Package ‘SPIAT’

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Type Package

Title Spatial Image Analysis of Tissues

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Description SPIAT (**Sp**atial **I**mage **A**nalysis of **T**issues) is an R package with a suite of data processing, quality control, visualization and data analysis tools. SPIAT is compatible with data generated from single-cell spatial proteomics platforms (e.g. OPAL, CODEX, MIBI, cellprofiler). SPIAT reads spatial data in the form of X and Y coordinates of cells, marker intensities and cell phenotypes. SPIAT includes six analysis modules that allow visualization, calculation of cell colocalization, categorization of the immune microenvironment relative to tumor areas, analysis of cellular neighborhoods, and the quantification of spatial heterogeneity, providing a comprehensive toolkit for spatial data analysis.

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`SPIAT-package` *SPIAT: Spatial Image Analysis of Tissues*

Description

SPIAT (**Sp**atial **I**mage **A**nalysis of **T**issues) is an R package with a suite of data processing, quality control, visualization and data analysis tools. SPIAT is compatible with data generated from single-cell spatial proteomics platforms (e.g. OPAL, CODEX, MIBI, cellprofiler). SPIAT reads spatial data in the form of X and Y coordinates of cells, marker intensities and cell phenotypes. SPIAT includes six analysis modules that allow visualization, calculation of cell colocalization, categorization of the immune microenvironment relative to tumor areas, analysis of cellular neighborhoods, and the quantification of spatial heterogeneity, providing a comprehensive toolkit for spatial data analysis.

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See Also

Useful links:

- https://trigosteam.github.io/SPIAT/
- Report bugs at https://github.com/trigosteam/SPIAT/issues

AUC_of_cross_function *The difference in AUC of the cross function curves*

Description

Calculate the difference of area under the curve (AUC) between two curves, normalised by the total area of the graph.

Usage

```
AUC_of_cross_function(df.cross)
```

Arguments

df.cross	Data.frame. The output of calculate_cross_functions. Containing the positions of the two curves. Columns contain "r", "border" and "theo".

Value

A number

Examples

```
df_cross <- calculate_cross_functions(SPIAT::defined_image, method = "Kcross",
              cell_types_of_interest = c("Tumour","Immune3"),
              feature_colname ="Cell.Type", dist = 100)
AUC_of_cross_function(df_cross)
```

average_marker_intensity_within_radius

average_marker_intensity_within_radius

Description

Calculates the average intensity of the target_marker within a radius from the cells positive for the reference marker. Note that it pools all cells with the target marker that are within the specific radius of any reference cell. Results represent the average intensities within a radius, but not a vector of metrics for each cell.

Usage

```
average_marker_intensity_within_radius(
  spe_object,
  reference_marker,
  target_marker,
  radius = 20
)
```

Arguments

spe_object	SpatialExperiment object in the form of the output of format_image_to_spe.
reference_marker	String specifying the marker that is used for reference cells.
target_marker	String specifying the marker to calculate its average intensity.
radius	Numeric specifying the radius of search for cells around the reference cells.

Value

A single number is returned

Examples

```
average_marker_intensity_within_radius(SPIAT::simulated_image,
                                       reference_marker ="Immune_marker3",
                                       target_marker = "Immune_marker2",
                                       radius=30)
```

average_minimum_distance

average_minimum_distance

Description

Calculates the average minimum distance of all cells to their nearest cells in the input image.

Usage

```
average_minimum_distance(spe_object)
```

Arguments

spe_object	SpatialExperiment object in the form of the output of format_image_to_spe.

Value

A single number is returned

Examples

```
average_minimum_distance(SPIAT::simulated_image)
```

average_nearest_neighbor_index

Average nearest neighbor index for point pattern (clustering or dispersion)

Description

Calculate the the average nearest neighbor (ANN) index of a specified type of cells. The index indicates the clustering effect of a point pattern. The pattern can be clustering, random or dispersion.

Usage

```
average_nearest_neighbor_index(
  spe_object,
  reference_celltypes,
  feature_colname,
  p_val = 5e-06
)
```

Arguments

- `spe_object` SpatialExperiment object in the form of the output of `format_image_to_spe`.
- `reference_celltypes` String Vector. Cells with these cell types will be used for ANNI calculation.
- `feature_colname` String. Specify the selected column for ‘reference_celltypes‘.
- `p_val` Numeric. The p value threshold to determine the significance of a pattern.

Details

ANN index is a statistical test to test for the presence of clusters of cells, (Clark and Evans, 1954). The ANN index evaluates the spatial aggregation or dispersion effect of objects based on the average distances between pairs of the nearest objects and can be used to test for the clustering of specific cell types (e.g. immune or tumor cells). Next, the z score and p-value of the ANN index is calculated to validate the significance of the pattern.

Value

A list with the ANN index, the pattern type and the corresponding p value

Examples

```
average_nearest_neighbor_index(SPIAT::defined_image, reference_celltypes =
"Tumour", feature_colname = "Cell.Type")
```

`average_percentage_of_cells_within_radius` *average_percentage_of_cells_within_radius*

Description

Calculates the average percentage of cells of a target cell type within a radius from the cells with a reference cell type. The calculation is done per reference cell, so runtime will depend on the number of reference cells present. Output is a single value (the mean for the image).

Usage

```
average_percentage_of_cells_within_radius(
  spe_object,
  reference_celltype,
  target_celltype,
  radius = 100,
  feature_colname
)
```

Arguments

- `spe_object` SpatialExperiment object in the form of the output of `format_image_to_spe`.
- `reference_celltype` String specifying the cell type of reference cells.
- `target_celltype` String specifying the cell type for target cells
- `radius` Integer specifying the radius of search for cells around the reference cells. Radii of ~100 are recommended. If too small, too few cells might be present.
- `feature_colname` String specifying the column with the desired cell type annotations.

Value

A numeric vector and a plot are returned

Examples

```
average_percentage_of_cells_within_radius(SPIAT::defined_image, "Tumour",
"Immune3", radius = 100, "Cell.Type")
```

---

calculate_cell_proportions *calculate_cell_proportions*

Description

Calculates the number and proportion of each cell type.

Usage

```
calculate_cell_proportions(
  spe_object,
  reference_celltypes = NULL,
  celltypes_to_exclude = NULL,
  feature_colname = "Phenotype",
  plot.image = TRUE
)
```

Arguments

- `spe_object` SpatialExperiment object in the form of the output of `format_image_to_spe`.
- `reference_celltypes` String Vector specifying reference cell types. If NULL (default), then the proportion of each cell type against all cells is returned. Alternatively, a custom vector of cell types can be used as input, and these will be used as the denominator in the calculation of the proportions.

`celltypes_to_exclude`
: String Vector specifying cell types to exclude. For example "OTHER" will exclude that celltype from the Total. If NULL, all cell types are included.

`feature_colname`
: String. Column of cells to choose the cell type from (e.g. Phenotype, Cell.Type, etc).

`plot.image`
: Boolean. Whether to plot the barplot of the cell percentages. By default is TRUE.

Value

A data.frame is returned

Examples

```
calculate_cell_proportions(SPIAT::defined_image, reference_celltypes = NULL,
celltypes_to_exclude = "Others", feature_colname="Cell.Type", plot.image = FALSE)
```

`calculate_cross_functions` *calculate_cross_functions*

Description

Compute and plot the cross functions between two specified cell types. This function implements the cross functions from [spatstat] package.

Usage

```
calculate_cross_functions(
  spe_object,
  method = "Kcross",
  cell_types_of_interest,
  feature_colname,
  plot_results = TRUE,
  dist = NULL
)
```

Arguments

`spe_object`
: SpatialExperiment object in the form of the output of `format_image_to_spe`.

`method`
: String that is the method for dependence calculation. Options: "Gcross", "Kcross", "Kcross.inhom", "Lcross", "Jcross". Default method is "Kcross".

`cell_types_of_interest`
: String Vector. Cell types of interest.

`feature_colname`
: String that is the name of the column of the types.

plot_results	Boolean. TRUE if result to be plotted, FALSE if not. In either case, an object with the results is returned
dist	Number (OPTIONAL) The largest distance between two cell types at which K function is evaluated. If NULL, use the default distances set by cross functions.

Value

An object of class "fv" defined in ‘spatstat‘ package.

