point (cell)
show_plot
                  show plot
                  return ggplot object
return_plot
save_plot
                  directly save the plot [boolean]
                  list of saving parameters, see showSaveParameters
save_param
default_save_name
                  default save name for saving, don't change, change save_name in save_param
```

#### **Details**

Description of parameters.

### Value

plotly

### See Also

```
Other reduced dimension visualizations: dimPlot2D(), dimPlot(), plotPCA_2D(), plotPCA_3D(), plotPCA(), plotTSNE_2D(), plotTSNE_3D(), plotUMAP_2D(), plotUMAP_3D(), plotUMAP()
```

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### **Examples**

```
dimPlot3D(gobject)
```

doHclust

doHclust

# **Description**

cluster cells using hierarchical clustering algorithm

# Usage

```
doHclust(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  genes_to_use = NULL,
  dim_reduction_to_use = c("cells", "pca", "umap", "tsne"),
  dim_reduction_name = "pca",
  dimensions_to_use = 1:10,
  distance_method = c("pearson", "spearman", "original", "euclidean", "maximum",
  "manhattan", "canberra", "binary", "minkowski"),
agglomeration_method = c("ward.D2", "ward.D", "single", "complete", "average",
    "mcquitty", "median", "centroid"),
  k = 10,
  h = NULL,
  name = "hclust",
  return_gobject = TRUE,
  set_seed = T,
  seed_number = 1234
)
```

```
gobject
                 giotto object
expression_values
                 expression values to use
genes_to_use
                 subset of genes to use
dim_reduction_to_use
                 dimension reduction to use
dim_reduction_name
                 dimensions reduction name
dimensions_to_use
                 dimensions to use
distance_method
                 distance method
agglomeration_method
                 agglomeration method for hclust
k
                 number of final clusters
```

doHMRF 91

```
h cut hierarchical tree at height = h
name name for hierarchical clustering
return_gobject boolean: return giotto object (default = TRUE)
set_seed set seed
seed_number number for seed
```

### **Details**

Description on how to use Kmeans clustering method.

#### Value

giotto object with new clusters appended to cell metadata

# See Also

hclust

# **Examples**

```
doHclust(gobject)
```

doHMRF

doHMRF

# **Description**

Run HMRF

```
doHMRF(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  spatial_network_name = "Delaunay_network",
  spatial_genes = NULL,
  spatial_dimensions = c("sdimx", "sdimy", "sdimz"),
  dim_reduction_to_use = NULL,
  dim_reduction_name = "pca",
  dimensions_to_use = 1:10,
  name = "test",
  k = 10,
  betas = c(0, 2, 50),
  tolerance = 1e-10,
  zscore = c("none", "rowcol", "colrow"),
  numinit = 100,
  python_path = NULL,
  output_folder = NULL,
  overwrite_output = TRUE
)
```

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# **Arguments**

gobject giotto object

expression\_values

expression values to use

spatial\_network\_name

name of spatial network to use for HMRF

spatial\_genes spatial genes to use for HMRF

spatial\_dimensions

select spatial dimensions to use, default is all possible dimensions

dim\_reduction\_to\_use

use another dimension reduction set as input

dim\_reduction\_name

name of dimension reduction set to use

dimensions\_to\_use

number of dimensions to use as input

name of HMRF run

k number of HMRF domains

betas betas to test for

tolerance tolerance

zscore zscore

numinit number of initializations

python\_path python path to use

output\_folder output folder to save results

overwrite\_output

overwrite output folder

### **Details**

Description of HMRF parameters ...

# Value

Creates a directory with results that can be viewed with viewHMRFresults

# **Examples**

doHMRF(gobject)

doKmeans 93

doKmeans

Description

# cluster cells using kmeans algorithm

doKmeans

# Usage

```
doKmeans(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  genes_to_use = NULL,
  dim_reduction_to_use = c("cells", "pca", "umap", "tsne"),
  dim_reduction_name = "pca",
  dimensions_to_use = 1:10,
  distance_method = c("original", "pearson", "spearman", "euclidean", "maximum",
    "manhattan", "canberra", "binary", "minkowski"),
  centers = 10,
  iter_max = 100,
  nstart = 1000,
  algorithm = "Hartigan-Wong",
  name = "kmeans",
  return_gobject = TRUE,
  set_seed = T,
  seed_number = 1234
)
```

```
gobject
                 giotto object
expression_values
                 expression values to use
                 subset of genes to use
genes_to_use
dim_reduction_to_use
                 dimension reduction to use
dim_reduction_name
                 dimensions reduction name
dimensions_to_use
                 dimensions to use
distance_method
                 distance method
                 number of final clusters
centers
                 kmeans maximum iterations
iter_max
nstart
                 kmeans nstart
                 kmeans algorithm
algorithm
name
                 name for kmeans clustering
return_gobject boolean: return giotto object (default = TRUE)
set_seed
                 set seed
seed_number
                 number for seed
```

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### **Details**

Description on how to use Kmeans clustering method.

### Value

giotto object with new clusters appended to cell metadata

#### See Also

kmeans

### **Examples**

```
doKmeans(gobject)
```

doLeidenCluster

doLeidenCluster

# **Description**

cluster cells using a NN-network and the Leiden community detection algorithm

# Usage

```
doLeidenCluster(
  gobject,
  name = "leiden_clus",
  nn_network_to_use = "sNN",
  network_name = "sNN.pca",
  python_path = NULL,
  resolution = 1,
  weight_col = "weight",
  partition_type = c("RBConfigurationVertexPartition", "ModularityVertexPartition"),
  init_membership = NULL,
  n_iterations = 1000,
  return_gobject = TRUE,
  set_seed = T,
  seed_number = 1234
)
```

doLeidenSubCluster 95

partition\_type The type of partition to use for optimisation.
init\_membership

initial membership of cells for the partition

n\_iterations number of interations to run the Leiden algorithm. If the number of iterations

is negative, the Leiden algorithm is run until an iteration in which there was no

improvement.

return\_gobject boolean: return giotto object (default = TRUE)

set\_seed set seed

seed\_number number for seed

#### **Details**

This function is a wrapper for the Leiden algorithm implemented in python, which can detect communities in graphs of millions of nodes (cells), as long as they can fit in memory. See the https://github.com/vtraag/leidenalgleidenalg github page or the https://leidenalg.readthedocs.io/en/stable/index.htmlreadthedocs page for more information.

Partition types available and information:

- RBConfigurationVertexPartition: Implements Reichardt and Bornholdt's Potts model with a configuration null model. This quality function is well-defined only for positive edge weights. This quality function uses a linear resolution parameter.
- Modularity Vertex Partition: Implements modularity. This quality function is well-defined only for positive edge weights. It does *not* use the resolution parameter

Set  $weight\_col = NULL$  to give equal weight (=1) to each edge.

### Value

giotto object with new clusters appended to cell metadata

### **Examples**

```
doLeidenCluster(gobject)
```

doLeidenSubCluster

doLeidenSubCluster

### **Description**

Further subcluster cells using a NN-network and the Leiden algorithm

```
doLeidenSubCluster(
  gobject,
  name = "sub_pleiden_clus",
  cluster_column = NULL,
  selected_clusters = NULL,
  hvg_param = list(reverse_log_scale = T, difference_in_cov = 1, expression_values =
    "normalized"),
```

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```
hvg_min_perc_cells = 5,
hvg_mean_expr_det = 1,
use_all_genes_as_hvg = FALSE,
min_nr_of_hvg = 5,
pca_param = list(expression_values = "normalized", scale_unit = T),
nn_param = list(dimensions_to_use = 1:20),
k_neighbors = 10,
resolution = 0.5,
n_iterations = 500,
python_path = NULL,
nn_network_to_use = "sNN",
network_name = "sNN.pca",
return_gobject = TRUE,
verbose = T
)
```

# **Arguments**

```
giotto object
gobject
                  name for new clustering result
name
cluster_column cluster column to subcluster
selected_clusters
                  only do subclustering on these clusters
hvg_param
                  parameters for calculateHVG
hvg_min_perc_cells
                  threshold for detection in min percentage of cells
hvg_mean_expr_det
                  threshold for mean expression level in cells with detection
use_all_genes_as_hvg
                  forces all genes to be HVG and to be used as input for PCA
                  minimum number of HVG, or all genes will be used as input for PCA
min_nr_of_hvg
                  parameters for runPCA
pca_param
                  parameters for parameters for createNearestNetwork
nn_param
k_neighbors
                  number of k for createNearestNetwork
resolution
                  resolution of Leiden clustering
n_iterations
                  number of interations to run the Leiden algorithm.
python_path
                  specify specific path to python if required
nn_network_to_use
                  type of NN network to use (kNN vs sNN)
                  name of NN network to use
network_name
return_gobject boolean: return giotto object (default = TRUE)
verbose
                  verbose
```

### **Details**

This function performs subclustering using the Leiden algorithm on selected clusters. The systematic steps are:

• 1. subset Giotto object

doLouvainCluster 97

- 2. identify highly variable genes
- 3. run PCA
- 4. create nearest neighbouring network
- 5. do Leiden clustering

### Value

giotto object with new subclusters appended to cell metadata

### See Also

doLeidenCluster

### **Examples**

```
doLeidenSubCluster(gobject)
```

doLouvainCluster

doLouvainCluster

# **Description**

cluster cells using a NN-network and the Louvain algorithm.

# Usage

```
doLouvainCluster(
  gobject,
  version = c("community", "multinet"),
  name = "louvain_clus",
  nn_network_to_use = "sNN",
  network_name = "sNN.pca",
  python_path = NULL,
  resolution = 1,
  weight_col = NULL,
  gamma = 1,
  omega = 1,
  louv_random = F,
  return_gobject = TRUE,
  set_seed = F,
  seed_number = 1234,
)
```

```
gobject giotto object

version implemented version of Louvain clustering to use

name name for cluster

nn_network_to_use

type of NN network to use (kNN vs sNN)
```

network\_name name of NN network to use [community] specify specific path to python if required python\_path resolution [community] resolution weight\_col weight column name [multinet] Resolution parameter for modularity in the generalized louvain method. gamma omega [multinet] Inter-layer weight parameter in the generalized louvain method louv\_random [community] Will randomize the node evaluation order and the community evaluation order to get different partitions at each call return\_gobject boolean: return giotto object (default = TRUE) set\_seed set seed seed\_number number for seed

# **Details**

. . .

Louvain clustering using the community or multinet implementation of the louvain clustering algorithm.

#### Value

giotto object with new clusters appended to cell metadata

additional parameters

#### See Also

doLouvainCluster\_community and doLouvainCluster\_multinet

# **Examples**

```
doLouvainCluster(gobject)
```

```
\label{lower} do Louvain Cluster\_community \\ do Louvain Cluster\_community
```

### Description

cluster cells using a NN-network and the Louvain algorithm from the community module in Python

```
doLouvainCluster_community(
  gobject,
  name = "louvain_clus",
  nn_network_to_use = "sNN",
  network_name = "sNN.pca",
  python_path = NULL,
  resolution = 1,
  weight_col = NULL,
  louv_random = F,
```

```
return_gobject = TRUE,
set_seed = F,
seed_number = 1234
)
```

### **Arguments**

giotto object gobject name for cluster name nn\_network\_to\_use type of NN network to use (kNN vs sNN) name of NN network to use network\_name specify specific path to python if required python\_path resolution resolution weight\_col weight column to use for edges Will randomize the node evaluation order and the community evaluation order louv\_random to get different partitions at each call return\_gobject boolean: return giotto object (default = TRUE) set\_seed set seed

# **Details**

seed\_number

This function is a wrapper for the Louvain algorithm implemented in Python, which can detect communities in graphs of nodes (cells). See the https://python-louvain.readthedocs.io/en/latest/index.htmlreadthedocs page for more information.

Set *weight\_col* = *NULL* to give equal weight (=1) to each edge.

### Value

giotto object with new clusters appended to cell metadata

number for seed

# **Examples**

```
doLouvainCluster_community(gobject)
```

```
\label{lower} do Louvain Cluster\_multinet \\ do Louvain Cluster\_multinet
```

# **Description**

cluster cells using a NN-network and the Louvain algorithm from the multinet package in R.

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### Usage

```
doLouvainCluster_multinet(
  gobject,
  name = "louvain_clus",
  nn_network_to_use = "sNN",
  network_name = "sNN.pca",
  gamma = 1,
  omega = 1,
  return_gobject = TRUE,
  set_seed = F,
  seed_number = 1234
)
```

### **Arguments**

gobject giotto object
name name for cluster
nn\_network\_to\_use

type of NN network to use (kNN vs sNN)

network\_name name of NN network to use

gamma Resolution parameter for modularity in the generalized louvain method.

omega Inter-layer weight parameter in the generalized louvain method.

return\_gobject boolean: return giotto object (default = TRUE)

set\_seed set seed

seed\_number number for seed

# **Details**

See glouvain\_ml from the multinet package in R for more information.

# Value

giotto object with new clusters appended to cell metadata

# **Examples**

```
doLouvainCluster_multinet(gobject)
```

 ${\tt doLouvainSubCluster} \qquad {\tt doLouvainSubCluster}$ 

# **Description**

subcluster cells using a NN-network and the Louvain algorithm

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### Usage

```
doLouvainSubCluster(
  gobject,
  name = "sub_louvain_clus",
  version = c("community", "multinet"),
  cluster_column = NULL,
  selected_clusters = NULL,
 hvg_param = list(reverse_log_scale = T, difference_in_cov = 1, expression_values =
    "normalized"),
  hvg_min_perc_cells = 5,
  hvg_mean_expr_det = 1,
  use_all_genes_as_hvg = FALSE,
  min_nr_of_hvg = 5,
  pca_param = list(expression_values = "normalized", scale_unit = T),
  nn_param = list(dimensions_to_use = 1:20),
  k_neighbors = 10,
  resolution = 0.5,
  gamma = 1,
  omega = 1,
  python_path = NULL,
  nn_network_to_use = "sNN",
  network_name = "sNN.pca",
  return_gobject = TRUE,
  verbose = T
)
```

```
gobject
                  giotto object
name
                  name for new clustering result
version
                  version of Louvain algorithm to use
cluster_column cluster column to subcluster
selected_clusters
                  only do subclustering on these clusters
                  parameters for calculateHVG
hvg_param
hvg_min_perc_cells
                  threshold for detection in min percentage of cells
hvg_mean_expr_det
                  threshold for mean expression level in cells with detection
use_all_genes_as_hvg
                  forces all genes to be HVG and to be used as input for PCA
                  minimum number of HVG, or all genes will be used as input for PCA
min_nr_of_hvg
                  parameters for runPCA
pca_param
                  parameters for parameters for createNearestNetwork
nn_param
k_neighbors
                  number of k for createNearestNetwork
resolution
                  resolution for community algorithm
gamma
                  gamma
omega
                  omega
```

### **Details**

This function performs subclustering using the Louvain algorithm on selected clusters. The systematic steps are:

- 1. subset Giotto object
- 2. identify highly variable genes
- 3. run PCA
- 4. create nearest neighbouring network
- 5. do Louvain clustering

#### Value

giotto object with new subclusters appended to cell metadata

### See Also

doLouvainCluster\_multinet and doLouvainCluster\_community

### **Examples**

```
doLouvainSubCluster(gobject)
```

```
\label{lower} do Louvain SubCluster\_community \\ do Louvain SubCluster\_community
```

# Description

subcluster cells using a NN-network and the Louvain community detection algorithm

```
doLouvainSubCluster_community(
   gobject,
   name = "sub_louvain_comm_clus",
   cluster_column = NULL,
   selected_clusters = NULL,
   hvg_param = list(reverse_log_scale = T, difference_in_cov = 1, expression_values =
        "normalized"),
   hvg_min_perc_cells = 5,
   hvg_mean_expr_det = 1,
   use_all_genes_as_hvg = FALSE,
```

```
min_nr_of_hvg = 5,
pca_param = list(expression_values = "normalized", scale_unit = T),
nn_param = list(dimensions_to_use = 1:20),
k_neighbors = 10,
resolution = 0.5,
python_path = NULL,
nn_network_to_use = "sNN",
network_name = "sNN.pca",
return_gobject = TRUE,
verbose = T
)
```

### **Arguments**

```
gobject
                  giotto object
name
                  name for new clustering result
cluster_column cluster column to subcluster
selected_clusters
                  only do subclustering on these clusters
                  parameters for calculateHVG
hvg_param
hvg_min_perc_cells
                  threshold for detection in min percentage of cells
hvg_mean_expr_det
                  threshold for mean expression level in cells with detection
use_all_genes_as_hvg
                  forces all genes to be HVG and to be used as input for PCA
min_nr_of_hvg
                 minimum number of HVG, or all genes will be used as input for PCA
                  parameters for runPCA
pca_param
nn_param
                  parameters for parameters for createNearestNetwork
k_neighbors
                  number of k for createNearestNetwork
resolution
                  resolution
                  specify specific path to python if required
python_path
nn_network_to_use
                  type of NN network to use (kNN vs sNN)
network_name
                  name of NN network to use
return_gobject boolean: return giotto object (default = TRUE)
verbose
                  verbose
```

### **Details**

This function performs subclustering using the Louvain community algorithm on selected clusters. The systematic steps are:

- 1. subset Giotto object
- 2. identify highly variable genes
- 3. run PCA
- 4. create nearest neighbouring network
- 5. do Louvain community clustering

#### Value

giotto object with new subclusters appended to cell metadata

#### See Also

```
doLouvainCluster_community
```

### **Examples**

```
doLouvainSubCluster_community(gobject)
```

```
doLouvainSubCluster_multinet
```

doLouvainSubCluster\_multinet

# Description

subcluster cells using a NN-network and the Louvain multinet detection algorithm

# Usage

```
doLouvainSubCluster_multinet(
  gobject,
  name = "sub_louvain_mult_clus",
  cluster_column = NULL,
  selected_clusters = NULL,
 hvg_param = list(reverse_log_scale = T, difference_in_cov = 1, expression_values =
    "normalized"),
  hvg_min_perc_cells = 5,
  hvg_mean_expr_det = 1,
  use_all_genes_as_hvg = FALSE,
  min_nr_of_hvg = 5,
  pca_param = list(expression_values = "normalized", scale_unit = T),
  nn_param = list(dimensions_to_use = 1:20),
  k_neighbors = 10,
  gamma = 1,
  omega = 1,
  nn_network_to_use = "sNN",
  network_name = "sNN.pca",
  return_gobject = TRUE,
  verbose = T
)
```

```
gobject giotto object

name name for new clustering result

cluster_column cluster column to subcluster

selected_clusters

only do subclustering on these clusters
```

hvg\_param parameters for calculateHVG

hvg\_min\_perc\_cells

threshold for detection in min percentage of cells

hvg\_mean\_expr\_det

threshold for mean expression level in cells with detection

use\_all\_genes\_as\_hvg

forces all genes to be HVG and to be used as input for PCA

min\_nr\_of\_hvg minimum number of HVG, or all genes will be used as input for PCA

pca\_param parameters for runPCA

nn\_param parameters for parameters for createNearestNetwork

k\_neighbors number of k for createNearestNetwork

gamma gamma
omega omega
nn\_network\_to\_use

type of NN network to use (kNN vs sNN)

network\_name name of NN network to use

return\_gobject boolean: return giotto object (default = TRUE)

verbose verbose

#### Details

This function performs subclustering using the Louvain multinet algorithm on selected clusters. The systematic steps are:

- 1. subset Giotto object
- 2. identify highly variable genes
- 3. run PCA
- 4. create nearest neighbouring network
- 5. do Louvain multinet clustering

#### Value

giotto object with new subclusters appended to cell metadata

### See Also

doLouvainCluster\_multinet

# **Examples**

doLouvainSubCluster\_multinet(gobject)

106 doRandomWalkCluster

doRandomWalkCluster

doRandomWalkCluster

# **Description**

Cluster cells using a random walk approach.

### Usage

```
doRandomWalkCluster(
  gobject,
  name = "random_walk_clus",
  nn_network_to_use = "sNN",
  network_name = "sNN.pca",
  walk_steps = 4,
  walk_clusters = 10,
  walk_weights = NA,
  return_gobject = TRUE,
  set_seed = F,
  seed_number = 1234
)
```

# **Arguments**

```
gobject
                 giotto object
name
                 name for cluster
nn_network_to_use
                 type of NN network to use (kNN vs sNN)
                 name of NN network to use
network_name
                 number of walking steps
walk_steps
walk_clusters
                 number of final clusters
walk_weights
                 cluster column defining the walk weights
return_gobject | boolean: return giotto object (default = TRUE)
set_seed
                 set seed
seed_number
                 number for seed
```

# **Details**

See cluster\_walktrap function from the igraph package in R for more information.

### Value

giotto object with new clusters appended to cell metadata

### **Examples**

```
doRandomWalkCluster(gobject)
```

doSNNCluster 107

doSNNCluster doSNNCluster

# **Description**

Cluster cells using a SNN cluster approach.

### Usage

```
doSNNCluster(
  gobject,
  name = "sNN_clus",
  nn_network_to_use = "kNN",
  network_name = "kNN.pca",
  k = 20,
  eps = 4,
  minPts = 16,
  borderPoints = TRUE,
  return_gobject = TRUE,
  set_seed = F,
  seed_number = 1234
)
```

# **Arguments**

gobject giotto object
name name for cluster
nn\_network\_to\_use

type of NN network to use (only works on kNN)

 $network\_name \qquad name \ of \ kNN \ network \ to \ use$ 

k Neighborhood size for nearest neighbor sparsification to create the shared NN

graph.

eps Two objects are only reachable from each other if they share at least eps nearest

neighbors.

minPts minimum number of points that share at least eps nearest neighbors for a point

to be considered a core points.

borderPoints should borderPoints be assigned to clusters like in DBSCAN?

return\_gobject boolean: return giotto object (default = TRUE)

set\_seed set seed

seed\_number number for seed

### **Details**

See sNNclust from dbscan package

### Value

giotto object with new clusters appended to cell metadata

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### **Examples**

```
doSNNCluster(gobject)
```

 $\verb"estimateImageBg"$ 

estimateImageBg

### **Description**

helps to estimate which color is the background color of your plot

### Usage

```
estimateImageBg(mg_object, top_color_range = 1:50)
```

# **Arguments**

```
mg_object magick image or Giotto image object top_color_range top possible background colors to return
```

### Value

vector of pixel color frequencies and an associated barplot

# **Examples**

```
estimateImageBg(mg_object)
```

exportGiottoViewer

*exportGiottoViewer* 

# Description

compute highly variable genes

```
exportGiottoViewer(
  gobject,
  output_directory = NULL,
  spat_enr_names = NULL,
  factor_annotations = NULL,
  numeric_annotations = NULL,
  dim_reductions,
  dim_reduction_names,
  expression_values = c("scaled", "normalized", "custom"),
  dim_red_rounding = NULL,
  dim_red_rescale = c(-20, 20),
  expression_rounding = 2,
  overwrite_dir = T,
  verbose = T
```

exprCellCellcom 109

#### **Arguments**

```
gobject
                  giotto object
output_directory
                  directory where to save the files
spat_enr_names spatial enrichment results to include for annotations
factor_annotations
                  giotto cell annotations to view as factor
numeric_annotations
                  giotto cell annotations to view as numeric
dim_reductions high level dimension reductions to view
dim_reduction_names
                  specific dimension reduction names
expression_values
                  expression values to use in Viewer
dim_red_rounding
                  numerical indicating how to round the coordinates
dim_red_rescale
                  numericals to rescale the coordinates
expression_rounding
                  numerical indicating how to round the expression data
                  overwrite files in the directory if it already existed
overwrite_dir
verbose
                  be verbose
```

### Details

Giotto Viewer expects the results from Giotto Analyzer in a specific format, which is provided by this function. To include enrichment results from createSpatialEnrich include the provided spatial enrichment name (default PAGE or rank) and add the gene signature names (.e.g cell types) to the numeric annotations parameter.

### Value

writes the necessary output to use in Giotto Viewer

### **Examples**

exportGiottoViewer(gobject)

exprCellCellcom exprCellCellcom

## Description

Cell-Cell communication scores based on expression only

110 exprCellCellcom

#### Usage

## **Arguments**

gobject giotto object to use cluster\_column cluster column with cell type information random\_iter number of iterations gene\_set\_1 first specific gene set from gene pairs gene\_set\_2 second specific gene set from gene pairs log2FC\_addendum addendum to add when calculating log2FC provide more detailed information (random variance and z-score) detailed which method to adjust p-values adjust\_method adjust\_target adjust multiple hypotheses at the cell or gene level verbose verbose

## **Details**

Statistical framework to identify if pairs of genes (such as ligand-receptor combinations) are expressed at higher levels than expected based on a reshuffled null distribution of gene expression values, without considering the spatial position of cells. More details will follow soon.

# Value

Cell-Cell communication scores for gene pairs based on expression only

```
exprCellCellcom(gobject)
```

fDataDT

fDataDT

fDataDT

## Description

show gene metadata

## Usage

```
fDataDT(gobject)
```

# Arguments

gobject

giotto object

### Value

data.table with gene metadata

# **Examples**

```
pDataDT(gobject)
```

filterCellProximityGenes

filter Cell Proximity Genes

## **Description**

Filter cell proximity gene scores.

# Usage

```
filterCellProximityGenes(
  cpgObject,
  min_cells = 4,
  min_cells_expr = 1,
  min_int_cells = 4,
  min_int_cells_expr = 1,
  min_fdr = 0.1,
  min_spat_diff = 0.2,
  min_log2_fc = 0.2,
  min_zscore = 2,
  zscores_column = c("cell_type", "genes"),
  direction = c("both", "up", "down")
)
```

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#### **Arguments**

```
cpgObject
                 cell proximity gene score object
min_cells
                 minimum number of source cell type
min_cells_expr minimum expression level for source cell type
min_int_cells
                 minimum number of interacting neighbor cell type
min_int_cells_expr
                 minimum expression level for interacting neighbor cell type
min_fdr
                 minimum adjusted p-value
min_spat_diff
                 minimum absolute spatial expression difference
                 minimum log2 fold-change
min_log2_fc
min_zscore
                 minimum z-score change
zscores_column calculate z-scores over cell types or genes
direction
                 differential expression directions to keep
```

#### Value

cpgObject that contains the filtered differential gene scores

### **Examples**

```
filterCellProximityGenes(gobject)
```

filterCombinations filterCombinations

## **Description**

Shows how many genes and cells are lost with combinations of thresholds.

## Usage

```
filterCombinations(
  gobject,
  expression_values = c("raw", "normalized", "scaled", "custom"),
  expression_thresholds = c(1, 2),
  gene_det_in_min_cells = c(5, 50),
  min_det_genes_per_cell = c(200, 400),
  scale_x_axis = "identity",
  x_axis_offset = 0,
  scale_y_axis = "identity",
  y_axis_offset = 0,
  show_plot = TRUE,
  return_plot = FALSE,
  save_plot = NA,
  save_param = list(),
  default_save_name = "filterCombinations")
```

filterCPG 113

### **Arguments**

```
gobject
                  giotto object
expression_values
                  expression values to use
{\tt expression\_thresholds}
                  all thresholds to consider a gene expressed
gene_det_in_min_cells
                  minimum number of cells that should express a gene to consider that gene fur-
                  ther
min_det_genes_per_cell
                  minimum number of expressed genes per cell to consider that cell further
scale_x_axis
                  ggplot transformation for x-axis (e.g. log2)
x_axis_offset
                  x-axis offset to be used together with the scaling transformation
scale_y_axis
                  ggplot transformation for y-axis (e.g. log2)
y_axis_offset
                  y-axis offset to be used together with the scaling transformation
show_plot
                  show plot
                  return only ggplot object
return_plot
                  directly save the plot [boolean]
save_plot
                  list of saving parameters from all_plots_save_function
save_param
default_save_name
                  default save name for saving, don't change, change save_name in save_param
```

# Details

Creates a scatterplot that visualizes the number of genes and cells that are lost with a specific combination of a gene and cell threshold given an arbitrary cutoff to call a gene expressed. This function can be used to make an informed decision at the filtering step with filterGiotto.

### Value

list of data.table and ggplot object

## **Examples**

filterCombinations(gobject)

filterCPG filterCPG

## **Description**

Filter cell proximity gene scores.

