# Package 'BayesSpace'

June 6, 2023

**Version** 1.10.1 **Date** 2021-11-03

Title Clustering and Resolution Enhancement of Spatial Transcriptomes

**Description** Tools for clustering and enhancing the resolution of spatial gene expression experiments. BayesSpace clusters a low-dimensional representation of the gene expression matrix, incorporating a spatial prior to encourage neighboring spots to cluster together. The method can enhance the resolution of the low-dimensional representation into ``sub-spots", for which features such as gene expression or cell type composition can be imputed.

**Depends** R (>= 4.0.0), SingleCellExperiment

Imports Rcpp (>= 1.0.4.6), stats, purrr, scater, scran, SummarizedExperiment, coda, rhdf5, S4Vectors, Matrix, assertthat, mclust, RCurl, DirichletReg, xgboost, utils, ggplot2, scales, BiocFileCache, BiocSingular

License MIT + file LICENSE

RoxygenNote 7.1.1

LinkingTo Rcpp, RcppArmadillo, RcppDist, RcppProgress

NeedsCompilation yes

SystemRequirements C++11

**Encoding** UTF-8

**Suggests** testthat, knitr, rmarkdown, igraph, spatialLIBD, dplyr, viridis, patchwork, RColorBrewer, Seurat

VignetteBuilder knitr

**biocViews** Software, Clustering, Transcriptomics, GeneExpression, SingleCell, ImmunoOncology, DataImport

BugReports https://github.com/edward130603/BayesSpace/issues

URL edward130603.github.io/BayesSpace

git\_url https://git.bioconductor.org/packages/BayesSpace

git\_branch RELEASE\_3\_17

git\_last\_commit 8af698b

git\_last\_commit\_date 2023-05-01

Date/Publication 2023-06-06

Author Edward Zhao [aut],
 Matt Stone [aut, cre],
 Xing Ren [ctb],
 Raphael Gottardo [ctb]

Maintainer Matt Stone <mstone@fredhutch.org>

## **R** topics documented:

adjust_hex_centers
.bsData
clean_chain
.compute_interspot_distances
find_neighbors
.flatten_matrix_list
.infer_param_dims
.init_cluster
.make_hex_spots
.make_index_names
.make_spot_vertices
.make_square_spots
.make_subspot_coldata
.make_subspot_offsets
.make_triangle_subspots
.make_vertices
prepare_inputs
read_chain
.select_spot_positions
.select_subspot_positions
BayesSpace
cluster
clusterPlot
deconvolve
enhanceFeatures
exampleSCE
featurePlot
find_neighbors
getRDS
mcmcChain
Mode
qTune
readVisium
spatialCluster
spatialEnhance
spatialPlot
spatialPreprocess

.adjust\_hex\_centers 3

Index 31

 $. \verb| adjust_hex_centers| & Adjust | hex| spot | positions| so | hexagons| are | adjacent| to | each| other| in | plot| \\$ 

## Description

Spots are regular hexagons with one unit of horizontal distance between centers

## Usage

```
.adjust_hex_centers(spot_positions)
```

#### Value

Shifted spot centers

.bsData

Access BayesSpace metadata

## Description

Access BayesSpace metadata

## Usage

```
.bsData(sce, name, default = NULL, warn = FALSE)
```

## Arguments

sce SingleCellExperiment

name Metadata name

#### Value

Requested metadata

.clean\_chain Tid

*Tidy C++ outputs before writing to disk.* 

#### **Description**

1) Convert each parameter to matrix (n\_iterations x n\_indices) 2) Add appropriate colnames 3) Thin evenly (for enhance)

#### Usage

```
.clean_chain(out, method = c("cluster", "enhance"), thin = 100)
```

#### **Arguments**

out List returned by cluster() or deconvolve().

method Whether the output came from clustering or enhancement. (Different params

are included in each.)

thin Thinning rate. Some enhanced parameters are thinned within C++ loop, others

(mu and Ychange) need to be thinned afterwards.

#### Value

List with standardized parameters

.compute\_interspot\_distances

Estimate the distance between two neighboring spots

#### **Description**

Fit linear models between each image pixel coordinate and its corresponding array coordinate to estimate the pixel distance between two spots along each axis. Add these distances to estimate the L1 distance between two spots, then add a small buffer.

#### Usage

```
.compute_interspot_distances(sce, scale.factor = 1.02)
```

## **Arguments**

sce SingleCellExperiment (must include row, col, imagerow, imagecol in colData)

scale.factor Scale estimated L1 difference up by this amount.

#### Value

doubles xdist, ydist, radius

.find\_neighbors 5

.find\_neighbors

Find neighboring spots based on array coordinates

#### **Description**

Find neighboring spots based on array coordinates

## Usage

```
.find_neighbors(sce, platform)
```

## Arguments

sce SingleCellExperiment

platform If "Visium", select six neighboring spots around center; if "ST", select four ad-

jacent spots.