Examples

```
df_cross <- calculate_cross_functions(SPIAT::defined_image,
method = "Kcross", cell_types_of_interest = c("Tumour","Immune3"),
feature_colname ="Cell.Type", dist = 100)
```

---

calculate_distance_to_margin

calculate the distances of each cell to the margin

Description

Returns a SPE object with the minimum distance from cells of interest (CoI) to the identified bordering cells.

Usage

```
calculate_distance_to_margin(spe_object)
```

Arguments

spe_object	SpatialExperiment object. It should contain information of the detected bordering cells (‘colData()‘ has ‘Region‘ column).

Value

An spe_object with a ‘Distance.To.Border‘ column is returned.

Examples

```
spe_border <- identify_bordering_cells(SPIAT::defined_image,
reference_cell = "Tumour", feature_colname = "Cell.Type", n_to_exclude = 10)
spe_dist <- calculate_distance_to_margin(spe_border)
```

calculate_entropy *calculate_entropy*

Description

If arg ‘radius‘ is not specified, the function returns the entropy of the cell types of interest for the whole image. If arg ‘radius‘ is specified, the function returns a data frame where each row is a reference cell and the columns stores the entropy of the cell types of interest in each circle of the reference cells.

Usage

```
calculate_entropy(
  spe_object,
  cell_types_of_interest,
  feature_colname = "Phenotype",
  radius = NULL
)
```

Arguments

`spe_object` SpatialExperiment object in the form of the output of `format_image_to_spe`.

`cell_types_of_interest` String Vector. Cell types of interest. If arg ‘radius‘ is not NULL, the first cell type is considered as reference cell type. Circles of the specified radius will be drawn around the reference cells and the entropy of cell types will be calculated for each of the reference cells.

`feature_colname` String specifying the column the cell types are from.

`radius` (OPTIONAL) Numeric. The maximum radius around a reference cell for another cell to be considered an interaction.

Value

A dataframe or a number depending on the argument radius

Examples

```
calculate_entropy(SPIAT::defined_image,
cell_types_of_interest = c("Immune1","Immune2"),
feature_colname = "Cell.Type")
```

calculate_minimum_distances_between_celltypes

calculate_minimum_distances_between_celltypes

Description

Returns the distance of the closest cell of a specific type from each reference cell.

Usage

```
calculate_minimum_distances_between_celltypes(
  spe_object,
  feature_colname,
  cell_types_of_interest = NULL
)
```

Arguments

`spe_object` SpatialExperiment object in the form of the output of `format_image_to_spe`.

`feature_colname` String of the feature column of cells to choose the cell types from (e.g. Cell.Type, Cell.Type2, etc).

`cell_types_of_interest` String Vector of marker combinations to consider is FALSE.

Value

A data.frame is returned

Examples

```
min_dists <- calculate_minimum_distances_between_celltypes(
SPIAT::defined_image, feature_colname = "Cell.Type",
cell_types_of_interest = c("Tumour","Immune1"))
```

calculate_pairwise_distances_between_celltypes

calculate_pairwise_distances_between_celltypes

Description

Returns the pairwise distances between cells of different types. If none of the cell types are found, it will print an error message and return a vector of NAs.

Usage

```
calculate_pairwise_distances_between_celltypes(
  spe_object,
  cell_types_of_interest = NULL,
  feature_colname
)
```

Arguments

`spe_object` SpatialExperiment object in the form of the output of `format_image_to_spe`.

`cell_types_of_interest` String Vector containing cell types to be considered, if NULL, all cell type combinations will be calculated.

`feature_colname` String of the name the feature column with the cell types of interest to be considered.

Value

A data.frame is returned.

Examples

```
calculate_pairwise_distances_between_celltypes(SPIAT::defined_image,
cell_types_of_interest = c("Tumour","Immune1"),
feature_colname = "Cell.Type")
```

---

calculate_percentage_of_grids *calculate_percentage_of_grids*

Description

Takes the result of `grid_metrics` (a RasterLayer object) and calculates the percentage of the grid squares whose values are above or below a specified threshold.

Usage

```
calculate_percentage_of_grids(raster_obj, threshold, above)
```

Arguments

`raster_obj` Raster object in the form of the output of `grid_metrics`.

`threshold` Numeric. The threshold for defining the pattern.

`above` Boolean. Indicating whether the pattern is above (TRUE) or below (FALSE) the threshold.

Value

A number is returned

Examples

```
grid <- grid_metrics(SPIAT::defined_image, FUN = calculate_entropy, n_split = 5,
cell_types_of_interest=c("Tumour","Immune3"), feature_colname = "Cell.Type")
calculate_percentage_of_grids(grid, threshold = 0.75, above = TRUE)
```

---

calculate_proportions_of_cells_in_structure

calculate_proportions_of_cells_in_structure

Description

Calculate the proportion of cells of interest in each defined tissue structure relative to all cells in each structure and relative to the same cell type in the whole image.

Usage

```
calculate_proportions_of_cells_in_structure(
  spe_object,
  cell_types_of_interest,
  feature_colname
)
```

Arguments

`spe_object` SpatialExperiment object in the form of the output of `format_image_to_spe`.

`cell_types_of_interest` String Vector of cell types to consider.

`feature_colname` String. The name of the column where the cell types of interest are under.

Value

A data.frame

Examples

```
spe_border <- identify_bordering_cells(SPIAT::defined_image,
reference_cell = "Tumour", feature_colname = "Cell.Type", n_to_exclude = 10)
spe_dist <- calculate_distance_to_margin(spe_border)
spe_structure <- define_structure(spe_dist,
cell_types_of_interest = c("Immune1","Immune2","Immune3"),
feature_colname = "Cell.Type", n_margin_layers = 5)
calculate_proportions_of_cells_in_structure(spe_structure,
cell_types_of_interest = c("Immune1","Immune3"),feature_colname="Cell.Type")
```

calculate_spatial_autocorrelation

calculate_spatial_autocorrelation

Description

Takes the result of grid_metrics (a RasterLayer object) and calculate its spatial autocorrelation.

Usage

```
calculate_spatial_autocorrelation(raster_obj, metric = "globalmoran", d = NULL)
```

Arguments

raster_obj	Raster object in the form of the output of grid_metrics.
metric	String. The method for calculating spatial autocorrelation. Choose from "globalmoran" and "GearyC".
d	Numeric. Upper bound local distance. The argument ‘d2‘ from function moran. Default is NULL and the distance will be calculated automatically from the number of splits and the extent of the grid image.

Value

A number is returned

Examples

```
grid <- grid_metrics(SPIAT::defined_image, FUN = calculate_entropy,
n_split = 5, cell_types_of_interest=c("Tumour","Immune3"),
feature_colname = "Cell.Type")
calculate_spatial_autocorrelation(grid, metric = "globalmoran")
```

calculate_summary_distances_between_celltypes

calculate_summary_distances_between_celltypes

Description

Returns the mean, median and standard deviation of the minimum/pairwise distances between phenotypes.

Usage

```
calculate_summary_distances_between_celltypes(df)
```

Arguments

df — Data.frame containing the distance output between cell types. The functions that generate the distances can be calculate_minimum_distances_between_celltypes and calculate_pairwise_distances_between_celltypes.

Value

A data frame is returned

Examples

```
# for pairwise dist
pairwise_dist <- calculate_pairwise_distances_between_celltypes(
SPIAT::defined_image, cell_types_of_interest = c("Tumour","Immune1"),
feature_colname = "Cell.Type")
summary_distances <- calculate_summary_distances_between_celltypes(pairwise_dist)

# for minimum dist
min_dists <- calculate_minimum_distances_between_celltypes(
SPIAT::defined_image, cell_types_of_interest = c("Tumour","Immune1"),
feature_colname = "Cell.Type")
summary_distances <- calculate_summary_distances_between_celltypes(min_dists)
```

---

calculate_summary_distances_of_cells_to_borders

calculate_summary_distances_of_cells_to_borders

Description

Returns the mean, median and standard deviation of the distances between a specified cell type to the border.

Usage

```
calculate_summary_distances_of_cells_to_borders(
  spe_object,
  cell_types_of_interest,
  feature_colname = "Cell.Type"
)
```

Arguments

spe_object — SpatialExperiment object. It should contain information of tissue structure and cell distances to the tissue region border (‘colData()‘ has ‘Region‘ and ‘Distance.To.Border‘ columns).

cell_types_of_interest — String Vector of cell types to consider.

feature_colname — String specifying which column the interested cell types are from.