114 filterDistributions

#### Usage

```
filterCPG(
  cpgObject,
  min_cells = 4,
  min_cells_expr = 1,
  min_int_cells = 4,
  min_int_cells_expr = 1,
  min_fdr = 0.1,
  min_spat_diff = 0.2,
  min_log2_fc = 0.2,
  min_zscore = 2,
  zscores_column = c("cell_type", "genes"),
  direction = c("both", "up", "down")
)
```

## **Arguments**

```
cell proximity gene score object
cpgObject
                 minimum number of source cell type
min_cells
min_cells_expr minimum expression level for source cell type
min_int_cells
                 minimum number of interacting neighbor cell type
min_int_cells_expr
                 minimum expression level for interacting neighbor cell type
min_fdr
                 minimum adjusted p-value
                 minimum absolute spatial expression difference
min_spat_diff
                 minimum log2 fold-change
min_log2_fc
min_zscore
                 minimum z-score change
zscores_column calculate z-scores over cell types or genes
direction
                 differential expression directions to keep
```

### Value

cpgObject that contains the filtered differential gene scores

## **Examples**

```
filterCPG(gobject)
```

 ${\it filter Distributions} \qquad {\it filter Distributions}$ 

## Description

show gene or cell distribution after filtering on expression threshold

filterDistributions 115

### Usage

```
filterDistributions(
  gobject,
  expression_values = c("raw", "normalized", "scaled", "custom"),
  expression_threshold = 1,
  detection = c("genes", "cells"),
  plot_type = c("histogram", "violin"),
  nr_bins = 30,
  fill_color = "lightblue",
  scale_axis = "identity",
  axis_offset = 0,
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "filterDistributions"
)
```

# **Arguments**

```
gobject
                  giotto object
expression_values
                  expression values to use
expression_threshold
                  threshold to consider a gene expressed
detection
                  consider genes or cells
plot_type
                  type of plot
nr_bins
                  number of bins for histogram plot
fill_color
                  fill color for plots
scale_axis
                  ggplot transformation for axis (e.g. log2)
axis_offset
                  offset to be used together with the scaling transformation
show_plot
                  show plot
return_plot
                  return ggplot object
save_plot
                  directly save the plot [boolean]
                  list of saving parameters from all_plots_save_function
save_param
default_save_name
                  default save name for saving, don't change, change save_name in save_param
```

### Value

ggplot object

```
filterDistributions(gobject)
```

116 filterGiotto

filterGiotto

filterGiotto

## Description

filter Giotto object based on expression threshold

## Usage

```
filterGiotto(
  gobject,
  expression_values = c("raw", "normalized", "scaled", "custom"),
  expression_threshold = 1,
  gene_det_in_min_cells = 100,
  min_det_genes_per_cell = 100,
  verbose = F
)
```

## **Arguments**

```
gobject giotto object

expression_values

expression values to use

expression_threshold

threshold to consider a gene expressed

gene_det_in_min_cells

minimum # of cells that need to express a gene

min_det_genes_per_cell

minimum # of genes that need to be detected in a cell

verbose

verbose
```

## **Details**

The function filterCombinations can be used to explore the effect of different parameter values.

## Value

giotto object

```
\verb|filterGiotto(gobject)|\\
```

```
findCellProximityGenes
```

findCellProximityGenes

## **Description**

Identifies genes that are differentially expressed due to proximity to other cell types.

## Usage

```
findCellProximityGenes(
 gobject,
 expression_values = "normalized",
  selected_genes = NULL,
 cluster_column,
 spatial_network_name = "Delaunay_network",
 minimum_unique_cells = 1,
 minimum_unique_int_cells = 1,
 diff_test = c("permutation", "limma", "t.test", "wilcox"),
 mean_method = c("arithmic", "geometric"),
 offset = 0.1,
 adjust_method = c("bonferroni", "BH", "holm", "hochberg", "hommel", "BY", "fdr",
    "none"),
 nr_permutations = 1000,
 exclude_selected_cells_from_test = T,
 do_parallel = TRUE,
  cores = NA
)
```

## **Arguments**

```
gobject
                  giotto object
expression_values
                  expression values to use
selected_genes subset of selected genes (optional)
cluster_column name of column to use for cell types
spatial_network_name
                  name of spatial network to use
minimum_unique_cells
                  minimum number of target cells required
minimum_unique_int_cells
                  minimum number of interacting cells required
diff_test
                  which differential expression test
mean_method
                  method to use to calculate the mean
                  offset value to use when calculating log2 ratio
offset
                  which method to adjust p-values
adjust_method
nr_permutations
                  number of permutations if diff_test = permutation
```

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#### Details

Function to calculate if genes are differentially expressed in cell types when they interact (approximated by physical proximity) with other cell types. The results data.table in the cpgObject contains - at least - the following columns:

- genes: All or selected list of tested genes
- sel: average gene expression in the interacting cells from the target cell type
- other: average gene expression in the NOT-interacting cells from the target cell type
- log2fc: log2 fold-change between sel and other
- · diff: spatial expression difference between sel and other
- p.value: associated p-value
- p.adj: adjusted p-value
- cell\_type: target cell type
- int\_cell\_type: interacting cell type
- nr\_select: number of cells for selected target cell type
- int\_nr\_select: number of cells for interacting cell type
- nr\_other: number of other cells of selected target cell type
- int\_nr\_other: number of other cells for interacting cell type
- unif\_int: cell-cell interaction

## Value

cpgObject that contains the differential gene scores

## **Examples**

findCellProximityGenes(gobject)

## **Description**

Identifies genes that are differentially expressed due to proximity to other cell types.

findCPG 119

#### Usage

```
findCPG(
  gobject,
  expression_values = "normalized",
  selected_genes = NULL,
  cluster_column,
  spatial_network_name = "Delaunay_network",
 minimum_unique_cells = 1,
 minimum_unique_int_cells = 1,
 diff_test = c("permutation", "limma", "t.test", "wilcox"),
 mean_method = c("arithmic", "geometric"),
 offset = 0.1,
 adjust_method = c("bonferroni", "BH", "holm", "hochberg", "hommel", "BY", "fdr",
    "none"),
 nr_permutations = 100,
 exclude_selected_cells_from_test = T,
 do_parallel = TRUE,
  cores = NA
)
```

## Arguments

```
gobject
                  giotto object
expression_values
                  expression values to use
selected_genes subset of selected genes (optional)
cluster_column name of column to use for cell types
spatial_network_name
                  name of spatial network to use
minimum_unique_cells
                  minimum number of target cells required
minimum_unique_int_cells
                  minimum number of interacting cells required
diff\_test
                  which differential expression test
mean_method
                  method to use to calculate the mean
offset
                  offset value to use when calculating log2 ratio
adjust_method
                  which method to adjust p-values
nr_permutations
                  number of permutations if diff_test = permutation
exclude_selected_cells_from_test
                  exclude interacting cells other cells
                  run calculations in parallel with mclapply
do_parallel
                  number of cores to use if do_parallel = TRUE
cores
```

## **Details**

Function to calculate if genes are differentially expressed in cell types when they interact (approximated by physical proximity) with other cell types. The results data.table in the cpgObject contains - at least - the following columns:

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- genes: All or selected list of tested genes
- sel: average gene expression in the interacting cells from the target cell type
- other: average gene expression in the NOT-interacting cells from the target cell type
- log2fc: log2 fold-change between sel and other
- diff: spatial expression difference between sel and other
- p.value: associated p-value
- p.adj: adjusted p-value
- cell\_type: target cell type
- int\_cell\_type: interacting cell type
- nr\_select: number of cells for selected target cell type
- int\_nr\_select: number of cells for interacting cell type
- nr\_other: number of other cells of selected target cell type
- int\_nr\_other: number of other cells for interacting cell type
- unif\_int: cell-cell interaction

#### Value

cpgObject that contains the differential gene scores

## **Examples**

```
findCPG(gobject)
```

findGiniMarkers

findGiniMarkers

## **Description**

Identify marker genes for selected clusters based on gini detection and expression scores.

## Usage

```
findGiniMarkers(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  cluster_column,
  subset_clusters = NULL,
  group_1 = NULL,
  group_2 = NULL,
  min_expr_gini_score = 0.2,
  min_det_gini_score = 0.2,
  detection_threshold = 0,
  rank_score = 1,
  min_genes = 5
)
```

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#### **Arguments**

```
gobject
                  giotto object
expression_values
                  gene expression values to use
cluster_column clusters to use
subset_clusters
                  selection of clusters to compare
                  group 1 cluster IDs from cluster_column for pairwise comparison
group_1
                  group 2 cluster IDs from cluster_column for pairwise comparison
group_2
min_expr_gini_score
                  filter on minimum gini coefficient for expression
min_det_gini_score
                  filter on minimum gini coefficient for detection
detection_threshold
                  detection threshold for gene expression
                  rank scores for both detection and expression to include
rank_score
                  minimum number of top genes to return
min_genes
```

#### **Details**

Detection of marker genes using the <a href="https://en.wikipedia.org/wiki/Gini\_coefficientginic

- 1. calculate average expression per cluster
- 2. calculate detection fraction per cluster
- 3. calculate gini-coefficient for av. expression values over all clusters
- 4. calculate gini-coefficient for detection fractions over all clusters
- 5. convert gini-scores to rank scores
- 6. for each gene create combined score = detection rank x expression rank x expr gini-coefficient x detection gini-coefficient
- 7. for each gene sort on expression and detection rank and combined score

As a results "top gini" genes are genes that are very selectivily expressed in a specific cluster, however not always expressed in all cells of that cluster. In other words highly specific, but not necessarily sensitive at the single-cell level.

To perform differential expression between cluster groups you need to specificy cluster IDs to the parameters *group\_1* and *group\_2*.

### Value

data.table with marker genes

## **Examples**

findGiniMarkers(gobject)

```
\label{limited} find \textit{GiniMarkers\_one\_vs\_all} \\ \textit{find GiniMarkers\_one\_vs\_all}
```

## Description

Identify marker genes for all clusters in a one vs all manner based on gini detection and expression scores.

### Usage

```
findGiniMarkers_one_vs_all(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  cluster_column,
  subset_clusters = NULL,
  min_expr_gini_score = 0.5,
  min_det_gini_score = 0.5,
  detection_threshold = 0,
  rank_score = 1,
  min_genes = 4,
  verbose = TRUE
)
```

## **Arguments**

```
gobject
                  giotto object
expression_values
                  gene expression values to use
cluster_column clusters to use
subset_clusters
                  selection of clusters to compare
min_expr_gini_score
                  filter on minimum gini coefficient on expression
min_det_gini_score
                  filter on minimum gini coefficient on detection
detection_threshold
                  detection threshold for gene expression
rank_score
                  rank scores for both detection and expression to include
                  minimum number of top genes to return
min_genes
verbose
                  be verbose
```

### Value

data.table with marker genes

### See Also

findGiniMarkers

findMarkers 123

#### **Examples**

```
findGiniMarkers_one_vs_all(gobject)
```

findMarkers

findMarkers

# Description

Identify marker genes for selected clusters.

## Usage

```
findMarkers(
 gobject,
  expression_values = c("normalized", "scaled", "custom"),
 cluster_column = NULL,
 method = c("scran", "gini", "mast"),
 subset_clusters = NULL,
 group_1 = NULL,
 group_2 = NULL,
 min_expr_gini_score = 0.5,
 min_det_gini_score = 0.5,
 detection_threshold = 0,
 rank_score = 1,
 min\_genes = 4,
 group_1_name = NULL,
 group_2_name = NULL,
 adjust_columns = NULL,
```

## **Arguments**

```
gobject
                  giotto object
expression_values
                  gene expression values to use
cluster_column clusters to use
method
                  method to use to detect differentially expressed genes
subset_clusters
                  selection of clusters to compare
group_1
                  group 1 cluster IDs from cluster_column for pairwise comparison
                  group 2 cluster IDs from cluster_column for pairwise comparison
group_2
min_expr_gini_score
                  gini: filter on minimum gini coefficient for expression
min\_det\_gini\_score
                  gini: filter minimum gini coefficient for detection
detection\_threshold
                  gini: detection threshold for gene expression
```

```
rank_score gini: rank scores to include
min_genes minimum number of top genes to return (for gini)
group_1_name mast: custom name for group_1 clusters
group_2_name mast: custom name for group_2 clusters
adjust_columns mast: column in pDataDT to adjust for (e.g. detection rate)
... additional parameters for the findMarkers function in scran or zlm function in MAST
```

## **Details**

Wrapper for all individual functions to detect marker genes for clusters.

### Value

data.table with marker genes

### See Also

findScranMarkers, findGiniMarkers and findMastMarkers

### **Examples**

```
findMarkers(gobject)
```

### **Description**

Identify marker genes for all clusters in a one vs all manner.

## Usage

```
findMarkers_one_vs_all(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  cluster_column,
  subset_clusters = NULL,
  method = c("scran", "gini", "mast"),
  pval = 0.01,
  logFC = 0.5,
  min_genes = 10,
  min_expr_gini_score = 0.5,
  min_det_gini_score = 0.5,
  detection_threshold = 0,
  rank_score = 1,
  adjust_columns = NULL,
  verbose = TRUE,
)
```

findMarkers\_one\_vs\_all 125

### Arguments

gobject giotto object

expression\_values

gene expression values to use

cluster\_column clusters to use

subset\_clusters

selection of clusters to compare

method method to use to detect differentially expressed genes

pval scran & mast: filter on minimal p-value

logFC scan & mast: filter on logFC

min\_genes minimum genes to keep per cluster, overrides pval and logFC

min\_expr\_gini\_score

gini: filter on minimum gini coefficient for expression

min\_det\_gini\_score

gini: filter minimum gini coefficient for detection

detection\_threshold

gini: detection threshold for gene expression

rank\_score gini: rank scores to include

adjust\_columns mast: column in pDataDT to adjust for (e.g. detection rate)

verbose be verbose

... additional parameters for the findMarkers function in scran or zlm function in

MAST

### **Details**

Wrapper for all one vs all functions to detect marker genes for clusters.

### Value

data.table with marker genes

### See Also

findScranMarkers\_one\_vs\_all, findGiniMarkers\_one\_vs\_all and findMastMarkers\_one\_vs\_all

```
{\tt findMarkers\_one\_vs\_all(gobject)}
```

126 findMastMarkers

findMastMarkers

findMastMarkers

## **Description**

Identify marker genes for selected clusters based on the MAST package.

## Usage

```
findMastMarkers(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  cluster_column,
  group_1 = NULL,
  group_1_name = NULL,
  group_2 = NULL,
  group_2_name = NULL,
  adjust_columns = NULL,
  verbose = FALSE,
  ...
)
```

## **Arguments**

```
gobject
                  giotto object
expression_values
                  gene expression values to use
cluster_column clusters to use
                  group 1 cluster IDs from cluster_column for pairwise comparison
group_1
                  custom name for group_1 clusters
group_1_name
group_2
                  group 2 cluster IDs from cluster_column for pairwise comparison
                  custom name for group_2 clusters
group_2_name
adjust_columns column in pDataDT to adjust for (e.g. detection rate)
verbose
                  be verbose
                  additional parameters for the zlm function in MAST
. . .
```

## **Details**

This is a minimal convenience wrapper around the zlm from the MAST package to detect differentially expressed genes.

#### Value

data.table with marker genes

```
findMastMarkers(gobject)
```

```
find {\it MastMarkers\_one\_vs\_all} \\ find {\it MastMarkers\_one\_vs\_all}
```

## **Description**

Identify marker genes for all clusters in a one vs all manner based on the MAST package.

## Usage

```
findMastMarkers_one_vs_all(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  cluster_column,
  subset_clusters = NULL,
  adjust_columns = NULL,
  pval = 0.001,
  logFC = 1,
  min_genes = 10,
  verbose = TRUE,
  ...
)
```

## **Arguments**

```
gobject
                  giotto object
expression_values
                  gene expression values to use
cluster_column clusters to use
subset_clusters
                  selection of clusters to compare
adjust_columns column in pDataDT to adjust for (e.g. detection rate)
                  filter on minimal p-value
pval
logFC
                  filter on logFC
min_genes
                  minimum genes to keep per cluster, overrides pval and logFC
                  be verbose
verbose
                  additional parameters for the zlm function in MAST
```

## Value

data.table with marker genes

## See Also

findMastMarkers

```
findMastMarkers_one_vs_all(gobject)
```

128 findScranMarkers

```
findNetworkNeighbors findNetworkNeighbors
```

## **Description**

Find the spatial neighbors for a selected group of cells within the selected spatial network.

## Usage

```
findNetworkNeighbors(
  gobject,
  spatial_network_name,
  source_cell_ids = NULL,
  name = "nb_cells"
)
```

## **Arguments**

### Value

data.table

## **Examples**

findNetworkNeighbors(gobject)

 ${\tt find Scran Markers}$ 

findScranMarkers

## **Description**

Identify marker genes for all or selected clusters based on scran's implementation of findMarkers.

## Usage

```
findScranMarkers(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  cluster_column,
  subset_clusters = NULL,
  group_1 = NULL,
  group_2 = NULL,
  verbose = FALSE,
  ...
)
```

### **Arguments**

#### **Details**

This is a minimal convenience wrapper around the findMarkers function from the scran package.

To perform differential expression between cluster groups you need to specificy cluster IDs to the parameters *group\_1* and *group\_2*.

### Value

data.table with marker genes

### **Examples**

```
findScranMarkers(gobject)
```

```
find Scran Markers\_one\_vs\_all \\ find Scran Markers\_one\_vs\_all
```

## **Description**

Identify marker genes for all clusters in a one vs all manner based on scran's implementation of findMarkers.

## Usage

```
findScranMarkers_one_vs_all(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  cluster_column,
  subset_clusters = NULL,
  pval = 0.01,
  logFC = 0.5,
  min_genes = 10,
  verbose = TRUE,
  ...
)
```

130 get10Xmatrix

### **Arguments**

gobject giotto object

expression\_values

gene expression values to use

cluster\_column clusters to use

subset\_clusters

subset of clusters to use

pval filter on minimal p-value

logFC filter on logFC

min\_genes minimum genes to keep per cluster, overrides pval and logFC

verbose be verbose

... additional parameters for the findMarkers function in scran

#### Value

data.table with marker genes

#### See Also

findScranMarkers

## **Examples**

findScranMarkers\_one\_vs\_all(gobject)

get10Xmatrix

get10Xmatrix

### **Description**

This function creates an expression matrix from a 10X structured folder

## Usage

```
get10Xmatrix(path_to_data, gene_column_index = 1)
```

## **Arguments**

```
\label{eq:path_to_data} \begin{array}{ll} \text{path to the } 10X \text{ folder} \\ \text{gene\_column\_index} \end{array}
```

which column from the features or genes .tsv file to use for row ids

### **Details**

A typical 10X folder is named raw\_feature\_bc\_matrix or raw\_feature\_bc\_matrix and tt has 3 files:

- barcodes.tsv(.gz)
- features.tsv(.gz) or genes.tsv(.gz)
- matrix.mtx(.gz)

By default the first column of the features or genes .tsv file will be used, however if multiple annotations are provided (e.g. ensembl gene ids and gene symbols) the user can select another column.

getClusterSimilarity 131

### Value

sparse expression matrix from 10X

```
getClusterSimilarity getClusterSimilarity
```

## Description

Creates data.table with pairwise correlation scores between each cluster.

## Usage

```
getClusterSimilarity(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  cluster_column,
  cor = c("pearson", "spearman")
)
```

## **Arguments**

```
gobject giotto object
expression_values
expression values to use
cluster_column name of column to use for clusters
cor correlation score to calculate distance
```

### **Details**

Creates data.table with pairwise correlation scores between each cluster and the group size (# of cells) for each cluster. This information can be used together with mergeClusters to combine very similar or small clusters into bigger clusters.

## Value

data.table

```
getClusterSimilarity(gobject)
```

132 getDendrogramSplits

```
getDendrogramSplits getDendrogramSplits
```

### **Description**

Split dendrogram at each node and keep the leave (label) information..

### Usage

```
getDendrogramSplits(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  cluster_column,
  cor = c("pearson", "spearman"),
  distance = "ward.D",
  h = NULL,
  h_color = "red",
  show_dend = TRUE,
  verbose = TRUE
)
```

## **Arguments**

```
gobject
                  giotto object
expression_values
                  expression values to use
cluster_column name of column to use for clusters
                  correlation score to calculate distance
cor
                  distance method to use for hierarchical clustering
distance
h
                  height of horizontal lines to plot
h_color
                  color of horizontal lines
show_dend
                  show dendrogram
verbose
                  be verbose
```

## **Details**

Creates a data.table with three columns and each row represents a node in the dendrogram. For each node the height of the node is given together with the two subdendrograms. This information can be used to determine in a hierarchical manner differentially expressed marker genes at each node.

## Value

data.table object

```
getDendrogramSplits(gobject)
```

getDistinctColors 133

getDistinctColors

getDistinctColors

# Description

Returns a number of distint colors based on the RGB scale

# Usage

```
getDistinctColors(n)
```

## **Arguments**

n

number of colors wanted

## Value

number of distinct colors

getGiottoImage

getGiottoImage

# Description

```
get get a giotto image from a giotto object
```

# Usage

```
getGiottoImage(gobject, image_name)
```

# Arguments

gobject

giotto object

 ${\tt image\_name}$ 

name of giotto image showGiottoImageNames

## Value

a giotto image

```
getGiottoImage(gobject)
```

134 giotto-class

getSpatialDataset
getSpatialDataset

## **Description**

This package will automatically download the spatial locations and expression matrix for the chosen dataset. These files are already in the right format to create a Giotto object.

## Usage

```
getSpatialDataset(
  dataset = c("ST_OB_1", "ST_OB_2", "codex_spleen", "cycif_PDAC", "starmap_3D_cortex",
        "osmfish_SS_cortex", "merfish_preoptic", "seqfish_SS_cortex", "seqfish_OB"),
        directory = getwd()
)
```

## **Arguments**

dataset dataset to download directory directory to save the data to

giotto-class

S4 giotto Class

## **Description**

Framework of giotto object to store and work with spatial expression data

### **Slots**

```
raw_exprs raw expression counts

norm_expr normalized expression counts

norm_scaled_expr normalized and scaled expression counts

custom_expr custom normalized counts

spatial_locs spatial location coordinates for cells

cell_metadata metadata for cells

gene_metadata metadata for genes

cell_ID unique cell IDs

gene_ID unique gene IDs

spatial_network spatial network in data.table/data.frame format

spatial_grid spatial grid in data.table/data.frame format

spatial_enrichment slot to save spatial enrichment-like results

dimension_reduction slot to save dimension reduction coordinates

nn_network nearest neighbor network in igraph format

images slot to store giotto images
```

heatmSpatialCorGenes 135

```
parameters slot to save parameters that have been used instructions slot for global function instructions offset_file offset file used to stitch together image fields OS_platform Operating System to run Giotto analysis on
```

 ${\tt heatmSpatialCorGenes} \quad \textit{heatmSpatialCorGenes}$ 

## Description

Create heatmap of spatially correlated genes

## Usage

```
heatmSpatialCorGenes(
  gobject,
  spatCorObject,
  use_clus_name = NULL,
  show_cluster_annot = TRUE,
  show_row_dend = T,
  show_column_dend = F,
  show_row_names = F,
  show_column_names = F,
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "heatmSpatialCorGenes",
  ...
)
```

### **Arguments**

```
giotto object
gobject
spatCorObject
                 spatial correlation object
                 name of clusters to visualize (from clusterSpatialCorGenes())
use_clus_name
show_cluster_annot
                 show cluster annotation on top of heatmap
                 show row dendrogram
show_row_dend
show_column_dend
                 show column dendrogram
show_row_names show row names
show_column_names
                 show column names
show_plot
                 show plot
return_plot
                 return ggplot object
save_plot
                 directly save the plot [boolean]
```

```
save_param list of saving parameters, see showSaveParameters

default_save_name

default save name for saving, don't change, change save_name in save_param

additional parameters to the Heatmap function from ComplexHeatmap
```

#### Value

Heatmap generated by ComplexHeatmap

## **Examples**

heatmSpatialCorGenes(gobject)

hyperGeometricEnrich hyperGeometricEnrich

## **Description**

Function to calculate gene signature enrichment scores per spatial position using a hypergeometric test.

### Usage

```
hyperGeometricEnrich(...)
```

### **Arguments**

Arguments passed on to runHyperGeometricEnrich
gobject Giotto object
sign\_matrix Matrix of signature genes for each cell type / process
expression\_values expression values to use
reverse\_log\_scale reverse expression values from log scale
logbase log base to use if reverse\_log\_scale = TRUE
top\_percentage percentage of cells that will be considered to have gene expression with matrix binarization
output\_enrichment how to return enrichment output
p\_value calculate p-values (boolean, default = FALSE)
name to give to spatial enrichment results, default = rank
return\_gobject return giotto object

### See Also

runHyperGeometricEnrich

```
insert {\tt CrossSectionGenePlot3D}
```

insertCrossSectionGenePlot3D

## **Description**

Visualize cells and gene expression in a virtual cross section according to spatial coordinates

### Usage

```
insertCrossSectionGenePlot3D(
 gobject,
 crossSection_obj = NULL,
 name = NULL,
 spatial_network_name = "Delaunay_network",
 mesh_grid_color = "#1f77b4",
 mesh_grid_width = 3,
 mesh_grid_style = "dot",
 sdimx = "sdimx",
  sdimy = "sdimy",
  sdimz = "sdimz",
  show_other_cells = F,
 axis_scale = c("cube", "real", "custom"),
  custom_ratio = NULL,
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
 default_save_name = "spatGenePlot3D_with_cross_section",
)
```

### **Arguments**

```
gobject
                  giotto object
crossSection_obj
                  cross section object as alternative input. default = NULL.
                  name of virtual cross section to use
name
spatial_network_name
                  name of spatial network to use
mesh_grid_color
                  color for the meshgrid lines
mesh_grid_width
                  width for the meshgrid lines
mesh_grid_style
                  style for the meshgrid lines
                  x-axis dimension name (default = 'sdimx')
sdimx
sdimy
                  y-axis dimension name (default = 'sdimy')
sdimz
                  z-axis dimension name (default = 'sdimy')
```

```
show_other_cells
                  display not selected cells
axis_scale
                  axis_scale
custom_ratio
                  custom_ratio
show_plot
                  show plots
                  return ggplot object
return_plot
save_plot
                  directly save the plot [boolean]
                  list of saving parameters from all_plots_save_function
save_param
default_save_name
                  default save name for saving, don't change, change save_name in save_param
                  parameters for spatGenePlot3D
. . .
```

#### **Details**

Description of parameters.

#### Value

ggplot

### **Examples**

insertCrossSectionGenePlot3D(gobject)

```
insertCrossSectionSpatPlot3D
```

### **Description**

Visualize the meshgrid lines of cross section together with cells

insertCrossSectionSpatPlot3D

### Usage

```
insertCrossSectionSpatPlot3D(
 gobject,
 crossSection_obj = NULL,
 name = NULL,
  spatial_network_name = "Delaunay_network",
 mesh_grid_color = "#1f77b4",
 mesh_grid_width = 3,
 mesh_grid_style = "dot",
 sdimx = "sdimx",
  sdimy = "sdimy",
 sdimz = "sdimz",
 show_other_cells = F,
 axis_scale = c("cube", "real", "custom"),
 custom_ratio = NULL,
 default_save_name = "spat3D_with_cross_section",
)
```

jackstrawPlot 139

## **Arguments**

```
gobject
                  giotto object
crossSection_obj
                  cross section object as alternative input. default = NULL.
                  name of virtual cross section to use
name
spatial_network_name
                  name of spatial network to use
mesh_grid_color
                  color for the meshgrid lines
mesh_grid_width
                  width for the meshgrid lines
mesh_grid_style
                  style for the meshgrid lines
sdimx
                  x-axis dimension name (default = 'sdimx')
sdimy
                  y-axis dimension name (default = 'sdimy')
                  z-axis dimension name (default = 'sdimy')
sdimz
show_other_cells
                  display not selected cells
axis_scale
                  axis_scale
custom_ratio
                  custom_ratio
default_save_name
                  default save name for saving, don't change, change save_name in save_param
                  parameters for spatPlot3D
```

### **Details**

Description of parameters.

## Value

ggplot

## Examples

 $insert {\tt CrossSectionSpatPlot3D(gobject)}$ 

jackstrawPlot jackstrawPlot

## **Description**

identify significant prinicipal components (PCs)

140 jackstrawPlot

#### Usage

```
jackstrawPlot(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  reduction = c("cells", "genes"),
  genes_to_use = NULL,
  center = FALSE,
  scale_unit = FALSE,
  ncp = 20,
  ylim = c(0, 1),
  iter = 10,
  threshold = 0.01,
  verbose = T,
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "jackstrawPlot"
)
```

### **Arguments**

```
gobject
                  giotto object
expression_values
                  expression values to use
                  cells or genes
reduction
                  subset of genes to use for PCA
genes_to_use
                  center data before PCA
center
scale_unit
                  scale features before PCA
ncp
                  number of principal components to calculate
                  y-axis limits on jackstraw plot
ylim
                  number of interations for jackstraw
iter
                  p-value threshold to call a PC significant
threshold
verbose
                  show progress of jackstraw method
                  show plot
show_plot
return_plot
                  return ggplot object
                  directly save the plot [boolean]
save_plot
save_param
                  list of saving parameters from all_plots_save_function()
default_save_name
```

### **Details**

The Jackstraw method uses the permutationPA function. By systematically permuting genes it identifies robust, and thus significant, PCs.

default save name for saving, don't change, change save\_name in save\_param

loadHMRF

## Value

ggplot object for jackstraw method

## **Examples**

```
jackstrawPlot(gobject)
```

loadHMRF

loadHMRF

# Description

load previous HMRF

## Usage

```
loadHMRF(
  name_used = "test",
  output_folder_used,
  k_used = 10,
  betas_used,
  python_path_used
)
```

# Arguments

```
name_used name of HMRF that was run
output_folder_used
output folder that was used

k_used number of HMRF domains that was tested
betas_used betas that were tested
python_path_used
python path that was used
```

## **Details**

Description of HMRF parameters ...