#### Value

df\_j a list of neighbor indices (zero-indexed) for each spot

## Description

Convert a list of matrices to a single matrix, where each row is a flattened matrix from the original list

## Usage

```
.flatten_matrix_list(xs, ...)
```

## Arguments

ΧS

List of matrices

#### Value

Matrix

6 .init\_cluster

 $. \verb|infer_param_dims| Infer original dimensions of parameter (per iteration) from colnames$ 

#### **Description**

Used to avoid writing colnames directly to HDF5 as attribute, which fails for large parameters (e.g. Y)

#### Usage

```
.infer_param_dims(cnames)
```

## Arguments

cnames List of column names

#### Value

Numeric vector (nrow, ncol)

## Description

Initialize cluster assignments

#### Usage

```
.init_cluster(Y, q, init = NULL, init.method = c("mclust", "kmeans"))
```

#### **Arguments**

q Number of clusters

init Vector of initial cluster assignmentsinit.method Initialization clustering algorithm

sce SingleCellExperiment

#### Value

Vector of cluster assignments.

.make\_hex\_spots 7

malca	hav	spots
·IIIake_	_HEX_	SDULS

Make vertices for each hex spot

## Description

Make vertices for each hex spot

#### Usage

```
.make_hex_spots(cdata, fill)
```

#### Value

Table of (x.pos, y.pos, spot, fill); where spot groups the vertices outlining the spot's border

.make\_index\_names

Make colnames for parameter indices.

## Description

Scalar parameters are named "name". Vector parameters are named "name[i]". Matrix parameters are named "name[i,j]".

## Usage

```
.make_index_names(name, m = NULL, n = NULL, dim = 1)
```

## Arguments

name Parame	eter name
name raram	ici namc

m, n Dimensions of parameter (m=nrow, n=ncol)

dim Dimensionality of parameter (0=scalar, 1=vector, 2=matrix)

## Value

List of names for parameter values

8 .make\_square\_spots

.make\_spot\_vertices

Compute vertex coordinates for each spot in frame of plot

## Description

Compute vertex coordinates for each spot in frame of plot

## Usage

```
.make_spot_vertices(spot_positions, vertex_offsets)
```

## Arguments

```
spot_positions Center for hex, top left for square
vertex_offsets Data frame of (x, y) offsets wrt spot position for each vertex of spot
```

#### Value

Cartesian product of positions and offsets, with coordinates computed as (pos + offset)

.make\_square\_spots

Make vertices for each square spot

## **Description**

Squares are simple, just mae a unit square at each array coordinate

#### Usage

```
.make_square_spots(cdata, fill = "spatial.cluster", scale.factor = 1)
```

#### Value

Table of (x.pos, y.pos, spot, fill); where spot groups the vertices outlining the spot's border

.make\_subspot\_coldata 9

.make\_subspot\_coldata Add subspot labels and offset row/col locations before making enhanced SCE.

## Description

```
Subspots are stored as (1.1, 2.1, 3.1, ..., 1.2, 2.2, 3.2, ...)
```

#### Usage

```
.make_subspot_coldata(positions, sce, n_subspots_per)
```

#### **Arguments**

sce Original sce (to obtain number of spots and original row/col)

n\_subspots\_per Number of subspots per spot

cdata Table of colData (imagerow and imagecol; from deconv\$positions)

#### Value

Data frame with added subspot names, parent spot indices, and offset row/column coordinates

 $. \verb|make_subspot_offsets|| \textit{Define offsets for each subspot layout.}|$ 

## **Description**

Hex spots are divided into 6 triangular subspots, square spots are divided into 9 squares. Offsets are relative to the spot center.

#### Usage

```
.make_subspot_offsets(n_subspots_per)
```

#### **Arguments**

n\_subspots\_per Number of subspots per spot

#### Value

Matrix of x and y offsets, one row per subspot

.make\_vertices

```
.make_triangle_subspots
```

Make vertices for each triangle subspot of a hex

## Description

Make vertices for each triangle subspot of a hex

#### Usage

```
.make_triangle_subspots(cdata, fill = "spatial.cluster")
```

#### Value

Table of (x.pos, y.pos, spot, fill); where spot groups the vertices outlining the spot's border

maka	vertices	
·IIIant	ACI LICES	

Make vertices outlining spots/subspots for geom\_polygon()

## Description

Make vertices outlining spots/subspots for geom\_polygon()

#### Usage

```
.make_vertices(sce, fill, platform, is.enhanced)
```

## Arguments

sce	SingleCellExperiment with row/col in colData
fill	Name of a column in colData(sce) or a vector of values to use as fill for each spot
platform	"Visium" or "ST", used to determine spot layout
is.enhanced	If true, see contains enhanced subspot data instead of spot-level expression. Used to determine spot layout.

