# Package: SpatialFeatureExperiment (via r-universe)

September 24, 2024

Type Package

Title Integrating SpatialExperiment with Simple Features in sf

Version 1.7.3

Description A new S4 class integrating Simple Features with the R package sf to bring geospatial data analysis methods based on vector data to spatial transcriptomics. Also implements management of spatial neighborhood graphs and geometric operations. This pakage builds upon SpatialExperiment and SingleCellExperiment, hence methods for these parent classes can still be used.

Imports Biobase, BiocGenerics, BiocNeighbors, BiocParallel, data.table, DropletUtils, EBImage, grDevices, lifecycle, Matrix, methods, rjson, rlang, S4Vectors, sf, sfheaders, SingleCellExperiment, SpatialExperiment, spdep (>= 1.1-7), SummarizedExperiment, stats, terra, utils, zeallot

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Collate 'AllGenerics.R' 'utils.R' 'SFE-class.R' 'aggregate.R' 'align.R' 'annotGeometries.R' 'cbind.R' 'changeSampleIDs.R' 'coerce.R' 'data.R' 'df2sf.R' 'dimGeometries.R' 'featureData.R' 'formatTxSpots.R' 'geometry\_operation.R' 'graph\_wrappers.R' 'image.R' 'int\_dimData.R' 'internal-Voyager.R' 'listw2sparse.R' 'localResults.R' 'read.R' 'reexports.R' 'saveRDS.R' 'spatialGraphs.R' 'split.R' 'subset.R' 'transformation.R' 'updateObject.R' 'validity.R'

**Suggests** arrow, BiocStyle, dplyr, knitr, RBioFormats, rhdf5, rmarkdown, scater, sfarrow, SFEData (>= 1.5.3), Seurat, SeuratObject, sparseMatrixStats, testthat (>= 3.0.0), tidyr, Voyager (>= 1.7.2), xml2

Remotes Voyager=github::pachterlab/voyager

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# Config/testthat/edition 3 Depends R (>= 4.2.0) VignetteBuilder knitr biocViews DataRepresentation, Transcriptomics, Spatial URL https://github.com/pachterlab/SpatialFeatureExperiment BugReports https://github.com/pachterlab/SpatialFeatureExperiment/issues Repository https://bioc.r-universe.dev RemoteUrl https://github.com/bioc/SpatialFeatureExperiment RemoteRef HEAD RemoteSha 4d13381edf27be2bf20daee1cdd2927f1298499b

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addVisiumSpotPoly	Add Visium spot polygons to colGeometry
add v 131 dill Spott O1 y	Than visium spot polygons to condecinelly

# **Description**

For adding the spot polygons to SFE objects converted from SPE.

# Usage

```
addVisiumSpotPoly(x, spotDiameter)
```

# **Arguments**

A SpatialFeatureExperiment object. Х

spotDiameter

Spot diameter for technologies with arrays of spots of fixed diameter per slide, such as Visium, ST, DBiT-seq, and slide-seq. The diameter must be in the same unit as the coordinates in the \*Geometry arguments. Ignored for geometries that

are not POINT or MULTIPOINT.

#### Value

A SFE object with a new colGeometry called spotPoly, which has polygons of the spots.

# **Examples**

```
library(SpatialExperiment)
example(read10xVisium)
# There can't be suplicate barcodes
colnames(spe) <- make.unique(colnames(spe), sep = "-")</pre>
rownames(spatialCoords(spe)) <- colnames(spe)</pre>
sfe <- toSpatialFeatureExperiment(spe)</pre>
# A hypothetical spot diameter; check the scalefactors_json.json file for
# actual diameter in pixels in full resolution image.
sfe <- addVisiumSpotPoly(sfe, spotDiameter = 80)</pre>
```

affineImg

Affine transformation of images

# **Description**

This function performs affine transformation on images, with any matrix and translation vector.

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# Usage

```
## S4 method for signature 'SpatRasterImage'
affineImg(x, M, v, maxcell = 1e+07, ...)
## S4 method for signature 'BioFormatsImage'
affineImg(x, M, v, ...)
## S4 method for signature 'ExtImage'
affineImg(x, M, v, ...)
```

# **Arguments**

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V	An object of class	. ★lmaαa ac ımnl	lemented in this	nackage
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M A 2x2 numeric matrix for the linear transformation in the xy plane.

v A numeric vector of length 2 for translation in the xy plane.

maxcell Max number of pixels to load SpatRasterImage into memory. The default

1e7 is chosen because this is the approximate number of pixels in the medium resolution image at resolution = 4L in Xenium OME-TIFF to make different

methods of this function consistent.

... Ignored. It's there so different methods can all be passed to the same lapply in

the method for SFE objects. Some methods have extra arguments.

# Value

SpatRasterImage will be converted to ExtImage. Otherwise \*Image object of the same class. For BioFormatsImage, the transformation info is stored and will be applied when the image is loaded into memory as ExtImage.

#### See Also

Other image methods: SFE-image, cropImg(), dim, BioFormatsImage-method, ext(), imgRaster(), imgSource(), mirrorImg(), rotateImg(), scaleImg(), translateImg(), transposeImg()

aggBboxes

Aggregate bounding boxes

# **Description**

To find the bounding box of multiple bounding boxes.

```
aggBboxes(bboxes)
```

# **Arguments**

bboxes

Either a matrix with 4 rows whose columns are the different bounding boxes, with row names "xmin", "xmax", "ymin", and "ymax" in any order, or a list of bounding boxes which are named numeric vectors.

# Value

A named numeric vector for the total bounding box.

# **Examples**

```
bboxes <- list(c(xmin = 5, xmax = 10, ymin = 2, ymax = 20),
c(xmin = 8, xmax = 18, ymin = 0, ymax = 15))
bbox_all <- aggBboxes(bboxes)</pre>
```

```
aggregate, SpatialFeatureExperiment-method

Aggregate data in SFE using geometry
```

# Description

Gene expression and numeric columns of colData will be aggregated with the function specified in FUN, according to another geometry supplied and a geometry predicate (such as st\_intersects). For example, when the predicate is st\_intersects and a spatial grid is used to aggregate, then the data associated with all cells that intersect with each grid cell will be aggregated with FUN, such as mean or sum. The categorical columns will be collected into list columns, and logical columns will be converted into numeric before applying FUN.

```
## S4 method for signature 'SpatialFeatureExperiment'
aggregate(
    x,
    by = NULL,
    FUN = sum,
    sample_id = "all",
    colGeometryName = 1L,
    rowGeometryName = NULL,
    cellsize = NULL,
    square = TRUE,
    flat_topped = FALSE,
    new_geometry_name = "bins",
    join = st_intersects,
    BPPARAM = SerialParam()
)
```

#### **Arguments**

x An SFE object to be aggregated.

A sf data frame whose geometry column is used for aggregation or sfc or for

multiple samples a list of sfc whose names are the sample IDs. For multiple samples, the sf data frame must have a column sample\_id to indicate which geometry for which sample. This argument is optional if cellsize is specified.

FUN Function to aggregate the numerical columns in colData and the gene count

matrix. This can be sum, mean, or any function that takes a numeric matrix as input and returns a numeric vector whose length is same as the number of rows in the input matrix, such as rowMedians. See package matrixStats. Depending on the function used for aggregation, numeric columns of colData may need to be interpreted differently after aggregation. Aggregation is not done when aggregating by transcript spots in rowGeometry. When it's sum or mean, matrix multiplication is used for aggregation rather than calling the sum or mean function itself; this is much faster than looping through the bins and calling the function on each of them

function on each of them.

sample\_id Which samples to aggregate, defaults to "all".

colGeometryName

Which colGeometry to spatially aggregate the data, by default the first one.

rowGeometryName

Which rowGeometry to spatially aggregate

cellsize numeric of length 1 or 2 with target cellsize: for square or rectangular cells the

width and height, for hexagonal cells the distance between opposite edges (edge length is cellsize/sqrt(3)). A length units object can be passed, or an area unit

object with area size of the square or hexagonal cell.

square logical; if FALSE, create hexagonal grid

flat\_topped logical; if TRUE generate flat topped hexagons, else generate pointy topped

new\_geometry\_name

Name to give to the new colGeometry in the output. Defaults to "bins".

join logical spatial predicate function to use if by is a simple features object or ge-

ometry; see st\_join

BPPARAM A BiocParallelParam object specifying parallel computing when aggregating

data with functions other than sum and mean. Defaults to SerialParam().

#### **Details**

For smFISH-based data where the transcript spots are available, the transcript spots can be used instead of cells to aggregate the gene count matrix, in which case all assays other than counts will be dropped and FUN only applies to colData because the transcript spots are simply counted.

What this function does is similar to SEraster but more general because any geometry and more aggregation function can be used, not just regular grids, and the aggregation can be performed on the transcript spots.

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# Value

An SFE object with colGeometry the same as the geometry specified in by or same as the grid specified in cellsize. rowGeometries and rowData remain the same as in the input x. reducedDims, localResults, colFeatureData (and its colGeometry, annotGeometry, and reducedDim counterparts), and spatialGraphs are dropped because those results no longer apply after aggregation.

# Note

For developers: When debugging this function after calling devtools::load\_all("."), you may get an error that comes from S3 dispatch of aggregate.Vector from the S4Vectors package. When that happens, either restart the R session, or run setGeneric("aggregate", function(x, ...) standardGeneric("aggregate")) in the console to make an S4 generic as done in the terra package to prioritize S4 dispatch.

# **Examples**

```
# example code
```

aggregateTx

Aggregate transcript spots from file

# Description

This function reads the transcript spot file from the standard output of the commercial technologies (not GeoParquet) for spatial aggregation where the spots are assigned to polygons such as cells or spatial bins. Presets for Xenium, MERFISH, and CosMX are available. For Vizgen and Xenium, the images can be added when add\_images = TRUE.

```
aggregateTx(
  file,
  df = NULL,
  by = NULL,
  sample_id = "sample01",
  spatialCoordsNames = c("X", "Y", "Z"),
  gene_col = "gene",
  phred_col = "qv",
 min_phred = 20,
  flip_geometry = FALSE,
  cellsize = NULL,
  square = TRUE,
  flat_topped = FALSE,
  new_geometry_name = "bins",
  unit = "micron"
)
```

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```
aggregateTxTech(
  data_dir,
  df = NULL,
  by = NULL,
  tech = c("Vizgen", "Xenium", "CosMX"),
  sample_id = "sample01",
  image = NULL,
  min_phred = 20,
  flip = c("geometry", "image", "none"),
  max_flip = "50 MB",
  cellsize = NULL,
  square = TRUE,
  flat_topped = FALSE,
  new_geometry_name = "bins"
)
```

# Arguments

file File with the transcript spot coordinates. Should be one row per spot when read

into R and should have columns for coordinates on each axis, gene the transcript is assigned to, and optionally cell the transcript is assigned to. Must be csv, tsv,

or parquet.

df If the file is already loaded into memory, a data frame (sf) with columns for the

x, y, and optionally z coordinates and gene assignment of each transcript spot.

If specified, then argument file will be ignored.

by A sfc or sf object for spatial aggregation.

sample\_id Which sample in the SFE object the transcript spots should be added to.

spatialCoordsNames

Column names for the x, y, and optionally z coordinates of the spots. The de-

faults are for Vizgen.

gene\_col Column name for genes.

phred\_col Column name for Phred scores of the spots.

min\_phred Minimum Phred score to keep spot. By default 20, the conventional threshold

indicating "acceptable", meaning that there's 1 chance that the spot was decoded

in error.

flip\_geometry Logical, whether to flip the transcript spot geometries to match the images if

added later.

cellsize numeric of length 1 or 2 with target cellsize: for square or rectangular cells the

width and height, for hexagonal cells the distance between opposite edges (edge length is cellsize/sqrt(3)). A length units object can be passed, or an area unit

object with area size of the square or hexagonal cell.

square logical; if FALSE, create hexagonal grid

flat\_topped logical; if TRUE generate flat topped hexagons, else generate pointy topped

new\_geometry\_name

Name to give to the new colGeometry in the output. Defaults to "bins".

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unit	Unit the coordinates are in, either microns or pixels in full resolution image.
data_dir	Top level output directory.
tech	Which technology whose output to read, must be one of "Vizgen", "Xenium", or "CosMX" though more technologies may be added later.
image	String, which image(s) to add to the output SFE object. Not applicable to CosMX. See readVizgen and readXenium for options and multiple images can be specified. If NULL, then the default from the read function for the technology will be used.
flip	Logical, whether to flip the geometry to match image. Here the y coordinates are simply set to -y, so the original bounding box is not preserved. This is consistent with readVizgen and readXenium.
max_flip	Maximum size of the image allowed to flip the image. Because the image will be loaded into memory to be flipped. If the image is larger than this size then the coordinates will be flipped instead.

#### Value

A SFE object with count matrix for number of spots of each gene in each geometry. Geometries with no spot are removed.

# Note

The resulting SFE object often includes geometries (e.g. grid cells) outside tissue, because there can be transcript spots detected outside the tissue. Also, bins at the edge of the tissue that don't fully overlap with the tissue will have lower transcript counts; this may have implications to downstream spatial analyses.

ion geometry methods
----------------------

# Description

"Annotation geometry" refers to Simple Feature (sf) geometries NOT associated with rows (features, genes) or columns (cells or spots) of the gene count matrix in the SpatialFeatureExperiment object. So there can be any number of rows in the sf data frame specifying the geometry. Examples of such geometries are tissue boundaries, pathologist annotation of histological regions, and objects not characterized by columns of the gene count matrix (e.g. nuclei segmentation in a Visium dataset where the columns are Visium spots). This page documents getters and setters for the annotation geometries. Internally, annotation geometries are stored in int\_metadata.