## Value

reloads a previous ran HMRF from doHRMF

```
loadHMRF(gobject)
```

142 makeSignMatrixRank

makeSignMatrixPAGE makeSignMatrixPAGE

### **Description**

Function to convert a list of signature genes (e.g. for cell types or processes) into a binary matrix format that can be used with the PAGE enrichment option. Each cell type or process should have a vector of cell-type or process specific genes. These vectors need to be combined into a list (sign\_list). The names of the cell types or processes that are provided in the list need to be given (sign\_names).

## Usage

```
makeSignMatrixPAGE(sign_names, sign_list)
```

### **Arguments**

sign\_names vector with names for each provided gene signature

sign\_list list of genes (signature)

#### Value

matrix

#### See Also

**PAGEEnrich** 

## **Examples**

makeSignMatrixPAGE()

 $make Sign Matrix Rank \qquad make Sign Matrix Rank$ 

# Description

Function to convert a single-cell count matrix and a corresponding single-cell cluster vector into a rank matrix that can be used with the Rank enrichment option.

### Usage

```
makeSignMatrixRank(sc_matrix, sc_cluster_ids, gobject = NULL)
```

### **Arguments**

sc\_matrix matrix of single-cell RNAseq expression data

sc\_cluster\_ids vector of cluster ids

gobject if giotto object is given then only genes present in both datasets will be consid-

ered

mergeClusters 143

### Value

matrix

#### See Also

rankEnrich

### **Examples**

makeSignMatrixRank()

mergeClusters

mergeClusters

## Description

Merge selected clusters based on pairwise correlation scores and size of cluster.

## Usage

```
mergeClusters(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  cluster_column,
  cor = c("pearson", "spearman"),
  new_cluster_name = "merged_cluster",
  min_cor_score = 0.8,
  max_group_size = 20,
  force_min_group_size = 10,
  max_sim_clusters = 10,
  return_gobject = TRUE,
  verbose = TRUE
)
```

# Arguments

```
gobject
                  giotto object
expression_values
                  expression values to use
cluster_column name of column to use for clusters
                  correlation score to calculate distance
{\tt new\_cluster\_name}
                  new name for merged clusters
min_cor_score min correlation score to merge pairwise clusters
max_group_size max cluster size that can be merged
force_min_group_size
                  size of clusters that will be merged with their most similar neighbor(s)
max_sim_clusters
                  maximum number of clusters to potentially merge to reach force_min_group_size
return_gobject return giotto object
verbose
                  be verbose
```

#### **Details**

Merge selected clusters based on pairwise correlation scores and size of cluster. To avoid large clusters to merge the max\_group\_size can be lowered. Small clusters can be forcibly merged with their most similar pairwise cluster by adjusting the force\_min\_group\_size parameter. Clusters smaller than this value will be merged independent on the provided min\_cor\_score value. The force\_min\_group\_size might not always be reached if clusters have already been merged before A giotto object is returned by default, if FALSE then the merging vector will be returned.

### Value

Giotto object

## **Examples**

```
mergeClusters(gobject)
```

```
mini_giotto_single_cell
```

mini Giotto object for spatial single-cell resolution data

## Description

Mini Giotto object created from the seqFISH+ data.

## Usage

```
data(mini_giotto_single_cell)
```

### **Format**

An object of class "giotto"; see createGiottoObject.

## References

```
Eng et al. (2019) Nature (PubMed)
```

```
data(mini_giotto_single_cell)
spatPlot2D(mini_giotto_single_cell,cell_color = 'cell_types', point_size = 5)
```

normalizeGiotto 145

normalizeGiotto	normalizeGiotto
-----------------	-----------------

## **Description**

fast normalize and/or scale expresion values of Giotto object

#### Usage

```
normalizeGiotto(
  gobject,
  norm_methods = c("standard", "osmFISH"),
  library_size_norm = TRUE,
  scalefactor = 6000,
  log_norm = TRUE,
  log_offset = 1,
  logbase = 2,
  scale_genes = T,
  scale_cells = T,
  scale_order = c("first_genes", "first_cells"),
  verbose = F
)
```

### **Arguments**

```
gobject
                  giotto object
norm_methods
                  normalization method to use
library_size_norm
                  normalize cells by library size
scalefactor
                  scale factor to use after library size normalization
                  transform values to log-scale
log_norm
log_offset
                  offset value to add to expression matrix, default = 1
logbase
                  log base to use to log normalize expression values
                  z-score genes over all cells
scale_genes
scale_cells
                  z-score cells over all genes
scale_order
                  order to scale genes and cells
verbose
                  be verbose
```

### **Details**

Currently there are two 'methods' to normalize your raw counts data.

A. The standard method follows the standard protocol which can be adjusted using the provided parameters and follows the following order:

- 1. Data normalization for total library size and scaling by a custom scale-factor.
- 2. Log transformation of data.
- 3. Z-scoring of data by genes and/or cells.

146 PAGEEnrich

- B. The normalization method as provided by the osmFISH paper is also implemented:
  - 1. First normalize genes, for each gene divide the counts by the total gene count and multiply by the total number of genes.
  - 2. Next normalize cells, for each cell divide the normalized gene counts by the total counts per cell and multiply by the total number of cells.

This data will be saved in the Giotto slot for custom expression.

#### Value

giotto object

## **Examples**

normalizeGiotto(gobject)

**PAGEEnrich** 

runPAGEEnrich

#### **Description**

Function to calculate gene signature enrichment scores per spatial position using PAGE.

## Usage

```
PAGEEnrich(...)
```

## Arguments

.. Arguments passed on to runPAGEEnrich

gobject Giotto object

sign\_matrix Matrix of signature genes for each cell type / process

 ${\tt expression\_values} \ \ expression \ values \ \ to \ use$ 

reverse\_log\_scale reverse expression values from log scale

logbase log base to use if reverse\_log\_scale = TRUE

 ${\tt output\_enrichment}$  how to return enrichment output

p\_value calculate p-values (boolean, default = FALSE)

n\_times number of permutations to calculate for p\_value

name to give to spatial enrichment results, default = PAGE

return\_gobject return giotto object

## See Also

runPAGEEnrich

pDataDT

pDataDT

pDataDT

### **Description**

show cell metadata

### Usage

```
pDataDT(gobject)
```

#### **Arguments**

gobject

giotto object

### Value

data.table with cell metadata

## **Examples**

```
pDataDT(gobject)
```

plotCCcomDotplot

plotCCcomDotplot

## Description

Plots dotplot for ligand-receptor communication scores in cell-cell interactions

```
plotCCcomDotplot(
  gobject,
  comScores,
  selected_LR = NULL,
  selected_cell_LR = NULL,
  show_LR_names = TRUE,
  show_cell_LR_names = TRUE,
  cluster_on = c("PI", "LR_expr", "log2fc"),
  cor_method = c("pearson", "kendall", "spearman"),
 aggl_method = c("ward.D", "ward.D2", "single", "complete", "average", "mcquitty",
    "median", "centroid"),
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "plotCCcomDotplot"
)
```

148 plotCCcomHeatmap

#### **Arguments**

```
gobject
                  giotto object
                  communinication scores from exprCellCellcom or spatCellCellcom
comScores
selected_LR
                  selected ligand-receptor combinations
selected_cell_LR
                  selected cell-cell combinations for ligand-receptor combinations
show_LR_names
                  show ligand-receptor names
show_cell_LR_names
                  show cell-cell names
                  values to use for clustering of cell-cell and ligand-receptor pairs
cluster_on
                  correlation method used for clustering
cor_method
                  agglomeration method used by hclust
aggl\_method
show_plot
                  show plots
return_plot
                  return plotting object
                  directly save the plot [boolean]
save_plot
                  list of saving parameters from all_plots_save_function
save_param
default_save_name
                  default save name for saving, don't change, change save_name in save_param
```

#### Value

ggplot

## **Examples**

```
plotCCcomDotplot(CPGscores)
```

plotCCcomHeatmap plotCCcomHeatmap

## **Description**

Plots heatmap for ligand-receptor communication scores in cell-cell interactions

```
show_plot = NA,
return_plot = NA,
save_plot = NA,
save_param = list(),
default_save_name = "plotCCcomHeatmap")
```

### **Arguments**

```
gobject
                 giotto object
                 communinication scores from exprCellCellcom or spatCellCellcom
comScores
selected_LR
                 selected ligand-receptor combinations
selected_cell_LR
                 selected cell-cell combinations for ligand-receptor combinations
show_LR_names
                 show ligand-receptor names
show_cell_LR_names
                 show cell-cell names
show
                 values to show on heatmap
                 correlation method used for clustering
cor_method
                 agglomeration method used by hclust
aggl_method
show_plot
                 show plots
return_plot
                 return plotting object
                 directly save the plot [boolean]
save_plot
                 list of saving parameters from all_plots_save_function
save_param
default_save_name
                 default save name for saving, don't change, change save_name in save_param
```

#### Value

ggplot

## **Examples**

```
plotCCcomHeatmap(CPGscores)
```

```
plot Cell Proximity Genes \\ plot Cell Proximity Genes
```

# Description

Create visualization for cell proximity gene scores

#### Usage

```
plotCellProximityGenes(
  gobject,
  cpgObject,
  method = c("volcano", "cell_barplot", "cell-cell", "cell_sankey", "heatmap",
    "dotplot"),
  min_cells = 4,
  min_cells_expr = 1,
  min_int_cells = 4,
  min_int_cells_expr = 1,
  min_fdr = 0.1,
  min_spat_diff = 0.2,
  min_log2_fc = 0.2,
  min_zscore = 2,
  zscores_column = c("cell_type", "genes"),
  direction = c("both", "up", "down"),
  cell_color_code = NULL,
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "plotCellProximityGenes"
```

### **Arguments**

```
gobject
                  giotto object
cpgObject
                  cell proximity gene score object
method
                  plotting method to use
min_cells
                  minimum number of source cell type
min_cells_expr minimum expression level for source cell type
min_int_cells
                  minimum number of interacting neighbor cell type
min_int_cells_expr
                  minimum expression level for interacting neighbor cell type
min_fdr
                  minimum adjusted p-value
min_spat_diff
                  minimum absolute spatial expression difference
                  minimum log2 fold-change
min_log2_fc
min_zscore
                  minimum z-score change
zscores_column calculate z-scores over cell types or genes
direction
                  differential expression directions to keep
cell_color_code
                  vector of colors with cell types as names
show_plot
                  show plots
return_plot
                  return plotting object
save_plot
                  directly save the plot [boolean]
                  list of saving parameters from all_plots_save_function
save_param
default_save_name
```

default save name for saving, don't change, change save\_name in save\_param

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#### Value

plot

### **Examples**

```
plotCellProximityGenes(CPGscores)
```

plotCombineCCcom

plotCombineCCcom

### **Description**

Create visualization for combined (pairwise) cell proximity gene scores

## Usage

```
plotCombineCCcom(
  gobject,
  combCCcom,
  selected_LR = NULL,
  selected_cell_LR = NULL,
  detail_plot = T,
  simple_plot = F,
  simple_plot_facet = c("interaction", "genes"),
  facet_scales = "fixed",
  facet_ncol = length(selected_LR),
  facet_nrow = length(selected_cell_LR),
  colors = c("#9932CC", "#FF8C00"),
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "plotCombineCCcom"
)
```

```
gobject
                  giotto object
{\sf combCCcom}
                  combined communcation scores, output from combCCcom()
selected_LR
                  selected ligand-receptor pair
selected_cell_LR
                  selected cell-cell interaction pair for ligand-receptor pair
detail_plot
                  show detailed info in both interacting cell types
simple_plot
                  show a simplified plot
simple_plot_facet
                  facet on interactions or genes with simple plot
facet_scales
                  ggplot facet scales paramter
facet_ncol
                  ggplot facet ncol parameter
```

```
facet_nrow ggplot facet nrow parameter

colors vector with two colors to use

show_plot show plots

return_plot return plotting object

save_plot directly save the plot [boolean]

save_param list of saving parameters from all_plots_save_function

default_save_name

default save name for saving, don't change, change save_name in save_param
```

#### Value

ggplot

## **Examples**

```
plotCombineCCcom(CPGscores)
```

```
plot {\tt Combine Cell Cell Communication} \\ plot {\tt Combine Cell Cell Communication} \\
```

## Description

Create visualization for combined (pairwise) cell proximity gene scores

```
plotCombineCellCellCommunication(
  gobject,
  combCCcom,
  selected_LR = NULL,
  selected_cell_LR = NULL,
  detail_plot = T,
  simple_plot = F,
  simple_plot_facet = c("interaction", "genes"),
  facet_scales = "fixed",
  facet_ncol = length(selected_LR),
  facet_nrow = length(selected_cell_LR),
  colors = c("#9932CC", "#FF8C00"),
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "plotCombineCellCellCommunication"
```

#### **Arguments**

```
giotto object
gobject
combCCcom
                  combined communcation scores, output from combCCcom()
selected_LR
                  selected ligand-receptor pair
selected_cell_LR
                  selected cell-cell interaction pair for ligand-receptor pair
detail_plot
                  show detailed info in both interacting cell types
                  show a simplified plot
simple_plot
simple_plot_facet
                  facet on interactions or genes with simple plot
facet_scales
                  ggplot facet scales paramter
                  ggplot facet ncol parameter
facet_ncol
facet_nrow
                  ggplot facet nrow parameter
colors
                  vector with two colors to use
show_plot
                  show plots
return_plot
                  return plotting object
                  directly save the plot [boolean]
save_plot
save_param
                  list of saving parameters from all_plots_save_function
default_save_name
                  default save name for saving, don't change, change save_name in save_param
```

### Value

ggplot

## **Examples**

```
plotCombineCellCellCommunication(CPGscores)
```

```
plot {\tt Combine Cell Proximity Genes} \\ plot {\tt Combine Cell Proximity Genes}
```

### **Description**

Create visualization for combined (pairwise) cell proximity gene scores

```
plotCombineCellProximityGenes(
  gobject,
  combCpgObject,
  selected_interactions = NULL,
  selected_gene_to_gene = NULL,
  detail_plot = T,
  simple_plot = F,
```

```
simple_plot_facet = c("interaction", "genes"),
facet_scales = "fixed",
facet_ncol = length(selected_gene_to_gene),
facet_nrow = length(selected_interactions),
colors = c("#9932CC", "#FF8C00"),
show_plot = NA,
return_plot = NA,
save_plot = NA,
save_plot = NA,
save_param = list(),
default_save_name = "plotCombineCPG"
```

### **Arguments**

```
gobject
                  giotto object
combCpgObject
                  CPGscores, output from combineCellProximityGenes()
selected_interactions
                  interactions to show
selected_gene_to_gene
                  pairwise gene combinations to show
detail_plot
                  show detailed info in both interacting cell types
simple_plot
                  show a simplified plot
simple_plot_facet
                  facet on interactions or genes with simple plot
facet_scales
                  ggplot facet scales paramter
facet_ncol
                  ggplot facet ncol parameter
facet_nrow
                  ggplot facet nrow parameter
colors
                  vector with two colors to use
show_plot
                  show plots
return_plot
                  return plotting object
                  directly save the plot [boolean]
save_plot
save_param
                  list of saving parameters from all_plots_save_function
default_save_name
                  default save name for saving, don't change, change save_name in save_param
```

#### Value

ggplot

```
plotCombineCellProximityGenes(CPGscores)
```

plotCombineCPG 155

plotCombineCPG plotCombineCPG

### **Description**

Create visualization for combined (pairwise) cell proximity gene scores

## Usage

```
plotCombineCPG(
  gobject,
  combCpgObject,
  selected_interactions = NULL,
  selected_gene_to_gene = NULL,
  detail_plot = T,
  simple_plot = F,
  simple_plot_facet = c("interaction", "genes"),
  facet_scales = "fixed",
  facet_ncol = length(selected_gene_to_gene),
  facet_nrow = length(selected_interactions),
  colors = c("#9932CC", "#FF8C00"),
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "plotCombineCPG"
)
```

## **Arguments**

```
gobject
                 giotto object
combCpgObject
                 CPGscores, output from combineCellProximityGenes()
selected_interactions
                 interactions to show
selected_gene_to_gene
                 pairwise gene combinations to show
detail_plot
                 show detailed info in both interacting cell types
simple_plot
                 show a simplified plot
simple_plot_facet
                 facet on interactions or genes with simple plot
facet_scales
                 ggplot facet scales paramter
facet_ncol
                 ggplot facet ncol parameter
facet_nrow
                 ggplot facet nrow parameter
colors
                 vector with two colors to use
show_plot
                 show plots
return_plot
                 return plotting object
                 directly save the plot [boolean]
save_plot
save_param
                 list of saving parameters from all_plots_save_function
default_save_name
```

default save name for saving, don't change, change save\_name in save\_param

156 plotCPG

#### Value

ggplot

#### **Examples**

```
plotCombineCPG(CPGscores)
```

plotCPG

plotCPG

### **Description**

Create visualization for cell proximity gene scores

## Usage

```
plotCPG(
  gobject,
  cpgObject,
  method = c("volcano", "cell_barplot", "cell-cell", "cell_sankey", "heatmap",
    "dotplot"),
  min_cells = 5,
  min_cells_expr = 1,
  min_int_cells = 3,
  min_int_cells_expr = 1,
  min_fdr = 0.05,
  min_spat_diff = 0.2,
  min_log2_fc = 0.2,
  min_zscore = 2,
  zscores_column = c("cell_type", "genes"),
  direction = c("both", "up", "down"),
  cell_color_code = NULL,
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "plotCPG"
)
```

```
gobject giotto object
cpgObject cell proximity gene score object
method plotting method to use
min_cells minimum number of source cell type
min_cells_expr minimum expression level for source cell type
min_int_cells minimum number of interacting neighbor cell type
min_int_cells_expr
minimum expression level for interacting neighbor cell type
```

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min\_fdr minimum adjusted p-value

min\_spat\_diff minimum absolute spatial expression difference

min\_log2\_fc minimum log2 fold-change min\_zscore minimum z-score change

zscores\_column calculate z-scores over cell types or genes direction differential expression directions to keep

cell\_color\_code

vector of colors with cell types as names

show\_plot show plots

return\_plot return plotting object

save\_plot directly save the plot [boolean]

save\_param list of saving parameters from all\_plots\_save\_function

default\_save\_name

default save name for saving, don't change, change save\_name in save\_param

## Value

plot

## **Examples**

plotCPG(CPGscores)

plotGiottoImage plotGiottoImage

## **Description**

get plot a giotto image from a giotto object

## Usage

```
plotGiottoImage(gobject, image_name)
```

## **Arguments**

gobject giotto object

image\_name name of giotto image showGiottoImageNames

## Value

plot

```
\verb|plotGiottoImage(gobject)|
```

158 plotHeatmap

plotHeatmap

plotHeatmap

#### **Description**

Creates heatmap for genes and clusters.

### Usage

```
plotHeatmap(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  genes,
  cluster_column = NULL,
  cluster_order = c("size", "correlation", "custom"),
  cluster_custom_order = NULL,
  cluster_color_code = NULL,
  cluster_cor_method = "pearson",
  cluster_hclust_method = "ward.D"
  gene_order = c("correlation", "custom"),
  gene_custom_order = NULL,
  gene_cor_method = "pearson",
  gene_hclust_method = "complete",
  show_values = c("rescaled", "z-scaled", "original"),
  size_vertical_lines = 1.1,
  gradient_colors = c("blue", "yellow", "red"),
  gene_label_selection = NULL,
  axis_text_y_size = NULL,
  legend_nrows = 1,
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "plotHeatmap"
)
```

```
gobject giotto object
expression_values
expression values to use
genes genes to use
cluster_column name of column to use for clusters
cluster_order method to determine cluster order
cluster_custom_order
custom order for clusters
cluster_color_code
color code for clusters
cluster_cor_method
method for cluster correlation
```

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```
cluster_hclust_method
                  method for hierarchical clustering of clusters
                  method to determine gene order
gene_order
gene_custom_order
                  custom order for genes
{\tt gene\_cor\_method}
                  method for gene correlation
gene_hclust_method
                  method for hierarchical clustering of genes
show_values
                  which values to show on heatmap
size_vertical_lines
                  sizes for vertical lines
gradient_colors
                  colors for heatmap gradient
gene_label_selection
                  subset of genes to show on y-axis
axis_text_y_size
                  size for y-axis text
legend_nrows
                  number of rows for the cluster legend
show_plot
                  show plot
return_plot
                  return ggplot object
                  directly save the plot [boolean]
save_plot
                  list of saving parameters, see showSaveParameters
save_param
default_save_name
                  default save name
```

### Details

If you want to display many genes there are 2 ways to proceed:

- 1. set axis\_text\_y\_size to a really small value and show all genes
- 2. provide a subset of genes to display to gene\_label\_selection

#### Value

ggplot

```
## Not run:
plotHeatmap(gobject)
## End(Not run)
```

160 plotICG

plotICG plotICG

### **Description**

Create barplot to visualize interaction changed genes

## Usage

```
plotICG(
   gobject,
   cpgObject,
   source_type,
   source_markers,
   ICG_genes,
   cell_color_code = NULL,
   show_plot = NA,
   return_plot = NA,
   save_plot = NA,
   save_param = list(),
   default_save_name = "plotICG"
)
```

## Arguments

```
gobject
                  giotto object
                  cell proximity gene score object
cpgObject
source_type
                  cell type of the source cell
source_markers markers for the source cell type
ICG_genes
                  named character vector of ICG genes
cell_color_code
                  cell color code for the interacting cell types
show_plot
                  show plots
return_plot
                  return plotting object
                  directly save the plot [boolean]
save_plot
save_param
                  list of saving parameters from all_plots_save_function
default_save_name
                  default save name for saving, don't change, change save_name in save_param
```

#### Value

plot

```
plotICG(CPGscores)
```

```
plotInteraction {\tt Changed Genes} \\ plotInteraction {\tt Changed Genes} \\
```

## Description

Create barplot to visualize interaction changed genes

### Usage

```
plotInteractionChangedGenes(
   gobject,
   cpgObject,
   source_type,
   source_markers,
   ICG_genes,
   cell_color_code = NULL,
   show_plot = NA,
   return_plot = NA,
   save_plot = NA,
   save_param = list(),
   default_save_name = "plotInteractionChangedGenes")
```

## **Arguments**

```
gobject
                  giotto object
cpgObject
                  cell proximity gene score object
                  cell type of the source cell
source_type
source_markers markers for the source cell type
ICG_genes
                  named character vector of ICG genes
cell_color_code
                  cell color code for the interacting cell types
show_plot
                  show plots
return_plot
                  return plotting object
save_plot
                  directly save the plot [boolean]
                  list of saving parameters from all_plots_save_function
save_param
default\_save\_name
                  default save name for saving, don't change, change save_name in save_param
```

## Value

plot

```
plotInteractionChangedGenes(CPGscores)
```

### **Description**

Creates heatmap for numeric cell metadata within aggregated clusters.

## Usage

```
plotMetaDataCellsHeatmap(
  gobject,
  metadata_cols = NULL,
  spat_enr_names = NULL,
  value_cols = NULL,
  first_meta_col = NULL,
  second_meta_col = NULL,
  show_values = c("zscores", "original", "zscores_rescaled"),
  custom_cluster_order = NULL,
  clus_cor_method = "pearson",
  clus_cluster_method = "complete",
  custom_values_order = NULL,
  values_cor_method = "pearson",
  values_cluster_method = "complete",
  midpoint = 0,
  x_{text_size} = 8,
  x_{text_angle} = 45,
  y_{text_size} = 8,
  strip_text_size = 8,
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "plotMetaDataCellsHeatmap"
)
```

```
clus_cor_method
```

correlation method for clusters

clus\_cluster\_method

hierarchical cluster method for the clusters

custom\_values\_order

custom values order (default = NULL)

values\_cor\_method

correlation method for values

values\_cluster\_method

hierarchical cluster method for the values

midpoint midpoint of show\_values

x\_text\_size size of x-axis text

x\_text\_angle angle of x-axis text

y\_text\_size size of y-axis text

strip\_text\_size

size of strip text

show\_plot show plot

return\_plot return ggplot object

save\_plot directly save the plot [boolean]

save\_param list of saving parameters, see showSaveParameters

default\_save\_name

default save name for saving, don't change, change save\_name in save\_param

# Details

Creates heatmap for the average values of selected value columns in the different annotation groups.

## Value

ggplot or data.table

## See Also

plotMetaDataHeatmap for gene expression instead of numeric cell annotation data.

## **Examples**

plotMetaDataCellsHeatmap(gobject)

```
plotMetaDataHeatmap
```

## Description

Creates heatmap for genes within aggregated clusters.

## Usage

```
plotMetaDataHeatmap(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  metadata_cols = NULL,
  selected_genes = NULL,
  first_meta_col = NULL,
  second_meta_col = NULL,
  show_values = c("zscores", "original", "zscores_rescaled"),
  custom_cluster_order = NULL,
  clus_cor_method = "pearson",
  clus_cluster_method = "complete",
  custom_gene_order = NULL,
  gene_cor_method = "pearson",
  gene_cluster_method = "complete",
  gradient_color = c("blue", "white", "red"),
  gradient_midpoint = 0,
  gradient_limits = NULL,
  x_{text_size} = 10,
  x_{text_angle} = 45,
  y_{text_size} = 10,
  strip_text_size = 8,
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "plotMetaDataHeatmap"
```

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```
clus_cor_method
                  correlation method for clusters
clus_cluster_method
                  hierarchical cluster method for the clusters
custom_gene_order
                  custom gene order (default = NULL)
gene_cor_method
                  correlation method for genes
gene_cluster_method
                  hierarchical cluster method for the genes
gradient_color vector with 3 colors for numeric data
gradient_midpoint
                  midpoint for color gradient
gradient_limits
                  vector with lower and upper limits
x_text_size
                  size of x-axis text
x_text_angle
                  angle of x-axis text
y_text_size
                  size of y-axis text
strip_text_size
                  size of strip text
show_plot
                  show plot
return_plot
                  return ggplot object
                  directly save the plot [boolean]
save_plot
save_param
                  list of saving parameters, see showSaveParameters
default_save_name
                  default save name
```

## Details

Creates heatmap for the average expression of selected genes in the different annotation/cluster groups. Calculation of cluster or gene order is done on the provided expression values, but visualization is by default on the z-scores. Other options are the original values or z-scores rescaled per gene (-1 to 1).

## Value

ggplot or data.table

### See Also

plotMetaDataCellsHeatmap for numeric cell annotation instead of gene expression.