## Value

Table of (x.pos, y.pos, spot, fill); where spot groups the vertices outlining the spot's border

.prepare\_inputs 11

.prepare\_inputs

Prepare cluster/deconvolve inputs from SingleCellExperiment object

## Description

Prepare cluster/deconvolve inputs from SingleCellExperiment object

## Usage

```
.prepare_inputs(
    sce,
    use.dimred = "PCA",
    d = 15,
    positions = NULL,
    position.cols = c("imagecol", "imagerow"),
    radius = NULL,
    xdist = NULL,
    ydist = NULL
```

#### Value

List of PCs, names of columns with x/y positions, and inter-spot distances

.read\_chain

Load saved chain from disk.

## **Description**

Load saved chain from disk.

## Usage

```
.read_chain(h5.fname, params = NULL, is.enhanced = FALSE)
```

## **Arguments**

h5. fname Path to hdf5 file containing chain

params List of parameters to read from file (will read all by default)

## Value

MCMC chain, represented as a coda::mcmc object

```
.select_spot_positions
```

Helper to extract x, y, fill ID from colData

#### **Description**

Helper to extract x, y, fill ID from colData

#### Usage

```
.select_spot_positions(cdata, x = "col", y = "row", fill = "spatial.cluster")
```

#### Value

Dataframe of (x.pos, y.pos, fill) for each spot

```
.select_subspot_positions
```

Helper to pull out subspot position columns Probably redundant with select\_spot\_positions above, but we need subspot.idx

## Description

Helper to pull out subspot position columns Probably redundant with select\_spot\_positions above, but we need subspot.idx

#### Usage

```
.select_subspot_positions(
  cdata,
  x = "spot.col",
  y = "spot.row",
  fill = "spatial.cluster")
```

#### Value

Dataframe of (x.pos, y.pos, fill) for each spot

BayesSpace 13

BayesSpace

BayesSpace: A package for processing spatial transcriptomes

#### **Description**

Tools for clustering and enhancing the resolution of spatial gene expression experiments. BayesS-pace clusters a low-dimensional representation of the gene expression matrix, incorporating a spatial prior to encourage neighboring spots to cluster together. The method can enhance the resolution of the low-dimensional representation into "sub-spots", for which features such as gene expression or cell type composition can be imputed.

#### **Details**

For an overview of the functionality provided by the package, please see the vignette: vignette("BayesSpace", package="BayesSpace")

cluster

Wrapper around C++ iterate\_\*() functions

#### Description

Wrapper around C++ iterate\_\*() functions

#### Usage

```
cluster(
    Y,
    q,
    df_j,
    init = rep(1, nrow(Y)),
    model = c("t", "normal"),
    precision = c("equal", "variable"),
    mu0 = colMeans(Y),
    lambda0 = diag(0.01, nrow = ncol(Y)),
    gamma = 3,
    alpha = 1,
    beta = 0.01,
    nrep = 1000
)
```

## Value

List of clustering parameter values at each iteration

14 clusterPlot

clusterPlot

Plot spatial cluster assignments.

## Description

Plot spatial cluster assignments.

## Usage

```
clusterPlot(
    sce,
    label = "spatial.cluster",
    palette = NULL,
    color = NULL,
    platform = NULL,
    is.enhanced = NULL,
    ...
)
```

## Arguments

sce	SingleCellExperiment. If fill is specified and is a string, it must exist as a column in colData(sce).
label	Labels used to color each spot. May be the name of a column in colData(sce), or a vector of discrete values.
palette	Optional vector of hex codes to use for discrete spot values.
color	Optional hex code to set color of borders around spots. Set to NA to remove borders.
platform	Spatial sequencing platform. If "Visium", the hex spot layout will be used, otherwise square spots will be plotted.  NOTE: specifying this argument is only necessary if see was not created by spatialCluster() or spatialEnhance().
is.enhanced	True if sce contains subspot-level data instead of spots. Spatial sequencing platform. If true, the respective subspot lattice for each platform will be plotted. NOTE: specifying this argument is only necessary if sce was not created by spatialCluster() or spatialEnhance().
• • •	Additional arguments for geom_polygon(). size, to specify the linewidth of these borders, is likely the most useful.

#### Value

Returns a ggplot object.

## See Also

Other spatial plotting functions: featurePlot()

deconvolve 15

#### **Examples**

```
sce <- exampleSCE()
clusterPlot(sce)</pre>
```

deconvolve

Wrapper around C++ iterate\_deconv() function

## Description

Wrapper around C++ iterate\_deconv() function

## Usage

```
deconvolve(
 Υ,
 positions,
 xdist,
 ydist,
 q,
  init,
 nrep = 1000,
 model = "normal",
 platform = c("Visium", "ST"),
 verbose = TRUE,
  jitter_scale = 5,
  jitter_prior = 0.01,
 mu0 = colMeans(Y),
 gamma = 2,
 lambda0 = diag(0.01, nrow = ncol(Y)),
 alpha = 1,
 beta = 0.01
)
```

#### Value

List of enhancement parameter values at each iteration

16 enhanceFeatures

enhanceFeatures

Predict feature vectors from enhanced PCs.