```
## S4 method for signature 'SpatialFeatureExperiment'
annotGeometries(x)
## S4 replacement method for signature 'SpatialFeatureExperiment'
```

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```
annotGeometries(x, translate = TRUE, ...) <- value

## S4 method for signature 'SpatialFeatureExperiment'
annotGeometryNames(x)

## S4 replacement method for signature 'SpatialFeatureExperiment, character'
annotGeometryNames(x) <- value

## S4 method for signature 'SpatialFeatureExperiment'
annotGeometry(x, type = 1L, sample_id = NULL)

## S4 replacement method for signature 'SpatialFeatureExperiment'
annotGeometry(x, type = 1L, sample_id = NULL, translate = TRUE, ...) <- value

tissueBoundary(x, sample_id = 1L)

tissueBoundary(x, sample_id = 1L, translate = TRUE, ...) <- value</pre>
```

# **Arguments**

X	A Spatia.	lFeatureExpe	riment object.

translate Logical. Only used if removeEmptySpace has been run of the SFE object. If that's the case, this argument indicates whether the new value to be assigned to

the geometry is in the coordinates prior to removal of empty space so it should be translated to match the new coordinates after removing empty space. Default

to TRUE.

... spatialCoordsNames, spotDiameter, geometryType passed to df2sf. De-

faults are the same as in df2sf. For dimGeometries<- only: geometryType can be a character vector of the geometry type of each data frame in the list of the same length as the list if the data frames specify different types of geome-

tries.

value Value to set. For annotGeometry, must be a sf data frame, or an ordinary data

frame that can be converted to a sf data frame (see df2sf). For annotGeometries, must be a list of such sf or ordinary data frames. There must be a column sample\_id to indicate the sample the geometries are for, and the sample\_id

must also appear in colData.

type An integer specifying the index or string specifying the name of the \*Geometry

to query or replace. If missing, then the first item in the \*Geometries will be

returned or replaced.

sample\_id Sample ID to get or set geometries.

# **Details**

Wrapper for getter and setter of special geometry:

**tisseuBoundary** Boundary of the tissue of interest, including holes. This is usually of geometry type MULTIPOLYGON, though geometries in annotGeometries can have any type supported by sf.

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# Value

Getters for multiple geometries return a named list. Getters for names return a character vector of the names. Getters for single geometries return an sf data frame. Setters return an SFE object.

# **Examples**

```
# Example dataset
library(SFEData)
sfe_small <- McKellarMuscleData(dataset = "small")</pre>
# Get all annotation geometries, returning a named list
annotGeometries(sfe_small)
# Set all annotation geometries, in a named list
toy <- readRDS(system.file("extdata/sfe_toy.rds",</pre>
    package = "SpatialFeatureExperiment"
))
ag <- readRDS(system.file("extdata/ag.rds",</pre>
    package = "SpatialFeatureExperiment"
))
annotGeometries(toy) <- list(hull = ag)</pre>
# Get names of annotation geometries
annotGeometryNames(sfe_small)
# Set names of annotation geometries
annotGeometryNames(toy) <- "foo"</pre>
# Get a specific annotation geometry by name
# sample_id is optional when there is only one sample present
nuclei <- annotGeometry(sfe_small, type = "nuclei", sample_id = "Vis5A")</pre>
# Get a specific annotation geometry by index
tb <- annotGeometry(sfe_small, type = 1L)</pre>
# Set a specific annotation geometry
annotGeometry(sfe_small, type = "nuclei2") <- nuclei</pre>
# Special convenience function for tissue boundaries
# Getter
tb <- tissueBoundary(sfe_small, sample_id = "Vis5A")</pre>
tissueBoundary(sfe_small, sample_id = "Vis5A") <- tb
```

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# **Description**

Just like annotPred, but performs the operation rather than predicate. For example, this function would return the geometry of the intersections between each Visium spot and the tissue boundary for each sample, rather than whether each Visium spot intersects the tissue boundary. In case one cell/spot gets broken up into multiple geometries, the union of those geometries will be taken, so each cell/spot will only get one geometry.

# Usage

```
annotOp(
   sfe,
   colGeometryName = 1L,
   annotGeometryName = 1L,
   sample_id = "all",
   op = st_intersection
)
```

# **Arguments**

```
sfe An SFE object.

colGeometryName

Name of column geometry for the predicate.

annotGeometryName

Name of annotation geometry for the predicate.

sample_id Which sample(s) to operate on. Can be "all" to indicate all samples.

op A binary operation function for the geometries. Defaults to st_intersection.
```

# Value

A sf data frame with geometry column containing the geometries and corresponding column names of sfe as row names. There is no guarantee that the returned geometries are valid or preserve the geometry class (e.g. when the intersection of polygons result into a line of a point).

#### See Also

annotPred

# **Examples**

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annotPred

Binary predicates for geometry of each cell/spot and annotation

# **Description**

This function finds binary predicates for the geometry of each cell/spot (i.e. colGeometry) and an annotation geometry for each sample. For example, whether each Visium spot intersects with the tissue boundary in each sample.

# Usage

```
annotPred(
   sfe,
   colGeometryName = 1L,
   annotGeometryName = 1L,
   sample_id = "all",
   pred = st_intersects
)

annotNPred(
   sfe,
   colGeometryName = 1L,
   annotGeometryName = 1L,
   sample_id = "all",
   pred = st_intersects
)
```

#### **Arguments**

```
sfe An SFE object.

colGeometryName
Name of column geometry for the predicate.

annotGeometryName
Name of annotation geometry for the predicate.

sample_id Which sample(s) to operate on. Can be "all" to indicate all samples.

pred Predicate function to use, defaults to st_intersects.
```

# Value

For annotPred, a logical vector of the same length as the number of columns in the sample(s) of interest, with barcodes (or corresponding column names of sfe) as names. For annotNPred, a numeric vector of the same length as the number of columns in the sample(s) of interest with barcodes as names, indicating the number of geometries in the annotGeometry of interest returns TRUE for the predicate for each each geometry in the colGeometry of interest.

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#### See Also

annotOp

# **Examples**

```
library(SFEData)
sfe <- McKellarMuscleData("small")
# Whether each spot is in tissue
in_tissue <- annotPred(sfe, "spotPoly", annotGeometryName = "tissueBoundary")
# How many nuclei are there in each Visium spot
n_nuclei <- annotNPred(sfe, "spotPoly", annotGeometryName = "nuclei")</pre>
```

annotSummary

Summarize attributes of an annotGeometry for each cell/spot

# **Description**

In SFE objects, the annotation geometries don't have to correspond to the dimensions of the gene count matrix, so there generally is no one to one mapping between annotation geometries and cells/spots. However, it may be interesting to relate attributes of annotation geometries to cell/spots so the attributes can be related to gene expression. This function summarizes attributes of an annotGeometry for each cell/spot by a geometric predicate with a colGeometry.

# Usage

```
annotSummary(
   sfe,
   colGeometryName = 1L,
   annotGeometryName = 1L,
   annotColNames = 1L,
   sample_id = "all",
   pred = st_intersects,
   summary_fun = mean
)
```

# **Arguments**

sfe An SFE object. colGeometryName

Name of column geometry for the predicate.

annotGeometryName

Name of annotation geometry for the predicate.

annotColNames Character, column names of the annotGeometry of interest, to indicate the

columns to summarize. Columns that are absent from the annotGeometry are

removed. The column cannot be "geometry" or "barcode".

sample\_id Which sample(s) to operate on. Can be "all" to indicate all samples.

pred Predicate function to use, defaults to st\_intersects.

summary\_fun Function for the summary, defaults to mean.

#### Value

A data frame whose row names are the relevant column names of sfe, and each column of which is the summary of each column specified in annotColName.

# **Examples**

bbox, SpatialFeatureExperiment-method

Find bounding box of SFE objects

# **Description**

Find bounding box of the union of all colGeometries and annotGeometries of each sample in the SFE object. This can be used to remove empty space so the tissue and geometries have one corner at the origin so all samples will be on comparable coordinates.

#### Usage

```
## S4 method for signature 'SpatialFeatureExperiment'
bbox(sfe, sample_id = "all", include_images = FALSE, include_row = TRUE)
```

# **Arguments**

Sample\_id Sample(s) whose bounding box(es) to find. The bounding box would be for the union of all colGeometries and annotGeometries associated with each sample.

include\_images Logical, whether the bounding boxes should include image extents. Defaults to FALSE because often the image has a lot of empty space surrounding the tissue.

include\_row Logical, whether the bounding boxes should include rowGeometries, defaults to TRUE.

# Value

For one sample, then a named vector with names xmin, ymin, xmax, and ymax specifying the bounding box. For multiple samples, then a matrix whose columns are samples and whose rows delineate the bounding box.

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# **Examples**

```
library(SFEData)
sfe <- McKellarMuscleData("small")
bbox(sfe, sample_id = "Vis5A")</pre>
```

bbox\_center

Find center of bounding box

# **Description**

Get x-y coordinates of the center of any bounding box

# Usage

```
bbox_center(bbox)
```

# **Arguments**

bbox

A numeric vector of length 4 with names xmin, xmax, ymin, ymax, in any order.

#### Value

A numeric vector of length 2.

# **Examples**

```
bbox <- c(xmin = 0, xmax = 100, ymin = 0, ymax = 80)
bbox_center(bbox)
```

BioFormatsImage

On disk representation of BioFormats images in SFE object

# Description

'r lifecycle::badge("experimental")' At present, the BioFormatsImage is designed for OME-TIFF from Xenium and has not been tested on other formats that can be read with BioFormats. The image is not loaded into memory, and when it is, the the BioFormatsImage object is converted into ExtImage because the loaded image is of a class that inherits from Image. The ExtImage class is a thin wrapper inheriting from VirtualSpatialImage so it's compatible with SpatialExperiment from which SFE is derived. This class might drastically change as it matures, say to accommodate other formats supported by BioFormats and to store the transformation matrix rather than loading image into memory upon transform.