### **Examples**

plotMetaDataHeatmap(gobject)

166 plotPCA

plotPCA

#### **Description**

Short wrapper for PCA visualization

plotPCA

#### Usage

```
plotPCA(gobject, dim_reduction_name = "pca", default_save_name = "PCA", ...)
```

```
gobject
                 giotto object
dim_reduction_name
                 name of PCA
default_save_name
                 default save name of PCA plot
                 Arguments passed on to dimPlot2D
. . .
                 group_by create multiple plots based on cell annotation column
                 group_by_subset subset the group_by factor column
                 dim1_to_use dimension to use on x-axis
                 dim2_to_use dimension to use on y-axis
                 spat_enr_names names of spatial enrichment results to include
                 show_NN_network show underlying NN network
                 nn_network_to_use type of NN network to use (kNN vs sNN)
                 network_name name of NN network to use, if show_NN_network = TRUE
                 cell_color color for cells (see details)
                 color_as_factor convert color column to factor
                 cell_color_code named vector with colors
                 cell_color_gradient vector with 3 colors for numeric data
                 gradient_midpoint midpoint for color gradient
                 gradient_limits vector with lower and upper limits
                 select_cell_groups select subset of cells/clusters based on cell_color param-
                 select_cells select subset of cells based on cell IDs
                  show_other_cells display not selected cells
                 other_cell_color color of not selected cells
                 other_point_size size of not selected cells
                 show_cluster_center plot center of selected clusters
                 show_center_label plot label of selected clusters
                 center_point_size size of center points
                 center_point_border_col border color of center points
                 center_point_border_stroke border stroke size of center points
                 label_size size of labels
                 label_fontface font of labels
```

plotPCA\_2D

```
edge_alpha column to use for alpha of the edges
point_shape point with border or not (border or no_border)
point_size size of point (cell)
point_alpha transparancy of point
point_border_col color of border around points
point_border_stroke stroke size of border around points
title title for plot, defaults to cell_color parameter
show_legend show legend
legend_text size of legend text
legend_symbol_size size of legend symbols
background_color color of plot background
axis_text size of axis text
axis_title size of axis title
cow_n_col cowplot param: how many columns
cow_rel_h cowplot param: relative height
cow_rel_w cowplot param: relative width
cow_align cowplot param: how to align
show_plot show plot
return_plot return ggplot object
save_plot directly save the plot [boolean]
save_param list of saving parameters, see showSaveParameters
```

### **Details**

Description of parameters, see dimPlot2D. For 3D plots see plotPCA\_3D

#### Value

ggplot

#### See Also

```
Other reduced dimension visualizations: dimPlot2D(), dimPlot3D(), dimPlot(), plotPCA_2D(), plotPCA_3D(), plotTSNE_3D(), plotTSNE(), plotUMAP_2D(), plotUMAP_3D(), plotUMAP()
```

#### **Examples**

plotPCA(gobject)

plotPCA\_2D

plotPCA\_2D

## **Description**

Short wrapper for PCA visualization

168 plotPCA\_2D

```
plotPCA_2D(
      gobject,
      dim_reduction_name = "pca",
      default_save_name = "PCA_2D",
    )
Arguments
    gobject
                     giotto object
    dim_reduction_name
                     name of PCA
    default_save_name
                     default save name of PCA plot
                      Arguments passed on to dimPlot2D
    . . .
                     group_by create multiple plots based on cell annotation column
                     group_by_subset subset the group_by factor column
                     dim1_to_use dimension to use on x-axis
                     dim2_to_use dimension to use on y-axis
                     spat_enr_names names of spatial enrichment results to include
                      show_NN_network show underlying NN network
                     nn_network_to_use type of NN network to use (kNN vs sNN)
                     network_name name of NN network to use, if show_NN_network = TRUE
                     cell_color color for cells (see details)
                     color_as_factor convert color column to factor
                     cell_color_code named vector with colors
                     cell_color_gradient vector with 3 colors for numeric data
                     gradient_midpoint midpoint for color gradient
                     gradient_limits vector with lower and upper limits
                      select_cell_groups select subset of cells/clusters based on cell_color param-
                          eter
                     select_cells select subset of cells based on cell IDs
                     show_other_cells display not selected cells
                     other_cell_color color of not selected cells
                     other_point_size size of not selected cells
                     show_cluster_center plot center of selected clusters
                     show_center_label plot label of selected clusters
                     center_point_size size of center points
                     center_point_border_col border color of center points
                     center_point_border_stroke border stroke size of center points
                     label_size size of labels
                     label_fontface font of labels
                     edge_alpha column to use for alpha of the edges
                     point_shape point with border or not (border or no_border)
                     point_size size of point (cell)
                     point_alpha transparancy of point
```

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```
point_border_col color of border around points
point_border_stroke stroke size of border around points
title title for plot, defaults to cell_color parameter
show_legend show legend
legend_text size of legend text
legend_symbol_size size of legend symbols
background_color color of plot background
axis_text size of axis text
axis_title size of axis title
cow_n_col cowplot param: how many columns
cow_rel_h cowplot param: relative height
cow_rel_w cowplot param: relative width
cow_align cowplot param: how to align
show_plot show plot
return_plot return ggplot object
save_plot directly save the plot [boolean]
save_param list of saving parameters, see showSaveParameters
```

#### **Details**

Description of parameters, see dimPlot2D. For 3D plots see plotPCA\_3D

#### Value

ggplot

## See Also

```
Other reduced dimension visualizations: dimPlot2D(), dimPlot3D(), dimPlot(), plotPCA_3D(), plotPCA(), plotTSNE_2D(), plotTSNE_3D(), plotUMAP_2D(), plotUMAP_3D(), plotUMAP()
```

## Examples

```
plotPCA_2D(gobject)
```

```
plotPCA_3D
```

plotPCA\_3D

# Description

Visualize cells according to 3D PCA dimension reduction

```
plotPCA_3D(
  gobject,
  dim_reduction_name = "pca",
  default_save_name = "PCA_3D",
   ...
)
```

plotPCA\_3D

#### **Arguments**

```
gobject
                  giotto object
dim_reduction_name
                  name of PCA
default_save_name
                  default save name of PCA plot
                  Arguments passed on to dimPlot3D
. . .
                  dim1_to_use dimension to use on x-axis
                  dim2_to_use dimension to use on y-axis
                  dim3_to_use dimension to use on z-axis
                  spat_enr_names names of spatial enrichment results to include
                  show_NN_network show underlying NN network
                  nn\_network\_to\_use \ type \ of \ NN \ network \ to \ use \ (kNN \ vs \ sNN)
                  network_name name of NN network to use, if show_NN_network = TRUE
                  cell_color color for cells (see details)
                  color_as_factor convert color column to factor
                  cell_color_code named vector with colors
                  select_cell_groups select subset of cells/clusters based on cell_color param-
                  select_cells select subset of cells based on cell IDs
                  show_other_cells display not selected cells
                  other_cell_color color of not selected cells
                  other_point_size size of not selected cells
                  show_cluster_center plot center of selected clusters
                  show_center_label plot label of selected clusters
                  center_point_size size of center points
                  label_size size of labels
                  edge_alpha column to use for alpha of the edges
                  point_size size of point (cell)
                  show_plot show plot
                  return_plot return ggplot object
                  save_plot directly save the plot [boolean]
                  save_param list of saving parameters, see showSaveParameters
```

### **Details**

Description of parameters.

#### Value

plotly

## See Also

```
Other reduced dimension visualizations: dimPlot2D(), dimPlot3D(), dimPlot(), plotPCA_2D(), plotPCA_(), plotTSNE_2D(), plotTSNE_3D(), plotTSNE(), plotUMAP_2D(), plotUMAP_3D(), plotUMAP()
```

```
plotPCA_3D(gobject)
```

plotRankSpatvsExpr 171

plotRankSpatvsExpr plotRankSpatvsExpr

#### **Description**

Plots dotplot to compare ligand-receptor rankings from spatial and expression information

## Usage

```
plotRankSpatvsExpr(
  gobject,
  combCC,
  expr_rnk_column = "LR_expr_rnk",
  spat_rnk_column = "LR_spat_rnk",
  midpoint = 10,
  size\_range = c(0.01, 1.5),
  xlims = NULL,
  ylims = NULL,
  selected_ranks = c(1, 10, 20),
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "plotRankSpatvsExpr"
)
```

```
giotto object
gobject
combCC
                  combined communinication scores from combCCcom
expr_rnk_column
                  column with expression rank information to use
spat_rnk_column
                  column with spatial rank information to use
midpoint
                  midpoint of colors
                  size ranges of dotplot
size_range
xlims
                  x-limits, numerical vector of 2
ylims
                  y-limits, numerical vector of 2
selected_ranks numerical vector, will be used to print out the percentage of top spatial ranks are
                  recovered
show_plot
                  show plots
                  return plotting object
return_plot
save_plot
                  directly save the plot [boolean]
                  list of saving parameters from all_plots_save_function
save_param
default_save_name
                  default save name for saving, don't change, change save_name in save_param
```

172 plotRecovery

#### Value

ggplot

### **Examples**

```
plotRankSpatvsExpr(CPGscores)
```

plotRecovery

plotRecovery

### **Description**

Plots recovery plot to compare ligand-receptor rankings from spatial and expression information

### Usage

```
plotRecovery(
   gobject,
   combCC,
   expr_rnk_column = "exprPI_rnk",
   spat_rnk_column = "spatPI_rnk",
   ground_truth = c("spatial", "expression"),
   show_plot = NA,
   return_plot = NA,
   save_plot = NA,
   save_param = list(),
   default_save_name = "plotRecovery"
)
```

## **Arguments**

```
giotto object
gobject
                  combined communinication scores from combCCcom
combCC
expr_rnk_column
                  column with expression rank information to use
spat_rnk_column
                  column with spatial rank information to use
ground_truth
                  what to consider as ground truth (default: spatial)
                  show plots
show_plot
return_plot
                  return plotting object
save_plot
                  directly save the plot [boolean]
                  list of saving parameters from all_plots_save_function
save_param
default_save_name
                  default save name for saving, don't change, change save_name in save_param
```

## Value

ggplot

plotRecovery\_sub 173

## **Examples**

```
plotRecovery(CPGscores)
```

```
plotRecovery_sub
```

plotRecovery\_sub

### Description

Plots recovery plot to compare ligand-receptor rankings from spatial and expression information

## Usage

```
plotRecovery_sub(combCC, first_col = "LR_expr_rnk", second_col = "LR_spat_rnk")
```

## Arguments

combCC combined communinication scores from combCCcom

first\_col first column to use second\_col second column to use

### **Examples**

```
plotRecovery_sub(CPGscores)
```

```
plotStatDelaunayNetwork
```

plot Stat Delaunay Network

### **Description**

Plots network statistics for a Delaunay network..

```
plotStatDelaunayNetwork(
  gobject,
  method = c("deldir", "delaunayn_geometry", "RTriangle"),
  dimensions = "all",
  maximum_distance = "auto",
  minimum_k = 0,
  options = "Pp",
  Y = TRUE,
  j = TRUE,
  S = 0,
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "plotStatDelaunayNetwork",
)
```

174 plotTSNE

### **Arguments**

gobject giotto object

method package to use to create a Delaunay network

dimensions which spatial dimensions to use (maximum 2 dimensions)

maximum\_distance

distance cuttof for Delaunay neighbors to consider

minimum\_k minimum neighbours if maximum\_distance != NULL

options (geometry) String containing extra control options for the underlying Qhull

command; see the Qhull documentation (../doc/qhull/html/qdelaun.html) for the

available options. (default = 'Pp', do not report precision problems)

Y (RTriangle) If TRUE prohibits the insertion of Steiner points on the mesh bound-

ary.

j (RTriangle) If TRUE jettisons vertices that are not part of the final triangulation

from the output.

S (RTriangle) Specifies the maximum number of added Steiner points.

show\_plot show plots

return\_plot return ggplot object

save\_plot directly save the plot [boolean]

save\_param list of saving parameters, see showSaveParameters

 ${\tt default\_save\_name}$ 

default save name for saving, don't change, change save\_name in save\_param

... Other parameters

#### Value

giotto object with updated spatial network slot

## **Examples**

 $\verb|plotStatDelaunayNetwork(gobject)|$ 

plotTSNE plotTSNE

### **Description**

Short wrapper for tSNE visualization

```
plotTSNE(gobject, dim_reduction_name = "tsne", default_save_name = "tSNE", ...)
```

plotTSNE 175

## Arguments

gobject giotto object dim\_reduction\_name name of TSNE default\_save\_name default save name of TSNE plot Arguments passed on to dimPlot2D . . . group\_by create multiple plots based on cell annotation column group\_by\_subset subset the group\_by factor column dim1\_to\_use dimension to use on x-axis dim2\_to\_use dimension to use on y-axis spat\_enr\_names names of spatial enrichment results to include show\_NN\_network show underlying NN network nn\_network\_to\_use type of NN network to use (kNN vs sNN) network\_name name of NN network to use, if show\_NN\_network = TRUE cell\_color color for cells (see details) color\_as\_factor convert color column to factor cell\_color\_code named vector with colors cell\_color\_gradient vector with 3 colors for numeric data gradient\_midpoint midpoint for color gradient gradient\_limits vector with lower and upper limits select\_cell\_groups select subset of cells/clusters based on cell\_color paramselect\_cells select subset of cells based on cell IDs show\_other\_cells display not selected cells other\_cell\_color color of not selected cells other\_point\_size size of not selected cells show\_cluster\_center plot center of selected clusters show\_center\_label plot label of selected clusters center\_point\_size size of center points center\_point\_border\_col border color of center points center\_point\_border\_stroke border stroke size of center points label\_size size of labels label\_fontface font of labels edge\_alpha column to use for alpha of the edges point\_shape point with border or not (border or no\_border) point\_size size of point (cell) point\_alpha transparancy of point point\_border\_col color of border around points point\_border\_stroke stroke size of border around points title title for plot, defaults to cell\_color parameter show\_legend show legend legend\_text size of legend text legend\_symbol\_size size of legend symbols background\_color color of plot background

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```
axis_text size of axis text

axis_title size of axis title

cow_n_col cowplot param: how many columns

cow_rel_h cowplot param: relative height

cow_rel_w cowplot param: relative width

cow_align cowplot param: how to align

show_plot show plot

return_plot return ggplot object

save_plot directly save the plot [boolean]

save_param list of saving parameters, see showSaveParameters
```

#### **Details**

Description of parameters, see dimPlot2D. For 3D plots see plotTSNE\_3D

#### Value

ggplot

#### See Also

```
Other reduced dimension visualizations: dimPlot2D(), dimPlot3D(), dimPlot(), plotPCA_2D(), plotPCA_3D(), plotPCA_3D(), plotTSNE_2D(), plotTSNE_3D(), plotUMAP_2D(), plotUMAP_3D(), plotUMAP()
```

## **Examples**

```
plotTSNE(gobject)
```

plotTSNE\_2D

plotTSNE\_2D

### **Description**

Short wrapper for tSNE visualization

```
plotTSNE_2D(
  gobject,
  dim_reduction_name = "tsne",
  default_save_name = "tSNE_2D",
   ...
)
```

plotTSNE\_2D 177

```
gobject
                 giotto object
dim_reduction_name
                 name of TSNE
default_save_name
                 default save name of TSNE plot
                  Arguments passed on to dimPlot2D
. . .
                  group_by create multiple plots based on cell annotation column
                 group_by_subset subset the group_by factor column
                 dim1_to_use dimension to use on x-axis
                 dim2_to_use dimension to use on y-axis
                  spat_enr_names names of spatial enrichment results to include
                  show_NN_network show underlying NN network
                 nn_network_to_use type of NN network to use (kNN vs sNN)
                 network_name name of NN network to use, if show_NN_network = TRUE
                 cell_color color for cells (see details)
                 color_as_factor convert color column to factor
                 cell_color_code named vector with colors
                 cell_color_gradient vector with 3 colors for numeric data
                 gradient_midpoint midpoint for color gradient
                 gradient_limits vector with lower and upper limits
                 select_cell_groups select subset of cells/clusters based on cell_color param-
                  select_cells select subset of cells based on cell IDs
                 show_other_cells display not selected cells
                 other_cell_color color of not selected cells
                  other_point_size size of not selected cells
                  show_cluster_center plot center of selected clusters
                 show_center_label plot label of selected clusters
                  center_point_size size of center points
                  center_point_border_col border color of center points
                  center_point_border_stroke border stroke size of center points
                 label_size size of labels
                 label_fontface font of labels
                  edge_alpha column to use for alpha of the edges
                 point_shape point with border or not (border or no_border)
                 point_size size of point (cell)
                 point_alpha transparancy of point
                 point_border_col color of border around points
                 point_border_stroke stroke size of border around points
                 title title for plot, defaults to cell_color parameter
                  show_legend show legend
                 legend_text size of legend text
                 legend_symbol_size size of legend symbols
                 background_color color of plot background
```

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```
axis_text size of axis text

axis_title size of axis title

cow_n_col cowplot param: how many columns

cow_rel_h cowplot param: relative height

cow_rel_w cowplot param: relative width

cow_align cowplot param: how to align

show_plot show plot

return_plot return ggplot object

save_plot directly save the plot [boolean]

save_param list of saving parameters, see showSaveParameters
```

#### **Details**

Description of parameters, see dimPlot2D. For 3D plots see plotTSNE\_3D

#### Value

ggplot

#### See Also

```
Other reduced dimension visualizations: dimPlot2D(), dimPlot3D(), dimPlot(), plotPCA_2D(), plotPCA_3D(), plotPCA(), plotTSNE_3D(), plotTSNE(), plotUMAP_2D(), plotUMAP_3D(), plotUMAP()
```

### **Examples**

```
plotTSNE_2D(gobject)
```

plotTSNE\_3D

plotTSNE\_3D

## Description

Visualize cells according to dimension reduction coordinates

```
plotTSNE_3D(
  gobject,
  dim_reduction_name = "tsne",
  default_save_name = "TSNE_3D",
   ...
)
```

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#### **Arguments**

```
gobject
                 giotto object
dim_reduction_name
                 name of TSNE
default_save_name
                 default save name of TSNE plot
                 Arguments passed on to dimPlot3D
                 dim1_to_use dimension to use on x-axis
                 dim2_to_use dimension to use on y-axis
                 dim3_to_use dimension to use on z-axis
                 spat_enr_names names of spatial enrichment results to include
                 show_NN_network show underlying NN network
                 nn_network_to_use type of NN network to use (kNN vs sNN)
                 network_name name of NN network to use, if show_NN_network = TRUE
                 cell_color color for cells (see details)
                 color_as_factor convert color column to factor
                 cell_color_code named vector with colors
                  select_cell_groups select subset of cells/clusters based on cell_color param-
                 select cells select subset of cells based on cell IDs
                 show_other_cells display not selected cells
                 other_cell_color color of not selected cells
                 other_point_size size of not selected cells
                 show_cluster_center plot center of selected clusters
                 show_center_label plot label of selected clusters
                 center_point_size size of center points
                 label_size size of labels
                 edge_alpha column to use for alpha of the edges
                 point_size size of point (cell)
                 show_plot show plot
                 return_plot return ggplot object
                 save_plot directly save the plot [boolean]
                  save_param list of saving parameters, see showSaveParameters
```

### Details

Description of parameters.

#### Value

plotly

### See Also

```
Other reduced dimension visualizations: dimPlot2D(), dimPlot3D(), dimPlot(), plotPCA_2D(), plotPCA_3D(), plotPCA(), plotTSNE_2D(), plotTSNE(), plotUMAP_2D(), plotUMAP_3D(), plotUMAP()
```

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#### **Examples**

```
plotTSNE_3D(gobject)
```

plotUMAP

plotUMAP

## Description

Short wrapper for UMAP visualization

## Usage

```
plotUMAP(gobject, dim_reduction_name = "umap", default_save_name = "UMAP", ...)
```

```
gobject
                 giotto object
dim_reduction_name
                 name of UMAP
default_save_name
                 default save name of UMAP plot
                 Arguments passed on to dimPlot2D
. . .
                 group_by create multiple plots based on cell annotation column
                 group_by_subset subset the group_by factor column
                 dim1_to_use dimension to use on x-axis
                 dim2_to_use dimension to use on y-axis
                 spat_enr_names names of spatial enrichment results to include
                 show_NN_network show underlying NN network
                 nn_network_to_use type of NN network to use (kNN vs sNN)
                 network_name name of NN network to use, if show_NN_network = TRUE
                 cell_color color for cells (see details)
                 color_as_factor convert color column to factor
                 cell_color_code named vector with colors
                 cell_color_gradient vector with 3 colors for numeric data
                 gradient_midpoint midpoint for color gradient
                 gradient_limits vector with lower and upper limits
                 select_cell_groups select subset of cells/clusters based on cell_color param-
                      eter
                 select_cells select subset of cells based on cell IDs
                 show_other_cells display not selected cells
                 other_cell_color color of not selected cells
                 other_point_size size of not selected cells
                 show_cluster_center plot center of selected clusters
                 show_center_label plot label of selected clusters
                 center_point_size size of center points
```

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```
center_point_border_col border color of center points
center_point_border_stroke border stroke size of center points
label_size size of labels
label_fontface font of labels
edge_alpha column to use for alpha of the edges
point_shape point with border or not (border or no_border)
point_size size of point (cell)
point_alpha transparancy of point
point_border_col color of border around points
point_border_stroke stroke size of border around points
title title for plot, defaults to cell_color parameter
show_legend show legend
legend_text size of legend text
legend_symbol_size size of legend symbols
background_color color of plot background
axis_text size of axis text
axis_title size of axis title
cow_n_col cowplot param: how many columns
cow_rel_h cowplot param: relative height
cow_rel_w cowplot param: relative width
cow_align cowplot param: how to align
show_plot show plot
return_plot return ggplot object
save_plot directly save the plot [boolean]
save_param list of saving parameters, see showSaveParameters
```

#### **Details**

Description of parameters, see dimPlot2D. For 3D plots see plotUMAP\_3D

### Value

ggplot

#### See Also

```
Other reduced dimension visualizations: dimPlot2D(), dimPlot3D(), dimPlot(), plotPCA_2D(), plotPCA_3D(), plotPCA(), plotTSNE_2D(), plotTSNE_3D(), plotTSNE(), plotUMAP_2D(), plotUMAP_3D()
```

### **Examples**

```
plotUMAP(gobject)
```

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plotUMAP\_2D

plotUMAP\_2D

#### **Description**

Short wrapper for UMAP visualization

# Usage

```
plotUMAP_2D(
  gobject,
  dim_reduction_name = "umap",
  default_save_name = "UMAP_2D",
   ...
)
```

### **Arguments**

```
giotto object
gobject
dim_reduction_name
                 name of UMAP
default_save_name
                 default save name of UMAP plot
                 Arguments passed on to dimPlot2D
                 group_by create multiple plots based on cell annotation column
                 group_by_subset subset the group_by factor column
                 dim1_to_use dimension to use on x-axis
                 dim2_to_use dimension to use on y-axis
                 spat_enr_names names of spatial enrichment results to include
                 show_NN_network show underlying NN network
                 nn_network_to_use type of NN network to use (kNN vs sNN)
                 network_name name of NN network to use, if show_NN_network = TRUE
                 cell_color color for cells (see details)
                 color_as_factor convert color column to factor
                 cell_color_code named vector with colors
                 cell_color_gradient vector with 3 colors for numeric data
                 gradient_midpoint midpoint for color gradient
                 gradient_limits vector with lower and upper limits
                  select_cell_groups select subset of cells/clusters based on cell_color param-
                      eter
                 select_cells select subset of cells based on cell IDs
                 show_other_cells display not selected cells
                 other_cell_color color of not selected cells
                 other_point_size size of not selected cells
                 show_cluster_center plot center of selected clusters
                 show_center_label plot label of selected clusters
                 center_point_size size of center points
```

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```
center_point_border_col border color of center points
center_point_border_stroke border stroke size of center points
label_size size of labels
label_fontface font of labels
edge_alpha column to use for alpha of the edges
point_shape point with border or not (border or no_border)
point_size size of point (cell)
point_alpha transparancy of point
point_border_col color of border around points
point_border_stroke stroke size of border around points
title title for plot, defaults to cell_color parameter
show_legend show legend
legend_text size of legend text
legend_symbol_size size of legend symbols
background_color color of plot background
axis_text size of axis text
axis_title size of axis title
cow_n_col cowplot param: how many columns
cow_rel_h cowplot param: relative height
cow_rel_w cowplot param: relative width
cow_align cowplot param: how to align
show_plot show plot
return_plot return ggplot object
save_plot directly save the plot [boolean]
save_param list of saving parameters, see showSaveParameters
```

#### **Details**

Description of parameters, see dimPlot2D. For 3D plots see plotUMAP\_3D

### Value

ggplot

#### See Also

```
Other reduced dimension visualizations: dimPlot2D(), dimPlot3D(), dimPlot(), plotPCA_2D(), plotPCA_3D(), plotPCA(), plotTSNE_2D(), plotTSNE_3D(), plotTSNE(), plotUMAP_3D(), plotUMAP()
```

### **Examples**

```
plotUMAP_2D(gobject)
```

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plotUMAP\_3D

plotUMAP\_3D

#### **Description**

Visualize cells according to dimension reduction coordinates

# Usage

```
plotUMAP_3D(
  gobject,
  dim_reduction_name = "umap"
  default_save_name = "UMAP_3D",
)
```

### **Arguments**

```
giotto object
gobject
dim_reduction_name
                 name of UMAP
default_save_name
                 default save name of UMAP plot
                 Arguments passed on to dimPlot3D
                 dim1_to_use dimension to use on x-axis
                 dim2_to_use dimension to use on y-axis
                 dim3_to_use dimension to use on z-axis
                 spat_enr_names names of spatial enrichment results to include
                 show_NN_network show underlying NN network
                 nn_network_to_use type of NN network to use (kNN vs sNN)
                 network_name name of NN network to use, if show_NN_network = TRUE
                 cell_color color for cells (see details)
                 color_as_factor convert color column to factor
                 cell_color_code named vector with colors
                 select_cell_groups select subset of cells/clusters based on cell_color param-
                 select cells select subset of cells based on cell IDs
                 show_other_cells display not selected cells
                 other_cell_color color of not selected cells
                 other_point_size size of not selected cells
                 show_cluster_center plot center of selected clusters
                 show_center_label plot label of selected clusters
                 center_point_size size of center points
                 label_size size of labels
                 edge_alpha column to use for alpha of the edges
                 point_size size of point (cell)
                 show_plot show plot
```

print.giotto 185

```
return_plot return ggplot object
save_plot directly save the plot [boolean]
save_param list of saving parameters, see showSaveParameters
```

#### **Details**

Description of parameters.

#### Value

plotly

#### See Also

```
Other reduced dimension visualizations: dimPlot2D(), dimPlot3D(), dimPlot(), plotPCA_2D(), plotPCA_3D(), plotPCA(), plotTSNE_2D(), plotTSNE_3D(), plotTSNE(), plotUMAP_2D(), plotUMAP()
```

### **Examples**

```
plotUMAP_3D(gobject)
```

print.giotto

Prints giotto object.

## Description

Prints giotto object

## Usage

```
## S3 method for class 'giotto'
print(object, nr_genes = 5, nr_cells = 5)
```

## **Arguments**

object giotto object

nr\_genes number of genes (rows) to print
nr\_cells number of cells (columns) to print

rankEnrich

rankEnrich

### **Description**

Function to calculate gene signature enrichment scores per spatial position using a rank based approach.

## Usage

```
rankEnrich(...)
```

### **Arguments**

... Arguments passed on to runRankEnrich

gobject Giotto object

sign\_matrix Matrix of signature genes for each cell type / process

expression\_values expression values to use

reverse\_log\_scale reverse expression values from log scale

logbase log base to use if reverse\_log\_scale = TRUE

 $\verb"output_enrichment" how to return enrichment output"$ 

p\_value calculate p-values (boolean, default = FALSE)
n\_times number of permutations to calculate for p\_value

name to give to spatial enrichment results, default = rank

return\_gobject return giotto object

## See Also

runRankEnrich

 ${\tt rankSpatialCorGroups} \quad {\tt rankSpatialCorGroups}$ 

### Description

Rank spatial correlated clusters according to correlation structure

# Usage

```
rankSpatialCorGroups(
  gobject,
  spatCorObject,
  use_clus_name = NULL,
  show_plot = NA,
  return_plot = FALSE,
  save_plot = NA,
  save_param = list(),
  default_save_name = "rankSpatialCorGroups"
)
```

readExprMatrix 187

### **Arguments**

gobject giotto object

spatCorObject spatial correlation object

use\_clus\_name name of clusters to visualize (from clusterSpatialCorGenes())

show\_plot show plot

return\_plot return ggplot object

save\_plot directly save the plot [boolean]

save\_param list of saving parameters, see showSaveParameters

default\_save\_name

default save name for saving, don't change, change save\_name in save\_param

#### Value

data.table with positive (within group) and negative (outside group) scores

## **Examples**

rankSpatialCorGroups(gobject)

readExprMatrix readExprMatrix

## Description

Function to read an expression matrix into a sparse matrix.

# Usage

```
readExprMatrix(path, cores = NA, transpose = FALSE)
```

# Arguments

path path to the expression matrix cores number of cores to use transpose matrix

#### **Details**

The expression matrix needs to have both unique column names and row names

#### Value

sparse matrix

### **Examples**

readExprMatrix()

188 removeCellAnnotation

```
{\tt readGiottoInstructions}
```

readGiottoInstrunctions

## Description

Retrieves the instruction associated with the provided parameter

## Usage

```
readGiottoInstructions(giotto_instructions, param = NULL)
```

### **Arguments**

```
giotto_instructions
```

giotto object or result from createGiottoInstructions()

param parameter to retrieve

#### Value

specific parameter

## **Examples**

readGiottoInstrunctions()

removeCellAnnotation removeCellAnnotation

# Description

removes cell annotation of giotto object

## Usage

```
removeCellAnnotation(gobject, columns = NULL, return_gobject = TRUE)
```

### **Arguments**

gobject giotto object

columns names of columns to remove

return\_gobject boolean: return giotto object (default = TRUE)

### **Details**

if return\_gobject = FALSE, it will return the cell metadata

#### Value

giotto object

removeGeneAnnotation 189

### **Examples**

```
removeCellAnnotation(gobject)
```

removeGeneAnnotation removeGeneAnnotation

## Description

removes gene annotation of giotto object

### Usage

```
removeGeneAnnotation(gobject, columns = NULL, return_gobject = TRUE)
```

### **Arguments**

gobject giotto object

columns names of columns to remove

return\_gobject boolean: return giotto object (default = TRUE)

#### **Details**

if return\_gobject = FALSE, it will return the gene metadata

### Value

giotto object

### **Examples**

removeGeneAnnotation(gobject)

replaceGiottoInstructions

replace Giot to Instructions

### **Description**

Function to replace all instructions from giotto object

#### Usage

```
replaceGiottoInstructions(gobject, instructions = NULL)
```

## Arguments

gobject giotto object

instructions new instructions (e.g. result from createGiottoInstructions)

runDWLSDeconv

#### Value

giotto object with replaces instructions

## **Examples**

```
replaceGiottoInstructions()
```

runDWLSDeconv

runDWLSDeconv

### **Description**

Function to perform DWLS deconvolution based on single cell expression data

## Usage

```
runDWLSDeconv(
  gobject,
  expression_values = c("normalized"),
  logbase = 2,
  cluster_column = "leiden_clus",
  sign_matrix,
  n_cell = 50,
  cutoff = 2,
  name = NULL,
  return_gobject = TRUE
)
```

### **Arguments**

```
gobject
                  giotto object
expression_values
                  expression values to use
logbase
                  base used for log normalization
cluster_column name of cluster column
                  sig matrix for deconvolution
sign_matrix
                  number of cells per spot
n_cell
cutoff
                  cut off (default = 2)
                  name to give to spatial deconvolution results, default = DWLS
name
return_gobject return giotto object
```

# Value

giotto object or deconvolution results