#### **Description**

Predict feature vectors from enhanced PCs.

#### Usage

```
enhanceFeatures(
    sce.enhanced,
    sce.ref,
    feature_names = NULL,
    model = c("xgboost", "dirichlet", "lm"),
    use.dimred = "PCA",
    assay.type = "logcounts",
    altExp.type = NULL,
    feature.matrix = NULL,
    nrounds = 0,
    train.n = round(ncol(sce.ref) * 2/3)
)
```

#### **Arguments**

sce.enhanced	SingleCellExperiment object with enhanced	$PC_{\varsigma}$
Sce.emanceu	Single Centraperiment object with emianced	rcs.

sce.ref SingleCellExperiment object with original PCs and expression.

feature\_names List of genes/features to predict expression/values for.

model Model used to predict enhanced values.

use.dimred Name of dimension reduction to use.

assay.type Expression matrix in assays(sce.ref) to predict.

altExp.type Expression matrix in altExps(sce.ref) to predict. Overrides assay.type if

specified.

feature.matrix Expression/feature matrix to predict, if not directly attached to sce.ref. Must

have columns corresponding to the spots in sce.ref. Overrides assay.type

and altExp. type if specified.

nrounds Nonnegative integer to set the nrounds parameter (max number of boosting

iterations) for xgboost. nrounds = 100 works reasonably well in most cases. If nrounds is set to 0, the parameter will be tuned using a train-test split. We recommend tuning nrounds for improved feature prediction, but note this will

increase runtime.

train.n Number of spots to use in the training dataset for tuning nrounds. By default,

2/3 the total number of spots are used.

exampleSCE 17

#### **Details**

Enhanced features are computed by fitting a predictive model to a low-dimensional representation of the original expression vectors. By default, a linear model is fit for each gene using the top 15 principal components from each spot, i.e. lm(gene ~ PCs), and the fitted model is used to predict the enhanced expression for each gene from the subspots' principal components.

Diagnostic measures, such as RMSE for xgboost or R.squared for linear regression, are added to the 'rowData' of the enhanced experiment if the features are an assay of the original experiment. Otherwise they are stored as an attribute of the returned matrix/altExp.

Note that feature matrices will be returned and are expected to be input as  $p \times n$  matrices of p-dimensional feature vectors over the n spots.

#### Value

If assay.type or altExp.type are specified, the enhanced features are stored in the corresponding slot of sce.enhanced and the modified SingleCellExperiment object is returned.

If feature.matrix is specified, or if a subset of features are requested, the enhanced features are returned directly as a matrix.

#### **Examples**

```
set.seed(149)
sce <- exampleSCE()
sce <- spatialCluster(sce, 7, nrep=100, burn.in=10)
enhanced <- spatialEnhance(sce, 7, init=sce$spatial.cluster, nrep=100, burn.in=10)
enhanced <- enhanceFeatures(enhanced, sce, feature_names=c("gene_1", "gene_2"))</pre>
```

exampleSCE	Create minimal	SingleCellExperiment	for	documentation	exam-
	ples.				

#### **Description**

Create minimal SingleCellExperiment for documentation examples.

#### **Usage**

```
exampleSCE(nrow = 8, ncol = 12, n_genes = 100, n_PCs = 10)
```

#### **Arguments**

nrow	Number of rows of spots
ncol	Number of columns of spots
n_genes	Number of genes to simulate
n_PCs	Number of principal components to include

18 featurePlot

#### **Details**

Inspired by scuttle's mockSCE().

#### Value

A SingleCellExperiment object with simulated counts, corresponding logcounts and PCs, and positional data in colData. Spots are distributed over an (nrow x ncol) rectangle.

## **Examples**

```
set.seed(149)
sce <- exampleSCE()</pre>
```

featurePlot

Plot spatial gene expression.

## Description

Plot spatial gene expression.

## Usage

```
featurePlot(
    sce,
    feature,
    assay.type = "logcounts",
    diverging = FALSE,
    low = NULL,
    high = NULL,
    mid = NULL,
    color = NULL,
    platform = NULL,
    is.enhanced = NULL,
    ...
)
```

## Arguments

sce	SingleCellExperiment. If feature is specified and is a string, it must exist as a row in the specified assay of sce.
feature	Feature vector used to color each spot. May be the name of a gene/row in an assay of sce, or a vector of continuous values.
assay.type	String indicating which assay in sce the expression vector should be taken from.
diverging	If true, use a diverging color gradient in featurePlot() (e.g. when plotting a fold change) instead of a sequential gradient (e.g. when plotting expression).

find\_neighbors 19

Optional hex codes for low, mid, and high values of the color gradient used for low, mid, high

continuous spot values.

color Optional hex code to set color of borders around spots. Set to NA to remove

platform Spatial sequencing platform. If "Visium", the hex spot layout will be used, oth-

erwise square spots will be plotted.

