Package 'SpotSweeper'

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Title Spatially-aware quality control for spatial transcriptomics

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Description Spatially-aware quality control (QC) software for both spot-level and artifact-level QC in spot-based spatial transcripomics, such as 10x Visium. These methods calculate local (nearest-neighbors) mean and variance of standard QC metrics (library size, unique genes, and mitochondrial percentage) to identify outliers spot and large technical artifacts.

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`biased_spots` *Biased Spots Data*

Description

The `biased_spots` dataset is a `data.frame` containing information about specific spatial spots identified as technical outliers in spatial transcriptomics experiments. Each entry represents a biased spot characterized by its spatial coordinates (row and column) and a unique barcode. This dataset is utilized by the `flagVisiumOutliers` function to flag and exclude these outlier spots from downstream analyses, thereby enhancing data quality and reliability.

Usage

```
data(biased_spots)
```

Format

A `data.frame` with the following columns:

row Integer. The row position of a biased spot within the spatial grid.

col Integer. The column position of a biased spot within the spatial grid.

barcode Character. A unique identifier corresponding to the spatial transcriptomics barcode of the biased spot.

Source

The `biased_spots.rds` file was generated in the analysis of local outliers. See https://github.com/boyiguo1/Manuscript-SpotSweeper/blob/main/code/03_Visium/figure_3.R for more details.

Examples

```
data(biased_spots)
```

`DLPFC_artifact` *human DLPFC dataset with a technical artifact (hangnail).*

Description

The `DLPFC_artifact` dataset is a `SpatialExperiment` object containing a single-sample subset of the human dorsolateral prefrontal cortex (DLPFC) dataset from Hukki-Myers et al. 2023. This particular sample ('Br2743_ant') is included to demonstrate the identification and removal of technical artifacts within spatial transcriptomics data. The dataset serves as an example for artifact detection using the 'SpotSweeper' workflow.

Usage

```
data(DLPFC_artifact)
```

Format

An SpatialExperiment object.

Source

spatialLIBD

References

Hukki-Myers et al. (2023) bioRxiv (bioRxiv)

Examples

```
data(DLPFC_artifact)
```

`findArtifacts` *Identify and annotate artifacts in spatial transcriptomics data*

Description

This function identifies and annotates potential artifacts in spatial transcriptomics data. Artifacts are detected based on local mito variance, and the results are added to the original SpatialExperiment (sce) object.

Usage

```
findArtifacts(
  spe,
  mito_percent = "expr_chrM_ratio",
  mito_sum = "expr_chrM",
  samples = "sample_id",
  n_order = 5,
  shape = "hexagonal",
  log = TRUE,
  name = "artifact",
  var_output = TRUE
)
```

Arguments

`spe`	A SingleCellExperiment object.
`mito_percent`	The column name representing the mitochondrial percent. Default is 'expr_chrM_ratio'.
`mito_sum`	The column name representing sum mitochondrial expression. Default is 'expr_chrM'.
`samples`	The column name representing sample IDs. Default is 'sample_id'.
`n_order`	The number of orders for local mito variance calculation. Default is 5.
`shape`	The shape of the neighborhood for local variance calculation. Can be either 'hexagonal' or 'square'. Default is 'hexagonal'.
`log`	Logical, indicating whether to log1p transform mito_percent. Default is TRUE.
`name`	Prefix for the local variance column names. Default is 'artifact'.
`var_output`	Logical, indicating whether to include local variances in the output. Default is TRUE.

Value

Returns the modified SingleCellExperiment object with artifact annotations.

See Also

`localVariance`

Examples

```
library(SpotSweeper)
library(SpatialExperiment)
library(escheR)

data(DLPFC_artifact)
spe <- DLPFC_artifact

# find artifacts
spe <- findArtifacts(spe,
    mito_percent = "expr_chrM_ratio",
    mito_sum = "expr_chrM",
    n_order = 2, # 5 recommended, using 2 for time
    shape = "hexagonal",
    name = "artifact"
)
```

`flagVisiumOutliers` *Flag Visium Outliers in SpatialExperiment Objects*

Description

The `flagVisiumoutliers` function identifies and flags Visium systematic outlier spots in a `SpatialExperiment` object based on barcodes. These outliers are marked in the `colData` of the `SpatialExperiment` object, allowing users to exclude them from downstream analyses to enhance data quality and reliability.

Usage

```
flagVisiumOutliers(spe)
```

Arguments

`spe`	A `SpatialExperiment` object containing spatial transcriptomics data. The object must include `array_row` and `array_col` columns in its `colData` that specify the spatial coordinates of each spot.