Value

A data.frame is returned

Examples

```
spe_border <- identify_bordering_cells(SPIAT::defined_image,
reference_cell = "Tumour", feature_colname = "Cell.Type", n_to_exclude = 10)
spe_dist <- calculate_distance_to_margin(spe_border)
spe_structure <- define_structure(spe_dist, cell_types_of_interest =
c("Immune1","Immune2","Immune3"), feature_colname = "Cell.Type",
n_margin_layers = 5)
calculate_summary_distances_of_cells_to_borders(spe_structure,
cell_types_of_interest = c("Immune1","Immune3"),feature_colname = "Cell.Type")
```

---

composition_of_neighborhoods *composition_of_neighborhoods*

Description

Returns a data.frame which contains the percentages of cells with a specific marker within each neighborhood. and the number of cells in the neighborhood.

Usage

```
composition_of_neighborhoods(spe_object, feature_colname)
```

Arguments

`spe_object`	SpatialExperiment that is the output of `identify_neighborhoods`.
`feature_colname`	String. Column with cell types.

Value

A data.frame is returned

Examples

```
neighborhoods <- identify_neighborhoods(image_no_markers,
method = "hierarchical", min_neighborhood_size = 100,
cell_types_of_interest = c("Immune", "Immune1", "Immune2"), radius = 50,
feature_colname = "Cell.Type")
neighborhoods_vis <- composition_of_neighborhoods(neighborhoods,
feature_colname="Cell.Type")
```

compute_gradient *compute_gradient*

Description

The function sweeps over circles of a range of radii surrounding reference cells and calculates the metrics at the radii. Metrics used with function need two conditions: 1) have a ‘radius‘ parameter. 2) return a single number. For metrics that do not return a single number, users can wrap them in a new function that returns a number and then pass the new function to ‘compute_gradient()‘.

Usage

```
compute_gradient(spe_object, radii, FUN, ...)
```

Arguments

spe_object	SpatialExperiment object in the form of the output of format_image_to_spe.
radii	Numeric Vector specifying the range of radii for the metrics to be calculated.
FUN	Variable name specifying the metric.
...	Arguments of FUN

Value

A list of the metrics under all radii

Examples

```
gradient_positions <- c(30, 50, 100)
gradient_entropy <- compute_gradient(SPIAT::defined_image,
radii = gradient_positions, FUN = calculate_entropy,
cell_types_of_interest = c("Immune1","Immune2"),
feature_colname = "Cell.Type")
```

crossing_of_crossK *crossing_of_crossK*

Description

Determine if there is a crossing in the cross K curves, to further detect the existence of potential immune rings.

Usage

```
crossing_of_crossK(df.cross)
```

Arguments

df.cross	Data.frame. The output of calculate_cross_functions. Containing the positions of the two curves. Columns contain "r", "border" and "theo".

Value

A number. The percentage of the crossing position of the specified distance. Returns NA when the crossing happens too close to the y axis (<4

Examples

```
df_cross <- calculate_cross_functions(SPIAT::defined_image, method="Kcross",
              cell_types_of_interest = c("Tumour","Immune3"),
              feature_colname ="Cell.Type", dist = 100)
crossing_of_crossK(df_cross)
```

---

defined_image — *SPE object of a simulated image with defined cell types based on marker combinations.*

Description

A dataset that contains a formatted spe object with cell ids, phenotypes, defined cell types in ‘colData()‘ and marker intensities in ‘assays()‘. (The cell locations are the same with the cells in simulated_image).

Usage

```
defined_image
```

Format

An spe object. Assay contains 5 rows (markers) and 4951 columns (cells); colData contains 4951 rows (cells) and 3 columns (features).

See Also

simulated_image image_no_markers

define_celltypes *define_celltypes*

Description

Define new cell types based on the existing cell types (categories) under a selected column (e.g. base on marker combinations under "Phenotype" column). This function will create a new column to store the new cell types.

Usage

```
define_celltypes(
  spe_object,
  categories = NULL,
  category_colname = "Phenotype",
  names = NULL,
  new_colname = "Cell.Type",
  print_names = FALSE
)
```

Arguments

spe_object	SpatialExperiment object in the form of the output of format_image_to_spe.
categories	Vector. Names of the old cell types to be defined; if NULL, the function will use predefined categories and names
category_colname	(Phenotype) String specifying the name of the column having the categories to be defined, by default "Phenotype".
names	Vector of new names assigned to the selected categories; if NULL, the function will use predefined categories and names. Should be of the same length of ‘categories‘.
new_colname	(Optional) String specifying the name of the column to be added, by default "Cell.Type".
print_names	(Optional) Boolean if the user wants the original and new names printed. Default is FALSE.

Details

Users need to specify the names of the old cell categories and under which column the old cell categories exist. Then the users specify the names of the new cell types and the name of the new column to store the new cell types. Any cell categories that are not specified in ‘categories‘ arg but present in the image will be defined as "Undefined" in the new column.

Value

An new SPE object is returned

Examples

```
# the selected column is:
category_colname = "Phenotype"
# define the following marker combinations:
categories <- c("Tumour_marker", "Immune_marker1,Immune_marker2",
"Immune_marker1,Immune_marker3",
"Immune_marker1,Immune_marker2,Immune_marker4", "OTHER")
# the new defined cell names:
names = c("Tumour", "Immune1", "Immune2","Immune3", "Others")
# the new names are stored under this column:
new_colname <- "Cell.Type"

defined_spe <- define_celltypes(SPIAT::simulated_image,
categories = categories, category_colname = category_colname, names = names,
new_colname = new_colname)
```

---

define_structure *define_structure*

Description

After identifying the bordering cells of tissue regions and calculating the distances of each cell to the bordering cells, this function further identifies the cells that are located in the inside and outside of the identified regions, and in the internal and external margins. It also identifies particular types of cells that are infiltrated, stromal, internal margin or external margin cells.

Usage

```
define_structure(
  spe_object,
  cell_types_of_interest,
  feature_colname = "Cell.Type",
  n_margin_layers = 5,
  margin_dist = NULL
)
```

Arguments

`spe_object` SpatialExperiment object that contains information of tumour bordering cells and cell distances to border (‘colData()‘ has ‘Region‘ and ‘Distance.To.Border‘ columns).

`cell_types_of_interest` String Vector of the names of the particular types of cells.

`feature_colname` String Specifying the column that contains the names of the immune cells.

`n_margin_layers` Integer. The number of layers of cells that compose the internal/external margins. Default is 5.

`margin_dist` Numeric. The width of the internal/external margins. Default is NULL. Only use when ‘n_margin_layers‘ is NULL.

Value

A new spe object is returned. Under the ‘Region‘ column, there will be potential categories including ‘Border‘ - the bordering cells, ‘Infiltrated.CoI‘ - cells of interest that present inside of the tissue regions, ‘Inside‘ - cells within the regiona excluding the ‘Infiltrated.CoI‘ cells and the cells at internal margin, ‘Stromal.CoI‘ - cells of interest that present outside of the tissue regions, ‘Outside‘ - cells outside of the tissue regions excluding the ‘Stromal.CoI‘ cells, ‘Internal.margin.CoI‘ - cells of interest that are in the internal margin of the tissue regions, ‘Internal.margin‘ - cells in the internal margin of the tissue regions excluding the ‘Internal.margin.CoI‘ cells, ‘External.margin.CoI‘ - cells of interest that are in the external margin of the tissue regions, ‘External.margin‘ - cells in the external margin of the tissue regions excluding the ‘External.margin.CoI‘ cells.

Examples

```
spe_border <- identify_bordering_cells(SPIAT::defined_image,
reference_cell = "Tumour", feature_colname = "Cell.Type", n_to_exclude = 10)
spe_dist <- calculate_distance_to_margin(spe_border)
spe_structure <- define_structure(spe_dist,
cell_types_of_interest = c("Immune1","Immune2","Immune3"),
feature_colname = "Cell.Type", n_margin_layers = 5)
plot_cell_categories(spe_structure, feature_colname = "Structure")
```

---

dimensionality_reduction_plot

Dimensionality reduction plot

Description

Generates the dimensionality reduction plots (UMAP or tSNE) based on marker intensities. Cells are grouped by the categories under the selected column.