NOTE: specifying this argument is only necessary if see was not created by

spatialCluster() or spatialEnhance().

is.enhanced True if sce contains subspot-level data instead of spots. Spatial sequencing

> platform. If true, the respective subspot lattice for each platform will be plotted. NOTE: specifying this argument is only necessary if see was not created by

spatialCluster() or spatialEnhance().

Additional arguments for geom\_polygon(). size, to specify the linewidth of

these borders, is likely the most useful.

#### Value

Returns a ggplot object.

#### See Also

Other spatial plotting functions: clusterPlot()

#### **Examples**

```
sce <- exampleSCE()</pre>
featurePlot(sce, "gene_2")
```

find\_neighbors

Compute pairwise distances between all spots and return list of neighbors for each spot.

#### **Description**

Compute pairwise distances between all spots and return list of neighbors for each spot.

#### **Usage**

```
find_neighbors(positions, radius, method = c("manhattan", "euclidean"))
```

## **Arguments**

(n x 2) matrix of spot coordinates. positions

radius The maximum distance for two spots to be considered neighbors.

method Distance metric to use. 20 mcmcChain

#### Value

List df\_j, where df\_j[[i]] is a vector of zero-indexed neighbors of i.

getRDS

Download a processed sample from our S3 bucket

#### **Description**

Datasets are cached locally using BiocFileCache. The first time using this function, you may need to consent to creating a BiocFileCache directory if one does not already exist.

#### Usage

```
getRDS(dataset, sample, cache = TRUE)
```

#### **Arguments**

dataset Dataset identifier sample Sample identifier

cache If true, cache the dataset locally with BiocFileCache. Otherwise, download

directly from our S3 bucket. Caching saves time on subsequent loads, but con-

sumes disk space.

#### Value

sce A SingleCellExperiment with positional information in colData and PCs based on the top  $2000\,\mathrm{HVGs}$ 

#### **Examples**

```
sce <- getRDS("2018_thrane_melanoma", "ST_mel1_rep2", cache=FALSE)</pre>
```

mcmcChain

Read MCMC chain associated with a BayesSpace clustering or enhancement

#### **Description**

BayesSpace stores the MCMC chain associated with a clustering or enhancement on disk in an HDF5 file. The mcmcChain() function reads any parameters specified by the user into a coda::mcmc object compatible with TidyBayes.

mcmcChain 21

#### Usage

```
mcmcChain(sce, params = NULL)
removeChain(sce)
```

#### **Arguments**

sce SingleCellExperiment with a file path stored in its metadata.

params List of model parameters to read

#### **Details**

To interact with the HDF5 file directly, obtain the filename from the SingleCellExperiment's metadata: metadata(sce)\$chain.h5. Each parameter is stored as a separate dataset in the file, and is represented as a matrix of size (n\_iterations x n\_parameter\_indices). Parameter choices for the spot-level clustering include:

- z (cluster assignments)
- weights  $(w_i)$
- mu (mean vectors)
- lambda (precision matrix)
- plogLik (pseudo-log-likelihood)

Parameter choices for the subspot-level enhanced clustering include:

- z (cluster assignments)
- weights  $(w_i)$
- Y (enhanced PCs)
- mu (mean vectors)
- lambda (precision matrix)
- Ychange (acceptance rate for the jittering of PCs)

#### Value

Returns an mcmc object containing the values of the requested parameters over the constructed chain.

#### **Examples**

```
set.seed(149)
sce <- exampleSCE()
sce <- spatialCluster(sce, 7, nrep=100, burn.in=10, save.chain=TRUE)
chain <- mcmcChain(sce)
removeChain(sce)</pre>
```

qTune

Mode	Find the mode

## Description

Used for finding the most frequent cluster for each z

## Usage

Mode(x)

#### **Arguments**

x Numeric vector

#### Value

mode Numeric scalar, most frequent element in x

qTune	Tuning the choice of q (number of clusters) before running spatial-
	Cluster

## Description

Before running spatialCluster(), we recommend tuning the choice of q by choosing the q that maximizes the model's negative log likelihood over early iterations. qTune() computes the average negative log likelihood for a range of q values over iterations 100:1000, and qPlot() displays the results.