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# Usage

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

BioFormatsImage(
  path,
  ext = NULL,
  isFull = TRUE,
  origin = c(0, 0),
  transformation = list()
)
```

#### **Arguments**

object A BioFormatsImage object.

path Path to an OME-TIFF image file.

ext Numeric vector with names "xmin", "xmax", "ymin", "ymax" in microns indi-

cating the spatial extent covered by the image. If NULL, then the extent will be inferred from the metadata, from physical pixel size and the number of pixels.

isFull Logical, if the extent specified in ext is the full extent. If ext = NULL so it will

be inferred from metadata then isFull = TRUE will be set internally.

origin Origin of the whole image in the x-y plane, defaults to c(0,0). This is shifted

when the image is translated. This is not the same as xmin and xmax. For example, when the extent is only part of the whole image and the whole image itself can be spatially translated, the origin is needed to determine which part of

the whole image this extent corresponds to.

transformation Named list specifying affine transformation. The list can have names "name"

and named parameter of the transformation, e.g. list(name = "mirror", direction = "vertical"), "rotate" and degrees = 90 (clockwise), and "scale" and factor = 2. The list can also have names "M" for a 2x2 linear transformation matrix in the xy plane and "v" for a translation vector of length 2 to specify general affine

transformation.

#### **Details**

Spatial extent is inferred from OME-TIFF metadata if not specified. Physical pixel size from the metadata is used to make the extent in micron space. If physical pixel size is absent from metadata, then the extent will be in pixel space, which might mean that the image will not align with the geometries because often the geometry coordinates are in microns, so a warning is issued in this case.

Affine transformations can be specified in the transformation argument, either by name or by directly specifying the matrix. The transformations specified by name will always preserve the center of the image. When named transformations are chained, name and parameter will be converted to matrix and translation vector the second time a transformation is specified. If the subsequent transformation happens to restore the image to its original place, then transformation specifications will be removed.

# Value

A BioFormatsImage object.

#### See Also

```
[isFull()], [origin()]
```

BioFormatsImage-getters

Other BioFormatsImage getters

# Description

isFULL indicates if the extent is the full extent of the image. origin gets the x-y coordinates of the origin of the image, i.e. the smallest possible x-y coordinate values within the full image.

# Usage

```
## S4 method for signature 'BioFormatsImage'
isFull(x)

## S4 method for signature 'BioFormatsImage'
origin(x)

## S4 method for signature 'BioFormatsImage'
transformation(x)
```

# **Arguments**

Х

A BioFormatsImage object.

# Value

For isFull: Logical scalar indicating whether the extent is the full extent. For origin: Numeric vector of length 2. For transformation, a list.

20 changeSampleIDs

cbind,SpatialFeatureExperiment-method

Concatenate SpatialFeatureExperiment objects

# **Description**

On top of the cbind method of SpatialExperiment, this method is needed to properly merge the spatialGraphs field in the different SFE objects. rowGeometries and annotGeometries also need to be combined properly.

# Usage

```
## S4 method for signature 'SpatialFeatureExperiment'
cbind(..., deparse.level = 1)
```

# **Arguments**

```
... SFE objects to cbind. departs e.level See ?rbind.
```

# Value

A combined SFE object.

# **Examples**

```
library(SFEData)
sfe_small <- McKellarMuscleData(dataset = "small")
sfe_small2 <- McKellarMuscleData(dataset = "small2")
sfe2 <- cbind(sfe_small, sfe_small2)</pre>
```

changeSampleIDs

Change sample IDs

# **Description**

Change sample IDs in all fields of the SFE object where sample IDs are present, not just the colData.

# Usage

```
changeSampleIDs(sfe, replacement)
```

# **Arguments**

sfe A SpatialFeatureExperiment object.

replacement A named character vector whose names are the existing sample IDs to be changed

and whose values are the corresponding replacements.

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# Value

An SFE object.

# **Examples**

```
library(SFEData)
sfe <- McKellarMuscleData(dataset = "small")
sfe <- changeSampleIDs(sfe, c(Vis5A = "sample01"))
sampleIDs(sfe)</pre>
```

colFeatureData

Get global spatial analysis results and metadata of colData, rowData, and geometries

# **Description**

Results of spatial analyses on columns in colData, rowData, and geometries are stored in their metadata. The colFeaturedata function allows the users to more directly access these results.

# Usage

```
colFeatureData(sfe)
rowFeatureData(sfe)
geometryFeatureData(sfe, type, MARGIN = 2L)
reducedDimFeatureData(sfe, dimred)
```

#### **Arguments**

sfe An SFE object.

type Which geometry, can be name (character) or index (integer)

MARGIN Integer, 1 means rowGeometry, 2 means colGeometry, and 3 means annotGe-

ometry. Defaults to 2, colGeometry.

dimred Name of a dimension reduction, can be seen in reducedDimNames.

# Value

A DataFrame.

#### See Also

getParams

22 colGeometries

# **Examples**

```
library(SpatialFeatureExperiment)
library(SingleCellExperiment)
library(SFEData)
library(Voyager)
sfe <- McKellarMuscleData("small")
colGraph(sfe, "visium") <- findVisiumGraph(sfe)
# Moran's I for colData
sfe <- colDataMoransI(sfe, "nCounts")
colFeatureData(sfe)</pre>
```

colGeometries

Column geometry getters and setters

# **Description**

colGeometries are geometries that correspond to columns of the gene count matrix, such as Visium spots or cells. Same as dimGeometry(x,MARGIN = 2L, ...), with convenience wrappers for getters and setters of special geometries:

spotPoly Polygons of spots from technologies such as Visium, ST, and slide-seq, which do not correspond to cells. Centroids of the polygons are stored in spatialCoords of the underlying SpatialExperiment object.

**ROIPoly** Polygons of regions of interest (ROIs) from technologies such as laser capture microdissection (LCM) and GeoMX DSP. These should correspond to columns of the gene count matrix.

**cellSeg** Cell segmentation polygons. If the columns of the gene count matrix are single cells, then this is stored in colGeometries. Otherwise, this is stored in annotGeometries.

**nucSeg** Similar to cellSeg, but for nuclei rather than whole cell.

```
colGeometry(x, type = 1L, sample_id = 1L, withDimnames = TRUE)

colGeometry(
    x,
    type = 1L,
    sample_id = 1L,
    withDimnames = TRUE,
    translate = TRUE
) <- value

colGeometries(x, withDimnames = TRUE)

colGeometries(x, withDimnames = TRUE, translate = TRUE) <- value</pre>
```

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```
colGeometryNames(x)
colGeometryNames(x) <- value
spotPoly(x, sample_id = 1L, withDimnames = TRUE)
spotPoly(x, sample_id = 1L, withDimnames = TRUE, translate = TRUE) <- value
centroids(x, sample_id = 1L, withDimnames = TRUE)
centroids(x, sample_id = 1L, withDimnames = TRUE, translate = TRUE) <- value
ROIPoly(x, sample_id = 1L, withDimnames = TRUE)
ROIPoly(x, sample_id = 1L, withDimnames = TRUE, translate = TRUE) <- value
cellSeg(x, sample_id = 1L, withDimnames = TRUE)
cellSeg(x, sample_id = 1L, withDimnames = TRUE, translate = TRUE) <- value
nucSeg(x, sample_id = 1L, withDimnames = TRUE)
nucSeg(x, sample_id = 1L, withDimnames = TRUE, translate = TRUE) <- value</pre>
```

#### **Arguments**

x A SpatialFeatureExperiment object.

type An integer specifying the index or string specifying the name of the \*Geometry

to query or replace. If missing, then the first item in the \*Geometries will be

returned or replaced.

sample\_id Sample ID to get or set geometries.

withDimnames Logical. If TRUE, then the dimnames (colnames or rownames) of the gene count

matrix should correspond to row names of the sf data frames of interest.

translate Logical. Only used if removeEmptySpace has been run of the SFE object. If

that's the case, this argument indicates whether the new value to be assigned to the geometry is in the coordinates prior to removal of empty space so it should be translated to match the new coordinates after removing empty space. Default

to TRUE.

value Value to set. For dimGeometry, must be a sf data frame with the same number

of rows as size in the dimension of interest, or an ordinary data frame that can be converted to such a sf data frame (see df2sf). For dimGeometries, must be

a list of such sf or ordinary data frames.

#### See Also

[dimGeometries()], [rowGeometries()]

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# **Examples**

```
library(SFEData)
sfe <- McKellarMuscleData(dataset = "small")
cgs <- colGeometries(sfe)
spots <- spotPoly(sfe)</pre>
```

crop

Crop an SFE object with a geometry

# **Description**

Returns an SFE object whose specified colGeometry returns TRUE with a geometric predicate function (usually intersects) with another geometry of interest. This can be used to subset an SFE object with a tissue boundary or histological region polygon, or crop away empty spaces. After cropping, not only will the cells/spots be subsetted, but also all geometries will be cropped.

# Usage

```
crop(
   x,
   y = NULL,
   colGeometryName = 1L,
   sample_id = "all",
   op = st_intersection,
   keep_whole = "none",
   cover = FALSE
)
```

# Arguments

у

x An SFE object.

An object of class sf, sfg, sfc with which to crop the SFE object, or a bounding box with the format of the output of bbox, SpatialFeatureExperiment-method.

colGeometryName

Column geometry to used to indicate which cells/spots to keep.

sample\_id

Samples to crop. Optional when only one sample is present. Can be multiple samples, or "all", which means all samples. For multiple samples, sf data frame y may have column sample\_id indicating which geometry subsets which sample or matrix y may indicate sample specific bounding boxes in its column names. Only samples included in the indicated sample IDs are subsetted. If sample is not indicated in y, then the same geometry or bounding box is used to subset all samples specified in the sample\_id argument.

ор

A geometric operation function to crop the geometries in the SFE object. Only st\_intersection and st\_difference are allowed. If "intersection", then only things inside y is kept after cropping. If "difference", then only things outside y is kept.

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keep\_whole Character vector, can be one or more of "col" and "annot" to keep whole items

from colGeometries or annotGeometries, keeping geometries that partially intersect with y whole. This can greatly speed up code while not breaking geometries into multiple pieces. Can also be "none" so all geometries are actually

cropped.

cover Logical, whether the geometries in x must be entirely covered by y if op =

st\_intersection or whether x must be entirely outside y if op = st\_difference.

Only relevant when keep\_whole != "none".

#### **Details**

3D geometries are allowed, but geometric operations can only be performed in x and y but not z.

#### Value

An SFE object. There is no guarantee that the geometries after cropping are still all valid or preserve the original geometry class.

# **Examples**

```
library(SFEData)
sfe <- McKellarMuscleData("small")
# Subset sfe to only keep spots on tissue
sfe_on_tissue <- crop(sfe, tissueBoundary(sfe),
        colGeometryName = "spotPoly",
        sample_id = "Vis5A"
)</pre>
```

cropImg

Crop images

# **Description**

Crop images of class \*Image in this package with a bounding box.

```
## S4 method for signature 'SpatRasterImage'
cropImg(x, bbox, filename = "")
## S4 method for signature 'BioFormatsImage'
cropImg(x, bbox)
## S4 method for signature 'ExtImage'
cropImg(x, bbox)
```

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# **Arguments**

x An object of class \*Image as implemented in this package.

bbox Numeric vector with names "xmin", "xmax", "ymin", "ymax", in any order, to

specify the bounding box.

filename Output file name for transformed SpatRaster.

#### Value

Image of the same class as input but cropped. For BioFormatsImage, the image is not loaded into memory; only the extent is changed.

#### See Also

```
Other image methods: SFE-image, affineImg(), dim, BioFormatsImage-method, ext(), imgRaster(), imgSource(), mirrorImg(), rotateImg(), scaleImg(), translateImg(), transposeImg()
```

df2sf

From ordinary data frame to sf to construct SFE object

# **Description**

While the SpatialFeatureExperiment constructor and \*Geometry replacement methods can convert properly formatted ordinary data frames into sf objects which are used to store the geometries internally, the user might want to do the conversion, check if the geometry is valid, and inspect and fix any invalid geometries.

# Usage

# **Arguments**

An ordinary data frame, i.e. not sf. Or a matrix that can be converted to a data frame.

spatialCoordsNames

Column names in df that specify spatial coordinates.

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spotDiameter Spot diameter for technologies with arrays of spots of fixed diameter per slide, such as Visium, ST, DBiT-seq, and slide-seq. The diameter must be in the same unit as the coordinates in the \*Geometry arguments. Ignored for geometries that are not POINT or MULTIPOINT. Type of geometry to convert the ordinary data frame to. If the geometry in df is geometryType de facto points, then this argument will be ignored and the returned sf will have geometry type POINT. Column to indicate which coordinates for which MULTI geometry, such as to group\_col identify which MULTIPOLYGON or MULTIPOINT. id\_col Column to indicate coordinates for which geometry, within a MULTI geometry if applicable, such as to identify which POLYGON or which polygon within a MULTIPOLYGON. subid\_col Column to indicate coordinates for holes in polygons. check Logical, whether to check the input data frame for issues related to constructing the geometry of interese such as number of vertices per geometry. If FALSE, it will save a bit of time, which is useful when the input is already known to be good. Other arguments passed to 'sf::st\_buffer', mainly to make polygon shapes, eg Visium spot 'endCapStyle = "ROUND" and VisiumHD bin 'endCapStyle = "SQUARE"

#### Value

An sf object.