Value

A `SpatialExperiment` object identical to the input `spe` but with an additional logical column `systematic_outliers` in its `colData`. This column indicates whether each spot is flagged as a technical outlier (`TRUE`) or not (`FALSE`).

Examples

```
library(SpotSweeper)
library(SpatialExperiment)

# load example data
spe <- STexampleData::Visium_humanDLPFC()

# Flag outlier spots
spe <- flagVisiumOutliers(spe)

# drop outlier spots
spe <- spe[, !colData(spe)$systematic_outliers]
```

`localOutliers` *localOutliers Function*

Description

This function detects local outliers in spatial transcriptomics data based on standard quality control metrics, such as library size, unique genes, and mitochondrial ratio. Local outliers are defined as spots with low/high quality metrics compared to their surrounding neighbors, based on a modified z-score statistic.

Usage

```
localOutliers(
  spe,
  metric = "detected",
  direction = "lower",
  n_neighbors = 36,
  samples = "sample_id",
  log = TRUE,
  cutoff = 3,
  workers = 1
)
```

Arguments

`spe`	SpatialExperiment or SingleCellExperiment object
`metric`	colData QC metric to use for outlier detection
`direction`	Direction of outlier detection (higher, lower, or both)
`n_neighbors`	Number of nearest neighbors to use for outlier detection
`samples`	Column name in colData to use for sample IDs
`log`	Logical indicating whether to log1p transform the features (default is TRUE)
`cutoff`	Cutoff for outlier detection (default is 3)
`workers`	Number of workers for parallel processing (default is 1)

Value

SpatialExperiment or SingleCellExperiment object with updated colData containing outputs

Examples

```
library(SpotSweeper)
library(SpatialExperiment)

# load example data
spe <- STexampleData::Visium_humanDLPFC()

# change from gene id to gene names
rownames(spe) <- rowData(spe)$gene_name

# drop out-of-tissue spots
spe <- spe[, spe$in_tissue == 1]
spe <- spe[, !is.na(spe$ground_truth)]

# Identifying the mitochondrial transcripts in our SpatialExperiment.
is.mito <- rownames(spe)[grepl("^MT-", rownames(spe))]

# Calculating QC metrics for each spot using scuttle
spe <- scuttle::addPerCellQCMetrics(spe, subsets = list(Mito = is.mito))
colnames(colData(spe))

# Identifying local outliers using SpotSweeper
spe <- localOutliers(spe,
                     metric = "sum",
                     direction = "lower",
                     log = TRUE
)
```

---

localVariance *localVariance Function*

Description

This function calculates the local variance based on kNN.

Usage

```
localVariance(
  spe,
  n_neighbors = 36,
  metric = c("expr_chrM_ratio"),
  samples = "sample_id",
  log = FALSE,
  name = NULL,
```

```
  workers = 1
)
```

Arguments

spe	SpatialExperiment object with the following columns in colData: sample_id, sum_umi, sum_gene
n_neighbors	Number of nearest neighbors to use for variance calculation
metric	Metric to use for variance calculation
samples	Column in colData to use for sample ID
log	Whether to log1p transform the metric
name	Name of the new column to add to colData
workers	Number of workers to use for parallel computation

Value

SpatialExperiment object with metric variance added to colData

Examples

```
# for more details see extended example in vignettes
library(SpotSweeper)
library(SpatialExperiment)
library(escheR)

# load example data
spe <- STexampleData::Visium_humanDLPFC()

# change from gene id to gene names
rownames(spe) <- rowData(spe)$gene_name

# show column data before SpotSweepR
colnames(colData(spe))

# drop out-of-tissue spots
spe <- spe[, spe$in_tissue == 1]
spe <- spe[, !is.na(spe$ground_truth)]

# Identifying the mitochondrial transcripts in our SpatialExperiment.
is.mito <- rownames(spe)[grepl("^MT-", rownames(spe))]

# Calculating QC metric for each spot using scuttle
spe <- scuttle::addPerCellQCMetrics(spe, subsets = list(Mito = is.mito))
colnames(colData(spe))

spe <- localVariance(spe,
    metric = "subsets_Mito_percent",
    n_neighbors = 36,
    name = "local_mito_variance_k36",
    workers = 1
```

```
)
```

`plotQCmetrics` *Plot QC metrics for a Single Sample in a SpatialExperiment object*

Description

This function generates a plot for a specified sample within a SpatialExperiment object, highlighting outliers based on a specified metric. The plot visualizes the metric of interest and indicates outliers with a distinct color.