## Usage

```
qPlot(sce, qs = seq(3, 7), force.retune = FALSE, ...)
qTune(sce, qs = seq(3, 7), burn.in = 100, nrep = 1000, ...)
```

## Arguments

sce	A SingleCellExperiment object containing the spatial data.
qs	The values of q to evaluate.
force.retune	If specified, existing tuning values in sce will be overwritten.
	Other parameters are passed to spatialCluster().
burn.in, nrep	Integers specifying the range of repetitions to compute.

readVisium 23

#### **Details**

qTune() takes the same parameters as spatialCluster() and will run the MCMC clustering algorithm up to nrep iterations for each value of q. The first burn. in iterations are discarded as burn-in and the log likelihood is averaged over the remaining iterations.

qPlot() plots the computed negative log likelihoods as a function of q. If qTune() was run previously, i.e. there exists an attribute of sce named "q.logliks", the pre-computed results are displayed. Otherwise, or if force.retune is specified, qplot() will automatically run qTune() before plotting (and can take the same parameters as spatialCluster().

#### Value

qTune() returns a modified sce with tuning log likelihoods stored as an attribute named "q.logliks". qPlot() returns a ggplot object.

#### **Examples**

```
set.seed(149)
sce <- exampleSCE()
sce <- qTune(sce, seq(3, 7), burn.in=10, nrep=100)
qPlot(sce)</pre>
```

readVisium

Load a Visium spatial dataset as a SingleCellExperiment.

#### **Description**

Load a Visium spatial dataset as a SingleCellExperiment.

#### Usage

```
readVisium(dirname)
```

#### **Arguments**

dirname

Path to spaceranger output directory (e.g. "sampleID/outs/"). This directory must contain the counts matrix and feature/barcode TSVs in filtered\_feature\_bc\_matrix/, and the spot positions at spatial/tissue\_positions\_list.csv. (These are default locations for spaceranger outputs.)

#### **Details**

We store two variables associated with downstream Bayes Space functions in a list called Bayes Space . data in the Single Cell Experiment's metadata.

- platform is set to "Visium", and is used to determine spot layout and neighborhood structure.
- is.enhanced is set to FALSE to denote the object contains spot-level data.

24 spatialCluster

#### Value

SingleCellExperiment containing the counts matrix in counts and spatial data in colData. Array coordinates for each spot are stored in columns row and col, while image coordinates are stored in columns imagerow and imagecol.

#### **Examples**

```
## Not run:
sce <- readVisium("path/to/outs/")</pre>
## End(Not run)
```

spatialCluster

Spatial clustering

## Description

Cluster a spatial expression dataset.

## Usage

```
spatialCluster(
  sce,
 use.dimred = "PCA",
 d = 15,
 platform = c("Visium", "ST"),
  init = NULL,
  init.method = c("mclust", "kmeans"),
 model = c("t", "normal"),
 precision = c("equal", "variable"),
 nrep = 50000,
 burn.in = 1000,
  gamma = NULL,
 mu0 = NULL,
 lambda0 = NULL,
  alpha = 1,
 beta = 0.01,
  save.chain = FALSE,
  chain.fname = NULL
)
```

## **Arguments**

A SingleCellExperiment object containing the spatial data. sce The number of clusters. q

spatialCluster 25

use.dimred Name of a reduced dimensionality result in reducedDims(sce). If provided,

cluster on these features directly.

d Number of top principal components to use when clustering.

platform Spatial transcriptomic platform. Specify 'Visium' for hex lattice geometry or

'ST' for square lattice geometry. Specifying this parameter is optional when analyzing SingleCellExperiments processed using readVisium or spatialPreprocess,

as this information is included in their metadata.

init Initial cluster assignments for spots.

init.method If init is not provided, cluster the top d PCs with this method to obtain initial

cluster assignments.

model Error model. ('normal' or 't')

precision Covariance structure. ('equal' or 'variable' for EEE and VVV covariance mod-

els, respectively.)

nrep The number of MCMC iterations.

burn.in The number of MCMC iterations to exclude as burn-in period.

gamma Smoothing parameter. Defaults to 2 for platform="ST" and 3 for platform="Visium".

(Values in range of 1-3 seem to work well.)

mu0 Prior mean hyperparameter for mu. If not provided, mu0 is set to the mean of

PCs over all spots.

lambda0 Prior precision hyperparam for mu. If not provided, lambda0 is set to a diagonal

matrix 0.01I.

alpha Hyperparameter for Wishart distributed precision lambda. beta Hyperparameter for Wishart distributed precision lambda.

save.chain If true, save the MCMC chain to an HDF5 file.

chain. fname File path for saved chain. Tempfile used if not provided.

#### **Details**

The input SCE must have row and col columns in its colData, corresponding to the array row and column coordinates of each spot. These are automatically parsed by readVisium or can be added manually when creating the SCE.

Cluster labels are stored in the spatial.cluster column of the SCE, and the cluster initialization is stored in cluster.init.

#### Value

Returns a modified sce with cluster assignments stored in colData under the name spatial.cluster.

#### See Also

spatialPreprocess for preparing the SCE for clustering, spatialEnhance for enhancing the clustering resolution, clusterPlot for visualizing the cluster assignments, featurePlot for visualizing expression levels in spatial context, and mcmcChain for examining the full MCMC chain associated with the clustering.