Usage

```
dimensionality_reduction_plot(
  spe_object,
  plot_type = "UMAP",
  scale = TRUE,
  perplexity = 30,
  feature_colname
)
```

Arguments

spe_object	SpatialExperiment object in the form of the output of format_image_to_spe.
plot_type	String. Choose from "UMAP" and "TSNE".
scale	Boolean. Whether scale the marker intensities.
perplexity	Numeric. Perplexity parameter of the Rtsne function (should be positive and no bigger than 3 * perplexity < n - 1, where n is the number of cells).
feature_colname	String. Specify the column name to group the cells.

Value

A plot

Examples

```
dimensionality_reduction_plot(SPIAT::simulated_image, plot_type = "TSNE",
feature_colname = "Phenotype")
```

---

entropy_gradient_aggregated

The aggregated gradient of entropy and the peak of the gradient

---

Description

This function first calculates the entropy within circles of each reference cell at each radius. Then at each radius, the entropy of all circles surrounding each cell are aggregated into one number. The function sweeps over the specified radii and calculates the aggregated entropy under each radius.

Usage

```
entropy_gradient_aggregated(
  spe_object,
  cell_types_of_interest,
  feature_colname,
  radii
)
```

Arguments

spe_object	SpatialExperiment object in the form of the output of format_image_to_spe.
cell_types_of_interest	String Vector. The cell types that the entropy is computed on.
feature_colname	String. The column name of the interested cell types.
radii	Numeric Vector. A vector of radii within a circle of a reference cell where the entropy is computed on.

Value

A list of the gradient of entropy and the peak

Examples

```
gradient_pos <- seq(50, 500, 50)
gradient_results <- entropy_gradient_aggregated(SPIAT::defined_image,
cell_types_of_interest = c("Tumour","Immune3"),
feature_colname = "Cell.Type", radii = gradient_pos)
plot(1:10,gradient_results$gradient_df[1, 3:12])
```

---

format_cellprofiler_to_spe

Format a cellprofiler image into a SpatialExperiment object

Description

Reads in spatial data in the form of cell coordinates, cell phenotypes (if available), and marker intensities and transforms to a SpatialExperiment object. The assay stores the intensity level of every marker (rows) for every cell (columns). Cell phenotype is stored under ‘colData()‘. Cell x and y coordinates are stored under ‘spatialCoords()‘ Note that if the data does not include these parameters, we recommend adding it to the output from cellprofiler with NAs in columns.

Usage

```
format_cellprofiler_to_spe(
  path = NULL,
  markers = NULL,
  intensity_columns_interest = NULL
)
```

Arguments

`path`	String of the path location cellprofiler csv file.
`markers`	String Vector containing the markers used for staining.
`intensity_columns_interest`	String Vector with the names of the columns with the level of each marker. Column names must match the order of the ’markers’ parameter.

Details

Note when specifying ‘markers‘, please use "DAPI" to replace "DNA" due to implementation. The output data will include "DAPI" instead of "DNA".

Value

A SpatialExperiment object is returned

Examples

```
path <- system.file("extdata", "tiny_cellprofiler.txt.gz", package = "SPIAT")
markers <- c("Marker1", "Marker2", "Marker3", "Marker4", "Marker5", "DAPI",
"Marker6")
intensity_columns_interest <- c("Intensity_MeanIntensity_Marker1_rs",
"Intensity_MeanIntensity_Marker2_rs", "Intensity_MeanIntensity_Marker3_rs",
"Intensity_MeanIntensity_Marker4_rs", "Intensity_MeanIntensity_Marker5_rs",
"Intensity_MeanIntensity_DAPI_rs", "Intensity_MeanIntensity_Marker6_rs")
formatted_cellprofiler <- format_cellprofiler_to_spe(path = path,
markers = markers, intensity_columns_interest = intensity_columns_interest)
```

---

format_codex_to_spe *Format a CODEX image into a SpatialExperiment object*

Description

Reads in spatial data in the form of cell coordinates, cell phenotypes (if available), and marker intensities and transforms to a ‘SpatialExperiment‘ object. The assay stores the intensity level of every marker (rows) for every cell (columns). Cell phenotype is stored under colData. Cell x and y coordinates are stored under ‘spatialCoords()‘ field.

Usage

```
format_codex_to_spe(path = NULL, markers, path_to_codex_cell_phenotypes = NULL)
```

Arguments

path	String of the path location of CODEX csv file.
markers	String Vector containing the markers used for staining.
path_to_codex_cell_phenotypes	String of the path to the Cluster ID/Cell type file.

Value

A SpatialExperiment object is returned

Examples

```
path <- system.file("extdata", "tiny_codex.csv.gz", package = "SPIAT")
path_to_codex_cell_phenotypes <- system.file("extdata",
"tiny_codex_phenotypes.txt.gz", package = "SPIAT")
markers <- c("CD45", "Ly6C", "CD27", "CD5", "CD79b")
formatted_codex <- format_codex_to_spe(path = path, markers = markers,
path_to_codex_cell_phenotypes = path_to_codex_cell_phenotypes)
```

format_colData_to_spe *format_colData_to_spe*

Description

Format a data frame into a SpatialExperiment class where the count assay is empty every cell (columns), cell phenotypes are stored under colData() and cell coordinates are stored under spatialCoords().

Usage

```
format_colData_to_spe(df)
```

Arguments

df	Data frame that contains cell coordinates, phenotypes (if available) and other cell properties. The rownames should be cell ID

Value

An SpatialExperiment object

Examples

```
df <- data.frame(row.names = c("Cell_1", "Cell_2"), Cell.X.Position = c(2,5),
Cell.Y.Position = c(3.3, 8), Phenotypes = c("CD3", "CD3,CD8"))
spe <- format_colData_to_spe(df)
```

format_halo_to_spe *Format a HALO image into a SpatialExperiment object*

Description

Reads in HALO data in the form of cell coordinates, cell phenotypes (if available), and marker intensities and transforms to a ‘SpatialExperiment‘ object. The assay stores the intensity level of every marker (rows) for every cell (columns). Cell x and y coordinates are stored under ‘spatialCoords()‘. Cell phenotype and other cell properties are stored under colData. The cell properties to be included are Cell.Area, Nucleus.Area and Cytoplasm.Area. Note that if the data does not include these parameters, we recommend adding it to the output from HALO with NAs in columns.

Usage

```
format_halo_to_spe(
  path = NULL,
  markers = NULL,
  locations = NULL,
  dye_columns_interest = NULL,
  intensity_columns_interest = NULL
)
```

Arguments

`path` String of the path location of HALO text file.

`markers` String Vector containing the markers used for staining.

`locations` (Optional) Vector containing the locations of markers used for staining. Location can be either "Nucleus", "Cytoplasm" or "Membrane". This is used to select the Intensity column and can be used instead of ‘intensity_columns_interest‘.

`dye_columns_interest` (Optional) Use if locations is not specified. Vector of names of the columns with the marker status (i.e. those indicating 1 or 0 for whether the cell is positive or negative for the marker). Column names must match the order of the ’markers’ parameter.

`intensity_columns_interest` (Optional) Use if locations is not specified. Vector with the names of the columns with the level of each marker. Column names must match the order of the ’markers’ parameter.

Value

A SpatialExperiment object is returned

Examples

```
raw_halo_data <- system.file("extdata", "tiny_halo.csv.gz", package="SPIAT")
markers <- c("DAPI", "CD3", "PDL-1", "CD4", "CD8", "AMACR")
intensity_columns_interest <- c("Dye 1 Nucleus Intensity",
"Dye 2 Cytoplasm Intensity","Dye 3 Membrane Intensity",
"Dye 4 Cytoplasm Intensity", "Dye 5 Cytoplasm Intensity",
"Dye 6 Cytoplasm Intensity")
dye_columns_interest <-c("Dye 1 Positive Nucleus","Dye 2 Positive Cytoplasm",
"Dye 3 Positive Membrane", "Dye 4 Positive Cytoplasm",
"Dye 5 Positive Cytoplasm", "Dye 6 Positive Cytoplasm")
formatted_HALO <- format_halo_to_spe(path=raw_halo_data,markers=markers,
intensity_columns_interest=intensity_columns_interest,
dye_columns_interest=dye_columns_interest)
```

format_image_to_spe *Format an image into a SpatialExperiment object*

Description

Reads in spatial data in the form of cell coordinates, cell phenotypes (if available), and marker intensities and transforms to a SpatialExperiment object. The assay stores the intensity level of every marker (rows) for every cell (columns). Cell phenotype is stored under ‘colData()‘. Cell x and y coordinates are stored under ‘spatialCoords()‘ field. The function can read in data in general format (manually constructed input), or data from other platforms including inForm, HALO, CODEX and cellprofiler. Alternatively, users can use the specific function for each format.