```
runHyperGeometricEnrich
```

runHyperGeometricEnrich

### **Description**

Function to calculate gene signature enrichment scores per spatial position using a hypergeometric test.

### Usage

```
runHyperGeometricEnrich(
  gobject,
  sign_matrix,
  expression_values = c("normalized", "scaled", "custom"),
  reverse_log_scale = TRUE,
  logbase = 2,
  top_percentage = 5,
  output_enrichment = c("original", "zscore"),
  p_value = FALSE,
  name = NULL,
  return_gobject = TRUE
)
```

## **Arguments**

```
gobject
                  Giotto object
                  Matrix of signature genes for each cell type / process
sign_matrix
expression_values
                  expression values to use
reverse_log_scale
                  reverse expression values from log scale
logbase
                  log base to use if reverse_log_scale = TRUE
top_percentage percentage of cells that will be considered to have gene expression with matrix
                  binarization
output_enrichment
                  how to return enrichment output
                  calculate p-values (boolean, default = FALSE)
p_value
                  to give to spatial enrichment results, default = rank
return_gobject return giotto object
```

#### **Details**

The enrichment score is calculated based on the p-value from the hypergeometric test,  $-\log 10$  (p-value).

#### Value

data.table with enrichment results

192 runPAGEEnrich

runPAGEEnrich

runPAGEEnrich

### **Description**

Function to calculate gene signature enrichment scores per spatial position using PAGE.

### Usage

```
runPAGEEnrich(
  gobject,
  sign_matrix,
  expression_values = c("normalized", "scaled", "custom"),
  reverse_log_scale = TRUE,
  logbase = 2,
  output_enrichment = c("original", "zscore"),
  p_value = FALSE,
  n_times = 1000,
  name = NULL,
  return_gobject = TRUE
)
```

#### **Arguments**

```
Giotto object
gobject
sign_matrix
                  Matrix of signature genes for each cell type / process
expression_values
                  expression values to use
reverse_log_scale
                  reverse expression values from log scale
logbase
                  log base to use if reverse_log_scale = TRUE
output_enrichment
                  how to return enrichment output
                  calculate p-values (boolean, default = FALSE)
p_value
                  number of permutations to calculate for p_value
n_times
                  to give to spatial enrichment results, default = PAGE
name
return_gobject return giotto object
```

#### **Details**

sign\_matrix: a binary matrix with genes as row names and cell-types as column names. Alternatively a list of signature genes can be provided to makeSignMatrixPAGE, which will create the matrix for you.

The enrichment Z score is calculated by using method (PAGE) from Kim SY et al., BMC bioinformatics, 2005 as  $Z=((Sm^{\vee}mu)*m^{(1/2)})/delta$ . For each gene in each spot, mu is the fold change values versus the mean expression and delta is the standard deviation. Sm is the mean fold change value of a specific marker gene set and m is the size of a given marker gene set.

runPatternSimulation 193

#### Value

data.table with enrichment results

#### See Also

```
makeSignMatrixPAGE
```

runPatternSimulation runPatternSimulation

### Description

Creates a known spatial pattern for selected genes one-by-one and runs the different spatial gene detection tests

## Usage

```
runPatternSimulation(
  gobject,
  pattern_name = "pattern",
  pattern_cell_ids = NULL,
  gene_names = NULL,
  spatial_probs = c(0.5, 1),
  reps = 2,
  save_plot = T,
  save_dir = "~",
  max_col = 4,
  height = 7,
  width = 7,
  ...
)
```

### **Arguments**

```
giotto object
gobject
pattern_name
                  name of spatial pattern
pattern_cell_ids
                  cell ids that make up the spatial pattern
                  selected genes
gene_names
                  probabilities to test for a high expressing gene value to be part of the spatial
spatial_probs
                  pattern
                  number of random simulation repititions
reps
save_plot
                  save intermediate random simulation plots or not
save_dir
                  directory to save results to
max_col
                  maximum number of columns for final plots
                  height of final plots
height
width
                  width of final plots
                  additional parameters for spatial gene detection tests
. . .
```

194 runPCA

#### Value

data.table with results

#### **Examples**

runPatternSimulation(gobject)

runPCA

runPCA

### **Description**

runs a Principal Component Analysis

### Usage

```
runPCA(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  reduction = c("cells", "genes"),
  name = "pca",
  genes_to_use = "hvg",
  return_gobject = TRUE,
  center = F,
  scale_unit = F,
  ncp = 100,
  method = c("irlba", "factominer"),
  rev = FALSE,
  verbose = TRUE,
  ...
)
```

### **Arguments**

```
gobject
                  giotto object
expression_values
                  expression values to use
reduction
                  cells or genes
                  arbitrary name for PCA run
name
                  subset of genes to use for PCA
genes_to_use
return_gobject boolean: return giotto object (default = TRUE)
                  center data first (default = FALSE)
center
scale_unit
                  scale features before PCA (default = FALSE)
                  number of principal components to calculate
ncp
method
                  which implementation to use
                  do a reverse PCA
rev
                  verbosity of the function
verbose
                  additional parameters for PCA (see details)
. . .
```

runRankEnrich 195

#### **Details**

See prcomp\_irlba and PCA for more information about other parameters.

- genes\_to\_use = NULL: will use all genes from the selected matrix
- genes\_to\_use = <hvg name>: can be used to select a column name of highly variable genes, created by (see calculateHVG)
- genes\_to\_use = c('geneA', 'geneB', ...): will use all manually provided genes

#### Value

giotto object with updated PCA dimension recuction

### **Examples**

```
# 1. create giotto object
expr_path = system.file("extdata", "seqfish_field_expr.txt", package = 'Giotto')
loc_path = system.file("extdata", "seqfish_field_locs.txt", package = 'Giotto')
VC_small <- createGiottoObject(raw_exprs = expr_path, spatial_locs = loc_path)

# 2. normalize giotto
VC_small <- normalizeGiotto(gobject = VC_small, scalefactor = 6000)
VC_small <- addStatistics(gobject = VC_small)

# 3. dimension reduction
VC_small <- calculateHVG(gobject = VC_small)
VC_small <- runPCA(gobject = VC_small)
plotPCA(VC_small)</pre>
```

 ${\tt runRankEnrich}$ 

runRankEnrich

#### **Description**

Function to calculate gene signature enrichment scores per spatial position using a rank based approach.

#### Usage

```
runRankEnrich(
  gobject,
  sign_matrix,
  expression_values = c("normalized", "scaled", "custom"),
  reverse_log_scale = TRUE,
  logbase = 2,
  output_enrichment = c("original", "zscore"),
  p_value = FALSE,
  n_times = 1000,
  name = NULL,
  return_gobject = TRUE
)
```

196 runSpatialDeconv

#### **Arguments**

gobject Giotto object

sign\_matrix Matrix of signature genes for each cell type / process

expression\_values

expression values to use

reverse\_log\_scale

reverse expression values from log scale

logbase log base to use if reverse\_log\_scale = TRUE

output\_enrichment

how to return enrichment output

p\_value calculate p-values (boolean, default = FALSE)

n\_times number of permutations to calculate for p\_value

name to give to spatial enrichment results, default = rank

return\_gobject return giotto object

#### **Details**

sign\_matrix: a rank-fold matrix with genes as row names and cell-types as column names. Alternatively a scRNA-seq matrix and vector with clusters can be provided to makeSignMatrixRank, which will create the matrix for you.

First a new rank is calculated as  $R = (R1*R2)^{(1/2)}$ , where R1 is the rank of fold-change for each gene in each spot and R2 is the rank of each marker in each cell type. The Rank-Biased Precision is then calculated as:  $RBP = (1 - 0.99) * (0.99)^{(R - 1)}$  and the final enrichment score is then calculated as the sum of top 100 RBPs.

### Value

data.table with enrichment results

### See Also

 ${\it make Sign Matrix Rank}$ 

runSpatialDeconv

runSpatialDeconv

## Description

Function to perform deconvolution based on single cell expression data

runSpatialEnrich 197

#### Usage

```
runSpatialDeconv(
  gobject,
  deconv_method = c("DWLS"),
  expression_values = c("normalized"),
  logbase = 2,
  cluster_column = "leiden_clus",
  sign_matrix,
  n_cell = 50,
  cutoff = 2,
  name = NULL,
  return_gobject = TRUE
)
```

#### **Arguments**

```
giotto object
gobject
                  method to use for deconvolution
deconv_method
expression_values
                  expression values to use
logbase
                  base used for log normalization
cluster_column name of cluster column
                  signature matrix for deconvolution
sign_matrix
n_cell
                  number of cells per spot
cutoff
                  cut off (default = 2)
                  name to give to spatial deconvolution results
name
return_gobject return giotto object
```

#### Value

giotto object or deconvolution results

 $run Spatial Enrich \\ run Spatial Enrich$ 

## Description

Function to calculate gene signature enrichment scores per spatial position using an enrichment test.

### Usage

```
runSpatialEnrich(
  gobject,
  enrich_method = c("PAGE", "rank", "hypergeometric"),
  sign_matrix,
  expression_values = c("normalized", "scaled", "custom"),
  reverse_log_scale = TRUE,
  logbase = 2,
```

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```
p_value = FALSE,
n_times = 1000,
top_percentage = 5,
output_enrichment = c("original", "zscore"),
name = NULL,
return_gobject = TRUE
)
```

### **Arguments**

gobject Giotto object method for gene signature enrichment calculation enrich\_method Matrix of signature genes for each cell type / process sign\_matrix expression\_values expression values to use reverse\_log\_scale reverse expression values from log scale logbase log base to use if reverse\_log\_scale = TRUE calculate p-value (default = FALSE) p\_value (page/rank) number of permutation iterations to calculate p-value n\_times (hyper) percentage of cells that will be considered to have gene expression with top\_percentage matrix binarization output\_enrichment how to return enrichment output to give to spatial enrichment results, default = PAGE

### **Details**

For details see the individual functions:

return\_gobject return giotto object

PAGE: runPAGEEnrichRank: runRankEnrich

• Hypergeometric: runHyperGeometricEnrich

## Value

Giotto object or enrichment results if return\_gobject = FALSE

runtSNE	runtSNE		

## Description

run tSNE

runtSNE 199

#### Usage

```
runtSNE(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  reduction = c("cells", "genes"),
 dim_reduction_to_use = "pca",
 dim_reduction_name = "pca",
 dimensions_to_use = 1:10,
 name = "tsne",
 genes_to_use = NULL,
 return_gobject = TRUE,
 dims = 2,
 perplexity = 30,
  theta = 0.5,
 do_PCA_first = F,
  set\_seed = T,
  seed_number = 1234,
 verbose = TRUE,
)
```

### **Arguments**

. . .

```
gobject
                 giotto object
expression_values
                 expression values to use
reduction
                 cells or genes
dim_reduction_to_use
                 use another dimension reduction set as input
dim_reduction_name
                 name of dimension reduction set to use
dimensions_to_use
                 number of dimensions to use as input
                 arbitrary name for tSNE run
name
                 if dim_reduction_to_use = NULL, which genes to use
genes_to_use
return_gobject boolean: return giotto object (default = TRUE)
dims
                 tSNE param: number of dimensions to return
                 tSNE param: perplexity
perplexity
                 tSNE param: theta
theta
                 tSNE param: do PCA before tSNE (default = FALSE)
do_PCA_first
set_seed
                 use of seed
                 seed number to use
seed_number
                 verbosity of the function
verbose
                 additional tSNE parameters
```

200 runUMAP

#### **Details**

See Rtsne for more information about these and other parameters.

- Input for tSNE dimension reduction can be another dimension reduction (default = 'pca')
- To use gene expression as input set dim\_reduction\_to\_use = NULL
- If dim\_reduction\_to\_use = NULL, genes\_to\_use can be used to select a column name of highly variable genes (see calculateHVG) or simply provide a vector of genes
- multiple tSNE results can be stored by changing the *name* of the analysis

#### Value

giotto object with updated tSNE dimension recuction

#### **Examples**

```
# 1. create giotto object
expr_path = system.file("extdata", "seqfish_field_expr.txt", package = 'Giotto')
loc_path = system.file("extdata", "seqfish_field_locs.txt", package = 'Giotto')
VC_small <- createGiottoObject(raw_exprs = expr_path, spatial_locs = loc_path)
# 2. normalize giotto
VC_small <- normalizeGiotto(gobject = VC_small, scalefactor = 6000)
VC_small <- addStatistics(gobject = VC_small)
# 3. dimension reduction
VC_small <- calculateHVG(gobject = VC_small)
VC_small <- runPCA(gobject = VC_small)
VC_small <- runTSNE(VC_small, dimensions_to_use = 1:5, n_threads = 2)
plotTSNE(gobject = VC_small)</pre>
```

runUMAP

runUMAP

#### **Description**

run UMAP

## Usage

```
runUMAP(
   gobject,
   expression_values = c("normalized", "scaled", "custom"),
   reduction = c("cells", "genes"),
   dim_reduction_to_use = "pca",
   dim_reduction_name = "pca",
   dimensions_to_use = 1:10,
   name = "umap",
   genes_to_use = NULL,
   return_gobject = TRUE,
   n_neighbors = 40,
```

runUMAP 201

```
n_components = 2,
n_epochs = 400,
min_dist = 0.01,
n_threads = 1,
spread = 5,
set_seed = T,
seed_number = 1234,
verbose = T,
...
)
```

#### **Arguments**

```
gobject
                 giotto object
expression_values
                 expression values to use
reduction
                 cells or genes
dim_reduction_to_use
                 use another dimension reduction set as input
dim_reduction_name
                 name of dimension reduction set to use
dimensions_to_use
                 number of dimensions to use as input
                 arbitrary name for UMAP run
name
                 if dim_reduction_to_use = NULL, which genes to use
genes_to_use
return_gobject boolean: return giotto object (default = TRUE)
n_neighbors
                 UMAP param: number of neighbors
n_components
                 UMAP param: number of components
                 UMAP param: number of epochs
n_epochs
min_dist
                 UMAP param: minimum distance
                 UMAP param: threads to use
n_threads
spread
                 UMAP param: spread
set_seed
                 use of seed
seed_number
                 seed number to use
verbose
                 verbosity of function
                 additional UMAP parameters
```

## Details

See umap for more information about these and other parameters.

- Input for UMAP dimension reduction can be another dimension reduction (default = 'pca')
- To use gene expression as input set dim\_reduction\_to\_use = NULL
- If dim\_reduction\_to\_use = NULL, genes\_to\_use can be used to select a column name of highly variable genes (see calculateHVG) or simply provide a vector of genes
- multiple UMAP results can be stored by changing the *name* of the analysis

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#### Value

giotto object with updated UMAP dimension recuction

### **Examples**

```
# 1. create giotto object
expr_path = system.file("extdata", "seqfish_field_expr.txt", package = 'Giotto')
loc_path = system.file("extdata", "seqfish_field_locs.txt", package = 'Giotto')
VC_small <- createGiottoObject(raw_exprs = expr_path, spatial_locs = loc_path)
# 2. normalize giotto
VC_small <- normalizeGiotto(gobject = VC_small, scalefactor = 6000)
VC_small <- addStatistics(gobject = VC_small)
# 3. dimension reduction
VC_small <- calculateHVG(gobject = VC_small)
VC_small <- runPCA(gobject = VC_small)
VC_small <- runUMAP(VC_small, dimensions_to_use = 1:5, n_threads = 2)
plotUMAP(gobject = VC_small)</pre>
```

screePlot

screePlot

### Description

identify significant prinicipal components (PCs) using an screeplot (a.k.a. elbowplot)

# Usage

```
screePlot(
  gobject,
  name = "pca",
  expression_values = c("normalized", "scaled", "custom"),
  reduction = c("cells", "genes"),
  method = c("irlba", "factominer"),
  rev = FALSE,
  genes_to_use = NULL,
  center = F,
  scale_unit = F,
  ncp = 100,
  ylim = c(0, 20),
  verbose = T,
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "screePlot",
)
```

screePlot 203

#### **Arguments**

gobject giotto object

name of PCA object if available

expression\_values

expression values to use

reduction cells or genes

method which implementation to use

rev do a reverse PCA

genes\_to\_use subset of genes to use for PCA

center center data before PCA
scale\_unit scale features before PCA

ncp number of principal components to calculate

ylim y-axis limits on scree plot

verbose verobsity show\_plot show plot

return\_plot return ggplot object

save\_plot directly save the plot [boolean]

save\_param list of saving parameters from all\_plots\_save\_function()

default\_save\_name

default save name for saving, don't change, change save\_name in save\_param

... additional arguments to pca function, see runPCA

#### **Details**

Screeplot works by plotting the explained variance of each individual PC in a barplot allowing you to identify which PC provides a significant contribution (a.k.a 'elbow method').

Screeplot will use an available pca object, based on the parameter 'name', or it will create it if it's not available (see runPCA)

#### Value

ggplot object for scree method

## **Examples**

screePlot(gobject)

204 selectPatternGenes

selectPatternGenes

selectPatternGenes

## Description

Select genes correlated with spatial patterns

### Usage

```
selectPatternGenes(
   spatPatObj,
   dimensions = 1:5,
   top_pos_genes = 10,
   top_neg_genes = 10,
   min_pos_cor = 0.5,
   min_neg_cor = -0.5,
   return_top_selection = FALSE
)
```

### **Arguments**

```
spatPatObj Output from detectSpatialPatterns
dimensions dimensions to identify correlated genes for.
top_pos_genes Top positively correlated genes.
top_neg_genes Top negatively correlated genes.
min_pos_cor Minimum positive correlation score to include a gene.
min_neg_cor Minimum negative correlation score to include a gene.
return_top_selection
only return selection based on correlation criteria (boolean)
```

### Details

Description.

#### Value

Data.table with genes associated with selected dimension (PC).

### **Examples**

```
selectPatternGenes(gobject)
```

show, giotto-method 205

show, giotto-method

show method for giotto class

## Description

show method for giotto class

### Usage

```
## S4 method for signature 'giotto'
show(object)
```

### **Arguments**

object giotto object

showClusterDendrogram showClusterDendrogram

### **Description**

Creates dendrogram for selected clusters.

### Usage

```
showClusterDendrogram(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  cluster_column,
  cor = c("pearson", "spearman"),
  distance = "ward.D",
  h = NULL,
  h_color = "red",
  rotate = FALSE,
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "showClusterDendrogram",
  ...
)
```

# Arguments

```
gobject giotto object
expression_values
expression values to use
cluster_column name of column to use for clusters
cor correlation score to calculate distance
```

206 showClusterHeatmap

```
distance method to use for hierarchical clustering
distance
h
                  height of horizontal lines to plot
h_color
                  color of horizontal lines
                  rotate dendrogram 90 degrees
rotate
show_plot
                  show plot
return_plot
                  return ggplot object
save_plot
                  directly save the plot [boolean]
                  list of saving parameters, see showSaveParameters
save_param
default_save_name
                  default save name for saving, don't change, change save_name in save_param
                  additional parameters for ggdendrogram()
```

#### **Details**

Expression correlation dendrogram for selected clusters.

#### Value

ggplot

### **Examples**

showClusterDendrogram(gobject)

 $show Cluster Heatmap \hspace{30mm} show Cluster Heatmap \\$ 

## Description

Creates heatmap based on identified clusters

## Usage

```
showClusterHeatmap(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  genes = "all",
  cluster_column,
  cor = c("pearson", "spearman"),
  distance = "ward.D",
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "showClusterHeatmap",
  ...
)
```

#### **Arguments**

gobject giotto object

expression\_values

expression values to use

genes vector of genes to use, default to 'all' cluster\_column name of column to use for clusters cor correlation score to calculate distance

distance distance method to use for hierarchical clustering

show\_plot show plot

return\_plot return ggplot object

save\_plot directly save the plot [boolean]

save\_param list of saving parameters, see showSaveParameters

default\_save\_name

default save name for saving, don't change, change save\_name in save\_param

... additional parameters for the Heatmap function from ComplexHeatmap

#### **Details**

Correlation heatmap of selected clusters.

#### Value

ggplot

#### **Examples**

showClusterHeatmap(gobject)

showGiottoImageNames showGiottoImageNames

## **Description**

Prints the available giotto images that are attached to the Giotto object

#### Usage

```
showGiottoImageNames(gobject, verbose = TRUE)
```

### **Arguments**

gobject a giotto object verbose verbosity of function

#### Value

a vector of giotto image names attached to the giotto object

#### **Examples**

showGiottoImageNames(gobject)

208 showGrids

```
showGiottoInstructions
```

show Giot to Instructions

# Description

Function to display all instructions from giotto object

# Usage

```
showGiottoInstructions(gobject)
```

## Arguments

gobject

giotto object

## Value

named vector with giotto instructions

## **Examples**

showGiottoInstructions()

showGrids

showGrids

## Description

Prints the available spatial grids that are attached to the Giotto object

# Usage

```
showGrids(gobject, verbose = TRUE)
```

## Arguments

gobject

a giotto object

verbose

verbosity of function#'

# Value

vector

# Examples

showGrids()

showNetworks 209

showNetworks

showNetworks

### **Description**

Prints the available spatial networks that are attached to the Giotto object

#### Usage

```
showNetworks(gobject, verbose = TRUE)
```

## Arguments

gobject a giotto object

verbose verbosity of function#'

### Value

vector

## **Examples**

showNetworks()

showPattern

showPattern

### Description

show patterns for 2D spatial data

## Usage

```
showPattern(gobject, spatPatObj, ...)
```

## Arguments

gobject giotto object

spatPatObj Output from detectSpatialPatterns

... Arguments passed on to showPattern2D

dimension dimension to plot trim Trim ends of the PC values.

background\_color background color for plot

grid\_border\_color color for grid
show\_legend show legend of ggplot

point\_size size of points
show\_plot show plot

return\_plot return ggplot object

210 showPattern2D

```
save_plot directly save the plot [boolean]
save_param list of saving parameters, see showSaveParameters
default_save_name default save name for saving, don't change, change save_name
in save_param
```

### Value

ggplot

#### See Also

showPattern2D

### **Examples**

showPattern(gobject)

 ${\it showPattern2D}$ 

showPattern2D

### **Description**

show patterns for 2D spatial data

### Usage

```
showPattern2D(
  gobject,
  spatPatObj,
  dimension = 1,
  trim = c(0.02, 0.98),
  background_color = "white",
  grid_border_color = "grey",
  show_legend = T,
  point_size = 1,
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "showPattern2D"
)
```

### Arguments

gobject giotto object

 ${\tt spatPatObj} \qquad {\tt Output} \ from \ detectSpatial Patterns$ 

dimension dimension to plot

trim Trim ends of the PC values.

background\_color

background color for plot

showPattern3D 211

```
grid_border_color
                  color for grid
show_legend
                  show legend of ggplot
point_size
                  size of points
show_plot
                  show plot
                  return ggplot object
return_plot
save_plot
                  directly save the plot [boolean]
save_param
                  list of saving parameters, see showSaveParameters
default_save_name
                  default save name for saving, don't change, change save_name in save_param
```

#### Value

ggplot

### **Examples**

showPattern2D(gobject)

showPattern3D

showPattern3D

## Description

show patterns for 3D spatial data

### Usage

```
showPattern3D(
 gobject,
 spatPatObj,
 dimension = 1,
  trim = c(0.02, 0.98),
 background_color = "white",
 grid_border_color = "grey",
 show_legend = T,
 point_size = 1,
 axis_scale = c("cube", "real", "custom"),
 custom_ratio = NULL,
 x_ticks = NULL,
 y_ticks = NULL,
 z_ticks = NULL,
 show_plot = NA,
 return_plot = NA,
 save_plot = NA,
 save_param = list(),
 default_save_name = "showPattern3D"
)
```

212 showPatternGenes

### **Arguments**

gobject giotto object

spatPatObj Output from detectSpatialPatterns

dimension dimension to plot

trim Trim ends of the PC values.

background\_color

background color for plot

grid\_border\_color

color for grid

show\_legend show legend of plot point\_size adjust the point size

axis\_scale scale the axis

custom\_ratio cutomize the scale of the axis

x\_ticks the tick number of x\_axis
y\_ticks the tick number of y\_axis
z\_ticks the tick number of z\_axis

show\_plot show plot

return\_plot return plot object

save\_plot directly save the plot [boolean]

save\_param list of saving parameters, see showSaveParameters

default\_save\_name

default save name for saving, don't change, change save\_name in save\_param

#### Value

plotly

## **Examples**

showPattern3D(gobject)

showPatternGenes

showPatternGenes

## Description

show genes correlated with spatial patterns

showPatternGenes 213

## Usage

```
showPatternGenes(
  gobject,
  spatPatObj,
  dimension = 1,
  top_pos_genes = 5,
  top_neg_genes = 5,
  point_size = 1,
  return_DT = FALSE,
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "showPatternGenes"
)
```

# Arguments

gobject	giotto object		
spatPatObj	Output from detectSpatialPatterns		
dimension	dimension to plot genes for.		
top_pos_genes	Top positively correlated genes.		
top_neg_genes	Top negatively correlated genes.		
point_size	size of points		
return_DT	if TRUE, it will return the data.table used to generate the plots		
show_plot	show plot		
return_plot	return ggplot object		
save_plot	directly save the plot [boolean]		
save_param	list of saving parameters, see showSaveParameters		
default_save_name			
	default save name for saving, don't change, change save_name in save_param		

### Value

ggplot

## **Examples**

```
showPatternGenes(gobject)
```

214 showSaveParameters

 $show Processing Steps \qquad \textit{show Processing Steps}$ 

## Description

shows the sequential processing steps that were performed in a summarized format

# Usage

```
showProcessingSteps(gobject)
```

## Arguments

gobject

giotto object

#### Value

list of processing steps and names

## **Examples**

showProcessingSteps(gobject)

showSaveParameters

showSaveParameters

# Description

Description of Giotto saving options, links to all\_plots\_save\_function

## Usage

```
showSaveParameters()
```

#### Value

Instruction on how to use the automatic plot saving options within Giotto

# **Examples**

showSaveParameters()

showSpatialCorGenes 215

showSpatialCorGenes showSpatialCorGenes

## Description

Shows and filters spatially correlated genes

### Usage

```
showSpatialCorGenes(
  spatCorObject,
  use_clus_name = NULL,
  selected_clusters = NULL,
  genes = NULL,
  min_spat_cor = 0.5,
  min_expr_cor = NULL,
  min_cor_diff = NULL,
  min_rank_diff = NULL,
  show_top_genes = NULL
)
```

### **Arguments**

```
spatCorObject
                  spatial correlation object
use_clus_name
                  cluster information to show
selected_clusters
                  subset of clusters to show
                  subset of genes to show
genes
                  filter on minimum spatial correlation
min_spat_cor
                  filter on minimum single-cell expression correlation
min_expr_cor
                  filter on minimum correlation difference (spatial vs expression)
min_cor_diff
                  filter on minimum correlation rank difference (spatial vs expression)
min_rank_diff
show_top_genes show top genes per gene
```

## Value

data.table with filtered information

# Examples

```
\verb|showSpatialCorGenes(gobject)|
```

216 signPCA

signPCA signPCA

#### **Description**

identify significant prinicipal components (PCs)

### Usage

```
signPCA(
 gobject,
 name = "pca",
 method = c("screeplot", "jackstraw"),
 expression_values = c("normalized", "scaled", "custom"),
 reduction = c("cells", "genes"),
 pca_method = c("irlba", "factominer"),
  rev = FALSE,
  genes_to_use = NULL,
  center = T,
  scale_unit = T,
 ncp = 50,
  scree_ylim = c(0, 10),
  jack_iter = 10,
  jack_threshold = 0.01,
  jack_ylim = c(0, 1),
  verbose = TRUE,
  show_plot = NA,
 return_plot = NA,
 save_plot = NA,
 save_param = list(),
 default_save_name = "signPCA"
)
```

## Arguments

gobject

name of PCA object if available name method to use to identify significant PCs method expression\_values expression values to use reduction cells or genes pca\_method which implementation to use do a reverse PCA rev subset of genes to use for PCA genes\_to\_use center center data before PCA scale features before PCA scale\_unit number of principal components to calculate ncp y-axis limits on scree plot scree\_ylim

giotto object

silhouetteRank 217

```
jack_iter
                  number of interations for jackstraw
jack_threshold p-value threshold to call a PC significant
jack_ylim
                  y-axis limits on jackstraw plot
verbose
                  verbosity
show_plot
                  show plot
return_plot
                  return ggplot object
                  directly save the plot [boolean]
save_plot
                  list of saving parameters from all_plots_save_function()
save_param
default_save_name
```

default save name for saving, don't change, change save\_name in save\_param

#### **Details**

Two different methods can be used to assess the number of relevant or significant prinicipal components (PC's).

- 1. Screeplot works by plotting the explained variance of each individual PC in a barplot allowing you to identify which PC provides a significant contribution (a.k.a. 'elbow method').
- 2. The Jackstraw method uses the permutationPA function. By systematically permuting genes it identifies robust, and thus significant, PCs.