26 spatialEnhance

#### **Examples**

```
set.seed(149)
sce <- exampleSCE()
sce <- spatialCluster(sce, 7, nrep=100, burn.in=10)</pre>
```

spatialEnhance

Enhance spot resolution

#### **Description**

Enhanced clustering of a spatial expression dataset to subspot resolution.

## Usage

```
spatialEnhance(
  sce,
  platform = c("Visium", "ST"),
  use.dimred = "PCA",
 d = 15,
  init = NULL,
  init.method = c("spatialCluster", "mclust", "kmeans"),
 model = c("t", "normal"),
 nrep = 2e + 05,
  gamma = NULL,
 mu0 = NULL,
  lambda0 = NULL,
  alpha = 1,
  beta = 0.01,
  save.chain = FALSE,
  chain.fname = NULL,
  burn.in = 10000,
  jitter_scale = 5,
  jitter_prior = 0.3,
  verbose = FALSE
)
```

#### Arguments

sce A SingleCellExperiment object containing the spatial data.

The number of clusters.

platform Spatial transcriptomic platform. Specify 'Visium' for hex lattice geometry or

'ST' for square lattice geometry. Specifying this parameter is optional when analyzing SingleCellExperiments processed using readVisium, spatialPreprocess,

or spatialCluster, as this information is included in their metadata.

spatialEnhance 27

use.dimred Name of a reduced dimensionality result in reducedDims(sce). If provided,

cluster on these features directly.

d Number of top principal components to use when clustering.

init Initial cluster assignments for spots.

init.method If init is not provided, cluster the top d PCs with this method to obtain initial

cluster assignments.

model Error model. ('normal' or 't')

nrep The number of MCMC iterations.

gamma Smoothing parameter. (Values in range of 1-3 seem to work well.)

mu0 Prior mean hyperparameter for mu. If not provided, mu0 is set to the mean of

PCs over all spots.

lambda0 Prior precision hyperparam for mu. If not provided, lambda0 is set to a diagonal

matrix 0.01I.

alpha Hyperparameter for Wishart distributed precision lambda. beta Hyperparameter for Wishart distributed precision lambda.

save.chain If true, save the MCMC chain to an HDF5 file.

chain. fname File path for saved chain. Tempfile used if not provided.

burn.in Number of iterations to exclude as burn-in period. The MCMC iterations are

currently thinned to every 100; accordingly burn.in is rounded down to the

nearest multiple of 100.

jitter\_scale Controls the amount of jittering. Small amounts of jittering are more likely to

be accepted but result in exploring the space more slowly. We suggest tuning

jitter\_scale so that Ychange is on average around 25%-40%.

jitter\_prior Scale factor for the prior variance, parameterized as the proportion (default =

0.3) of the mean variance of the PCs. We suggest making jitter\_prior smaller if the jittered values are not expected to vary much from the overall mean of the

spot.

verbose Log progress to stderr.

#### Details

The enhanced SingleCellExperiment has most of the properties of the input SCE - rowData, colData, reducedDims - but does not include expression data in counts or logcounts. To impute enhanced expression vectors, please use [enhanceFeatures()] after running spatialEnhance.

The colData of the enhanced SingleCellExperiment includes the following columns to permit referencing the subspots in spatial context and linking back to the original spots:

- spot.idx: Index of the spot this subspot belongs to (with respect to the input SCE).
- subspot.idx: Index of the subspot within its parent spot.
- spot.row: Array row of the subspot's parent spot.
- spot.col: Array col of the subspot's parent spot.
- row: Array row of the subspot. This is the parent spot's row plus an offset based on the subspot's position within the spot.

28 spatialPlot

• col: Array col of the subspot. This is the parent spot's col plus an offset based on the subspot's position within the spot.

- imagerow: Pixel row of the subspot. This is the parent spot's row plus an offset based on the subspot's position within the spot.
- imagecol: Pixel col of the subspot. This is the parent spot's col plus an offset based on the subspot's position within the spot.

#### Value

Returns a new SingleCellExperiment object. By default, the assays of this object are empty, and the enhanced resolution PCs are stored as a reduced dimensionality result accessible with reducedDim(sce, 'PCA').

#### See Also

spatialCluster for clustering at the spot level before enhancing, clusterPlot for visualizing the cluster assignments, enhanceFeatures for imputing enhanced expression, and mcmcChain for examining the full MCMC chain associated with the enhanced clustering.