Usage

```
format_image_to_spe(
  format = "general",
  intensity_matrix = NULL,
  phenotypes = NULL,
  coord_x = NULL,
  coord_y = NULL,
  path = NULL,
  markers = NULL,
  locations = NULL,
  intensity_columns_interest = NULL,
  dye_columns_interest = NULL,
  path_to_codex_cell_phenotypes = NULL
)
```

Arguments

`format` String specifying the format of the data source. Default is "general" (RECOMMENDED), where the cell phenotypes, coordinates and marker intensities are imported manually by the user. Other formats include "inForm", "HALO", "cellprofiler" and "CODEX".

`intensity_matrix` (Optional) For "general" format. A matrix of marker intensities or gene expression where the column names are the Cell IDs, and the rownames the marker.

`phenotypes` (Optional) For "general" format. String Vector of cell phenotypes in the same order in which they appear in ‘intensity_matrix‘. If no phenotypes available, then a vector of NAs can be used as input. Note that the combination of markers (e.g. CD3,CD4) needs to be used instead of the cell type name (e.g. helper T cells).

`coord_x` (Optional) For "general" format. Numeric Vector with the X coordinates of the cells. The cells must be in the same order as in the ‘intensity_matrix‘.

`coord_y` (Optional) For "general" format. Numeric Vector with the Y coordinates of the cells. The cells must be in the same order as in the ‘intensity_matrix‘.

`path` (Optional) For formats other than "general". String of the path location of the source file.

`markers` For formats other than "general". String Vector containing the markers used for staining. These must be in the same order as the marker columns in the input file, and must match the marker names used in the input file. One of the markers must be "DAPI".

`locations` (Optional) For "inForm" and "HALO". String Vector containing the locations of markers used for staining. Location can be either "Nucleus", "Cytoplasm" or "Membrane". This is used to select the Intensity column and can be used instead of ‘intensity_columns_interest‘.

`intensity_columns_interest` (Optional) For "inForm" and "HALO", use if ‘locations‘ is not specified. For "cellprofiler", mandatory. Vector with the names of the columns with the level of each marker. Column names must match the order of the ’markers’ parameter.

`dye_columns_interest` (Optional) For "HALO". Use if locations is not specified. Vector of names of the columns with the marker status (i.e. those indicating 1 or 0 for whether the cell is positive or negative for the marker). Column names must match the order of the ’markers’ parameter.

`path_to_codex_cell_phenotypes` (Optional) For "CODEX".String of the path to the Cluster ID/Cell type file.

Details

If the user inputs ‘intensity_matrix‘, please make sure the ‘colnames‘ of the intensity matrix are the cell IDs. If the ‘intensity_matrix‘ is ‘NULL‘, the function will automatically assign IDs to the cells.

Note for "cellprofiler" format, when specifying ‘markers‘, please use "DAPI" to replace "DNA" due to implementation. The output data will include "DAPI" instead of "DNA".

The format of "Phenotype" column: For example, a cell positive for both "CD3" and "CD4" markers has the "CD3,CD4" **cell phenotype**. The phenotype has to be strictly formatted in such way where each positive marker has to be separated by a coma, with no space in between, and the order of the positive markers has to be the same as the order in the assay.

Value

A SpatialExperiment object is returned

See Also

`format_inform_to_spe` `format_halo_to_spe` `format_codex_to_spe` `format_cellprofiler_to_spe`

Examples

```
#Construct a marker intensity matrix (rows are markers, columns are cells)
intensity_matrix <- matrix(c(14.557, 0.169, 1.655, 0.054, 17.588, 0.229,
1.188, 2.074, 21.262, 4.206,  5.924, 0.021), nrow = 4, ncol = 3)
# define marker names as rownames
rownames(intensity_matrix) <- c("DAPI", "CD3", "CD4", "AMACR")
```

```
# define cell IDs as colnames
colnames(intensity_matrix) <- c("Cell_1", "Cell_2", "Cell_3")
# Construct a dummy metadata (phenotypes, x/y coordinates)
# the order of the elements in these vectors correspond to the cell order
# in `intensity matrix`
phenotypes <- c("OTHER",  "AMACR", "CD3,CD4")
coord_x <- c(82, 171, 184)
coord_y <- c(30, 22, 38)

formatted_image <- format_image_to_spe(intensity_matrix=intensity_matrix,
phenotypes = phenotypes, coord_x = coord_x,coord_y = coord_y)
```

`format_inform_to_spe` *Format an inForm image into a SpatialExperiment object*

Description

Reads in inForm data in the form of cell coordinates, cell phenotypes (if available), and marker intensities and transforms to a SpatialExperiment object. The assay stores the intensity level of every marker (rows) for every cell (columns). Cell phenotype, x and y coordinates and other cell properties are stored under colData. The cell properties to include are Cell.Area, Nucleus.Area, Nucleus.Compactness, Nucleus.Axis.Ratio, and Cell.Axis.Ratio. Note that if the data does not include these parameters, we recommend adding it to the output from inForm with NAs in columns.

Usage

```
format_inform_to_spe(
  path,
  markers,
  locations = NULL,
  intensity_columns_interest = NULL
)
```

Arguments

`path`	String of the path location of inForm text file.
`markers`	String Vector containing the markers used for staining.
`locations`	(Optional) String Vector containing the locations of markers used for staining. Location can be either "Nucleus", "Cytoplasm" or "Membrane". This is used to select the Intensity column and can be used instead of ‘intensity_columns_interest‘.
`intensity_columns_interest`	(Optional) Use if ‘locations‘ is not specified. Vector with the names of the columns with the level of each marker. Column names must match the order of the ’markers’ parameter.

Value

A SpatialExperiment object is returned

Examples

```
raw_inform_data<-system.file("extdata","tiny_inform.txt.gz",package="SPIAT")
markers <- c("DAPI", "CD3", "PD-L1", "CD4", "CD8", "AMACR")
locations <- c("Nucleus", "Cytoplasm", "Membrane", "Cytoplasm", "Cytoplasm",
"Cytoplasm")
formatted_inForm <- format_inform_to_spe(path=raw_inform_data,
markers=markers, locations=locations)
```

---

format_spe_to_ppp *Format SPE object as a ppp object (‘spatstat‘ package)*

Description

Formats an spe object into a ppp object which has the x,y coordinates, phenotypes as markers window specifies the range of x and y coordinates

Usage

```
format_spe_to_ppp(
  spe_object,
  window_pol = FALSE,
  feature_colname = "Phenotype"
)
```

Arguments

spe_object	SpatialExperiment object in the form of the output of format_image_to_spe.
window_pol	Optional Boolean Specifying if the window is polygon.
feature_colname	String specifying the feature column of interest.

Value

A ppp object is returned (defined in ‘spatstat‘ package)

Examples

```
ppp_object<-format_spe_to_ppp(SPIAT::defined_image,
feature_colname = "Cell.Type")
```

grid_metrics *Split an image into grid and calculates a metric for each grid square*

Description

Calculates a specified metric for each grid tile in the image and plots the metrics for the grid tiles.

Usage

```
grid_metrics(spe_object, FUN, n_split, ...)
```

Arguments

spe_object	SpatialExperiment object in the form of the output of format_image_to_spe.
FUN	Variable name specifying the metric to be calculated.
n_split	Integer specifying the number of splits for the calculation of metrics. This number is the splits on each side (e.g. ‘n_split‘ = 3 means the image will be split into 9 tiles.)
...	Arguments of FUN

Value

A list of the metrics of all grid tiles

Examples

```
grid <- grid_metrics(SPIAT::defined_image, FUN = calculate_entropy, n_split = 5,
cell_types_of_interest=c("Tumour","Immune3"), feature_colname = "Cell.Type")
```

identify_bordering_cells *identify_bordering_cells*

Description

Identify the cells bordering a group of cells of a particular phenotype, and calculate the number of clustered groups of this cell type.