#### Value

ggplot object for scree method and maxtrix of p-values for jackstraw

# **Examples**

```
signPCA(gobject)
```

silhouetteRank

silhouetteRank

## **Description**

Previously: calculate\_spatial\_genes\_python. This method computes a silhouette score per gene based on the spatial distribution of two partitions of cells (expressed L1, and non-expressed L0). Here, rather than L2 Euclidean norm, it uses a rank-transformed, exponentially weighted function to represent the local physical distance between two cells.

```
silhouetteRank(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  metric = "euclidean",
  subset_genes = NULL,
  rbp_p = 0.95,
  examine_top = 0.3,
  python_path = NULL
)
```

#### **Arguments**

```
gobject giotto object
expression_values
expression values to use

metric distance metric to use

subset_genes only run on this subset of genes
rbp_p fractional binarization threshold
examine_top top fraction to evaluate with silhouette
python_path specify specific path to python if required
```

#### Value

data.table with spatial scores

#### **Examples**

```
silhouetteRank(gobject)
```

```
simulate One Gene Pattern Giotto Object \\ simulate One Gene Pattern Giotto Object
```

# Description

Create a simulated spatial pattern for one selected gnee

#### Usage

```
simulateOneGenePatternGiottoObject(
  gobject,
  pattern_name = "pattern",
  pattern_cell_ids = NULL,
  gene_name = NULL,
  spatial_prob = 0.95,
  gradient_direction = NULL,
  show_pattern = TRUE,
  pattern_colors = c(`in` = "green", out = "red"),
  ...
)
```

# Arguments

spark 219

```
gradient_direction
direction of gradient

show_pattern show the discrete spatial pattern

pattern_colors 2 color vector for the spatial pattern

... additional parameters for (re-)normalizing
```

#### Value

Reprocessed Giotto object for which one gene has a forced spatial pattern

#### **Examples**

```
simulateOneGenePatternGiottoObject(gobject)
```

spark spark

#### **Description**

Compute spatially expressed genes with SPARK method

# Usage

```
spark(
  gobject,
  percentage = 0.1,
  min_count = 10,
  expression_values = "raw",
  num_core = 5,
  covariates = NULL,
  return_object = "data.table",
  ...
)
```

# Arguments

gobject giotto object percentage The percentage of cells that are expressed for analysis minimum number of counts for a gene to be included min\_count expression\_values type of values to use (raw by default) number of cores to use num\_core The covariates in experiments, i.e. confounding factors/batch effect. Column covariates name of giotto cell metadata. return\_object type of result to return (data.table or spark object) Additional parameters to the spark.vc function

220 spatCellCellcom

#### **Details**

This function is a wrapper for the method implemented in the SPARK package:

- 1. CreateSPARKObject create a SPARK object from a Giotto object
- 2. spark.vc Fits the count-based spatial model to estimate the parameters, see spark.vc for additional parameters
- 3. spark.test Testing multiple kernel matrices

#### Value

data.table with SPARK spatial genes results or the SPARK object

spatCellCellcom

spatCellCellcom

#### **Description**

Spatial Cell-Cell communication scores based on spatial expression of interacting cells

## Usage

```
spatCellCellcom(
 gobject,
  spatial_network_name = "Delaunay_network",
  cluster_column = "cell_types",
 random_iter = 1000,
 gene_set_1,
  gene_set_2,
  log2FC_addendum = 0.1,
 min_observations = 2,
 detailed = FALSE,
 adjust_method = c("fdr", "bonferroni", "BH", "holm", "hochberg", "hommel", "BY",
    "none"),
  adjust_target = c("genes", "cells"),
 do_parallel = TRUE,
 cores = NA,
  verbose = c("a little", "a lot", "none")
)
```

#### **Arguments**

spatCellCellcom 221

log2FC\_addendum

addendum to add when calculating log2FC

min\_observations

minimum number of interactions needed to be considered

detailed provide more detailed information (random variance and z-score)

adjust\_method which method to adjust p-values

adjust\_target adjust multiple hypotheses at the cell or gene level

do\_parallel run calculations in parallel with mclapply cores number of cores to use if do\_parallel = TRUE

verbose verbose

#### **Details**

Statistical framework to identify if pairs of genes (such as ligand-receptor combinations) are expressed at higher levels than expected based on a reshuffled null distribution of gene expression values in cells that are spatially in proximity to eachother..

- LR\_comb:Pair of ligand and receptor
- lig\_cell\_type: cell type to assess expression level of ligand
- lig\_expr: average expression of ligand in lig\_cell\_type
- · ligand: ligand name
- rec\_cell\_type: cell type to assess expression level of receptor
- rec\_expr: average expression of receptor in rec\_cell\_type
- receptor: receptor name
- LR\_expr: combined average ligand and receptor expression
- lig\_nr: total number of cells from lig\_cell\_type that spatially interact with cells from rec\_cell\_type
- rec\_nr: total number of cells from rec\_cell\_type that spatially interact with cells from lig\_cell\_type
- rand\_expr: average combined ligand and receptor expression from random spatial permutations
- av\_diff: average difference between LR\_expr and rand\_expr over all random spatial permutations
- sd\_diff: (optional) standard deviation of the difference between LR\_expr and rand\_expr over all random spatial permutations
- z\_score: (optinal) z-score
- log2fc: log2 fold-change (LR\_expr/rand\_expr)
- pvalue: p-value
- LR\_cell\_comb: cell type pair combination
- p.adj: adjusted p-value
- PI: significanc score: log2fc \* -log10(p.adj)

## Value

Cell-Cell communication scores for gene pairs based on spatial interaction

## **Examples**

spatCellCellcom(gobject)

222 spatCellPlot

spatCellPlot

spatCellPlot

#### **Description**

Visualize cells according to spatial coordinates

#### Usage

```
spatCellPlot(...)
```

# Arguments

```
Arguments passed on to spatCellPlot2D
gobject giotto object
show_image show a tissue background image
gimage a giotto image
image_name name of a giotto image
sdimx x-axis dimension name (default = 'sdimx')
sdimy y-axis dimension name (default = 'sdimy')
spat_enr_names names of spatial enrichment results to include
cell_annotation_values numeric cell annotation columns
cell_color_gradient vector with 3 colors for numeric data
gradient_midpoint midpoint for color gradient
gradient_limits vector with lower and upper limits
select_cell_groups select subset of cells/clusters based on cell_color param-
select_cells select subset of cells based on cell IDs
point_shape shape of points (border, no_border or voronoi)
point_size size of point (cell)
point_alpha transparancy of spatial points
point_border_col color of border around points
point_border_stroke stroke size of border around points
show_cluster_center plot center of selected clusters
show_center_label plot label of selected clusters
center_point_size size of center points
center_point_border_col border color of center points
center_point_border_stroke border stroke size of center points
label_size size of labels
label_fontface font of labels
show_network show underlying spatial network
spatial_network_name name of spatial network to use
network_color color of spatial network
network_alpha alpha of spatial network
show_grid show spatial grid
spatial_grid_name name of spatial grid to use
```

spatCellPlot 223

```
grid_color color of spatial grid
show_other_cells display not selected cells
other_cell_color color of not selected cells
other_point_size point size of not selected cells
other_cells_alpha alpha of not selected cells
coord_fix_ratio fix ratio between x and y-axis
show_legend show legend
legend_text size of legend text
legend_symbol_size size of legend symbols
background_color color of plot background
vor_border_color border colorr for voronoi plot
vor_max_radius maximum radius for voronoi 'cells'
vor_alpha transparancy of voronoi 'cells'
axis_text size of axis text
axis_title size of axis title
cow_n_col cowplot param: how many columns
cow_rel_h cowplot param: relative height
cow_rel_w cowplot param: relative width
cow_align cowplot param: how to align
show_plot show plot
return_plot return ggplot object
save_plot directly save the plot [boolean]
save_param list of saving parameters, see showSaveParameters
default_save_name default save name for saving, don't change, change save_name
    in save_param
```

## **Details**

Description of parameters.

#### Value

ggplot

#### See Also

Other spatial cell annotation visualizations: spatCellPlot2D()

## **Examples**

```
spatCellPlot(gobject)
```

224 spatCelIPlot2D

spatCellPlot2D

spatCellPlot2D

## **Description**

Visualize cells according to spatial coordinates

```
spatCellPlot2D(
 gobject,
  show_image = F,
  gimage = NULL,
  image_name = "image",
  sdimx = "sdimx",
  sdimy = "sdimy"
  spat_enr_names = NULL,
  cell_annotation_values = NULL,
 cell_color_gradient = c("blue", "white", "red"),
 gradient_midpoint = NULL,
 gradient_limits = NULL,
 select_cell_groups = NULL,
  select_cells = NULL,
 point_shape = c("border", "no_border", "voronoi"),
 point_size = 3,
 point_alpha = 1,
 point_border_col = "black",
 point_border_stroke = 0.1,
  show_cluster_center = F,
  show\_center\_label = F,
  center_point_size = 4,
  center_point_border_col = "black",
  center_point_border_stroke = 0.1,
  label_size = 4,
  label_fontface = "bold",
  show_network = F,
  spatial_network_name = "Delaunay_network",
 network_color = NULL,
 network_alpha = 1,
  show_grid = F,
  spatial_grid_name = "spatial_grid",
 grid_color = NULL,
  show_other_cells = T,
 other_cell_color = "lightgrey",
 other_point_size = 1,
 other_cells_alpha = 0.1,
  coord_fix_ratio = NULL,
  show_legend = T,
  legend_text = 8,
  legend_symbol_size = 1,
 background_color = "white",
```

```
vor_border_color = "white",
      vor_max_radius = 200,
      vor_alpha = 1,
      axis_text = 8,
      axis_title = 8,
      cow_n_col = 2,
      cow_rel_h = 1,
      cow_rel_w = 1,
      cow_align = "h",
      show_plot = NA,
      return_plot = NA,
      save_plot = NA,
      save_param = list(),
      default_save_name = "spatCellPlot2D"
    )
Arguments
   gobject
                     giotto object
    show_image
                     show a tissue background image
    gimage
                     a giotto image
    image_name
                     name of a giotto image
    sdimx
                     x-axis dimension name (default = 'sdimx')
                     y-axis dimension name (default = 'sdimy')
    sdimy
    spat_enr_names names of spatial enrichment results to include
    cell_annotation_values
                     numeric cell annotation columns
    cell_color_gradient
                     vector with 3 colors for numeric data
    gradient_midpoint
                     midpoint for color gradient
    gradient_limits
                     vector with lower and upper limits
    select_cell_groups
                     select subset of cells/clusters based on cell_color parameter
                     select subset of cells based on cell IDs
    select_cells
                     shape of points (border, no_border or voronoi)
    point_shape
   point_size
                     size of point (cell)
    point_alpha
                     transparancy of spatial points
   point_border_col
                     color of border around points
   point_border_stroke
                     stroke size of border around points
    show_cluster_center
                     plot center of selected clusters
```

plot label of selected clusters

show\_center\_label

center\_point\_size

size of center points

center\_point\_border\_col

border color of center points

center\_point\_border\_stroke

border stroke size of center points

label\_size size of labels
label\_fontface font of labels

show\_network show underlying spatial network

spatial\_network\_name

name of spatial network to use

network\_color color of spatial network network\_alpha alpha of spatial network show\_grid show spatial grid

spatial\_grid\_name

name of spatial grid to use

grid\_color color of spatial grid

show\_other\_cells

display not selected cells

other\_cell\_color

color of not selected cells

other\_point\_size

point size of not selected cells

other\_cells\_alpha

alpha of not selected cells

coord\_fix\_ratio

fix ratio between x and y-axis

show\_legend show legend
legend\_text size of legend text

legend\_symbol\_size

size of legend symbols

background\_color

color of plot background

vor\_border\_color

border colorr for voronoi plot

vor\_max\_radius maximum radius for voronoi 'cells' vor\_alpha transparancy of voronoi 'cells'

axis\_text size of axis text
axis\_title size of axis title

cow\_n\_col cowplot param: how many columns
cow\_rel\_h cowplot param: relative height
cow\_rel\_w cowplot param: relative width
cow\_align cowplot param: how to align

show\_plot show plot

return\_plot return ggplot object

```
save_plot directly save the plot [boolean]
```

save\_param list of saving parameters, see showSaveParameters

default\_save\_name

default save name for saving, don't change, change save\_name in save\_param

#### **Details**

Description of parameters.

#### Value

ggplot

#### See Also

Other spatial cell annotation visualizations: spatCellPlot()

## **Examples**

```
spatCellPlot2D(gobject)
```

spatDimCellPlot

spatDimCellPlot

# **Description**

Visualize numerical features of cells according to spatial AND dimension reduction coordinates in 2D

## Usage

```
spatDimCellPlot(...)
```

## **Arguments**

arguments passed on to spatDimCellPlot2D

gobject giotto object

show\_image show a tissue background image

gimage a giotto image

image\_name name of a giotto image

plot\_alignment direction to align plot

spat\_enr\_names names of spatial enrichment results to include

cell\_annotation\_values numeric cell annotation columns

dim\_reduction\_to\_use dimension reduction to use

dim\_reduction\_name dimension reduction name

dim1\_to\_use dimension to use on x-axis

dim2\_to\_use dimension to use on y-axis

sdimx = spatial dimension to use on y-axis

sdimy = spatial dimension to use on y-axis

```
cell_color_gradient vector with 3 colors for numeric data
gradient_midpoint midpoint for color gradient
gradient_limits vector with lower and upper limits
select_cell_groups select subset of cells/clusters based on cell_color param-
select_cells select subset of cells based on cell IDs
dim_point_shape dim reduction points with border or not (border or no_border)
dim_point_size size of points in dim. reduction space
dim_point_alpha transparancy of dim. reduction points
dim_point_border_col border color of points in dim. reduction space
dim_point_border_stroke border stroke of points in dim. reduction space
spat_point_shape shape of points (border, no_border or voronoi)
spat_point_size size of spatial points
spat_point_alpha transparancy of spatial points
spat_point_border_col border color of spatial points
spat_point_border_stroke border stroke of spatial points
dim_show_cluster_center show the center of each cluster
dim_show_center_label provide a label for each cluster
dim_center_point_size size of the center point
dim_center_point_border_col border color of center point
dim_center_point_border_stroke stroke size of center point
dim_label_size size of the center label
dim_label_fontface font of the center label
spat_show_cluster_center show the center of each cluster
spat_show_center_label provide a label for each cluster
spat_center_point_size size of the spatial center points
spat_center_point_border_col border color of the spatial center points
spat_center_point_border_stroke stroke size of the spatial center points
spat_label_size size of the center label
spat_label_fontface font of the center label
show_NN_network show underlying NN network
nn_network_to_use type of NN network to use (kNN vs sNN)
nn_network_name name of NN network to use, if show_NN_network = TRUE
dim_edge_alpha column to use for alpha of the edges
spat_show_network show spatial network
spatial_network_name name of spatial network to use
spat_network_color color of spatial network
spat_network_alpha alpha of spatial network
spat_show_grid show spatial grid
spatial_grid_name name of spatial grid to use
spat_grid_color color of spatial grid
show_other_cells display not selected cells
other_cell_color color of not selected cells
dim_other_point_size size of not selected dim cells
spat_other_point_size size of not selected spat cells
spat_other_cells_alpha alpha of not selected spat cells
```

```
coord_fix_ratio ratio for coordinates
cow_n_col cowplot param: how many columns
cow_rel_h cowplot param: relative height
cow_rel_w cowplot param: relative width
cow_align cowplot param: how to align
show_legend show legend
legend_text size of legend text
legend_symbol_size size of legend symbols
dim_background_color background color of points in dim. reduction space
spat_background_color background color of spatial points
vor_border_color border colorr for voronoi plot
vor_max_radius maximum radius for voronoi 'cells'
vor_alpha transparancy of voronoi 'cells'
axis_text size of axis text
axis_title size of axis title
show_plot show plot
return_plot return ggplot object
save_plot directly save the plot [boolean]
save_param list of saving parameters, see showSaveParameters
default_save_name default save name for saving, don't change, change save_name
    in save_param
```

## **Details**

Description of parameters.

# Value

ggplot

# See Also

Other spatial and dimension reduction cell annotation visualizations: spatDimCellPlot2D()

#### **Examples**

spatDimCellPlot(gobject)

spatDimCellPlot2D

spatDimCellPlot2D

# Description

Visualize numerical features of cells according to spatial AND dimension reduction coordinates in 2D

```
spatDimCellPlot2D(
  gobject,
  show_image = F,
 gimage = NULL,
  image_name = "image",
 plot_alignment = c("vertical", "horizontal"),
 spat_enr_names = NULL,
 cell_annotation_values = NULL,
 dim_reduction_to_use = "umap",
 dim_reduction_name = "umap",
 dim1_to_use = 1,
 dim2\_to\_use = 2,
  sdimx = "sdimx",
  sdimy = "sdimy",
  cell_color_gradient = c("blue", "white", "red"),
  gradient_midpoint = NULL,
  gradient_limits = NULL,
  select_cell_groups = NULL,
  select_cells = NULL,
  dim_point_shape = c("border", "no_border"),
  dim_point_size = 1,
 dim_point_alpha = 1,
 dim_point_border_col = "black",
 dim_point_border_stroke = 0.1,
  spat_point_shape = c("border", "no_border", "voronoi"),
  spat_point_size = 1,
  spat_point_alpha = 1,
  spat_point_border_col = "black",
  spat_point_border_stroke = 0.1,
 dim_show_cluster_center = F,
  dim_show_center_label = T,
  dim_center_point_size = 4,
  dim_center_point_border_col = "black",
 dim_center_point_border_stroke = 0.1,
  dim_label_size = 4,
  dim_label_fontface = "bold",
  spat_show_cluster_center = F,
  spat_show_center_label = F,
  spat_center_point_size = 4,
  spat_center_point_border_col = "black",
  spat_center_point_border_stroke = 0.1,
  spat_label_size = 4,
  spat_label_fontface = "bold",
  show_NN_network = F,
 nn_network_to_use = "sNN",
 nn_network_name = "sNN.pca",
  dim_edge_alpha = 0.5,
  spat\_show\_network = F,
  spatial_network_name = "Delaunay_network",
  spat_network_color = "red",
  spat_network_alpha = 0.5,
```

```
spat_show_grid = F,
  spatial_grid_name = "spatial_grid",
  spat_grid_color = "green",
  show_other_cells = TRUE,
  other_cell_color = "grey",
  dim_other_point_size = 0.5,
  spat_other_point_size = 0.5,
  spat_other_cells_alpha = 0.5,
  show_legend = T,
  legend_text = 8,
  legend_symbol_size = 1,
  dim_background_color = "white",
  spat_background_color = "white",
  vor_border_color = "white",
  vor_max_radius = 200,
  vor_alpha = 1,
  axis_text = 8,
  axis_title = 8,
  coord_fix_ratio = NULL,
  cow_n_col = 2,
  cow_rel_h = 1,
  cow_rel_w = 1,
  cow_align = "h",
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "spatDimCellPlot2D"
)
```

#### **Arguments**

```
gobject
                  giotto object
                  show a tissue background image
show_image
gimage
                  a giotto image
                  name of a giotto image
image_name
plot_alignment direction to align plot
spat_enr_names names of spatial enrichment results to include
cell_annotation_values
                  numeric cell annotation columns
dim_reduction_to_use
                  dimension reduction to use
dim_reduction_name
                  dimension reduction name
                  dimension to use on x-axis
dim1_to_use
dim2_to_use
                  dimension to use on y-axis
                  = spatial dimension to use on x-axis
sdimx
sdimy
                  = spatial dimension to use on y-axis
cell_color_gradient
                  vector with 3 colors for numeric data
```

gradient\_midpoint midpoint for color gradient gradient\_limits vector with lower and upper limits select\_cell\_groups select subset of cells/clusters based on cell\_color parameter select subset of cells based on cell IDs select\_cells dim\_point\_shape dim reduction points with border or not (border or no\_border) dim\_point\_size size of points in dim. reduction space dim\_point\_alpha transparancy of dim. reduction points dim\_point\_border\_col border color of points in dim. reduction space dim\_point\_border\_stroke border stroke of points in dim. reduction space spat\_point\_shape shape of points (border, no\_border or voronoi) spat\_point\_size size of spatial points spat\_point\_alpha transparancy of spatial points spat\_point\_border\_col border color of spatial points spat\_point\_border\_stroke border stroke of spatial points dim\_show\_cluster\_center show the center of each cluster dim\_show\_center\_label provide a label for each cluster dim\_center\_point\_size size of the center point dim\_center\_point\_border\_col border color of center point dim\_center\_point\_border\_stroke stroke size of center point dim\_label\_size size of the center label dim\_label\_fontface font of the center label spat\_show\_cluster\_center show the center of each cluster spat\_show\_center\_label provide a label for each cluster spat\_center\_point\_size size of the spatial center points  ${\tt spat\_center\_point\_border\_col}$ border color of the spatial center points

spat\_center\_point\_border\_stroke stroke size of the spatial center points spat\_label\_size size of the center label spat\_label\_fontface font of the center label show\_NN\_network show underlying NN network nn\_network\_to\_use type of NN network to use (kNN vs sNN) nn\_network\_name name of NN network to use, if show\_NN\_network = TRUE dim\_edge\_alpha column to use for alpha of the edges spat\_show\_network show spatial network spatial\_network\_name name of spatial network to use spat\_network\_color color of spatial network spat\_network\_alpha alpha of spatial network spat\_show\_grid show spatial grid spatial\_grid\_name name of spatial grid to use spat\_grid\_color color of spatial grid show\_other\_cells display not selected cells other\_cell\_color color of not selected cells  ${\tt dim\_other\_point\_size}$ size of not selected dim cells spat\_other\_point\_size size of not selected spat cells spat\_other\_cells\_alpha alpha of not selected spat cells show legend show\_legend size of legend text legend\_text legend\_symbol\_size size of legend symbols dim\_background\_color background color of points in dim. reduction space spat\_background\_color background color of spatial points vor\_border\_color border colorr for voronoi plot vor\_max\_radius maximum radius for voronoi 'cells'

```
vor_alpha
                  transparancy of voronoi 'cells'
axis_text
                  size of axis text
axis_title
                  size of axis title
coord_fix_ratio
                  ratio for coordinates
                  cowplot param: how many columns
cow_n_col
cow_rel_h
                  cowplot param: relative height
cow_rel_w
                  cowplot param: relative width
                  cowplot param: how to align
cow_align
show_plot
                  show plot
return_plot
                  return ggplot object
save_plot
                  directly save the plot [boolean]
                  list of saving parameters, see showSaveParameters
save_param
default_save_name
                  default save name for saving, don't change, change save_name in save_param
```

## **Details**

Description of parameters.

#### Value

ggplot

# See Also

Other spatial and dimension reduction cell annotation visualizations: spatDimCellPlot()

# Examples

```
{\tt spatDimCellPlot2D(gobject)}
```

```
spatDimGenePlot spatDimGenePlot
```

## **Description**

Visualize cells according to spatial AND dimension reduction coordinates in ggplot mode

```
spatDimGenePlot(...)
```

#### **Arguments**

Arguments passed on to spatDimGenePlot2D gobject giotto object show\_image show a tissue background image gimage a giotto image image\_name name of a giotto image expression\_values gene expression values to use plot\_alignment direction to align plot genes genes to show dim\_reduction\_to\_use dimension reduction to use dim\_reduction\_name dimension reduction name dim1\_to\_use dimension to use on x-axis dim2\_to\_use dimension to use on y-axis dim\_point\_shape dim reduction points with border or not (border or no\_border) dim\_point\_size dim reduction plot: point size dim\_point\_alpha transparancy of dim. reduction points dim\_point\_border\_col color of border around points dim\_point\_border\_stroke stroke size of border around points show\_NN\_network show underlying NN network show\_spatial\_network show underlying spatial netwok nn\_network\_to\_use type of NN network to use (kNN vs sNN) network\_name name of NN network to use, if show\_NN\_network = TRUE dim\_network\_color color of NN network dim\_edge\_alpha dim reduction plot: column to use for alpha of the edges scale\_alpha\_with\_expression scale expression with ggplot alpha parameter sdimx spatial x-axis dimension name (default = 'sdimx') sdimy spatial y-axis dimension name (default = 'sdimy') spatial\_network\_name name of spatial network to use spatial\_network\_color color of spatial network show\_spatial\_grid show spatial grid grid\_color color of spatial grid spatial\_grid\_name name of spatial grid to use spat\_point\_shape spatial points with border or not (border or no\_border) spat\_point\_size spatial plot: point size spat\_point\_alpha transparancy of spatial points spat\_point\_border\_col color of border around points spat\_point\_border\_stroke stroke size of border around points spat\_edge\_alpha edge alpha cell\_color\_gradient vector with 3 colors for numeric data gradient\_midpoint midpoint for color gradient gradient\_limits vector with lower and upper limits show\_legend show legend legend\_text\_size of legend text dim\_background\_color color of plot background for dimension plot spat\_background\_color color of plot background for spatial plot

```
vor_border_color border colorr for voronoi plot
vor_max_radius maximum radius for voronoi 'cells'
vor_alpha transparancy of voronoi 'cells'
axis_text size of axis text
axis_title size of axis title
cow_n_col cowplot param: how many columns
cow_rel_h cowplot param: relative height
cow_rel_w cowplot param: relative width
cow_align cowplot param: how to align
show_plot show plots
return_plot return ggplot object
save_plot directly save the plot [boolean]
save_param list of saving parameters, see showSaveParameters
default_save_name default save name for saving, don't change, change save_name
in save_param
```

## **Details**

Description of parameters.

#### Value

ggplot

#### See Also

```
spatDimGenePlot3D
```

Other spatial and dimension reduction gene expression visualizations: spatDimGenePlot2D(), spatDimGenePlot3D()

## **Examples**

```
spatDimGenePlot(gobject)
```

spatDimGenePlot2D

spatDimGenePlot2D

# Description

Visualize cells according to spatial AND dimension reduction coordinates in ggplot mode

```
spatDimGenePlot2D(
  gobject,
  show_image = F,
  gimage = NULL,
  image_name = "image",
  expression_values = c("normalized", "scaled", "custom"),
  plot_alignment = c("vertical", "horizontal"),
```

```
genes,
dim_reduction_to_use = "umap",
dim_reduction_name = "umap",
dim1_to_use = 1,
dim2_to_use = 2,
dim_point_shape = c("border", "no_border"),
dim_point_size = 1,
dim_point_alpha = 1,
dim_point_border_col = "black",
dim_point_border_stroke = 0.1,
show_NN_network = F,
show_spatial_network = F,
dim_network_color = "gray",
nn_network_to_use = "sNN",
network_name = "sNN.pca",
dim_edge_alpha = NULL,
scale_alpha_with_expression = FALSE,
sdimx = "sdimx",
sdimy = "sdimy",
spatial_network_name = "Delaunay_network",
spatial_network_color = NULL,
show_spatial_grid = F,
grid_color = NULL,
spatial_grid_name = "spatial_grid",
spat_point_shape = c("border", "no_border", "voronoi"),
spat_point_size = 1,
spat_point_alpha = 1,
spat_point_border_col = "black",
spat_point_border_stroke = 0.1,
spat_edge_alpha = NULL,
cell_color_gradient = c("blue", "white", "red"),
gradient_midpoint = NULL,
gradient_limits = NULL,
cow_n_col = 2,
cow_rel_h = 1,
cow_rel_w = 1,
cow_align = "h",
show_legend = T,
legend_text = 8,
dim_background_color = "white",
spat_background_color = "white",
vor_border_color = "white",
vor_max_radius = 200,
vor_alpha = 1,
axis_text = 8,
axis_title = 8,
show_plot = NA,
return_plot = NA,
save_plot = NA,
save_param = list(),
default_save_name = "spatDimGenePlot2D"
```

#### **Arguments**

gobject giotto object show a tissue background image show\_image gimage a giotto image name of a giotto image image\_name expression\_values gene expression values to use plot\_alignment direction to align plot genes genes to show  ${\tt dim\_reduction\_to\_use}$ dimension reduction to use dim\_reduction\_name dimension reduction name dimension to use on x-axis dim1\_to\_use dimension to use on y-axis dim2\_to\_use dim\_point\_shape dim reduction points with border or not (border or no\_border) dim\_point\_size dim reduction plot: point size dim\_point\_alpha transparancy of dim. reduction points dim\_point\_border\_col color of border around points dim\_point\_border\_stroke stroke size of border around points show\_NN\_network show underlying NN network show\_spatial\_network show underlying spatial netwok dim\_network\_color color of NN network nn\_network\_to\_use type of NN network to use (kNN vs sNN) name of NN network to use, if show\_NN\_network = TRUE network\_name dim\_edge\_alpha dim reduction plot: column to use for alpha of the edges scale\_alpha\_with\_expression scale expression with ggplot alpha parameter sdimx spatial x-axis dimension name (default = 'sdimx') spatial y-axis dimension name (default = 'sdimy') sdimy spatial\_network\_name name of spatial network to use spatial\_network\_color color of spatial network show\_spatial\_grid show spatial grid color of spatial grid grid\_color

```
spatial_grid_name
                  name of spatial grid to use
spat_point_shape
                  spatial points with border or not (border or no_border)
spat_point_size
                  spatial plot: point size
spat_point_alpha
                  transparancy of spatial points
spat_point_border_col
                  color of border around points
spat_point_border_stroke
                  stroke size of border around points
spat_edge_alpha
                  edge alpha
cell_color_gradient
                  vector with 3 colors for numeric data
gradient_midpoint
                  midpoint for color gradient
gradient_limits
                  vector with lower and upper limits
cow_n_col
                  cowplot param: how many columns
cow_rel_h
                  cowplot param: relative height
                  cowplot param: relative width
cow_rel_w
cow_align
                  cowplot param: how to align
show_legend
                  show legend
legend_text
                  size of legend text
dim_background_color
                  color of plot background for dimension plot
spat_background_color
                  color of plot background for spatial plot
vor_border_color
                  border colorr for voronoi plot
vor_max_radius maximum radius for voronoi 'cells'
vor_alpha
                  transparancy of voronoi 'cells'
axis_text
                  size of axis text
                  size of axis title
axis_title
show_plot
                  show plots
return_plot
                  return ggplot object
save_plot
                  directly save the plot [boolean]
save_param
                  list of saving parameters, see showSaveParameters
default_save_name
                  default save name for saving, don't change, change save_name in save_param
```

## **Details**

Description of parameters.