#### **Examples**

```
set.seed(149)
sce <- exampleSCE()
sce <- spatialCluster(sce, 7, nrep=100, burn.in=10)
enhanced <- spatialEnhance(sce, 7, nrep=100, burn.in=10)</pre>
```

spatialPlot

Spatial plotting functions

#### **Description**

Spatial plotting functions

## **Arguments**

olor	Optional hex code to set color of borders around spots. Set to NA to remove borders.
••	Additional arguments for geom_polygon(). size, to specify the linewidth of these borders, is likely the most useful.
latform	Spatial sequencing platform. If "Visium", the hex spot layout will be used, otherwise square spots will be plotted.  NOTE: specifying this argument is only necessary if see was not created by
	<pre>spatialCluster() or spatialEnhance().</pre>
s.enhanced	True if sce contains subspot-level data instead of spots. Spatial sequencing platform. If true, the respective subspot lattice for each platform will be plotted. NOTE: specifying this argument is only necessary if sce was not created by spatialCluster() or spatialEnhance().
	olor  latform s.enhanced

spatialPreprocess 29

cnat	i - 1	Dron	rocess
spat	1a1	.Preb	rocess

Preprocess a spatial dataset for BayesSpace

## Description

Adds metadata required for downstream analyses, and (optionally) performs PCA on log-normalized expression of top HVGs.

#### Usage

```
spatialPreprocess(
   sce,
   platform = c("Visium", "ST"),
   n.PCs = 15,
   n.HVGs = 2000,
   skip.PCA = FALSE,
   log.normalize = TRUE,
   assay.type = "logcounts",
   BSPARAM = ExactParam()
)
```

## Arguments

sce	SingleCellExperiment to preprocess
platform	Spatial sequencing platform. Used to determine spot layout and neighborhood structure (Visium = hex, ST = square).
n.PCs	Number of principal components to compute. We suggest using the top 15 PCs in most cases.
n.HVGs	Number of highly variable genes to run PCA upon.
skip.PCA	Skip PCA (if dimensionality reduction was previously computed.)
log.normalize	Whether to log-normalize the input data with scater. May be omitted if log-normalization previously computed.
assay.type	Name of assay in sce containing normalized counts. Leave as "logcounts" unless you explicitly pre-computed a different normalization and added it to sce under another assay. Note that we do not recommend running BayesSpace on PCs computed from raw counts.
BSPARAM	A BiocSingularParam object specifying which algorithm should be used to perform the PCA. By default, an exact PCA is performed, as current spatial datasets are generally small (<10,000 spots). To perform a faster approximate PCA, please specify FastAutoParam() and set a random seed to ensure reproducibility.

## Value

SingleCellExperiment with PCA and BayesSpace metadata

30 spatialPreprocess

## Examples

```
sce <- exampleSCE()
sce <- spatialPreprocess(sce)</pre>
```

# **Index**

* internal	.make_index_names,7	
.adjust_hex_centers, 3	.make_spot_vertices, 8	
. bsData, 3	.make_square_spots, 8	
.clean_chain, 4	.make_subspot_coldata, 9	
.compute_interspot_distances, 4	.make_subspot_offsets, 9	
. find_neighbors, 5	.make_triangle_subspots, 10	
.flatten_matrix_list, 5	.make_vertices, 10	
.infer_param_dims, 6	.prepare_inputs, 11	
.init_cluster, 6	.read_chain, 11	
.make_hex_spots, 7	.select_spot_positions, 12	
.make_index_names, 7	<pre>.select_subspot_positions, 12</pre>	
.make_spot_vertices, 8	_ ' _'	
.make_square_spots, 8	BayesSpace, 13	
.make_subspot_coldata, 9	BiocSingularParam, 29	
.make_subspot_offsets, 9		
.make_triangle_subspots, 10	cluster, 13	
.make_triangre_subspects, 10	clusterPlot, 14, <i>19</i> , <i>25</i> , <i>28</i>	
.prepare_inputs, 11	deconvolve, 15	
read_chain, 11	deconvoive, 13	
.select_spot_positions, 12	enhanceFeatures, 16, 28	
.select_subspot_positions, 12	exampleSCE, 17	
BayesSpace, 13	o. ap20002, 17	
cluster, 13	featurePlot, <i>14</i> , 18, <i>25</i>	
deconvolve, 15	find_neighbors, 19	
find_neighbors, 19		
Mode, 22	getRDS, 20	
spatialPlot, 28		
* spatial plotting functions	mcmcChain, 20, 25, 28	
clusterPlot, 14	Mode, 22	
featurePlot, 18	qPlot (qTune), 22	
.adjust_hex_centers, 3	qTune, 22	
.bsData, 3	qrane, <u>22</u>	
.clean_chain, 4	readVisium, 23, 25, 26	
.compute_interspot_distances, 4	removeChain (mcmcChain), 20	
.find_neighbors, 5		
.flatten_matrix_list, 5	spatialCluster, 24, 26, 28	
.infer_param_dims, 6	spatialEnhance, 25, 26	
.init_cluster, 6	spatialPlot, 28	
.make_hex_spots, 7	spatialPreprocess, 25, 26, 29	