# **Examples**

```
# Points, use spotDiameter to convert to circle polygons
# This is done to Visium spots
pts_df <- readRDS(system.file("extdata/pts_df.rds",</pre>
    package = "SpatialFeatureExperiment"
sf_use <- df2sf(pts_df, geometryType = "POINT", spotDiameter = 0.1)</pre>
# Linestring
ls_df <- readRDS(system.file("extdata/ls_df.rds",</pre>
    package = "SpatialFeatureExperiment"
))
sf_use <- df2sf(ls_df, geometryType = "LINESTRING")</pre>
# Polygon
pol_df <- readRDS(system.file("extdata/pol_df.rds",</pre>
    package = "SpatialFeatureExperiment"
))
sf_use <- df2sf(pol_df,
    geometryType = "POLYGON",
    spatialCoordsNames = c("V1", "V2")
# Multipolygon
mpol_df <- readRDS(system.file("extdata/mpol_df.rds",</pre>
    package = "SpatialFeatureExperiment"
```

```
))
sf_use <- df2sf(mpol_df,
        geometryType = "MULTIPOLYGON",
        spatialCoordsNames = c("V1", "V2")
)
# Multiple sample_ids present
multipts_df <- readRDS(system.file("extdata/multipts_df.rds",
        package = "SpatialFeatureExperiment"
))
sf_use <- df2sf(multipts_df, geometryType = "MULTIPOINT")</pre>
```

dim,BioFormatsImage-method

Find dimension of BioFormatsImage

# **Description**

This is different from other classes. The metadata is read where the dimensions in pixels can be found. The image itself is not read into memory here.

# Usage

```
## S4 method for signature 'BioFormatsImage'
dim(x)
```

# Arguments

Х

A BioFormatsImage object.

#### Value

An integer vector of length 5 showing the number of rows and columns in the full resolution image. The 5 dimensions are in the order of XYCZT: x, y, channel, z, and time. This is not changed by transformations. Use ext to see the extent after transformation.

# See Also

```
Other image methods: SFE-image, affineImg(), cropImg(), ext(), imgRaster(), imgSource(), mirrorImg(), rotateImg(), scaleImg(), translateImg(), transposeImg()
```

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dimGeometries

Dimension geometry methods

#### **Description**

"Dimension geometry" refers to Simple Feature (sf) geometries associated with rows (features, genes) or columns (cells or spots) of the gene count matrix in the SpatialFeatureExperiment object. For each dimension, the number of rows in the sf data frame specifying the geometries must match the size of the dimension of interest. For example, there must be the same number of rows in the sf data frame describing cells as there are cells in the gene count matrix. This page documents getters and setters for the dimension geometries. The getters and setters are implemented in a way similar to those of reducedDims in SingleCellExperiment.

# Usage

```
## S4 method for signature 'SpatialFeatureExperiment'
dimGeometries(x, MARGIN = 2, withDimnames = TRUE)
## S4 replacement method for signature 'SpatialFeatureExperiment'
dimGeometries(x, MARGIN, withDimnames = TRUE, translate = TRUE, ...) <- value</pre>
## S4 method for signature 'SpatialFeatureExperiment'
dimGeometryNames(x, MARGIN)
## S4 replacement method for signature 'SpatialFeatureExperiment,numeric,character'
dimGeometryNames(x, MARGIN) <- value</pre>
## S4 method for signature 'SpatialFeatureExperiment'
dimGeometry(x, type = 1L, MARGIN, sample_id = 1L, withDimnames = TRUE)
## S4 replacement method for signature 'SpatialFeatureExperiment'
dimGeometry(
  Х,
  type = 1L,
 MARGIN,
  sample_id = 1L,
 withDimnames = TRUE,
  translate = TRUE,
) <- value
```

# **Arguments**

x A SpatialFeatureExperiment object.

MARGIN As in apply. 1 stands for rows and 2 stands for columns.

matrix should correspond to row names of the sf data frames of interest.

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translate Logical. Only used if removeEmptySpace has been run of the SFE object. If that's the case, this argument indicates whether the new value to be assigned to the geometry is in the coordinates prior to removal of empty space so it should be translated to match the new coordinates after removing empty space. Default to TRUE. spatialCoordsNames, spotDiameter, geometryType passed to df2sf. Defaults are the same as in df2sf. For dimGeometries<- only: geometryType can be a character vector of the geometry type of each data frame in the list of the same length as the list if the data frames specify different types of geometries. value Value to set. For dimGeometry, must be a sf data frame with the same number of rows as size in the dimension of interest, or an ordinary data frame that can be converted to such a sf data frame (see df2sf). For dimGeometries, must be a list of such sf or ordinary data frames. An integer specifying the index or string specifying the name of the \*Geometry type to query or replace. If missing, then the first item in the \*Geometries will be

returned or replaced.

Sample ID to get or set geometries.

#### Value

sample\_id

Getters for multiple geometries return a named list. Getters for names return a character vector of the names. Getters for single geometries return an sf data frame. Setters return an SFE object.

#### See Also

```
[colGeometries()], [rowGeometries()]
```

# Examples

```
library(SFEData)
sfe <- McKellarMuscleData(dataset = "small")</pre>
# Get all column geometries as a named list
# Use MARGIN = 1 or rowGeometry/ies for rowGeometries
cgs <- dimGeometries(sfe, MARGIN = 2)</pre>
# Or equivalently
cgs <- colGeometries(sfe)</pre>
# Set all column geometries with a named list
dimGeometries(sfe, MARGIN = 2) <- cgs</pre>
# Or equivalently
colGeometries(sfe) <- cgs</pre>
# Get names of column geometries
cgns <- dimGeometryNames(sfe, MARGIN = 2)</pre>
cgns <- colGeometryNames(sfe)</pre>
# Set column geometry names
dimGeometryNames(sfe, MARGIN = 2) <- cgns</pre>
```

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```
colGeometryNames(sfe) <- cgns</pre>
# Get a specific column geometry by name
spots <- dimGeometry(sfe, "spotPoly", MARGIN = 2)</pre>
spots <- colGeometry(sfe, "spotPoly")</pre>
# Or equivalently, the wrapper specifically for Visium spot polygons,
# for the name "spotPoly"
spots <- spotPoly(sfe)</pre>
# Other colGeometry wrappers for specific names:
# ROIPoly (for LCM and GeoMX DSP), cellSeg and nucSeg (for MERFISH; would
# query annotGeometries for Visium)
# rowGeometry wrappers for specific names: txSpots (MERFISH transcript spots)
# By index
spots <- colGeometry(sfe, 1L)</pre>
# Multiple samples, only get geometries for one sample
sfe2 <- McKellarMuscleData("small2")</pre>
sfe_combined <- cbind(sfe, sfe2)</pre>
spots1 <- colGeometry(sfe, "spotPoly", sample_id = "Vis5A")</pre>
spots2 <- spotPoly(sfe_combined, sample_id = "sample02")</pre>
# Get geometries for multiple samples
spots3 <- spotPoly(sfe_combined, sample_id = c("Vis5A", "sample02"))</pre>
# All samples
spots3 <- spotPoly(sfe_combined, sample_id = "all")</pre>
# Set specific column geometry by name
colGeometry(sfe, "foobar") <- spots</pre>
# Or use wrapper
spotPoly(sfe) <- spots</pre>
# Specify sample_id
colGeometry(sfe_combined, "foobar", sample_id = "Vis5A") <- spots1</pre>
# Only entries for the specified sample are set.
foobar <- colGeometry(sfe_combined, "foobar", sample_id = "sample02")</pre>
```

ext

Get and set extent of image objects

# **Description**

Unlike in SpatialExperiment, images in SFE have extents which are used to align them to the geometries and in geometric operations on SFE objects. These functions get or set the extent for S4 image classes inheriting from VirtualSpatialImage implemented in the SFE package.

```
## S4 method for signature 'BioFormatsImage'
ext(x)

## S4 method for signature 'ExtImage'
ext(x)
```

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```
## S4 method for signature 'SpatRasterImage'
ext(x)

## S4 replacement method for signature 'BioFormatsImage,numeric'
ext(x) <- value

## S4 replacement method for signature 'ExtImage,numeric'
ext(x) <- value

## S4 replacement method for signature 'SpatRasterImage,numeric'
ext(x) <- value</pre>
```

# **Arguments**

x A \*Image object.

value A numeric vector with names "xmin", "xmax", "ymin", "ymax" specifying the

extent to use.

#### Value

Getters return a numeric vector specifying the extent. Setters return a \*Image object of the same class as the input.

#### Note

For SpatRasterImage, the image may be may not be loaded into memory. You can check if the image is loaded into memory with terra::inMemory(imgRaster(x)), and check the original file path with imgSource. If the image is not loaded into memory, then the original file must be present at the path indicated by imgSource in order for any code using the image to work, which includes this function ext.

For BioFormatsImage, internally only the pre-transform extent is stored. The ext getter will apply the transformation on the fly. The setter sets the pre-transformation extent.

# See Also

```
Other image methods: SFE-image, affineImg(), cropImg(), dim,BioFormatsImage-method, imgRaster(),imgSource(),mirrorImg(),rotateImg(),scaleImg(),translateImg(),transposeImg()
```

ExtImage

Use the EBImage Image class in SFE objects

# **Description**

This is a thin wrapper around the Image class in the EBImage package so it inherits from VirtualSpatialImage to be compatible with SpatialExperiment from which SFE inherits. An ext field is added to specify the spatial extent of the image in microns to facilitate geometric operations on the SFE object (including the images) and plotting with Voyager.

# Usage

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

ExtImage(img, ext = NULL)
```

# **Arguments**

object An ExtImage object.

img An Image object or anything that inherits from Image such as AnnotatedImage

in RBioFormats.

ext Numeric vector with names "xmin", "xmax", "ymin", "ymax" in microns indi-

cating the spatial extent covered by the image. If NULL, then the extent will be inferred from the metadata, from physical pixel size and the number of pixels.

#### Value

An ExtImage object.

# Description

This function wraps all spatial neighborhood graphs implemented in the package spdep for the SpatialFeatureExperiment (SFE) class, to find spatial neighborhood graphs for the entities represented by columns or rows of the gene count matrix in the SFE object or spatial entities in the annotGeometries field of the SFE object. Results are stored as listwobjects in the spatialGraphs field of the SFE object, as listw is used in many methods that facilitate the spatial neighborhood graph in the spdep, spatialreg, and adespatial. The edge weights of the graph in the listwobject are by default style W (see nb2listw) and the unweighted neighbor list is in the neighbours field of the listwobject.