Usage

```
identify_bordering_cells(
  spe_object,
  reference_cell,
  feature_colname = "Cell.Type",
  ahull_alpha = NULL,
  n_to_exclude = 10,
  plot_final_border = TRUE
)
```

Arguments

- `spe_object` SpatialExperiment object in the form of the output of `format_image_to_spe`.
- `reference_cell` String. Cells of this cell type will be used for border detection.
- `feature_colname` String that specifies the column of ‘reference_cell‘.
- `ahull_alpha` Number specifying the parameter for the alpha hull algorithm. The larger the number, the more cells will be included in one cell cluster.
- `n_to_exclude` Integer. Clusters with cell count under this number will be deleted.
- `plot_final_border` Boolean if plot the identified bordering cells.

Details

The bordering cell detection algorithm is based on computing an alpha hull (Hemmer et al., 2020), a generalization of convex hull (Green and Silverman, 1979). The cells detected to be on the alpha hull are identified as the bordering cells.

Value

A new SPE object is returned. The SPE object has a ‘Region‘ column with "Border", "Inside" and "Outside" categories. The returned object also has an attribute saving the number of clusters.

Examples

```
spe_border <- identify_bordering_cells(SPIAT::defined_image,
reference_cell = "Tumour", feature_colname = "Cell.Type", n_to_exclude = 10)
n_clusters <- attr(spe_border, "n_of_clusters") # get the number of clusters
n_clusters
```

---

identify_neighborhoods *identify_neighborhoods*

Description

Uses Euclidean distances to identify neighborhoods of cells. Three clustering methods are available, including hierarchical clustering, dbscan, and Rphenograph (Note: While Rphenograph option cannot be run directly in this package, the code is available and commented out.)

Usage

```
identify_neighborhoods(
  spe_object,
  method = "hierarchical",
  cell_types_of_interest,
  radius,
```

```
  min_neighborhood_size = 10,
  k = 100,
  feature_colname,
  no_pheno = NULL
)
```

Arguments

- `spe_object` SpatialExperiment object in the form of the output of `format_image_to_spe`.
- `method` String. The clustering method. Choose from "hierarchical", "dbscan" and "Rphenograph". (Note Rphenograph function is not available for this version yet).
- `cell_types_of_interest` String Vector of phenotypes to consider.
- `radius` Numeric specifying the radius of search. Need to specify when ‘method‘ is "hierarchical" or "dbscan".
- `min_neighborhood_size` Numeric. The minimum number of cells within each cluster. Need to specify when ‘method‘ is "hierarchical" or "dbscan".
- `k` Numeric. The parameter for "Rphenograph" method.
- `feature_colname` String. Column from which the cell types are selected.
- `no_pheno` Cell type corresponding to cells without a known phenotype (e.g. "None", "Other")

Value

An spe object and a plot is returned. The spe object contains information of the defined neighborhood under "Neighborhood" column. The cells of interest that do not form clusters are labelled "Free_cell", cells not of interest are labelled ‘NA‘.

Examples

```
neighborhoods <- identify_neighborhoods(image_no_markers, method = "hierarchical",
min_neighborhood_size = 100, cell_types_of_interest = c("Immune", "Immune1", "Immune2"),
radius = 50, feature_colname = "Cell.Type")
```

`image_no_markers` *SPE object of a formatted image without marker intensities (simulated by ‘spaSim‘ package)*

Description

A dataset that contains a formatted spe object with cell ids and cell types in ‘colData()‘ and cell coordinates in ‘spatialCoords()‘. This dataset does not contain assays (marker intensities).

Usage

```
image_no_markers
```

Format

An spe object. colData contains 4951 rows (cells) and 3 columns (features).

See Also

defined_image simulated_image

---

image_splitter *Split a large image into sub images*

Description

Takes in an image in SpatialExperiment format, splits the image into specified sections and returns a list of SpatialExperiment objects. Users can choose to plot the cell positions in each sub image. Note that this function does not split the assay.

Usage

```
image_splitter(
  spe_object,
  number_of_splits,
  plot = FALSE,
  cut_labels = TRUE,
  colour_vector = NULL,
  minX = NULL,
  maxX = NULL,
  minY = NULL,
  maxY = NULL,
  feature_colname = "Phenotype"
)
```

Arguments

spe_object	‘SpatialExperiment‘ object in the form of the output of format_image_to_spe.
number_of_splits	Numeric. specifying the number of segments (e.g. 2 = 2x2, 3 = 3x3).
plot	Boolean. Specifies whether the splitted images should be printed in a pdf.
cut_labels	Boolean. Specifies whether to plot where the image had been segmented.
colour_vector	String Vector. If specified, the colours will be used for plotting. If NULL, colors will be generated automatically.
minX	Integer used to specify the minimum x boundary to be splitted.

maxX	Integer used to specify the maximum x boundary to be splitted.
minY	Integer used to specify the minimum y boundary to be splitted.
maxY	Integer used to specify the maximum y boundary to be splitted.
feature_colname	String specifying which column the colouring should be based on. Specify when ‘plot‘ is TRUE. Default is "Phenotype".

Value

A list of spe objects is returned. Each data frame represents an image without assay data.

Examples

```
split_image <- image_splitter(SPIAT::simulated_image, number_of_splits=3,
plot = FALSE)
```

---

marker_intensity_boxplot

marker_intensity_boxplot

Description

Produces boxplots of marker levels for cells phenotyped as being positive for the marker, and those that where phenotyped as being negative.

Usage

```
marker_intensity_boxplot(spe_object, marker, feature_colname = "Phenotype")
```

Arguments

spe_object	SpatialExperiment object in the form of the output of format_image_to_spe.
marker	String. Marker being queried.
feature_colname	String. Column containing marker information

Value

A plot is returned

Examples

```
marker_intensity_boxplot(SPIAT::simulated_image, "Immune_marker1")
```

marker_prediction_plot *marker_prediction_plot*

Description

Takes in the returned dataframe from marker_threshold_plot and generates a .pdf file containing scatter plots of actual intensity and predicted intensity for every marker.

Usage

```
marker_prediction_plot(predicted_data, marker)
```

Arguments

predicted_data	Output from predict_phenotypes.
marker	String. Marker to plot

Value

A plot is returned

Examples

```
predicted_result <- predict_phenotypes(spe_object = simulated_image, thresholds = NULL,
tumour_marker = "Tumour_marker",baseline_markers = c("Immune_marker1", "Immune_marker2",
"Immune_marker3", "Immune_marker4"), reference_phenotypes = TRUE)
marker_prediction_plot(predicted_result, marker = "Tumour_marker")
```

marker_surface_plot *marker_surface_plot*

Description

Generates a 3D surface plot of the level of the selected marker. Note that the image is blurred based on the 'num_splits' parameter.

Usage

```
marker_surface_plot(
  spe_object,
  num_splits,
  marker,
  x_position_min = NULL,
  x_position_max = NULL,
  y_position_min = NULL,
  y_position_max = NULL
)
```

Arguments

spe_object	SpatialExperiment object in the form of the output of format_image_to_spe.
num_splits	Integer specifying the number of splits on the image, higher splits equal to higher resolution. Recommendation: 10-100
marker	Marker to plot
x_position_min	Integer specifying the minimum x boundary to be splitted
x_position_max	Integer specifying the maximum x boundary to be splitted
y_position_min	Integer specifying the minimum y boundary to be splitted
y_position_max	Integer specifying the maximum y boundary to be splitted

Value

A plot is returned

Examples

```
marker_surface_plot(SPIAT::simulated_image, num_splits=15, marker="Immune_marker1")
```

---

marker_surface_plot_stack

marker_surface_plot_stack

Description

Generates stacked 3D surface plots showing normalized intensity level of specified markers.