Usage

```
plotQCmetrics(
  spe,
  sample_id = "sample_id",
  sample = unique(spe$sample_id)[1],
  metric = "detected",
  outliers = NULL,
  point_size = 2,
  colors = c("white", "black"),
  stroke = 1
)
```

Arguments

`spe`	A SpatialExperiment object containing the data to be plotted.
`sample_id`	A character string specifying the column name in `colData(spe)` that contains unique sample identifiers. Default is "sample_id".
`sample`	A character string or numeric value specifying the sample to be plotted. By default, it plots the first unique sample found in `spe$sample_id`.
`metric`	A character string specifying the metric to be visualized in the plot. This metric should be a column name in `colData(spe)`.
`outliers`	A character string specifying the column name in `colData(spe)` that indicates whether a data point is considered an outlier. Default is NULL.
`point_size`	A numeric value specifying the size of the points in the plot. Default is 2.
`colors`	A character vector specifying the colors to be used for the gradient scale. If length is 2, the gradient will be a single color gradient.
`stroke`	A numeric value specifying the border thickness for outlier points. Default is 1.

Value

The function returns a plot object created by `make_escheR` and modified with additional layers for visualizing the specified metric and outliers. The plot is not explicitly printed by the function and should be printed by the caller.

Examples

```
library(SpotSweeper)
library(SpatialExperiment)
library(escheR)

# load example data
spe <- STexampleData::Visium_humanDLPFC()

# change from gene id to gene names
rownames(spe) <- rowData(spe)$gene_name

# drop out-of-tissue spots
spe <- spe[, spe$in_tissue == 1]
spe <- spe[, !is.na(spe$ground_truth)]

# Identifying the mitochondrial transcripts in our SpatialExperiment.
is.mito <- rownames(spe)[grepl("^MT-", rownames(spe))]

# Calculating QC metrics for each spot using scuttle
spe <- scuttle::addPerCellQCMetrics(spe, subsets = list(Mito = is.mito))
colnames(colData(spe))

# Identifying local outliers using SpotSweeper
spe <- localOutliers(spe,
                     metric = "sum",
                     direction = "lower",
                     log = TRUE
)

plotQCmetrics(spe, metric="sum", outliers="sum_outliers")
```

`plotQCpdf` *Plot Outlier Metrics to PDF*

Description

This function generates a PDF file containing plots for each sample in the SpatialExperiment object, highlighting outliers based on specified metrics. Each plot visualizes outlier metrics for a single sample, allowing for easy comparison and analysis across samples.

Usage

```
plotQCpdf(
  spe,
  sample_id = "sample_id",
  metric = "detected",
  outliers = "local_outliers",
  colors = c("white", "black"),
```

```
  stroke = 1,
  point_size = 2,
  width = 5,
  height = 5,
  fname
)
```

Arguments

spe	A SpatialExperiment object containing the data to be plotted.
sample_id	A character string specifying the column name in colData(spe) that contains unique sample identifiers. Default is 'sample_id'.
metric	A character string specifying the metric to be visualized in the plot. This metric should be a column name in colData(spe).
outliers	A character string specifying the column name in colData(spe) that indicates whether a data point is considered an outlier. Default is local_outliers'.
colors	A character vector specifying the colors to be used for the gradient scale. If length is 2, the gradient will be a single color gradient
stroke	A numeric value specifying the border thickness for outlier points. Default is 1.
point_size	A numeric value specifying the size of the points in the plot. Default is 2.
width	A numeric value indicating the width of the plot. Default is 5.
height	A numeric value indicating the height of the plot. Default is 5.
fname	A character string specifying the path and name of the output PDF file.

Value

ggplot object if specified. Generates a plot otherwise.

Examples

```
library(SpotSweeper)
library(SpatialExperiment)
library(escheR)

# load example data
spe <- STexampleData::Visium_humanDLPFC()

tempFilePath <- file.path(tempdir(), "examplePlot.pdf")

# change from gene id to gene names
rownames(spe) <- rowData(spe)$gene_name

# drop out-of-tissue spots
spe <- spe[, spe$in_tissue == 1]
spe <- spe[, !is.na(spe$ground_truth)]

# Identifying the mitochondrial transcripts in our SpatialExperiment.
is.mito <- rownames(spe)[grepl("^MT-", rownames(spe))]
```

```
# Calculating QC metrics for each spot using scuttle
spe <- scuttle::addPerCellQCMetrics(spe, subsets = list(Mito = is.mito))
colnames(colData(spe))

# Identifying local outliers using SpotSweeper
spe <- localOutliers(spe,
                     metric = "sum",
                     direction = "lower",
                     log = TRUE
)

plotQCpdf(spe,
          metric="sum",
          outliers="sum_outliers",
          fname=tempFilePath)
```

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