```
style = c("raw", "W", "B", "C", "U", "minmax", "S"),
nn_method = c("bioc", "spdep"),
alpha = 1,
dmax = NULL,
BPPARAM = SerialParam(),
BNPARAM = KmknnParam(),
zero.policy = TRUE,
...
)
```

# Arguments

Х	A SpatialFeatureExperiment object.
sample_id	Which sample(s) in the SFE object to use for the graph. Can also be "all", which means this function will compute the graph for all samples independently.
type	Name of the geometry associated with the MARGIN of interest for which to compute the graph.
MARGIN	Just like in apply, where 1 stands for row, 2 stands for column. Here, in addition, 3 stands for annotation, to query the annotGeometries, such as nuclei segmentation in a Visium data
method	Name of function in the package spdep to use to find the spatial neighborhood graph.
dist_type	Type of distance-based weight. "none" means not using distance-based weights; the edge weights of the spatial neighborhood graph will be entirely determined by the style argument. "idw" means inverse distance weighting. "exp" means exponential decay. "dpd" means double-power distance weights. See nb2listwdist for details.
glist	list of general weights corresponding to neighbours
style	style can take values "W", "B", "C", "U", "minmax" and "S"
nn_method	Method to find k nearest neighbors and distance based neighbors. Can be either "bioc" or "spdep". For "bioc", methods from BiocNeighbors are used. For "spdep", methods from the spdep package are used. The "bioc" option is more scalable to larger datasets and supports multithreading.
alpha	Only relevant when dist_type = "dpd".
dmax	Only relevant when dist_type = "dpd".
BPPARAM	A BiocParallelParam object for multithreading. Only used for k nearest neighbor and distance based neighbor with nn_method = "bioc".
BNPARAM	A BiocNeighborParam object specifying the algorithm to find k nearest neighbors and distance based neighbors with nn_method = "bioc". For distance based neighbors, only KmknnParam and VptreeParam are applicable.
zero.policy	default NULL, use global option value; if FALSE stop with error for any empty neighbour sets, if TRUE permit the weights list to be formed with zero-length weights vectors
•••	Extra arguments passed to the spdep function stated in the method argument, such as k, use_kd_tree, d1, d2, nnmult, sym, and quadsegs. Note that any arguments about using longitude and latitude, which are irrelevant, are ignored.

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#### Value

For one sample, then a listw object representing the graph, with an attribute "method" recording the function used to build the graph, its arguments, and information about the geometry for which the graph was built. The attribute is used to reconstruct the graphs when the SFE object is subsetted since some nodes in the graph will no longer be present. If sample\_id = "all" or has length > 1, then a named list of listw objects, whose names are the sample\_ids. To add the list for multiple samples to a SFE object, specify the name argument in the spatialGraphs replacement method, so graph of the same name will be added to the SFE object for each sample.

#### Note

style = "raw" is only applicable when dist\_type is not "none". If dist\_type = "none" and style = "raw", then style will default to "W". Using distance based weights does not supplant finding a spatial neighborhood graph. The spatial neighborhood graph is first found and then its edges weighted based on distance in this function.

# **Examples**

```
library(SFEData)
sfe <- McKellarMuscleData(dataset = "small")
# sample_id is optional when only one sample is present
g <- findSpatialNeighbors(sfe, sample_id = "Vis5A")
attr(g, "method")
# Returns named list for multiple samples
sfe2 <- McKellarMuscleData(dataset = "small2")
sfe_combined <- cbind(sfe, sfe2)
gs <- findSpatialNeighbors(sfe, sample_id = "all")</pre>
```

findVisiumGraph

Find spatial neighborhood graphs for Visium spots

# Description

Visium spots are arranged in a hexagonal grid. This function uses the known locations of the Visium barcodes to construct a neighborhood graph, so adjacent spots are connected by edges. Since the known rows and columns of the spots are used, the unit the spot centroid coordinates are in does not matter.

# Usage

```
findVisiumGraph(x, sample_id = "all", style = "W", zero.policy = NULL)
```

#### **Arguments**

Х

A SpatialFeatureExperiment object with Visium data. Column names of the gene count matrix must be Visium barcodes, which may have a numeric suffix to distinguish between samples (e.g. "AAACAACGAATAGTTC-1").

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sample_id	Which sample(s) in the SFE object to use for the graph. Can also be "all", which means this function will compute the graph for all samples independently.
style	style can take values "W", "B", "C", "U", "minmax" and "S"
zero.policy	default NULL, use global option value; if FALSE stop with error for any empty neighbour sets, if TRUE permit the weights list to be formed with zero-length weights vectors

#### Value

For one sample, then a listw object representing the graph, with an attribute "method" recording the function used to build the graph, its arguments, and information about the geometry for which the graph was built. The attribute is used to reconstruct the graphs when the SFE object is subsetted since some nodes in the graph will no longer be present. If sample\_id = "all" or has length > 1, then a named list of listw objects, whose names are the sample\_ids. To add the list for multiple samples to a SFE object, specify the name argument in the spatialGraphs replacement method, so graph of the same name will be added to the SFE object for each sample.

# Examples

```
library(SFEData)
sfe <- McKellarMuscleData(dataset = "small")
g <- findVisiumGraph(sfe)
# For multiple samples, returns named list
sfe2 <- McKellarMuscleData(dataset = "small2")
sfe_combined <- cbind(sfe, sfe2)
gs <- findVisiumGraph(sfe, sample_id = "all")</pre>
```

formatTxSpots

Read and process transcript spots geometry for SFE

# **Description**

The function 'formatTxSpots' reads the transcript spot coordinates of smFISH-based data and formats the data. The data is not added to an SFE object. If the file specified in 'file\_out' already exists, then this file will be read instead of the original file in the 'file' argument, so the processing is not run multiple times. The function 'addTxSpots' adds the data read and processed in 'formatTxSpots' to the SFE object, and reads all transcript spot data. To only read a subset of transcript spot data, first use 'formatTxSpots' to write the re-formatted data to disk. Then read the specific subset and add them separately to the SFE object with the setter functions.

```
formatTxSpots(
  file,
  dest = c("rowGeometry", "colGeometry"),
  spatialCoordsNames = c("global_x", "global_y", "global_z"),
  gene_col = "gene",
```

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```
cell_col = "cell_id",
  z = "all",
  phred_col = "qv",
 min_phred = 20,
  split_col = NULL,
  not_in_cell_id = c("-1", "UNASSIGNED"),
  z_{option} = c("3d", "split"),
  flip = FALSE,
  file_out = NULL,
 BPPARAM = SerialParam(),
  return = TRUE
)
addTxSpots(
  sfe,
  file,
  sample_id = 1L,
  spatialCoordsNames = c("global_x", "global_y", "global_z"),
  gene_col = "gene",
  z = "all",
  phred_col = "qv",
 min_phred = 20,
  split_col = NULL,
  z_option = c("3d", "split"),
  flip = FALSE,
  file_out = NULL,
 BPPARAM = SerialParam()
)
```

## Arguments

file

File with the transcript spot coordinates. Should be one row per spot when read into R and should have columns for coordinates on each axis, gene the transcript is assigned to, and optionally cell the transcript is assigned to. Must be csv, tsv, or parquet.

dest

Where in the SFE object to store the spot geometries. This affects how the data is processed. Options:

**rowGeometry** All spots for each gene will be a 'MULTIPOINT' geometry, regardless of whether they are in cells or which cells they are assigned to.

**colGeometry** The spots for each gene assigned to a cell of interest will be a 'MULTIPOINT' geometry; since the gene count matrix is sparse, the geometries are NOT returned to memory.

## spatialCoordsNames

Column names for the x, y, and optionally z coordinates of the spots. The defaults are for Vizgen.

gene\_col Column name for genes.

cell\_col

Column name for cell IDs, ignored if 'dest = "rowGeometry"'. Can have length > 1 when multiple columns are needed to uniquely identify cells, in which case

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the contents of the columns will be concatenated, such as in CosMX data where

cell ID is only unique within the same FOV. Default "cell\_id" is for Vizgen MERFISH. Should be 'c("cell\_ID", "fov")' for CosMX. Index of z plane to read. Can be "all" to read all z-planes into MULTIPOINT z geometries with XYZ coordinates. If z values are not integer, then spots with all z values will be read. Column name for Phred scores of the spots. phred\_col min\_phred Minimum Phred score to keep spot. By default 20, the conventional threshold indicating "acceptable", meaning that there's 1 chance that the spot was decoded in error. split\_col Categorical column to split the geometries, such as cell compartment the spots are assigned to as in the "CellComp" column in CosMX output. Value of cell ID indicating that the spot is not assigned to any cell, such as "-1" not\_in\_cell\_id in Vizgen MERFISH and "0" in CosMX. When there're multiple columns for 'cell\_col', the first column is used to identify spots that are not in cells. What to do with z coordinates. "3d" is to construct 3D geometries. "split" is to z\_option create a separate 2D geometry for each z-plane so geometric operations are fully supported but some data wrangling is required to perform 3D analyses. When the z coordinates are not integers, 3D geometries will always be constructed since there are no z-planes to speak of. This argument does not apply when 'spatialCoordsNames' has length 2. flip Logical, whether to flip the geometry to match image. Here the y coordinates are simply set to -y, so the original bounding box is not preserved. This is consistent with readVizgen and readXenium. file out Name of file to save the geometry or raster to disk. Especially when the geometries are so large that it's unwieldy to load everything into memory. If this file (or directory for multiple files) already exists, then the existing file(s) will be read, skipping the processing. When writing the file, extensions supplied are

**BPPARAM** 

BiocParallelParam object to specify multithreading to convert raw char in

some parquet files to R objects. Not used otherwise.

ignored and extensions are determined based on 'dest'.

return Logical, whether to return the geometries in memory. This does not depend

on whether the geometries are written to file. Always 'FALSE' when 'dest =

"colGeometry"'.

sfe A 'SpatialFeatureExperiment' object.

sample\_id Which sample in the SFE object the transcript spots should be added to.

## Value

A sf data frame for vector geometries if 'file\_out' is not set. 'SpatRaster' for raster. If there are multiple files written, such as when splitting by cell compartment or when 'dest = "colGeometry"', then a directory with the same name as 'file\_out' will be created (but without the extension) and the files are written to that directory with informative names. 'parquet' files that can be read with 'st\_read' is written for vector geometries. When 'return = FALSE', the file name or directory (when there're multiple files) is returned.

The 'sf' data frame, or path to file where geometries are written if 'return = FALSE'.

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## Note

When 'dest = "colGeometry"', the geometries are always written to disk and not returned in memory, because this is essentially the gene count matrix, which is sparse. This kind of reformatting is implemented so users can read in MULTIPOINT geometries with transcript spots for each gene assigned to each cell for spatial point process analyses, where not all genes are loaded at once.

## **Examples**

```
# Default arguments are for MERFISH
fp <- tempfile()</pre>
dir_use <- SFEData::VizgenOutput(file_path = fp)</pre>
g <- formatTxSpots(file.path(dir_use, "detected_transcripts.csv"))</pre>
unlink(dir_use, recursive = TRUE)
# For CosMX, note the colnames, also dest = "colGeometry"
# Results are written to the tx_spots directory
dir_use <- SFEData::CosMXOutput(file_path = fp)</pre>
cg <- formatTxSpots(file.path(dir_use, "Run5642_S3_Quarter_tx_file.csv"),</pre>
dest = "colGeometry", z = "all",
cell_col = c("cell_ID", "fov"),
gene_col = "target", not_in_cell_id = "0",
spatialCoordsNames = c("x_global_px", "y_global_px", "z"),
file_out = file.path(dir_use, "tx_spots"))
# Cleanup
unlink(dir_use, recursive = TRUE)
```

formatTxTech

Read and process transcript spots for specific commercial technologies

## Description

To preset parameters such as spatialCoordsNames, gene\_col, cell\_col, and phred\_col that are standard for the output of the technology.

```
formatTxTech(
  data_dir,
  tech = c("Vizgen", "Xenium", "CosMX"),
  dest = c("rowGeometry", "colGeometry"),
  z = "all",
  min_phred = 20,
  split_cell_comps = FALSE,
  z_option = c("3d", "split"),
  flip = FALSE,
  file_out = NULL,
  BPPARAM = SerialParam(),
```