#### Value

ggplot

#### See Also

```
spatDimGenePlot3D
Other spatial and dimension reduction gene expression visualizations: spatDimGenePlot3D(),
spatDimGenePlot()
```

# **Examples**

```
spatDimGenePlot2D(gobject)
```

spatDimGenePlot3D

spatDimGenePlot3D

## **Description**

Visualize cells according to spatial AND dimension reduction coordinates in ggplot mode

```
spatDimGenePlot3D(
 gobject,
  expression_values = c("normalized", "scaled", "custom"),
 plot_alignment = c("horizontal", "vertical"),
 dim_reduction_to_use = "umap",
 dim_reduction_name = "umap",
 dim1_to_use = 1,
 dim2\_to\_use = 2,
 dim3_to_use = NULL,
  sdimx = "sdimx",
  sdimy = "sdimy",
  sdimz = "sdimz",
  genes,
  cluster_column = NULL,
  select_cell_groups = NULL,
  select_cells = NULL,
  show_other_cells = T,
 other_cell_color = "lightgrey",
 other_point_size = 1.5,
 show_NN_network = FALSE,
 nn_network_to_use = "sNN",
 nn_network_color = "lightgrey",
 nn_network_alpha = 0.5,
 network_name = "sNN.pca",
 label_size = 16,
  genes_low_color = "blue",
 genes_mid_color = "white",
 genes_high_color = "red",
  dim_point_size = 3,
```

```
show_spatial_network = FALSE,
      spatial_network_name = "Delaunay_network",
      spatial_network_color = "lightgray",
      spatial_network_alpha = 0.5,
      show_spatial_grid = FALSE,
      spatial_grid_name = "spatial_grid",
      spatial_grid_color = NULL,
      spatial_grid_alpha = 0.5,
      spatial_point_size = 3,
      legend_text_size = 12,
      axis_scale = c("cube", "real", "custom"),
      custom_ratio = NULL,
      x_ticks = NULL,
      y_ticks = NULL,
      z_ticks = NULL,
      show_plot = NA,
      return_plot = NA,
      save_plot = NA,
      save_param = list(),
      default_save_name = "spatDimGenePlot3D"
    )
Arguments
                     giotto object
    gobject
    expression_values
                     gene expression values to use
    plot_alignment direction to align plot
    dim_reduction_to_use
                     dimension reduction to use
    dim_reduction_name
                     dimension reduction name
                     dimension to use on x-axis
    dim1_to_use
    dim2_to_use
                     dimension to use on y-axis
    dim3_to_use
                     dimension to use on z-axis
    sdimx
                     spatial dimension to use on x-axis
    sdimy
                     spatial dimension to use on y-axis
    sdimz
                     spatial dimension to use on z-axis
    genes
                     genes to show
    cluster_column cluster column to select groups
    select_cell_groups
                     select subset of cells/clusters based on cell_color parameter
                     select subset of cells based on cell IDs
    select_cells
    show_other_cells
                     display not selected cells
    other_cell_color
                     color of not selected cells
    other_point_size
                     size of not selected cells
```

show\_NN\_network

show underlying NN network

nn\_network\_to\_use

type of NN network to use (kNN vs sNN)

nn\_network\_color

color of NN network

nn\_network\_alpha

alpha of NN network

network\_name name of NN network to use, if show\_NN\_network = TRUE

label\_size size of labels

genes\_low\_color

color for low expression levels

genes\_mid\_color

color for medium expression levels

genes\_high\_color

color for high expression levels

dim\_point\_size dim reduction plot: point size

show\_spatial\_network

show spatial network (boolean)

spatial\_network\_name

name of spatial network to use

spatial\_network\_color

color of spatial network

spatial\_network\_alpha

alpha of spatial network

show\_spatial\_grid

show spatial grid (boolean)

spatial\_grid\_name

name of spatial grid to use

spatial\_grid\_color

color of spatial grid

spatial\_grid\_alpha

alpha of spatial grid

spatial\_point\_size

spatial plot: point size

the way to scale the axis

legend\_text\_size

axis\_scale

size of legend

custom\_ratio customize the scale of the plot
x\_ticks set the number of ticks on the x-axis
y\_ticks set the number of ticks on the y-axis
z\_ticks set the number of ticks on the z-axis

show\_plot show plots

return\_plot return plotly object

save\_plot directly save the plot [boolean]

save\_param list of saving parameters, see showSaveParameters

default\_save\_name

default save name for saving, don't change, change save\_name in save\_param

#### **Details**

Description of parameters.

#### Value

plotly

#### See Also

Other spatial and dimension reduction gene expression visualizations: spatDimGenePlot2D(), spatDimGenePlot()

spatDimPlot

spatDimPlot

#### **Description**

Visualize cells according to spatial AND dimension reduction coordinates 2D

#### Usage

```
spatDimPlot(...)
```

#### **Arguments**

Arguments passed on to spatDimPlot2D gobject giotto object show\_image show a tissue background image gimage a giotto image image\_name name of a giotto image plot\_alignment direction to align plot dim\_reduction\_to\_use dimension reduction to use dim\_reduction\_name dimension reduction name dim1\_to\_use dimension to use on x-axis dim2\_to\_use dimension to use on y-axis sdimx = spatial dimension to use on x-axissdimy = spatial dimension to use on y-axis spat\_enr\_names names of spatial enrichment results to include cell\_color color for cells (see details) color\_as\_factor convert color column to factor cell\_color\_code named vector with colors cell\_color\_gradient vector with 3 colors for numeric data gradient\_midpoint midpoint for color gradient gradient\_limits vector with lower and upper limits select\_cell\_groups select subset of cells/clusters based on cell\_color parameter select\_cells select subset of cells based on cell IDs dim\_point\_shape point with border or not (border or no\_border)

```
dim_point_size size of points in dim. reduction space
dim_point_alpha transparancy of point in dim. reduction space
dim_point_border_col border color of points in dim. reduction space
dim_point_border_stroke border stroke of points in dim. reduction space
spat_point_shape shape of points (border, no border or voronoi)
spat_point_size size of spatial points
spat_point_alpha transparancy of spatial points
spat_point_border_col border color of spatial points
spat_point_border_stroke border stroke of spatial points
dim_show_cluster_center show the center of each cluster
dim_show_center_label provide a label for each cluster
dim_center_point_size size of the center point
dim_center_point_border_col border color of center point
dim_center_point_border_stroke stroke size of center point
dim label size size of the center label
dim_label_fontface font of the center label
spat_show_cluster_center show the center of each cluster
spat_show_center_label provide a label for each cluster
spat_center_point_size size of the center point
spat_center_point_border_col border color of spatial center points
spat_center_point_border_stroke border strike size of spatial center points
spat_label_size size of the center label
spat_label_fontface font of the center label
show_NN_network show underlying NN network
nn_network_to_use type of NN network to use (kNN vs sNN)
network_name name of NN network to use, if show_NN_network = TRUE
nn_network_alpha column to use for alpha of the edges
show_spatial_network show spatial network
spat_network_name name of spatial network to use
spat_network_color color of spatial network
spat_network_alpha alpha of spatial network
show_spatial_grid show spatial grid
spat_grid_name name of spatial grid to use
spat_grid_color color of spatial grid
show_other_cells display not selected cells
other_cell_color color of not selected cells
dim_other_point_size size of not selected dim cells
spat_other_point_size size of not selected spat cells
spat_other_cells_alpha alpha of not selected spat cells
dim_show_legend show legend of dimension reduction plot
spat_show_legend show legend of spatial plot
legend_text size of legend text
legend_symbol_size size of legend symbols
dim_background_color background color of points in dim. reduction space
spat_background_color background color of spatial points
```

```
vor_border_color border colorr for voronoi plot
vor_max_radius maximum radius for voronoi 'cells'
vor_alpha transparancy of voronoi 'cells'
axis_text size of axis text
axis_title size of axis title
show_plot show plot
return_plot return ggplot object
save_plot directly save the plot [boolean]
save_param list of saving parameters, see showSaveParameters
default_save_name default save name for saving, don't change, change save_name
in save_param
```

#### **Details**

Description of parameters.

#### Value

ggplot

#### See Also

```
spatDimPlot2D and spatDimPlot3D for 3D visualization.

Other spatial and dimension reduction visualizations: spatDimPlot2D(), spatDimPlot3D()
```

# **Examples**

```
spatDimPlot(gobject)
```

 ${\tt spatDimPlot2D}$ 

spatDimPlot2D

## **Description**

Visualize cells according to spatial AND dimension reduction coordinates 2D

```
spatDimPlot2D(
  gobject,
  show_image = F,
  gimage = NULL,
  image_name = "image",
  plot_alignment = c("vertical", "horizontal"),
  dim_reduction_to_use = "umap",
  dim_reduction_name = "umap",
  dim1_to_use = 1,
  dim2_to_use = 2,
  sdimx = "sdimx",
  sdimy = "sdimy",
```

```
spat_enr_names = NULL,
cell_color = NULL,
color_as_factor = T,
cell_color_code = NULL,
cell_color_gradient = c("blue", "white", "red"),
gradient_midpoint = NULL,
gradient_limits = NULL,
select_cell_groups = NULL,
select_cells = NULL,
dim_point_shape = c("border", "no_border"),
dim_point_size = 1,
dim_point_alpha = 1,
dim_point_border_col = "black",
dim_point_border_stroke = 0.1,
spat_point_shape = c("border", "no_border", "voronoi"),
spat_point_size = 1,
spat_point_alpha = 1,
spat_point_border_col = "black",
spat_point_border_stroke = 0.1,
dim_show_cluster_center = F,
dim_show_center_label = T,
dim_center_point_size = 4,
dim_center_point_border_col = "black",
dim_center_point_border_stroke = 0.1,
dim_label_size = 4,
dim_label_fontface = "bold"
spat_show_cluster_center = F,
spat_show_center_label = F,
spat_center_point_size = 4,
spat_center_point_border_col = "blue",
spat_center_point_border_stroke = 0.1,
spat_label_size = 4,
spat_label_fontface = "bold",
show_NN_network = F,
nn_network_to_use = "sNN",
network_name = "sNN.pca",
nn_network_alpha = 0.05,
show_spatial_network = F,
spat_network_name = "Delaunay_network",
spat_network_color = "blue",
spat_network_alpha = 0.5,
show_spatial_grid = F,
spat_grid_name = "spatial_grid",
spat_grid_color = "blue",
show_other_cells = T,
other_cell_color = "lightgrey",
dim_other_point_size = 1,
spat_other_point_size = 1,
spat_other_cells_alpha = 0.5,
dim_show_legend = F,
spat_show_legend = F,
legend_text = 8,
```

```
legend_symbol_size = 1,
      dim_background_color = "white",
      spat_background_color = "white",
      vor_border_color = "white",
      vor_max_radius = 200,
      vor_alpha = 1,
      axis_text = 8,
      axis_title = 8,
      show_plot = NA,
      return_plot = NA,
      save_plot = NA,
      save_param = list(),
      default_save_name = "spatDimPlot2D"
   )
Arguments
   gobject
                     giotto object
    show_image
                     show a tissue background image
                     a giotto image
    gimage
                     name of a giotto image
    image\_name
    plot_alignment direction to align plot
    dim_reduction_to_use
                     dimension reduction to use
    dim_reduction_name
                     dimension reduction name
   dim1_to_use
                     dimension to use on x-axis
   dim2_to_use
                     dimension to use on y-axis
    sdimx
                     = spatial dimension to use on x-axis
    sdimy
                     = spatial dimension to use on y-axis
    spat_enr_names names of spatial enrichment results to include
                     color for cells (see details)
    cell_color
    color_as_factor
                     convert color column to factor
    cell_color_code
                     named vector with colors
    cell_color_gradient
                     vector with 3 colors for numeric data
    gradient_midpoint
                     midpoint for color gradient
    gradient_limits
                     vector with lower and upper limits
    select_cell_groups
                     select subset of cells/clusters based on cell_color parameter
                     select subset of cells based on cell IDs
    select cells
    dim_point_shape
                     point with border or not (border or no_border)
```

dim\_point\_size size of points in dim. reduction space

dim\_point\_alpha transparancy of point in dim. reduction space dim\_point\_border\_col border color of points in dim. reduction space dim\_point\_border\_stroke border stroke of points in dim. reduction space spat\_point\_shape shape of points (border, no\_border or voronoi) spat\_point\_size size of spatial points spat\_point\_alpha transparancy of spatial points spat\_point\_border\_col border color of spatial points spat\_point\_border\_stroke border stroke of spatial points dim\_show\_cluster\_center show the center of each cluster dim\_show\_center\_label provide a label for each cluster dim\_center\_point\_size size of the center point dim\_center\_point\_border\_col border color of center point dim\_center\_point\_border\_stroke stroke size of center point dim\_label\_size size of the center label dim\_label\_fontface font of the center label spat\_show\_cluster\_center show the center of each cluster spat\_show\_center\_label provide a label for each cluster spat\_center\_point\_size size of the center point spat\_center\_point\_border\_col border color of spatial center points spat\_center\_point\_border\_stroke border strike size of spatial center points spat\_label\_size size of the center label spat\_label\_fontface font of the center label show\_NN\_network show underlying NN network nn\_network\_to\_use

type of NN network to use (kNN vs sNN)

network\_name

name of NN network to use, if show\_NN\_network = TRUE

nn\_network\_alpha column to use for alpha of the edges show\_spatial\_network show spatial network spat\_network\_name name of spatial network to use spat\_network\_color color of spatial network spat\_network\_alpha alpha of spatial network show\_spatial\_grid show spatial grid spat\_grid\_name name of spatial grid to use spat\_grid\_color color of spatial grid show\_other\_cells display not selected cells other\_cell\_color color of not selected cells dim\_other\_point\_size size of not selected dim cells spat\_other\_point\_size size of not selected spat cells spat\_other\_cells\_alpha alpha of not selected spat cells dim\_show\_legend show legend of dimension reduction plot spat\_show\_legend show legend of spatial plot size of legend text legend\_text legend\_symbol\_size size of legend symbols dim\_background\_color background color of points in dim. reduction space spat\_background\_color background color of spatial points vor\_border\_color border colorr for voronoi plot vor\_max\_radius maximum radius for voronoi 'cells' vor\_alpha transparancy of voronoi 'cells' size of axis text axis\_text axis\_title size of axis title show plot show\_plot return\_plot return ggplot object save\_plot directly save the plot [boolean] save\_param list of saving parameters, see showSaveParameters default\_save\_name default save name for saving, don't change, change save\_name in save\_param

#### **Details**

Description of parameters.

#### Value

ggplot

#### See Also

```
spatDimPlot3D
```

Other spatial and dimension reduction visualizations: spatDimPlot3D(), spatDimPlot()

# **Examples**

```
spatDimPlot2D(gobject)
```

spatDimPlot3D

spatDimPlot3D

## Description

Visualize cells according to spatial AND dimension reduction coordinates in plotly mode

```
spatDimPlot3D(
  gobject,
  plot_alignment = c("horizontal", "vertical"),
  dim_reduction_to_use = "umap",
  dim_reduction_name = "umap",
  dim1_to_use = 1,
  dim2_to_use = 2,
  dim3_to_use = 3,
  sdimx = "sdimx",
  sdimy = "sdimy",
  sdimz = "sdimz",
  spat_enr_names = NULL,
  show_NN_network = FALSE,
  nn_network_to_use = "sNN",
  network_name = "sNN.pca",
  nn_network_color = "lightgray",
  nn_network_alpha = 0.5,
  show_cluster_center = F,
  show_center_label = T,
  center_point_size = 4,
  label_size = 16,
  select_cell_groups = NULL,
  select_cells = NULL,
  show_other_cells = T,
  other_cell_color = "lightgrey",
  other_point_size = 1.5,
```

```
cell_color = NULL,
      color_as_factor = T,
      cell_color_code = NULL,
      dim_point_size = 3,
      show_spatial_network = F,
      spatial_network_name = "Delaunay_network",
      spatial_network_color = "lightgray",
      spatial_network_alpha = 0.5,
      show_spatial_grid = F,
      spatial_grid_name = "spatial_grid",
      spatial_grid_color = NULL,
      spatial_grid_alpha = 0.5,
      spatial_point_size = 3,
      axis_scale = c("cube", "real", "custom"),
      custom_ratio = NULL,
      x_ticks = NULL,
      y_ticks = NULL,
      z_ticks = NULL,
      legend_text_size = 12,
      show_plot = NA,
      return_plot = NA,
      save_plot = NA,
      save_param = list(),
      default_save_name = "spatDimPlot3D"
    )
Arguments
                    giotto object
   gobject
   plot_alignment direction to align plot
    dim_reduction_to_use
                    dimension reduction to use
   dim_reduction_name
                    dimension reduction name
   dim1_to_use
                    dimension to use on x-axis
                    dimension to use on y-axis
   dim2_to_use
   dim3_to_use
                    dimension to use on z-axis
    sdimx
                    = spatial dimension to use on x-axis
    sdimy
                    = spatial dimension to use on y-axis
    sdimz
                    = spatial dimension to use on z-axis
    spat_enr_names names of spatial enrichment results to include
    show_NN_network
                    show underlying NN network
    nn_network_to_use
                    type of NN network to use (kNN vs sNN)
                    name of NN network to use, if show_NN_network = TRUE
   network_name
    nn_network_color
                    color of nn network
   nn_network_alpha
```

column to use for alpha of the edges

show\_cluster\_center show the center of each cluster show\_center\_label provide a label for each cluster center\_point\_size size of the center point label size size of the center label select\_cell\_groups select subset of cells/clusters based on cell\_color parameter select subset of cells based on cell IDs select\_cells show\_other\_cells display not selected cells other\_cell\_color color of not selected cells other\_point\_size size of not selected cells cell\_color color for cells (see details) color\_as\_factor convert color column to factor cell\_color\_code named vector with colors dim\_point\_size size of points in dim. reduction space show\_spatial\_network show spatial network spatial\_network\_name name of spatial network to use spatial\_network\_color color of spatial network spatial\_network\_alpha alpha of spatial network show\_spatial\_grid show spatial grid spatial\_grid\_name name of spatial grid to use spatial\_grid\_color color of spatial grid  $spatial\_grid\_alpha$ alpha of spatial grid spatial\_point\_size size of spatial points the way to scale the axis axis\_scale customize the scale of the plot custom\_ratio set the number of ticks on the x-axis x\_ticks y\_ticks set the number of ticks on the y-axis set the number of ticks on the z-axis z\_ticks legend\_text\_size

size of legend

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```
show_plot show plot
```

return\_plot return ggplot object

save\_plot directly save the plot [boolean]

save\_param list of saving parameters, see showSaveParameters

default\_save\_name

default save name for saving, don't change, change save\_name in save\_param

#### **Details**

Description of parameters.

#### Value

plotly

#### See Also

Other spatial and dimension reduction visualizations: spatDimPlot2D(), spatDimPlot()

#### **Examples**

```
spatDimPlot3D(gobject)
```

spatGenePlot

spatGenePlot

## **Description**

Visualize cells and gene expression according to spatial coordinates

#### Usage

```
spatGenePlot(...)
```

### **Arguments**

... Arguments passed on to spatGenePlot2D

gobject giotto object

show\_image show a tissue background image

gimage a giotto image

image\_name name of a giotto image

sdimx x-axis dimension name (default = 'sdimx')

sdimy y-axis dimension name (default = 'sdimy')

expression\_values gene expression values to use

genes genes to show

cell\_color\_gradient vector with 3 colors for numeric data

gradient\_midpoint midpoint for color gradient
gradient\_limits vector with lower and upper limits

show\_network show underlying spatial network

254 spatGenePlot

```
network_color color of spatial network
spatial_network_name name of spatial network to use
edge_alpha alpha of edge
show_grid show spatial grid
grid_color color of spatial grid
spatial_grid_name name of spatial grid to use
midpoint expression midpoint
scale_alpha_with_expression scale expression with ggplot alpha parameter
point_shape shape of points (border, no_border or voronoi)
point_size size of point (cell)
point_alpha transparancy of points
point_border_col color of border around points
point_border_stroke stroke size of border around points
cow_n_col cowplot param: how many columns
cow_rel_h cowplot param: relative height
cow_rel_w cowplot param: relative width
cow_align cowplot param: how to align
show_legend show legend
legend_text size of legend text
background_color color of plot background
vor_border_color border colorr for voronoi plot
vor_max_radius maximum radius for voronoi 'cells'
vor_alpha transparancy of voronoi 'cells'
axis_text size of axis text
axis_title size of axis title
show_plot show plots
return_plot return ggplot object
save_plot directly save the plot [boolean]
save_param list of saving parameters, see showSaveParameters
default_save_name default save name for saving, don't change, change save_name
    in save_param
```

# Details

Description of parameters.

# Value

ggplot

### See Also

```
spatGenePlot3D and spatGenePlot2D
```

Other spatial gene expression visualizations: spatGenePlot2D(), spatGenePlot3D()

#### **Examples**

```
spatGenePlot(gobject)
```

spatGenePlot2D 255

spatGenePlot2D

spatGenePlot2D

## **Description**

Visualize cells and gene expression according to spatial coordinates

## Usage

```
spatGenePlot2D(
 gobject,
  show_image = F,
 gimage = NULL,
  image_name = "image",
  sdimx = "sdimx",
  sdimy = "sdimy",
  expression_values = c("normalized", "scaled", "custom"),
 genes,
 cell_color_gradient = c("blue", "white", "red"),
 gradient_midpoint = NULL,
 gradient_limits = NULL,
 show_network = F,
 network_color = NULL,
  spatial_network_name = "Delaunay_network",
  edge_alpha = NULL,
 show\_grid = F,
 grid_color = NULL,
  spatial_grid_name = "spatial_grid",
 midpoint = 0,
  scale_alpha_with_expression = FALSE,
 point_shape = c("border", "no_border", "voronoi"),
 point_size = 1,
 point_alpha = 1,
 point_border_col = "black",
 point_border_stroke = 0.1,
  show_legend = T,
 legend_text = 8,
 background_color = "white",
 vor_border_color = "white",
  vor_alpha = 1,
  vor_max_radius = 200,
 axis_text = 8,
 axis_title = 8,
 cow_n_col = 2,
 cow_rel_h = 1,
 cow_rel_w = 1,
  cow_align = "h",
  show_plot = NA,
 return_plot = NA,
  save_plot = NA,
  save_param = list(),
```

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```
default_save_name = "spatGenePlot2D"
)
```

## **Arguments**

gobject giotto object

show\_image show a tissue background image

gimage a giotto image

image\_name name of a giotto image

sdimx x-axis dimension name (default = 'sdimx')
sdimy y-axis dimension name (default = 'sdimy')

expression\_values

gene expression values to use

genes genes to show

cell\_color\_gradient

vector with 3 colors for numeric data

gradient\_midpoint

midpoint for color gradient

gradient\_limits

vector with lower and upper limits

show\_network show underlying spatial network

network\_color color of spatial network

spatial\_network\_name

name of spatial network to use

edge\_alpha alpha of edge show\_grid show spatial grid grid\_color color of spatial grid

spatial\_grid\_name

name of spatial grid to use

midpoint expression midpoint
scale\_alpha\_with\_expression

scale expression with ggplot alpha parameter

point\_shape shape of points (border, no\_border or voronoi)

point\_size size of point (cell)

point\_alpha transparancy of points

point\_border\_col

color of border around points

point\_border\_stroke

stroke size of border around points

show\_legend show legend

legend\_text size of legend text

background\_color

color of plot background

vor\_border\_color

border colorr for voronoi plot

spatGenePlot3D 257

```
vor_alpha
                  transparancy of voronoi 'cells'
vor_max_radius maximum radius for voronoi 'cells'
axis_text
                  size of axis text
axis_title
                  size of axis title
                  cowplot param: how many columns
cow_n_col
cow_rel_h
                  cowplot param: relative height
                  cowplot param: relative width
cow_rel_w
                  cowplot param: how to align
cow_align
show_plot
                  show plots
return_plot
                  return ggplot object
save_plot
                  directly save the plot [boolean]
save_param
                  list of saving parameters, see showSaveParameters
default_save_name
                  default save name for saving, don't change, change save_name in save_param
```

#### **Details**

Description of parameters.

## Value

ggplot

# See Also

```
spatGenePlot3D
```

Other spatial gene expression visualizations: spatGenePlot3D(), spatGenePlot()

# **Examples**

spatGenePlot2D(gobject)

# Description

Visualize cells and gene expression according to spatial coordinates

258 spatGenePlot3D

#### Usage

```
spatGenePlot3D(
 gobject,
 expression_values = c("normalized", "scaled", "custom"),
  genes,
  show_network = FALSE,
 network_color = NULL,
  spatial_network_name = "Delaunay_network",
 edge_alpha = NULL,
 cluster_column = NULL,
  select_cell_groups = NULL,
 select_cells = NULL,
  show_other_cells = T,
 other_cell_color = "lightgrey",
 other_point_size = 1,
 genes_high_color = NULL,
 genes_mid_color = "white",
 genes_low_color = "blue",
  show_grid = FALSE,
  spatial_grid_name = "spatial_grid",
  point_size = 2,
  show_legend = TRUE,
  axis_scale = c("cube", "real", "custom"),
 custom_ratio = NULL,
 x_ticks = NULL,
 y_ticks = NULL,
 z_ticks = NULL,
  show_plot = NA,
 return_plot = NA,
 save_plot = NA,
 save_param = list(),
 default_save_name = "spatGenePlot3D"
)
```

```
gobject
                  giotto object
expression_values
                  gene expression values to use
genes
                  genes to show
                  show underlying spatial network
show_network
network_color
                  color of spatial network
spatial_network_name
                  name of spatial network to use
edge_alpha
                  alpha of edges
cluster_column cluster column to select groups
select_cell_groups
                  select subset of cells/clusters based on cell_color parameter
                  select subset of cells based on cell IDs
select_cells
```

spatGenePlot3D 259

```
show_other_cells
                  display not selected cells
other_cell_color
                  color of not selected cells
other_point_size
                  size of not selected cells
genes_high_color
                  color represents high gene expression
genes_mid_color
                  color represents middle gene expression
genes_low_color
                  color represents low gene expression
show_grid
                  show spatial grid
spatial_grid_name
                  name of spatial grid to use
point_size
                  size of point (cell)
show_legend
                  show legend
axis_scale
                  the way to scale the axis
custom_ratio
                  customize the scale of the plot
x_ticks
                  set the number of ticks on the x-axis
y_ticks
                  set the number of ticks on the y-axis
z_ticks
                  set the number of ticks on the z-axis
show_plot
                  show plots
return_plot
                  return ggplot object
save_plot
                  directly save the plot [boolean]
                  list of saving parameters, see showSaveParameters
save_param
default_save_name
                  default save name for saving, don't change, change save_name in save_param
```

## **Details**

Description of parameters.

#### Value

ggplot

## See Also

Other spatial gene expression visualizations: spatGenePlot2D(), spatGenePlot()

#### **Examples**

```
spatGenePlot3D(gobject)
```

260 spatialAEH

spatialAEH spatialAEH

# Description

Compute spatial variable genes with spatialDE method

## Usage

```
spatialAEH(
  gobject = NULL,
  SpatialDE_results = NULL,
  name_pattern = "AEH_patterns",
  expression_values = c("raw", "normalized", "scaled", "custom"),
  pattern_num = 6,
  l = 1.05,
  python_path = NULL,
  return_gobject = TRUE
)
```

## **Arguments**

# Details

This function is a wrapper for the SpatialAEH method implemented in the ...

# Value

An updated giotto object

spatialDE 261

spatialDE	spatialDE
Spatiaide	spanan

## **Description**

Compute spatial variable genes with spatialDE method

## Usage

```
spatialDE(
  gobject = NULL,
  expression_values = c("raw", "normalized", "scaled", "custom"),
  size = c(4, 2, 1),
  color = c("blue", "green", "red"),
  sig_alpha = 0.5,
  unsig_alpha = 0.5,
  python_path = NULL,
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "SpatialDE"
)
```

# Arguments

```
gobject
                  Giotto object
expression_values
                  gene expression values to use
size
                  size of plot
color
                  low/medium/high color scheme for plot
sig_alpha
                  alpha value for significance
unsig_alpha
                  alpha value for unsignificance
                  specify specific path to python if required
python_path
show_plot
                  show plot
                  return ggplot object
return_plot
save_plot
                  directly save the plot [boolean]
save_param
                  list of saving parameters, see showSaveParameters
default_save_name
                  default save name for saving, don't change, change save_name in save_param
```

## **Details**

This function is a wrapper for the SpatialDE method implemented in the ...