Usage

```
marker_surface_plot_stack(
  spe_object,
  num_splits,
  markers_to_plot,
  sep = 1,
  x_position_min = NULL,
  x_position_max = NULL,
  y_position_min = NULL,
  y_position_max = NULL
)
```

Arguments

spe_object	SpatialExperiment object in the form of the output of format_image_to_spe.
num_splits	Integer specifying the number of splits on the image, higher splits equal to higher resolution. Recommendation: 10-100.
markers_to_plot	Vector of marker names for plotting.
sep	Integer specifying the distance separation between each surface plot. We recommend values in the 1-2 range.
x_position_min	Integer specifying the minimum x boundary to be splitted.
x_position_max	Integer specifying the maximum x boundary to be splitted.
y_position_min	Integer specifying the minimum y boundary to be splitted.
y_position_max	Integer specifying the maximum y boundary to be splitted.

Value

A plot is returned

Examples

```
marker_surface_plot_stack(SPIAT::simulated_image, num_splits=15,
markers=c("Tumour_marker", "Immune_marker4"))
```

---

measure_association_to_cell_properties

measure_association_to_cell_properties

Description

Plots the density or boxplot of a property of two cell celltypes or compares using t test/wilcoxon rank sum test.

Usage

```
measure_association_to_cell_properties(
  spe_object,
  property = "Cell.Area",
  celltypes,
  feature_colname = "Cell.Type",
  method = "density",
  Nucleus.Ratio = FALSE,
  log.scale = FALSE
)
```

Arguments

spe_object	SpatialExperiment object in the form of the output of format_image_to_spe.
property	String that is the name of the column of interest.
celltypes	String Vector of celltypes of interest.
feature_colname	String that speficies the column of the cell types.
method	String. The analysis to perform on the selected cell types and property. Options are "density", "box", "t", "wilcox".
Nucleus.Ratio	Boolean whether the ratio of the nucleus size is of interest.
log.scale	Boolean whether to log the data.

Value

With method "box" or "density a plot is returned. With method "t" or "wilcox", the text output from the test are returned.

Examples

```
measure_association_to_cell_properties(image_no_markers,
                                       celltypes = c("Tumour", "Immune1"),
                                       feature_colname = "Cell.Type",
                                       property = "Cell.Size",
                                       method = "box")
measure_association_to_cell_properties(image_no_markers,
                                       celltypes = c("Tumour", "Immune2"),
                                       feature_colname="Cell.Type",
                                       property = "Cell.Size",
                                       method = "t")
```

---

mixing_score_summary *Calculate the (normalised) mixing score for interested cell types*

Description

Produces a data.frame with mixing scores of input reference and target cells from a SpatialExperiment object. It calculates reference-target interactions and reference-reference interactions based on a radius. It derives the mixing score and the normalised mixing score. Function returns NA if the mixing score is being calculated between cells of the same type.

Usage

```
mixing_score_summary(
  spe_object,
  reference_celltype,
  target_celltype,
  radius = 20,
  feature_colname
)
```

Arguments

- `spe_object` SpatialExperiment object in the form of the output of `format_image_to_spe`.
- `reference_celltype` String Vector. Cell types of the reference cells.
- `target_celltype` String Vector. Cell types of the target cells.
- `radius` The maximum radius around a reference cell type for another cell to be considered an interaction.
- `feature_colname` String specifying the column with the desired cell type annotations.

Details

The mixing score was originally defined as the number of immune-tumour interactions divided by the number of immune-immune interactions within a defined radius (Keren et al., 2018). The normalised mixing score normalises the immune-tumour interactions and immune-immune interactions within radius by the total number of immune-tumour and immune-immune interactions in the image, respectively. We have generalized this score to allow calculation of any two cell phenotypes defined by the user.

Value

A data.frame of cell numbers, number of cell interactions, mixing scores, and normalised mixing scores. If there are no reference or target cells found in the image, or there are no reference cells found within the specified radius of any reference cells,the returned (normalised) mixing scores will be NA. If there are no target cells found within the radius of any refernece cells, the returned (normalised) mixing scores will be 0.

Examples

```
mixing_score_summary(SPIAT::defined_image, reference_celltype = "Tumour", target_celltype="Immune1",
radius = 50, feature_colname = "Cell.Type")
```

---

number_of_cells_within_radius

Number of cells within a radius

Description

Calculates the number of cells of a target cell type within a pre-defined radius around cells of a reference cell type.

Usage

```
number_of_cells_within_radius(
  spe_object,
  reference_celltype,
  target_celltype,
  radius = 20,
  feature_colname
)
```

Arguments

`spe_object` SpatialExperiment object in the form of the output of `format_image_to_spe`.

`reference_celltype` String. Cell type to be used for reference cells.

`target_celltype` String. Cell type to be used for target cells.

`radius` Numeric. Radius around the reference cells.

`feature_colname` String specifying the column with the desired cell type annotations.

Value

A list of dataframes with the number of target cells of each of the reference cells

Examples

```
n_in_radius <- number_of_cells_within_radius(SPIAT::defined_image,
reference_celltype = "Tumour", target_celltype="Immune1", radius = 50,
feature_colname = "Cell.Type")
```

---

plot_average_intensity *plot_average_intensity*

Description

Takes in a vector or radii and calculates the average intensity of a target marker using average_intensity function. It plots the intensity level as a line graph.

Usage

```
plot_average_intensity(spe_object, reference_marker, target_marker, radii)
```

Arguments

- `spe_object` SpatialExperiment object in the form of the output of `format_image_to_spe`.
- `reference_marker` String specifying the reference marker.
- `target_marker` String specifying the marker to calculate its average intensity.
- `radii` Numeric Vector specifying the search radius around reference cells.

Value

A plot is returned

Examples

```
plot_average_intensity(SPIAT::simulated_image, reference_marker="Immune_marker3",
target_marker="Immune_marker2", c(30, 35, 40, 45, 50, 75, 100))
```

`plot_cell_categories` *plot_cell_categories*

Description

Produces a scatter plot of the cells of their x-y positions in the tissue. Cells are coloured categorically by phenotype. Cells not part of the phenotypes of interest will be coloured "lightgrey".

Usage

```
plot_cell_categories(
  spe_object,
  categories_of_interest = NULL,
  colour_vector = NULL,
  feature_colname = "Cell.Type",
  cex = 1,
  layered = FALSE
)
```

Arguments

- `spe_object` SpatialExperiment object in the form of the output of `format_image_to_spe`.
- `categories_of_interest` Vector of cell categories to be coloured.
- `colour_vector` Vector specifying the colours of each cell phenotype.
- `feature_colname` String specifying the column the cell categories belong to.
- `cex` Numeric. The size of the plot points. Default is 1.
- `layered` Boolean. Whether to plot the cells layer by layer (cell categories). By default is FALSE.

Value

A plot is returned

Examples

```
categories_of_interest <- c("Tumour", "Immune1","Immune2","Immune3")
colour_vector <- c("red","darkblue","blue","darkgreen")
plot_cell_categories(SPIAT::defined_image, categories_of_interest, colour_vector,
feature_colname = "Cell.Type")
```

---

plot_cell_distances_violin *plot_cell_distances_violin*

Description

Plots distances between cells as a violin plot

Usage

```
plot_cell_distances_violin(cell_to_cell_dist)
```

Arguments

`cell_to_cell_dist`
: Data.frame containing the distance output between cell types. The functions that generate the distances can be `calculate_minimum_distances_between_celltypes` and `calculate_pairwise_distances_between_celltypes`.

Value

A plot is returned

Examples

```
distances <- calculate_pairwise_distances_between_celltypes(SPIAT::defined_image,
cell_types_of_interest = c("Immune1", "Immune2"), feature_colname="Cell.Type")
plot_cell_distances_violin(distances)
```

plot_cell_marker_levels *plot_cell_marker_levels*

Description

Produces a scatter plot of the level of a marker in each cell. The level of the marker in all cells is shown, at x-y positions, no matter if cells are phenotyped as being positive or negative for the particular marker.

Usage

```
plot_cell_marker_levels(spe_object, marker, feature_colname = "Phenotype")
```

Arguments

spe_object	SpatialExperiment object in the form of the output of format_image_to_spe.
marker	String. Marker to plot.
feature_colname	String. Column containing marker information

Value

A plot is returned

Examples

```
plot_cell_marker_levels(SPIAT::simulated_image, "Immune_marker1")
```

plot_cell_percentages *plot_cell_percentages*

Description

Plots cells proportions as barplots.

Usage

```
plot_cell_percentages(
  cell_proportions,
  cells_to_exclude = NULL,
  cellprop_colname = "Proportion_name"
)
```

Arguments

`cell_proportions`
: Data Frame. Output from `calculate_cell_proportions`.