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```
return = TRUE
)
addTxTech(
  sfe,
  data_dir,
  sample_id = 1L,
  tech = c("Vizgen", "Xenium", "CosMX"),
  z = "all"
 min_phred = 20,
  split_cell_comps = FALSE,
  z_option = c("3d", "split"),
  flip = FALSE,
  file_out = NULL,
 BPPARAM = SerialParam()
)
```

## **Arguments**

data\_dir Top level output directory.

tech Which technology whose output to read, must be one of "Vizgen", "Xenium",

or "CosMX" though more technologies may be added later.

dest Where in the SFE object to store the spot geometries. This affects how the data

is processed. Options:

rowGeometry All spots for each gene will be a 'MULTIPOINT' geometry, regardless of whether they are in cells or which cells they are assigned to.

colGeometry The spots for each gene assigned to a cell of interest will be a 'MULTIPOINT' geometry; since the gene count matrix is sparse, the ge-

ometries are NOT returned to memory. Which z-planes to read. Always "all" for Xenium where the z coordinates are

not discrete.

min\_phred Minimum Phred score to keep spot. By default 20, the conventional threshold

indicating "acceptable", meaning that there's 1 chance that the spot was decoded in error.

split\_cell\_comps

Only relevant to CosMX whose transcript spot file assigns the spots to cell com-

ponents. Setting this argument to TRUE

What to do with z coordinates. "3d" is to construct 3D geometries. "split" is to

create a separate 2D geometry for each z-plane so geometric operations are fully supported but some data wrangling is required to perform 3D analyses. When the z coordinates are not integers, 3D geometries will always be constructed since there are no z-planes to speak of. This argument does not apply when

'spatialCoordsNames' has length 2.

flip Logical, whether to flip the geometry to match image. Here the y coordinates are simply set to -y, so the original bounding box is not preserved. This is consistent

with readVizgen and readXenium.

Z

z\_option

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file_out	Name of file to save the geometry or raster to disk. Especially when the geometries are so large that it's unwieldy to load everything into memory. If this file (or directory for multiple files) already exists, then the existing file(s) will be read, skipping the processing. When writing the file, extensions supplied are ignored and extensions are determined based on 'dest'.
BPPARAM	BiocParallelParam object to specify multithreading to convert raw char in some parquet files to R objects. Not used otherwise.
return	Logical, whether to return the geometries in memory. This does not depend on whether the geometries are written to file. Always 'FALSE' when 'dest = "colGeometry"'.
sfe	A 'SpatialFeatureExperiment' object.
sample_id	Which sample in the SFE object the transcript spots should be added to.

#### Value

The 'sf' data frame, or path to file where geometries are written if 'return = FALSE'.

## **Examples**

gdalParquetAvailable Check if Parquet GDAL driver is available

## **Description**

The GeoParquet files for geometries are typically written and read with the sfarrow package, but to add only a select few genes to the SFE object say for visualization purposes, the Parquet GDAL driver is required in order to use GDAL's SQL to query the GeoParquet file to only load the few genes requested. The transcript spots from a large dataset can take up a lot of memory if all loaded.

#### Usage

```
gdalParquetAvailable()
```

#### **Details**

The Parquet driver has been supported since GDAL 3.5.0. The arrow C++ library must be installed in order to make the Parquet driver available. When arrow is installed, newer versions of GDAL installed from Homebrew (Mac) should have the Parquet driver. For Linux, the binary from apt-get's default repo is 3.4.1 (as of April 2024). To use the Parquet driver, GDAL may need to be installed from source. See script from the geospatial rocker. A Voyager docker container with the Parquet driver will soon be provided.

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## Value

Logical, indicating whether the Parquet driver is present.

## **Examples**

```
gdalParquetAvailable()
```

getParams

Get parameters used in spatial methods

# Description

The getParams function allows users to access the parameters used to compute the results that may be stored in colFeatureData.

# Usage

```
getParams(
   sfe,
   name,
   local = FALSE,
   colData = FALSE,
   colGeometryName = NULL,
   annotGeometryName = NULL,
   reducedDimName = NULL
)
```

## **Arguments**

sfe A SpatialFeatureExperiment object.

name Name used to store the results.

Logical, whether the results of interest come from a local spatial method.

colData

Logical, whether the results were computed for a column of colData(sfe).

colGeometryName

To get results for a colGeometry.

 $\verb"annotGeometryName"$ 

To get results for an annotGeometry; colGeometry has precedence so this ar-

gument is ignored if colGeometryName is specified.

reducedDimName Name of a dimension reduction, can be seen in reducedDimNames. colGeometryName

and annot GeometryName have precedence over reduced DimName.

## Value

A named list showing the parameters

getPixelSize 43

## **Examples**

```
library(SFEData)
library(scater)
library(Voyager)
sfe <- McKellarMuscleData("small")
colGraph(sfe, "visium") <- findVisiumGraph(sfe)
sfe <- colDataMoransI(sfe, "nCounts")
getParams(sfe, "moran", colData = TRUE)</pre>
```

getPixelSize

Get physical size of pixels

## **Description**

This function gets physical size of pixels in each resolution of a OME-TIFF pyramid in BioFormatsImage.

## Usage

```
getPixelSize(file, resolution = 1L)
```

## **Arguments**

file Path to an OME-TIFF file.

resolution Which resolution to query; 1 means the highest resolution. The pixels will be

larger for the lower resolutions.

#### Value

Numeric vector of length 2 of pixel size in x and y. Usually they're the same.

## **Examples**

```
library(SFEData)
fp <- tempfile()
dir_use <- XeniumOutput("v1", file_path = fp)
# RBioFormats null pointer error
try(getPixelSize(file.path(dir_use, "morphology_focus.ome.tif")))
getPixelSize(file.path(dir_use, "morphology_focus.ome.tif"))
unlink(dir_use, recursive = TRUE)</pre>
```

imageIDs

getTechTxFields

Get relevant fields and file paths for transcript spots

## **Description**

Get column names for x, y, and z coordinates, gene IDs, and cell IDs from the transcript file and get file paths for transcript spot coordinates given technology.

# Usage

```
getTechTxFields(tech, data_dir = NULL)
```

#### **Arguments**

Name of the commercial technology, must be one of Vizgen, Xenium, and

CosMX.

data\_dir Top level directory of the output.

#### Value

A named list with elements:

spatialCoordsNames A character vector for column names for the xyz coordinates of the transcript spots.

gene\_col Column name for gene IDs.

cell\_col Column name for cell IDs.

fn File path of the transcript spot file.

imageIDs

Show all image\_ids in the SFE object

## **Description**

The title is self-explanatory. Some functions require image\_id to get or set images.

## Usage

```
imageIDs(sfe)
```

## **Arguments**

sfe

A SpatialFeatureExperiment object.

## Value

A character vector of image\_ids.

## **Examples**

```
fp <- system.file(file.path("extdata", "sample01"),
package = "SpatialFeatureExperiment")
sfe <- read10xVisiumSFE(fp, type = "sparse")
imageIDs(sfe)</pre>
```

## **Description**

Modify or replace images stored in a SpatialExperiment object. This is different from addImg which adds the image from files and can't replace existing images, which is there to be consistent with SpatialExperiment. This setter here can replace existing images with another object that inherits from VirtualSpatialImage, including SpatRasterImage, BioFormatsImage, and ExtImage.

## Usage

```
## S4 replacement method for signature 'SpatialExperiment'
Img(x, sample_id = 1L, image_id, scale_fct = 1) <- value</pre>
```

## Arguments

X	A SpatialExperiment object, which includes SFE.
sample_id	Which sample the image is associated with. Use sampleIDs to get sample IDs present in the SFE object.
image_id	Image ID, such as "lowres" and "hires" for Visium data and "DAPI" and "PolyT" for Vizgen MERFISH data.
scale_fct	Scale factor to convert pixels in lower resolution to those in the full resolution. Only relevant to image classes implemented in SpatialExperiment but not SpatialFeatureExperiment because the spatial extent of images in SFE takes precedence.
value	New version of image to add, must inherit from VirtualSpatialImage.

## Value

SFE object with the new image added.

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## **Examples**

```
library(EBImage)
library(SFEData)
library(RBioFormats)
fp <- tempfile()
fn <- XeniumOutput("v2", file_path = fp)
# Weirdly the first time I get the null pointer error
try(sfe <- readXenium(fn))
sfe <- readXenium(fn)
img <- getImg(sfe) |> toExtImage(resolution = 1L)
img <- img[,,1] > 500
Img(sfe, image_id = "mask") <- img
imageIDs(sfe)
unlink(fn, recursive = TRUE)</pre>
```

imgRaster

Get the image from \*Image class

## **Description**

In SFE, S4 classes inheriting from VirtualSpatialImage have been implemented to make these image classes compatible with SpatialExperiment. The imgRaster methods in SFE are meant to extract the original image from the \*Image classes, such as SpatRaster from SpatRasterImage, and Image from ExtImage and BioFormatsImage. For BioFormatsImage, the image of the specified resolution will be read into memory as AnnotatedImage, which inherits from EBImage::Image.

## **Arguments**

x An object of class \*Image as implemented in this package.

resolution Resolution to read in from OME-TIFF, defaults to 4, which is a medium resolu-

tion in Xenium.

## Value

SpatRaster from SpatRasterImage, and Image from ExtImage and BioFormatsImage. For BioFormatsImage, the image of the specified resolution will be read into memory as AnnotatedImage and ExtImage, which both inherit from EBImage::Image.

## See Also

```
Other image methods: SFE-image, affineImg(), cropImg(), dim,BioFormatsImage-method, ext(), imgSource(), mirrorImg(), rotateImg(), scaleImg(), translateImg(), transposeImg()
```

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imgSource

Source of images that are on disk

# Description

Get the file path of images that are on disk and not read into memory. Only applies to SpatRasterImage and BioFormatsImage.

## Usage

```
## S4 method for signature 'SpatRasterImage'
imgSource(x)

## S4 method for signature 'BioFormatsImage'
imgSource(x)

## S4 method for signature 'ExtImage'
imgSource(x)
```

## **Arguments**

Х

An object of class \*Image as implemented in this package.

## Value

String, file path to the original image on disk. For SpatRasterImage, if the image is loaded into memory, then NULL.

#### See Also

```
Other image methods: SFE-image, affineImg(), cropImg(), dim,BioFormatsImage-method, ext(), imgRaster(), mirrorImg(), rotateImg(), scaleImg(), translateImg(), transposeImg()
```

listw2sparse

Convert listw into sparse adjacency matrix

# Description

Edge weights are used in the adjacency matrix. Because most elements of the matrix are 0, using sparse matrix greatly reduces memory use.

```
listw2sparse(listw)
```

## Arguments

listw

A listw object for spatial neighborhood graph.

#### Value

A sparse dgCMatrix, whose row represents each cell or spot and whose columns represent the neighbors. The matrix does not have to be symmetric. If region.id is present in the listwobject, then it will be the row and column names of the output matrix.

## **Examples**

```
library(SFEData)
sfe <- McKellarMuscleData("small")
g <- findVisiumGraph(sfe)
mat <- listw2sparse(g)</pre>
```

localResults

Get and set results from local spatial statistics

## **Description**

Local spatial statics like local Moran's I, local Geary's C, Getis-Ord Gi\*, and geographically weighted summary statistics return values at each spatial location. Just like dimension reductions, these results are clearly associated with the broader SFE object, so they should have a place within the object. However, a separate field is needed because these analyses are conceptually distinct from dimension reduction. Also, each feature (e.g. gene) can have its own results with values at each location. The localResults field in the SFE object stores these results that has a value for each spatial location.