# Value

a list of data.frames with results and plot (optional)

262 spatNetwDistributions

```
{\tt spatNetwDistributions} \ \textit{spatNetwDistributionsDistance}
```

#### **Description**

This function return histograms displaying the distance distribution for each spatial k-neighbor

# Usage

```
spatNetwDistributions(
  gobject,
  spatial_network_name = "spatial_network",
  distribution = c("distance", "k_neighbors"),
  hist_bins = 30,
  test_distance_limit = NULL,
  ncol = 1,
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "spatNetwDistributions"
)
```

# **Arguments**

```
gobject
                  Giotto object
spatial_network_name
                  name of spatial network
distribution
                  show the distribution of cell-to-cell distance or number of k neighbors
hist_bins
                  number of binds to use for the histogram
test_distance_limit
                  effect of different distance threshold on k-neighbors
                  number of columns to visualize the histograms in
ncol
show_plot
                  show plot
return_plot
                  return ggplot object
save_plot
                  directly save the plot [boolean]
save_param
                  list of saving parameters from all_plots_save_function
default_save_name
                  default save name for saving, alternatively change save_name in save_param
```

#### **Details**

The **distance** option shows the spatial distance distribution for each nearest neighbor rank (1st, 2nd, 3th, ... neigbor). With this option the user can also test the effect of a distance limit on the spatial network. This distance limit can be used to remove neigbor cells that are considered to far away. The **k\_neighbors** option shows the number of k neighbors distribution over all cells.

#### Value

```
ggplot plot
```

#### **Examples**

```
spatNetwDistributionsDistance(gobject)
```

```
spat {\tt NetwDistributionsDistance} \\ spat {\tt NetwDistributionsDistance}
```

# **Description**

This function return histograms displaying the distance distribution for each spatial k-neighbor

## Usage

```
spatNetwDistributionsDistance(
  gobject,
  spatial_network_name = "spatial_network",
  hist_bins = 30,
  test_distance_limit = NULL,
  ncol = 1,
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "spatNetwDistributionsDistance")
```

# **Arguments**

```
gobject
                  Giotto object
spatial_network_name
                  name of spatial network
                  number of binds to use for the histogram
hist_bins
test_distance_limit
                  effect of different distance threshold on k-neighbors
ncol
                  number of columns to visualize the histograms in
show_plot
                  show plot
return_plot
                  return ggplot object
                  directly save the plot [boolean]
save_plot
                  list of saving parameters from all_plots_save_function
save_param
default_save_name
                  default save name for saving, alternatively change save_name in save_param
```

#### Value

```
ggplot plot
```

#### **Examples**

```
spatNetwDistributionsDistance(gobject)
```

```
spat Netw Distributions Kneighbors \\ spat Netw Distributions Kneighbors
```

## **Description**

This function returns a histogram displaying the number of k-neighbors distribution for each cell

## Usage

```
spatNetwDistributionsKneighbors(
  gobject,
  spatial_network_name = "spatial_network",
  hist_bins = 30,
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "spatNetwDistributionsKneighbors")
```

# **Arguments**

```
Giotto object
gobject
spatial_network_name
                  name of spatial network
hist_bins
                  number of binds to use for the histogram
show_plot
                  show plot
return_plot
                  return ggplot object
                  directly save the plot [boolean]
save_plot
                  list of saving parameters from all_plots_save_function
save_param
default_save_name
                  default save name for saving, alternatively change save_name in save_param
```

# Value

ggplot plot

### **Examples**

```
spatNetwDistributionsKneighbors(gobject)
```

spatPlot 265

spatPlot spatPlot

#### **Description**

Visualize cells according to spatial coordinates

#### Usage

```
spatPlot(...)
```

# Arguments

```
Arguments passed on to spatPlot2D
gobject giotto object
show_image show a tissue background image
gimage a giotto image
image_name name of a giotto image
group_by create multiple plots based on cell annotation column
group_by_subset subset the group_by factor column
sdimx x-axis dimension name (default = 'sdimx')
sdimy y-axis dimension name (default = 'sdimy')
spat_enr_names names of spatial enrichment results to include
cell_color color for cells (see details)
color_as_factor convert color column to factor
cell_color_code named vector with colors
cell_color_gradient vector with 3 colors for numeric data
gradient_midpoint midpoint for color gradient
gradient_limits vector with lower and upper limits
select_cell_groups select subset of cells/clusters based on cell_color param-
    eter
select_cells select subset of cells based on cell IDs
point_shape shape of points (border, no_border or voronoi)
point_size size of point (cell)
point_alpha transparancy of point
point_border_col color of border around points
point_border_stroke stroke size of border around points
show_cluster_center plot center of selected clusters
show_center_label plot label of selected clusters
center_point_size size of center points
center_point_border_col border color of center points
center_point_border_stroke border stroke size of center points
label_size size of labels
label_fontface font of labels
show_network show underlying spatial network
```

spatial\_network\_name name of spatial network to use

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```
network_color color of spatial network
network_alpha alpha of spatial network
show_grid show spatial grid
spatial_grid_name name of spatial grid to use
grid_color color of spatial grid
show_other_cells display not selected cells
other_cell_color color of not selected cells
other_point_size point size of not selected cells
other_cells_alpha alpha of not selected cells
coord_fix_ratio fix ratio between x and y-axis
title title of plot
show_legend show legend
legend_text size of legend text
legend_symbol_size size of legend symbols
background_color color of plot background
vor_border_color border colorr for voronoi plot
vor_max_radius maximum radius for voronoi 'cells'
vor_alpha transparancy of voronoi 'cells'
axis_text size of axis text
axis_title size of axis title
cow_n_col cowplot param: how many columns
cow_rel_h cowplot param: relative height
cow_rel_w cowplot param: relative width
cow_align cowplot param: how to align
show_plot show plot
return_plot return ggplot object
save_plot directly save the plot [boolean]
save_param list of saving parameters, see showSaveParameters
default_save_name default save name for saving, don't change, change save_name
    in save_param
```

#### **Details**

Description of parameters.

#### Value

ggplot

#### See Also

```
spatPlot3D
```

Other spatial visualizations: spatPlot2D(), spatPlot3D()

#### **Examples**

```
spatPlot(gobject)
```

spatPlot2D 267

spatPlot2D

spatPlot2D

#### **Description**

Visualize cells according to spatial coordinates

## Usage

```
spatPlot2D(
 gobject,
  show_image = F,
  gimage = NULL,
  image_name = "image",
 group_by = NULL,
 group_by_subset = NULL,
  sdimx = "sdimx",
  sdimy = "sdimy",
  spat_enr_names = NULL,
 cell_color = NULL,
 color_as_factor = T,
 cell_color_code = NULL,
 cell_color_gradient = c("blue", "white", "red"),
  gradient_midpoint = NULL,
  gradient_limits = NULL,
 select_cell_groups = NULL,
  select_cells = NULL,
 point_shape = c("border", "no_border", "voronoi"),
 point_size = 3,
 point_alpha = 1,
 point_border_col = "black",
  point_border_stroke = 0.1,
  show_cluster_center = F,
  show_center_label = F,
  center_point_size = 4,
  center_point_border_col = "black",
  center_point_border_stroke = 0.1,
  label_size = 4,
 label_fontface = "bold",
  show_network = F,
  spatial_network_name = "Delaunay_network",
 network_color = NULL,
 network_alpha = 1,
  show_grid = F,
  spatial_grid_name = "spatial_grid",
 grid_color = NULL,
  show_other_cells = T,
 other_cell_color = "lightgrey",
 other_point_size = 1,
 other_cells_alpha = 0.1,
  coord_fix_ratio = NULL,
```

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```
title = NULL,
  show_legend = T,
  legend_text = 8,
  legend_symbol_size = 1,
  background_color = "white",
  vor_border_color = "white",
  vor_max_radius = 200,
  vor_alpha = 1,
  axis_text = 8,
  axis_title = 8,
  cow_n_col = 2,
  cow_rel_h = 1,
  cow_rel_w = 1,
  cow_align = "h",
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
 save_param = list(),
 default_save_name = "spatPlot2D"
)
```

```
gobject
                  giotto object
                  show a tissue background image
show_image
                  a giotto image
gimage
                  name of a giotto image
image_name
                  create multiple plots based on cell annotation column
group_by
group_by_subset
                  subset the group_by factor column
                  x-axis dimension name (default = 'sdimx')
sdimx
                  y-axis dimension name (default = 'sdimy')
sdimy
spat_enr_names names of spatial enrichment results to include
cell_color
                  color for cells (see details)
color_as_factor
                  convert color column to factor
cell_color_code
                  named vector with colors
cell_color_gradient
                  vector with 3 colors for numeric data
gradient_midpoint
                  midpoint for color gradient
gradient_limits
                  vector with lower and upper limits
select_cell_groups
                  select subset of cells/clusters based on cell_color parameter
select_cells
                  select subset of cells based on cell IDs
                  shape of points (border, no_border or voronoi)
point_shape
point_size
                  size of point (cell)
```

spatPlot2D 269

point\_alpha transparancy of point point\_border\_col color of border around points point\_border\_stroke stroke size of border around points show\_cluster\_center plot center of selected clusters show\_center\_label plot label of selected clusters center\_point\_size size of center points center\_point\_border\_col border color of center points  ${\tt center\_point\_border\_stroke}$ border stroke size of center points label\_size size of labels label\_fontface font of labels show\_network show underlying spatial network spatial\_network\_name name of spatial network to use color of spatial network network\_color alpha of spatial network network\_alpha show\_grid show spatial grid spatial\_grid\_name name of spatial grid to use grid\_color color of spatial grid show\_other\_cells display not selected cells other\_cell\_color color of not selected cells other\_point\_size point size of not selected cells other\_cells\_alpha alpha of not selected cells coord\_fix\_ratio fix ratio between x and y-axis title title of plot show\_legend show legend legend\_text size of legend text legend\_symbol\_size size of legend symbols background\_color color of plot background vor\_border\_color border colorr for voronoi plot vor\_max\_radius maximum radius for voronoi 'cells' 270 spatPlot3D

```
vor_alpha
                  transparancy of voronoi 'cells'
                  size of axis text
axis_text
                  size of axis title
axis_title
cow_n_col
                  cowplot param: how many columns
                  cowplot param: relative height
cow_rel_h
                  cowplot param: relative width
cow_rel_w
                  cowplot param: how to align
cow_align
show_plot
                  show plot
return_plot
                  return ggplot object
save_plot
                  directly save the plot [boolean]
                  list of saving parameters, see showSaveParameters
save_param
default_save_name
                  default save name for saving, don't change, change save_name in save_param
```

#### **Details**

Description of parameters.

#### Value

ggplot

#### See Also

```
spatPlot3D
```

Other spatial visualizations: spatPlot3D(), spatPlot()

## **Examples**

```
spatPlot2D(gobject)
```

spatPlot3D spatPlot3D

## **Description**

Visualize cells according to spatial coordinates

# Usage

```
spatPlot3D(
  gobject,
  sdimx = "sdimx",
  sdimy = "sdimy",
  sdimz = "sdimz",
  spat_enr_names = NULL,
  point_size = 3,
  cell_color = NULL,
```

spatPlot3D 271

```
cell_color_code = NULL,
  select_cell_groups = NULL,
  select_cells = NULL,
  show_other_cells = T,
  other_cell_color = "lightgrey",
  other_point_size = 0.5,
  other_cell_alpha = 0.5,
  show_network = F,
  spatial_network_name = "Delaunay_network",
  network_color = NULL,
  network_alpha = 1,
  show\_grid = F,
  spatial_grid_name = "spatial_grid",
  grid_color = NULL,
  grid_alpha = 1,
  title = "",
  show_legend = T,
  axis_scale = c("cube", "real", "custom"),
  custom_ratio = NULL,
  x_ticks = NULL,
  y_ticks = NULL,
  z_ticks = NULL,
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "spat3D"
)
```

```
gobject
                  giotto object
sdimx
                  x-axis dimension name (default = 'sdimx')
sdimy
                  y-axis dimension name (default = 'sdimy')
sdimz
                  z-axis dimension name (default = 'sdimy')
spat_enr_names names of spatial enrichment results to include
                  size of point (cell)
point_size
cell_color
                  color for cells (see details)
cell_color_code
                  named vector with colors
select_cell_groups
                  select subset of cells/clusters based on cell_color parameter
                  select subset of cells based on cell IDs
select_cells
show_other_cells
                  display not selected cells
other_cell_color
                  color of not selected cells
other_point_size
                  size of not selected cells
```

272 spatPlot3D

other\_cell\_alpha

alpha of not selected cells

show\_network show underlying spatial network

spatial\_network\_name

name of spatial network to use

network\_color color of spatial network

network\_alpha opacity of spatial network

show\_grid show spatial grid

spatial\_grid\_name

name of spatial grid to use

grid\_color color of spatial grid grid\_alpha opacity of spatial grid

title title of plot show\_legend show legend

axis\_scale the way to scale the axis

custom\_ratio customize the scale of the plot

x\_ticks set the number of ticks on the x-axis y\_ticks set the number of ticks on the y-axis

z\_ticks set the number of ticks on the z-axis

show\_plot show plot

return\_plot return ggplot object

save\_plot directly save the plot [boolean]

save\_param list of saving parameters, see showSaveParameters

 ${\tt default\_save\_name}$ 

default save name for saving, don't change, change save\_name in save\_param

## Value

ggplot

#### See Also

Other spatial visualizations: spatPlot2D(), spatPlot()

#### **Examples**

spatPlot3D(gobject)

```
specific Cell Cell communication Scores\\ specific Cell Cell communication Scores
```

# Description

Specific Cell-Cell communication scores based on spatial expression of interacting cells

#### Usage

```
specificCellCellcommunicationScores(
  gobject,
  spatial_network_name = "Delaunay_network",
  cluster_column = "cell_types",
  random_iter = 100,
  cell_type_1 = "astrocyte",
  cell_type_2 = "endothelial",
  gene_set_1,
  gene_set_2,
  log2FC_addendum = 0.1,
 min_observations = 2,
 detailed = FALSE,
 adjust_method = c("fdr", "bonferroni", "BH", "holm", "hochberg", "hommel", "BY",
    "none"),
  adjust_target = c("genes", "cells"),
  verbose = T
)
```

```
giotto object to use
gobject
spatial_network_name
                  spatial network to use for identifying interacting cells
cluster_column cluster column with cell type information
random_iter
                  number of iterations
cell_type_1
                  first cell type
cell_type_2
                  second cell type
                  first specific gene set from gene pairs
gene_set_1
gene_set_2
                  second specific gene set from gene pairs
log2FC_addendum
                  addendum to add when calculating log2FC
min_observations
                  minimum number of interactions needed to be considered
                  provide more detailed information (random variance and z-score)
detailed
                  which method to adjust p-values
adjust_method
                  adjust multiple hypotheses at the cell or gene level
adjust_target
verbose
                  verbose
```

#### **Details**

Statistical framework to identify if pairs of genes (such as ligand-receptor combinations) are expressed at higher levels than expected based on a reshuffled null distribution of gene expression values in cells that are spatially in proximity to eachother.

- LR\_comb:Pair of ligand and receptor
- lig\_cell\_type: cell type to assess expression level of ligand
- lig\_expr: average expression of ligand in lig\_cell\_type
- · ligand: ligand name
- rec\_cell\_type: cell type to assess expression level of receptor
- rec\_expr: average expression of receptor in rec\_cell\_type
- · receptor: receptor name
- LR\_expr: combined average ligand and receptor expression
- lig\_nr: total number of cells from lig\_cell\_type that spatially interact with cells from rec\_cell\_type
- rec\_nr: total number of cells from rec\_cell\_type that spatially interact with cells from lig\_cell\_type
- rand\_expr: average combined ligand and receptor expression from random spatial permutations
- av\_diff: average difference between LR\_expr and rand\_expr over all random spatial permutations
- sd\_diff: (optional) standard deviation of the difference between LR\_expr and rand\_expr over all random spatial permutations
- z\_score: (optinal) z-score
- log2fc: log2 fold-change (LR\_expr/rand\_expr)
- pvalue: p-value
- LR\_cell\_comb: cell type pair combination
- p.adj: adjusted p-value
- PI: significanc score: log2fc \* -log10(p.adj)

#### Value

Cell-Cell communication scores for gene pairs based on spatial interaction

#### **Examples**

 ${\tt specificCellCellcommunicationScores(gobject)}$ 

# Description

Merge selected clusters based on pairwise correlation scores and size of cluster.

# Usage

```
split_dendrogram_in_two(dend)
```

# Arguments

dend

dendrogram object

## Value

list of two dendrograms and height of node

## **Examples**

```
split_dendrogram_in_two(dend)
```

stitchFieldCoordinates

stitchFieldCoordinates

# Description

Helper function to stitch field coordinates together to form one complete picture

## Usage

```
stitchFieldCoordinates(
  location_file,
  offset_file,
  cumulate_offset_x = F,
  cumulate_offset_y = F,
  field_col = "Field of View",
  X_coord_col = "X",
  Y_coord_col = "Y",
  reverse_final_x = F,
  reverse_final_y = T
)
```

276 stitchTileCoordinates

## **Arguments**

```
location dataframe with X and Y coordinates
location_file
offset_file
                  dataframe that describes the offset for each field (see details)
cumulate_offset_x
                  (boolean) Do the x-axis offset values need to be cumulated?
cumulate_offset_y
                  (boolean) Do the y-axis offset values need to be cumulated?
                  column that indicates the field within the location_file
field_col
X_coord_col
                  column that indicates the x coordinates
Y_coord_col
                  column that indicates the x coordinates
reverse_final_x
                  (boolean) Do the final x coordinates need to be reversed?
reverse_final_y
                  (boolean) Do the final y coordinates need to be reversed?
```

#### **Details**

Stitching of fields:

- 1. have cell locations: at least 3 columns: field, X, Y
- 2. create offset file: offset file has 3 columns: field, x\_offset, y\_offset
- 3. create new cell location file by stitching original cell locations with stitchFieldCoordinates
- 4. provide new cell location file to createGiottoObject

#### Value

Updated location dataframe with new X ['X\_final'] and Y ['Y\_final'] coordinates

```
stitchTileCoordinates stitchTileCoordinates
```

## **Description**

Helper function to stitch tile coordinates together to form one complete picture

#### Usage

```
stitchTileCoordinates(location_file, Xtilespan, Ytilespan)
```

```
 \begin{array}{ll} \mbox{location\_file} & \mbox{location dataframe with $X$ and $Y$ coordinates} \\ \mbox{Xtilespan} & \mbox{numerical value specifying the width of each tile} \\ \mbox{Ytilespan} & \mbox{numerical value specifying the height of each tile} \\ \end{array}
```

subClusterCells 277

subClusterCells

subClusterCells

#### **Description**

subcluster cells

## Usage

```
subClusterCells(
  gobject,
 name = "sub_clus",
 cluster_method = c("leiden", "louvain_community", "louvain_multinet"),
 cluster_column = NULL,
  selected_clusters = NULL,
 hvg_param = list(reverse_log_scale = T, difference_in_cov = 1, expression_values =
    "normalized"),
 hvg_min_perc_cells = 5,
 hvg_mean_expr_det = 1,
 use_all_genes_as_hvg = FALSE,
 min_nr_of_hvg = 5,
 pca_param = list(expression_values = "normalized", scale_unit = T),
 nn_param = list(dimensions_to_use = 1:20),
 k_neighbors = 10,
 resolution = 1,
 n_{iterations} = 1000,
 gamma = 1,
 omega = 1,
 python_path = NULL,
 nn_network_to_use = "sNN",
 network_name = "sNN.pca",
 return_gobject = TRUE,
  verbose = T
)
```

```
gobject
                  giotto object
                  name for new clustering result
name
cluster_method clustering method to use
cluster_column cluster column to subcluster
selected_clusters
                  only do subclustering on these clusters
                  parameters for calculateHVG
hvg_param
hvg_min_perc_cells
                  threshold for detection in min percentage of cells
hvg_mean_expr_det
                  threshold for mean expression level in cells with detection
use_all_genes_as_hvg
                  forces all genes to be HVG and to be used as input for PCA
```

278 subClusterCells

min\_nr\_of\_hvg minimum number of HVG, or all genes will be used as input for PCA

pca\_param parameters for runPCA

nn\_param parameters for parameters for createNearestNetwork

k\_neighbors number of k for createNearestNetwork

resolution resolution

n\_iterations number of interations to run the Leiden algorithm.

gamma gamma omega omega

python\_path specify specific path to python if required

nn\_network\_to\_use

type of NN network to use (kNN vs sNN)

network\_name name of NN network to use

return\_gobject boolean: return giotto object (default = TRUE)

verbose verbose

#### **Details**

This function performs subclustering on selected clusters. The systematic steps are:

- 1. subset Giotto object
- 2. identify highly variable genes
- 3. run PCA
- 4. create nearest neighbouring network
- 5. do clustering

#### Value

giotto object with new subclusters appended to cell metadata

# See Also

doLouvainCluster\_multinet, doLouvainCluster\_community and @seealso doLeidenCluster

# **Examples**

subClusterCells(gobject)

subsetGiotto 279

subsetGiotto

subsetGiot to

# Description

subsets Giotto object including previous analyses.

# Usage

```
subsetGiotto(gobject, cell_ids = NULL, gene_ids = NULL, verbose = FALSE)
```

# Arguments

```
gobject giotto object
cell_ids cell IDs to keep
gene_ids gene IDs to keep
verbose be verbose
```

#### Value

giotto object

## **Examples**

```
subsetGiotto(gobject)
```

 ${\tt subsetGiottoLocs}$ 

subsetGiottoLocs

# **Description**

subsets Giotto object based on spatial locations

# Usage

```
subsetGiottoLocs(
  gobject,
  x_max = NULL,
  x_min = NULL,
  y_max = NULL,
  y_min = NULL,
  z_max = NULL,
  z_min = NULL,
  return_gobject = T,
  verbose = FALSE
)
```

280 trendSceek

# **Arguments**

gobject	giotto object
x_max	maximum x-coordinate
x_min	minimum x-coordinate
y_max	maximum y-coordinate
y_min	minimum y-coordinate
z_max	maximum z-coordinate
z_min	minimum z-coordinate
return_gobject	return Giotto object
verbose	be verbose

#### **Details**

if return\_gobject = FALSE, then a filtered combined metadata data.table will be returned

#### Value

giotto object

## **Examples**

```
subsetGiottoLocs(gobject)
```

trendSceek trendSceek

# Description

Compute spatial variable genes with trendsceek method

# Usage

```
trendSceek(
  gobject,
  expression_values = c("normalized", "raw"),
  subset_genes = NULL,
  nrand = 100,
  ncores = 8,
  ...
)
```

```
gobject Giotto object
expression_values
gene expression values to use
subset_genes subset of genes to run trendsceek on
nrand An integer specifying the number of random resamplings of the mark distribution as to create the null-distribution.
ncores An integer specifying the number of cores to be used by BiocParallel
... Additional parameters to the trendsceek_test function
```

updateGiottoImage 281

#### **Details**

This function is a wrapper for the trendsceek\_test method implemented in the trendsceek package

#### Value

data.frame with trendsceek spatial genes results

updateGiottoImage

updateGiottoImage

## **Description**

Updates the boundaries of a giotto image attached to a giotto object

## Usage

```
updateGiottoImage(
  gobject,
  image_name,
  xmax_adj = 0,
  xmin_adj = 0,
  ymax_adj = 0,
  ymin_adj = 0,
  return_gobject = TRUE
)
```

# **Arguments**

```
gobject giotto object
image_name spatial locations

xmax_adj adjustment of the maximum x-value to align the image

xmin_adj adjustment of the minimum x-value to align the image

ymax_adj adjustment of the maximum y-value to align the image

ymin_adj adjustment of the minimum y-value to align the image

return_gobject return a giotto object
```

#### Value

```
a giotto object or an updated giotto image if return_gobject = F
```

# **Examples**

```
updateGiottoImage(gobject)
```

282 viewHMRFresults2D

viewHMRFresults

viewHMRFresults

# Description

View results from doHMRF.

# Usage

```
viewHMRFresults(
  gobject,
  HMRFoutput,
  k = NULL,
  betas_to_view = NULL,
  third_dim = FALSE,
  ...
)
```

# Arguments

gobject giotto object

HMRF output from doHMRF k number of HMRF domains

betas\_to\_view results from different betas that you want to view

third\_dim 3D data (boolean)

... additional paramters (see details)

## Value

spatial plots with HMRF domains

# See Also

```
spatPlot2D and spatPlot3D
```

viewHMRFresults2D

viewHMRFresults2D

# Description

View results from doHMRF.

# Usage

```
viewHMRFresults2D(gobject, HMRFoutput, k = NULL, betas_to_view = NULL, ...)
```

viewHMRFresults3D 283

## **Arguments**

gobject giotto object

HMRF output from doHMRF k number of HMRF domains

betas\_to\_view results from different betas that you want to view

... additional parameters to spatPlot2D()

#### Value

spatial plots with HMRF domains

#### See Also

spatPlot2D

viewHMRFresults3D v

viewHMRFresults3D

# Description

View results from doHMRF.

# Usage

```
viewHMRFresults3D(gobject, HMRFoutput, k = NULL, betas_to_view = NULL, ...)
```

# Arguments

gobject giotto object

HMRF output from doHMRF k number of HMRF domains

betas\_to\_view results from different betas that you want to view

... additional parameters to spatPlot3D()

## Value

spatial plots with HMRF domains

# See Also

spatPlot3D

284 violinPlot

violinPlot

violinPlot

## **Description**

Creates violinplot for selected clusters

# Usage

```
violinPlot(
  gobject,
  expression_values = c("normalized", "scaled", "custom"),
  genes,
  cluster_column,
  cluster_custom_order = NULL,
  color_violin = c("genes", "cluster"),
  cluster_color_code = NULL,
  strip_position = c("top", "right", "left", "bottom"),
  strip\_text = 7,
  axis_text_x_size = 10,
  axis_text_y_size = 6,
  show_plot = NA,
  return_plot = NA,
  save_plot = NA,
  save_param = list(),
  default_save_name = "violinPlot"
)
```

```
gobject
                  giotto object
expression_values
                  expression values to use
                  genes to plot
genes
cluster_column name of column to use for clusters
cluster_custom_order
                  custom order of clusters
color_violin
                  color violin according to genes or clusters
cluster_color_code
                  color code for clusters
strip_position position of gene labels
strip_text
                  size of strip text
\verb"axis_text_x_size"
                  size of x-axis text
axis_text_y_size
                  size of y-axis text
show_plot
                  show plot
return_plot
                  return ggplot object
```

writeHMRFresults 285

## Value

ggplot

## **Examples**

```
violinPlot(gobject)
```

writeHMRFresults

writeHMRFresults

## **Description**

write results from doHMRF to a data.table.

# Usage

```
writeHMRFresults(
  gobject,
  HMRFoutput,
  k = NULL,
  betas_to_view = NULL,
  print_command = F
)
```

# Arguments

gobject giotto object

HMRF output from doHMRF

k k to write results for

betas\_to\_view results from different betas that you want to view

print\_command see the python command

## Value

data.table with HMRF results for each b and the selected k

## **Examples**

```
writeHMRFresults(gobject)
```

```
write\_giotto\_viewer\_annotation \\ write\_giotto\_viewer\_annotation
```

## **Description**

write out factor-like annotation data from a giotto object for the Viewer

# Usage

```
write_giotto_viewer_annotation(
  annotation,
  annot_name = "test",
  output_directory = getwd()
)
```

# Arguments

```
annotation annotation from the data.table from giotto object
annot_name name of the annotation
output_directory
directory where to save the files
```

## Value

write a .txt and .annot file for the selection annotation

```
write\_giotto\_viewer\_dim\_reduction \\ write\_giotto\_viewer\_dim\_reduction
```

# Description

write out dimensional reduction data from a giotto object for the Viewer

#### Usage

```
write_giotto_viewer_dim_reduction(
  dim_reduction_cell,
  dim_red = NULL,
  dim_red_name = NULL,
  dim_red_rounding = NULL,
  dim_red_rescale = c(-20, 20),
  output_directory = getwd()
)
```

### **Arguments**

```
dim_reduction_cell

dimension reduction slot from giotto object

dim_red high level name of dimension reduction

dim_red_name specific name of dimension reduction to use

dim_red_rounding

numerical indicating how to round the coordinates

dim_red_rescale

numericals to rescale the coordinates

output_directory

directory where to save the files
```

#### Value

write a .txt and .annot file for the selection annotation

## **Description**

write out numeric annotation data from a giotto object for the Viewer

# Usage

```
write_giotto_viewer_numeric_annotation(
  annotation,
  annot_name = "test",
  output_directory = getwd()
)
```

## **Arguments**

```
annotation annotation from the data.table from giotto object
annot_name name of the annotation
output_directory
directory where to save the files
```

## Value

write a .txt and .annot file for the selection annotation

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