`cells_to_exclude`
: String Vector. Markers to exclude.

`cellprop_colname`
: String. Column to use for y axis names. Default is "Proportion_name".

Value

A plot is returned

Examples

```
p_cells <- calculate_cell_proportions(SPIAT::simulated_image)
plot_cell_percentages(p_cells)
```

---

plot_composition_heatmap *plot_composition_heatmap*

Description

Produces a heatmap showing the marker percentages within each cluster and the cluster sizes.

Usage

```
plot_composition_heatmap(
  composition,
  pheno_to_exclude = NULL,
  log_values = FALSE,
  feature_colname
)
```

Arguments

`composition`
: Data.frame. Output from `composition_of_neighborhoods`.

`pheno_to_exclude`
: String Vector of phenotype to exclude.

`log_values`
: Boolean. TRUE if the percentages should be logged (base 10).

`feature_colname`
: String. Column with cell types.

Value

A plot is returned

Examples

```
neighborhoods <- identify_neighborhoods(image_no_markers, method = "hierarchical",
min_neighborhood_size = 100, cell_types_of_interest = c("Immune", "Immune1", "Immune2"),
radius = 50, feature_colname = "Cell.Type")
neighborhoods_vis <- composition_of_neighborhoods(neighborhoods, feature_colname="Cell.Type")
plot_composition_heatmap(neighborhoods_vis, feature_colname="Cell.Type")
```

---

plot_distance_heatmap *plot_distance_heatmap*

Description

Takes the output of cell_distances and plot the distances as a heatmap.

Usage

```
plot_distance_heatmap(phenotype_distances_result, metric = "mean")
```

Arguments

phenotype_distances_result	Dataframe output from ‘calculate_summary_distances_between_celltypes‘ or ‘calculate_minimum_distances_between_celltypes‘.
metric	Metric to be plotted. One of "mean", "std.dev", "median", "min" or "max".

Value

A plot is returned

Examples

```
dists <- calculate_pairwise_distances_between_celltypes(SPIAT::defined_image,
cell_types_of_interest = c("Tumour","Immune1"), feature_colname = "Cell.Type")
summary_distances <- calculate_summary_distances_between_celltypes(dists)
plot_distance_heatmap(summary_distances)
```

plot_marker_level_heatmap *plot_marker_level_heatmap*

Description

Blurs the image by splitting the images into small squares. The marker levels are then averaged within each square. All cells are considered, regardless of phenotype status.

Usage

```
plot_marker_level_heatmap(spe_object, num_splits, marker)
```

Arguments

spe_object	SpatialExperiment object in the form of the output of format_image_to_spe.
num_splits	Integer specifying the blurring level (number of splits) for the image. Higher numbers result in higher resolution.
marker	String. Marker to plot.

Value

A plot is returned

Examples

```
plot_marker_level_heatmap(SPIAT::simulated_image, num_splits = 100, "Tumour_marker")
```

predict_phenotypes *predict_phenotypes*

Description

Predicts cell phenotypes based on marker intensity levels. If no prior cell phenotypes are available, it adds the phenotypes to the SpaitalExperiment object used as input. If reference cell phenotypes are available, it produces a density plot showing predicted cutoff of a positive reading for marker intensity and it returns a dataframe containing the predicted intensity status for a particular marker.

Usage

```
predict_phenotypes(
  spe_object,
  thresholds = NULL,
  tumour_marker,
  baseline_markers,
  nuclear_marker = NULL,
  reference_phenotypes = FALSE,
  markers_to_phenotype = NULL,
  plot_distribution = TRUE
)
```

Arguments

`spe_object` SpatialExperiment object in the form of the output of `format_image_to_spe`.

`thresholds` (Optional) Numeric Vector specifying the cutoff of a positive reading. The order must match the marker order, and it should be NA for DAPI.

`tumour_marker` String containing the tumour_marker used for the image. If tumor cells are known, annotate tumor cells as 1 and non-tumor cells as 0, and include the rowname.

`baseline_markers` String Vector. Markers not found on tumour cells to refine the threshold used for tumour cell phenotying.

`nuclear_marker` String. Nuclear marker used.

`reference_phenotypes` Boolean. TRUE or FALSE value whether there are reference phenotypes for the sample obtained by the user through other means (e.g. HALO or InForm). If there are reference phenotypes available, a matrix of predicted phenotypes, intensities, and reference phenotypes will be returned, which can be used as input to "marker_prediction_plot". If no reference phenotype available, the result of the function will be added to the spe object used in the input. Note that if a reference phenotype is to be used, the phenotypes must be an explicit combination of positive markers (e.g. AMACR,PDL1), as opposed to descriptive (PDL1+ tumour cells).

`markers_to_phenotype` String Vector. Markers to be included in the phenotyping. If NULL, then all markers will be used. DAPI needs to be excluded.

`plot_distribution` Boolean. If TRUE, plots of the marker intensities distributions and cutoffs are plotted.

Value

An updated spe object with cell phenotypes or a data.frame of predicted phenotypes

Examples

```
# keep the original phenotypes
predicted_result <- predict_phenotypes(spe_object = simulated_image, thresholds = NULL,
tumour_marker = "Tumour_marker",baseline_markers = c("Immune_marker1", "Immune_marker2",
"Immune_marker3", "Immune_marker4"), reference_phenotypes = TRUE)
# update the predicted phenotypes
predicted_spe_image <- predict_phenotypes(spe_object = simulated_image, thresholds = NULL,
tumour_marker = "Tumour_marker",baseline_markers = c("Immune_marker1", "Immune_marker2",
"Immune_marker3", "Immune_marker4"), reference_phenotypes = FALSE)
```

R_BC *The ratio of border cell count to cluster cell count*

Description

Calculates the ratio of the bordering cell count and the total to-be-clustered cell count in an image. The bordering cells are detected by the default `identify_bordering_cells` function. If the ratio is high, it means that most cells to be clustered are identified as bordering cells. This means there is no clear clusters.

Usage

```
R_BC(spe_object, cell_type_of_interest, feature_colname)
```

Arguments

`spe_object` SpatialExperiment object in the form of the output of `format_image_to_spe`.

`cell_type_of_interest` String. The cell type that the user wants to determine a cluster of.

`feature_colname` String. The column that contains the cell type to be clustered.

Value

A number is returned.

Examples

```
R_BC(SPIAT::defined_image, cell_type_of_interest = "Tumour", "Cell.Type")
```

select_celltypes *select_celltypes*

Description

Select cell types to keep or exclude in the analysis. The output of this function also includes the original image size and cell count.

Usage

```
select_celltypes(
  spe_object,
  celltypes,
  feature_colname = "Phenotype",
  keep = TRUE
)
```

Arguments

spe_object	SpatialExperiment object in the form of the output of format_image_to_spe.
celltypes	String Vector of celltypes of keep or exclude.
feature_colname	String. The column that has the interested cell types. If the cells ids are used to select cells, use "Cell.ID" for this arg.
keep	Boolean. TRUE if vector of ‘celltypes‘ are the cells that are going to be kept, FALSE if they are to be removed.

Value

A SpatialExperiment object is returned. The original image size and cell count can be accessed by ‘attr(slim_spe, "original_cell_number")‘ and ‘attr(slim_spe, "range_of_coords")‘, where ‘slim_spe‘ is the output of this function.

Examples

```
data_subset <- select_celltypes(SPIAT::simulated_image,
celltypes = c("Tumour_marker","Immune_marker1","Immune_marker2",
"Immune_marker3","Immune_marker4"),
feature_colname = "Phenotype", keep=TRUE)
attr(data_subset, "original_cell_number") #cell number in the original image
attr(data_subset, "range_of_coords")
dim(data_subset)[2] # this is the new image cell number
```

`simulated_image` *SPE object of a formatted image (simulated by ‘spaSim‘ package)*

Description

A dataset that contains a formatted spe object with cell ids and phenotypes in ‘colData()‘ and marker intensities in ‘assays()‘.

Usage

```
simulated_image
```

Format

An SpatialExperiment object. Assay contains 5 rows (markers) and 4951 columns (cells); colData contains 4951 rows (cells) and 3 columns.

See Also

`defined_image` `image_no_markers`

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