```
## S4 method for signature 'SpatialFeatureExperiment'
localResults(
    x,
    sample_id = "all",
    name = "all",
    features = NULL,
    colGeometryName = NULL,
    annotGeometryName = NULL,
    withDimnames = TRUE,
    swap_rownames = NULL,
    ...
)

## S4 replacement method for signature 'SpatialFeatureExperiment'
localResults(
    x,
```

```
sample_id = "all",
  name = "all",
  features = NULL,
  colGeometryName = NULL,
  annotGeometryName = NULL,
 withDimnames = TRUE,
 swap_rownames = NULL,
) <- value
## S4 method for signature 'SpatialFeatureExperiment'
localResultNames(x)
## S4 replacement method for signature 'SpatialFeatureExperiment,character'
localResultNames(x) \leftarrow value
## S4 method for signature 'SpatialFeatureExperiment'
localResultFeatures(
 Χ,
  type = 1L,
 colGeometryName = NULL,
 annotGeometryName = NULL,
  swap\_rownames = NULL
)
## S4 method for signature 'SpatialFeatureExperiment'
localResultAttrs(
 Х,
  type = 1L,
  feature,
  colGeometryName = NULL,
  annotGeometryName = NULL,
  swap_rownames = NULL
)
## S4 method for signature 'SpatialFeatureExperiment'
localResult(
  х,
  type = 1L,
  feature,
  colGeometryName = NULL,
  annotGeometryName = NULL,
  sample_id = 1L,
 withDimnames = TRUE,
 simplify = TRUE,
  swap\_rownames = NULL
)
```

```
## S4 replacement method for signature 'SpatialFeatureExperiment'
localResult(
    x,
    type = 1L,
    feature,
    colGeometryName = NULL,
    annotGeometryName = NULL,
    sample_id = 1L,
    withDimnames = TRUE
) <- value</pre>
```

## **Arguments**

x A SpatialFeatureExperiment object.

sample\_id Sample ID to get or set geometries.

name Name of the spatial method used, such as "localmoran".

features Features whose local results to get or set, for localResults getter and setter for

multiple features at a time.

colGeometryName

Which colGeometry to get or set local results.

annotGeometryName

Which annotGeometry to get or set local results.

withDimnames Logical. If TRUE, then the dimnames (colnames or rownames) of the gene count

matrix should correspond to row names of the sf data frames of interest.

swap\_rownames Name of a column in rowData to identify features instead of the row names of

the SFE object. For example, if the row names of the SFE object are Ensembl IDs and gene symbols are in the "symbol" column in rowData, then putting "symbol" for this argument will use the gene symbols to identify which gene's

local results to get or set.

... Ignored

value Values to set, should be either a matrix or a data frame.

type Name or index of the spatial method used, such as "localmoran".

feature Feature whose local results to get or set, for localResult getter and setter for

one feature at a time.

simplify Basically whether to return the content of the list rather than a list when the list

only has one element, such as results for one type and one feature.

#### Value

localResults returns a named list each element of which is a set of local results of interest. localResult returns a matrix or a data frame, whichever the original is when it's set. localResultNames returns a character vector. Setters return an SFE object with the desired field set. For genes and colData columns, the local results are stored in the localResults field in int\_colData, whereas for colGeometries and annotGeometries, the local results are stored as columns in the same sf data frames. localResultFeatures returns a character vector of names of features for which local results are available. localResultAttrs returns a character vector of the column names of the local results of one type for one feature. It returns NULL if the results are a vector.

## **Examples**

```
# Toy example
sfe <- readRDS(system.file("extdata/sfe_toy.rds",</pre>
    package = "SpatialFeatureExperiment"
))
# localResults functions are written for organizing results from local
# spatial statistics (see the Voyager package). But for the examples here,
# random toy matrices are used. The real results are often matrices, with a
# matrix for each feature.
library(S4Vectors)
set.seed(29)
toy_res1 <- matrix(rnorm(10),</pre>
    nrow = 5, ncol = 2,
    dimnames = list(colnames(sfe), c("meow", "purr"))
toy_res1b <- matrix(rgamma(10, shape = 2),</pre>
    nrow = 5, ncol = 2,
    dimnames = list(colnames(sfe), c("meow", "purr"))
toy_df1 <- DataFrame(gene1 = I(toy_res1), gene2 = I(toy_res1b))</pre>
toy_res2 <- matrix(rpois(10, lambda = 2),</pre>
    nrow = 5, ncol = 2,
    dimnames = list(colnames(sfe), c("sassy", "tortitude"))
toy_df2 <- DataFrame(gene1 = I(toy_res2))</pre>
# Set all local results
localResults(sfe) <- list(localmoran = toy_df1, Gistar = toy_df2)</pre>
# Get all local results
lrs <- localResults(sfe)</pre>
# Set results of the same type for multiple genes
localResults(sfe, name = "localmoran") <- toy_df1</pre>
# Can also use a list
localResults(sfe, name = "localmoran") <- as.list(toy_df1)</pre>
# Get results of the same type for multiple genes
lrs <- localResults(sfe, name = "localmoran", features = c("gene1", "gene2"))</pre>
# Set results for one type and one gene
localResult(sfe, "localmoran", feature = "gene1") <- toy_res1</pre>
# Get results for one type and one gene
lr <- localResult(sfe, "localmoran", feature = "gene1")</pre>
# Set results for a feature in colGeometries
cg_toy <- readRDS(system.file("extdata/cg_toy.rds",</pre>
    package = "SpatialFeatureExperiment"
colGeometry(sfe, "cg") <- cg_toy</pre>
localResult(sfe, "localmoran",
    feature = "gene1",
    colGeometryName = "cg"
) <- toy_res1
```

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```
# Get results for a feature in colGeometries
lr <- localResult(sfe, "localmoran", "gene1", colGeometryName = "cg")</pre>
```

mirrorImg

Mirror/flip images

## **Description**

Flip images along the middle horizontal or vertical axis.

# Usage

```
## S4 method for signature 'SpatRasterImage'
mirrorImg(
    X,
    direction = c("vertical", "horizontal"),
    filename = "",
    maxcell = NULL,
    ...
)

## S4 method for signature 'BioFormatsImage'
mirrorImg(x, direction = c("vertical", "horizontal"), ...)

## S4 method for signature 'ExtImage'
mirrorImg(x, direction = c("vertical", "horizontal"), ...)
```

## **Arguments**

X	SpatRaster or SpatVector
direction	character. Should (partially) match "vertical" to flip by rows, or "horizontal" to flip by columns
filename	character. Output filename
maxcell	Max number of pixels to load SpatRasterImage into memory. The default 1e7 is chosen because this is the approximate number of pixels in the medium resolution image at resolution = 4L in Xenium OME-TIFF to make different methods of this function consistent.
• • •	additional arguments for writing files as in writeRaster

## Value

\*Image object of the same class.

## See Also

```
Other image methods: SFE-image, affineImg(), cropImg(), dim,BioFormatsImage-method, ext(), imgRaster(), imgSource(), rotateImg(), scaleImg(), translateImg(), transposeImg()
```

multi\_listw2sparse 53

multi\_listw2sparse

Convert multiple listw graphs into a single sparse adjacency matrix

## **Description**

Each sample in the SFE object has a separate spatial neighborhood graph. Spatial analyses performed jointly on multiple samples require a combined spatial neighborhood graph from the different samples, where the different samples would be disconnected components of the graph. This combined adjacency matrix can be used in MULTISPATI PCA.

## Usage

```
multi_listw2sparse(listws)
```

## **Arguments**

listws

A list of listw objects.

#### Value

A sparse dgCMatrix of the combined spatial neighborhood graph, with the original spatial neighborhood graphs of the samples on the diagonal. When the input is an SFE object, the rows and columns will match the column names of the SFE object.

## **Examples**

# example code

read10xVisiumSFE

Read 10X Visium data as SpatialFeatureExperiment

## **Description**

Read Space Ranger output as a SpatialFeatureExperiment object, where spots are represented with polygons in the colGeometry called "spotPoly". Other geometries can be added later after the dataset is read. If data = "filtered", then spatial neighborhood graphs of the spots are also computed and stored in the colGraph called "visium" in all samples for downstream spatial analyses.

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## **Usage**

```
read10xVisiumSFE(
  samples = "",
  dirs = file.path(samples, "outs"),
  sample_id = paste0("sample", sprintf("%02d", seq_along(samples))),
  bin_size = NULL,
  type = c("HDF5", "sparse"),
  data = c("filtered", "raw"),
  images = c("lowres", "hires"),
  unit = c("full_res_image_pixel", "micron"),
  add_Graph = TRUE,
  style = "W",
  zero.policy = NULL,
  load = FALSE,
  row.names = c("id", "symbol")
)
```

## **Arguments**

guileits		
samples	a character vector specifying one or more directories, each corresponding to a 10x Genomics Visium sample (see Details); if provided, names will be used as sample identifiers	
dirs	Directory for each sample that contains the spatial and raw/filtered_featues_bc_matrix directories. By default, the outs directory under the directory specified in the samples argument, as in Space Ranger output. Change the dirs argument if you have moved or renamed the output directory.	
sample_id	character string specifying unique sample identifiers, one for each directory specified via samples; ignored if !is.null(names(samples))	
bin_size	c(character), use this only when loading VisiumHD. Specify which bin resolution to load, default is NULL which assumes that data is standard Visium. Eg, single resolution is c("8"), if to load all three resolutions, use c("2", "8", "16").	
type	Either "HDF5", and the matrix will be represented as TENxMatrix, or "sparse", and the matrix will be read as a dgCMatrix.	
data	character string specifying whether to read in filtered (spots mapped to tissue) or raw data (all spots).	
images	character vector specifying which images to include. Valid values are "lowres", "hires", "fullres", "detected", "aligned"	
unit	Whether to use pixels in full resolution image or microns as the unit. If using	

Whether to use pixels in full resolution image or microns as the unit. If using microns, then spacing between spots in pixels will be used to convert the coordinates into microns, as the spacing is known to be 100 microns. This is used to

plot scale bar.

add\_Graph c(local), if to add spatial neighborhood graph for spots and only if c(data =

"filtered"). Default is c(TRUE)

style style can take values "W", "B", "C", "U", "minmax" and "S"

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zero.policy default NULL, use global option value; if FALSE stop with error for any empty

neighbour sets, if TRUE permit the weights list to be formed with zero-length

weights vectors

load Not used, kept for backward compatibility.

row.names String specifying whether to use Ensembl IDs ("ID") or gene symbols ("Sym-

bol") as row names. If using symbols, the Ensembl ID will be appended to disambiguate in case the same symbol corresponds to multiple Ensembl IDs.

#### Value

A SpatialFeatureExperiment object. The images might need to be manually transposed and/or mirrored to match the spots in this version of this package.

#### Note

The as(<dgTMatrix>, "dgCMatrix") is deprecated warning comes from the DropletUtils package which is used by SpatialExperiment to read 10X outputs. This will be fixed when SpatialExperiment switches to TENxIO.

It is assumed that the images have not been cropped. Otherwise the images might not align with the spots.

## **Examples**

```
dir <- system.file("extdata", package = "SpatialFeatureExperiment")</pre>
sample_ids <- c("sample01", "sample02")</pre>
samples <- file.path(dir, sample_ids)</pre>
list.files(samples[1])
list.files(file.path(samples[1], "spatial"))
(sfe <- read10xVisiumSFE(samples, sample_id = sample_ids,</pre>
    type = "sparse", data = "filtered",
    load = FALSE
))
# load VisiumHD
# path to "binned_outputs" directory containing:
# |-- binned_outputs
      |-- square_002um
      |-- square_008um
      |-- square_016um
dir_hd <- "~/Downloads/Visium_HD_Mouse_Brain/binned_outputs/"</pre>
# this is public dataset ->
# https://www.10xgenomics.com/datasets/visium-hd-cytassist-gene-expression-libraries-of-mouse-brain-he
sfe_hd <-
read10xVisiumSFE(samples = list.files(dir_hd), # 1:3 resolutions
                 dirs = dir_hd,
                 bin_size = c("8", "16"), # this defines which of 1:3 resolutions to load
                 type = "HDF5", # Note, "sparse" -> takes longer to load
                 data = "filtered", # spots under tissue
                 images = c("lowres"), # for now low res. image only
```

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```
add_Graph = FALSE # Note, if VisiumHD this can take time for 2 or 8\mu m res. )
```

readCosMX

Read CosMX data into SFE

#### **Description**

This function reads the standard CosMX output into an SFE object, as in "Basic Data Files" on the Nanostring website.

## Usage

```
readCosMX(
  data_dir,
  z = "all",
  sample_id = "sample01",
  add_molecules = FALSE,
  split_cell_comps = FALSE,
  BPPARAM = SerialParam(),
  file_out = file.path(data_dir, "tx_spots.parquet"),
  z_option = c("3d", "split")
)
```

#### **Arguments**

data\_dir Top level output directory.

\_

Integer z index or "all" to indicate which z-planes to read for the transcript spots.

sample\_id

A character sample identifier, which matches the sample\_id in imgData. The sample\_id will also be stored in a new column in colData, if not already present. Default = sample01.

Logical, whether to split transcript spot geometries by cell compartment. Only relevant when 'add\_molecules = TRUE'.

**BPPARAM** 

A BiocParallelParam object specifying parallel processing backend and number of threads to use for parallelizable tasks:

- To load cell segmentation from HDF5 files from different fields of view (FOVs) with multiple cores. A progress bar can be configured in the BiocParallelParam object. When there are numerous FOVs, reading in the geometries can be time consuming, so we recommend using a server and larger number of threads. This argument is not used if use\_cellpose = TRUE and the parquet file is present.
- 2. To get the largest piece and see if it's larger than min\_area when there are multiple pieces in the cell segmentation for one cell.

readSelectTx 57

file\_out

Name of file to save the geometry or raster to disk. Especially when the geometries are so large that it's unwieldy to load everything into memory. If this file (or directory for multiple files) already exists, then the existing file(s) will be read, skipping the processing. When writing the file, extensions supplied are ignored and extensions are determined based on 'dest'.

z\_option

What to do with z coordinates. "3d" is to construct 3D geometries. "split" is to create a separate 2D geometry for each z-plane so geometric operations are fully supported but some data wrangling is required to perform 3D analyses. When the z coordinates are not integers, 3D geometries will always be constructed since there are no z-planes to speak of. This argument does not apply when 'spatialCoordsNames' has length 2.

#### Value

An SFE object. Cell polygons are written to 'cell\_boundaries\_sf.parquet' in 'data\_dir'. If reading transcript spots ('add\_molecules = TRUE'), then the reformatted transcript spots are saved to file specified in the 'file\_out' argument, which is by default 'tx\_spots.parquet' in the same directory as the rest of the data.

## **Examples**

```
fp <- tempfile()
dir_use <- SFEData::CosMXOutput(file_path = fp)
sfe <- readCosMX(dir_use, z = "all", add_molecules = TRUE)
# Clean up
unlink(dir_use, recursive = TRUE)</pre>
```

readSelectTx

Read transcript spots of select genes

## **Description**

I speculate that in practice, the most common use of the transcript spots is visualization, and only a few genes can be visualized at a time or the spots will overcrowd. Then it doesn't make sense to load the transcript spots of all genes into memory as they can take up a lot of memory. The function readSelectTx reads transcript spots of select genes into R, and the function addSelectTx adds them to rowGeometries of the SFE object.

```
readSelectTx(file, gene_select, z = "all", z_option = c("3d", "split"))
addSelectTx(
    sfe,
    file,
    gene_select,
    sample_id = 1L,
```

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```
z = "all",
z_option = c("3d", "split"),
swap_rownames = NULL
)
```

#### **Arguments**

z

file File path of a GeoParquet file (e.g. already reformatted with the formatTxSpots

or addTxSpots function, should have already flipped to match image if neces-

sary).

gene\_select Character vector of a subset of genes. If NULL, then all genes that have transcript

spots are added. Only relevant when reading data from formatted files on disk.

If specified, then return = TRUE.

Index of z plane to read. Can be "all" to read all z-planes into MULTIPOINT

geometries with XYZ coordinates. If z values are not integer, then spots with all

z values will be read.

z\_option What to do with z coordinates. "3d" is to construct 3D geometries. "split" is to

create a separate 2D geometry for each z-plane so geometric operations are fully supported but some data wrangling is required to perform 3D analyses. When the z coordinates are not integers, 3D geometries will always be constructed since there are no z-planes to speak of. This argument does not apply when

'spatialCoordsNames' has length 2.

sfe A 'SpatialFeatureExperiment' object.

sample\_id Which sample in the SFE object the transcript spots should be added to.

swap\_rownames Name of a column in rowData(sfe) to use as gene identifiers in place of the

actual row names. In some cases this may be needed to match each transcript

spot MULTIPOINT geometry to rows of sfe.

#### Value

When there are multipel parquet files to be read, a list of sf data frames with MULTIPOINT geometry for genes selected. When there is only one file, then one sf data frame. For addSelectTx, an SFE object with the transcript spots of the selected genes added.

#### Note

The GDAL Parquet driver is required for this function, though not for other functions that work with GeoParquet files. GDAL Parquet driver has been supported since GDAL 3.5.0, but is not part of the default installation. The z and z\_option arguments are there since the file names contain z-plane information when relevant. See the GDAL documentation page for the Parquet driver.

## **Examples**

```
library(SFEData)
if (gdalParquetAvailable()) {
   fp <- tempfile()
   dir_use <- XeniumOutput("v2", file_path = fp)
   fn_tx <- formatTxTech(dir_use, tech = "Xenium", flip = TRUE, return = FALSE,</pre>
```

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```
file_out = file.path(dir_use, "tx_spots.parquet"))
gene_select <- c("ACE2", "BMX")
df <- readSelectTx(fn_tx, gene_select)
# RBioFormats null pointer error the first time
try(sfe <- readXenium(dir_use))
sfe <- readXenium(dir_use)
sfe <- addSelectTx(sfe, fn_tx, head(rownames(sfe), 5), swap_rownames = "Symbol")
unlink(dir_use, recursive = TRUE)
}</pre>
```

readVizgen

Read Vizgen MERFISH output as SpatialFeatureExperiment

#### **Description**

This function reads the standard Vizgen MERFISH output into an SFE object. The coordinates are in microns. Cell centroids are read into colGeometry "centroids", and cell segmentations are read into colGeometry "cellSeg". The image(s) (polyT, DAPI, and cell boundaries) are also read as SpatRaster objects so they are not loaded into memory unless necessary. Because the image's origin is the top left while the geometry's origin is bottom left, either the image or the geometry needs to be flipped. Because the image accompanying MERFISH datasets are usually very large, the coordinates will be flipped so the flipping operation won't load the entire image into memory. Large datasets with hundreds of thousands of cells can take a while to read if reading transcript spots as it takes a while to convert the spots to MULTIPOINT geometries.

## Usage

```
readVizgen(
  data_dir,
  z = "all"
  sample_id = "sample01",
 min_area = 15,
  image = c("DAPI", "PolyT", "Cellbound"),
  flip = c("geometry", "image", "none"),
 max_flip = "50 MB",
  filter_counts = FALSE,
  add_molecules = FALSE,
  use_bboxes = FALSE,
  use_cellpose = TRUE,
 BPPARAM = SerialParam(),
  file_out = file.path(data_dir, "detected_transcripts.parquet"),
  z_{option} = c("3d", "split")
)
```

## Arguments

data\_dir Top level output directory.

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Integer, z index to read, or "all", indicating z-planes of the images and transcript Z spots to read. While cell segmentation seems to have multiple z-planes, the segmentation in all z-planes are the same so in effect the cell segmentatio is only in 2D. sample\_id A character sample identifier, which matches the sample\_id in imgData. The sample\_id will also be stored in a new column in colData, if not already present. Default = sample01. Minimum cell area in square microns. Anything smaller will be considered min\_area artifact or debris and removed. Which image(s) to load, can be "DAPI", "PolyT", "Cellbound" or any combinaimage tion of them. flip To flip the image, geometry coordinates, or none. Because the image has the origin at the top left while the geometry has origin at the bottom left, one of them needs to be flipped for them to match. If one of them is already flipped, then use "none". The image will not be flipped if it's GeoTIFF. Maximum size of the image allowed to flip the image. Because the image will max\_flip be loaded into memory to be flipped. If the image is larger than this size then the coordinates will be flipped instead. Logical, whether to keep cells with counts > 0. filter\_counts add\_molecules Logical, whether to add transcripts coordinates to an object. use\_bboxes If no segmentation output is present, use cell\_metadata to make bounding boxes instead. Whether to read the parquet files from CellPose cell segmentation. If FALSE, use\_cellpose cell segmentation will be read from the HDF5 files. Note that reading HDF5 files for numerous FOVs is very slow. A BiocParallelParam object specifying parallel processing backend and num-**BPPARAM** ber of threads to use for parallelizable tasks: 1. To load cell segmentation from HDF5 files from different fields of view (FOVs) with multiple cores. A progress bar can be configured in the BiocParallelParam object. When there are numerous FOVs, reading in the geometries can be time consuming, so we recommend using a server and larger number of threads. This argument is not used if use\_cellpose = TRUE and the parquet file is present. 2. To get the largest piece and see if it's larger than min\_area when there are multiple pieces in the cell segmentation for one cell. file\_out Name of file to save the geometry or raster to disk. Especially when the geometries are so large that it's unwieldy to load everything into memory. If this file (or directory for multiple files) already exists, then the existing file(s) will be read, skipping the processing. When writing the file, extensions supplied are ignored and extensions are determined based on 'dest'. What to do with z coordinates. "3d" is to construct 3D geometries. "split" is to  $z_{option}$ create a separate 2D geometry for each z-plane so geometric operations are fully supported but some data wrangling is required to perform 3D analyses. When the z coordinates are not integers, 3D geometries will always be constructed since there are no z-planes to speak of. This argument does not apply when 'spatialCoordsNames' has length 2.

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#### Value

A SpatialFeatureExperiment object.

#### Note

Since the transcript spots file is often very large, we recommend only using add\_molecules = TRUE on servers with a lot of memory. If reading all z-planes, conversion of transcript spot geometry to parquet file might fail due to arrow data length limit. In a future version, when the transcript spot geometry is large, it will be written to multiple separate parquet files which are then concatenated with DuckDB. Also, in a future version, the transcript spot processing function might be rewritten in C++ to stream the original CSV file so it's not entirely loaded into memory.

#### **Examples**

```
fp <- tempfile()
dir_use <- SFEData::VizgenOutput(file_path = fp)
sfe <- readVizgen(dir_use, z = 3L, image = "PolyT",
flip = "geometry")
## Filtering of counts, and addition of molecule coordinates..
sfe <- readVizgen(dir_use, z = 3L, image = "PolyT", filter_counts = TRUE,
add_molecules = TRUE, flip = "geometry")
unlink(dir_use, recursive = TRUE)</pre>
```

readXenium

Read 10X Xenium output as SpatialFeatureExperiment

#### **Description**

This function reads the standard 10X Xenium output into an SFE object.

```
readXenium(
  data_dir,
  sample_id = "sample01",
  image = c("morphology_focus", "morphology_mip"),
  segmentations = c("cell", "nucleus"),
  row.names = c("id", "symbol"),
  flip = c("geometry", "image", "none"),
  max_flip = "50 MB",
  filter_counts = FALSE,
  add_molecules = FALSE,
  min_phred = 20,
  BPPARAM = SerialParam(),
  file_out = file.path(data_dir, "tx_spots.parquet")
)
```

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#### **Arguments**

data\_dir Top level output directory.

sample\_id A character sample identifier, which matches the sample\_id in imgData. The

sample\_id will also be stored in a new column in colData, if not already

present. Default = sample01.

Which image(s) to load, can be "morphology\_mip", "morphology\_focus" or image

> both. Note that in Xenium Onboarding Analysis (XOA) v2, there is no longer "morphology\_mip" and "morphology\_focus" is a directory with 4 images corresponding to 4 channels: DAPI, "Cadherin", 18S, and Vimentin. So this argument

is ignored for XOA v2.

Which segmentation outputs to read, can be "cell", "nucleus", or both. segmentations

String specifying whether to use Ensembl IDs ("id") or gene symbols ("symrow.names

> bol") as row names. If using symbols, the Ensembl ID will be appended to disambiguate in case the same symbol corresponds to multiple Ensembl IDs. Always "symbol" if 'add\_molecules = TRUE' because only gene symbols are

used in the transcript spot files.

To flip the image, geometry coordinates, or none. Because the image has the

origin at the top left while the geometry has origin at the bottom left, one of them needs to be flipped for them to match. If one of them is already flipped,

then use "none". The image will not be flipped if it's GeoTIFF.

Maximum size of the image allowed to flip the image. Because the image will max\_flip be loaded into memory to be flipped. If the image is larger than this size then

the coordinates will be flipped instead.

filter\_counts Logical, whether to keep cells with counts > 0.

add\_molecules Logical, whether to add transcripts coordinates to an object.

min\_phred Minimum Phred score to keep spot. By default 20, the conventional threshold

indicating "acceptable", meaning that there's 1 chance that the spot was decoded

**BPPARAM** A BiocParallelParam object specifying parallel processing backend and num-

ber of threads to use for parallelizable tasks:

1. To load cell segmentation from HDF5 files from different fields of view (FOVs) with multiple cores. A progress bar can be configured in the BiocParallelParam object. When there are numerous FOVs, reading in the geometries can be time consuming, so we recommend using a server and larger number of

threads. This argument is not used if use\_cellpose = TRUE and the parquet file is present.

2. To get the largest piece and see if it's larger than min\_area when there are multiple pieces in the cell segmentation for one cell.

file\_out Name of file to save the geometry or raster to disk. Especially when the geometries are so large that it's unwieldy to load everything into memory. If this file (or directory for multiple files) already exists, then the existing file(s) will be read, skipping the processing. When writing the file, extensions supplied are

ignored and extensions are determined based on 'dest'.

flip

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#### Value

An SFE object. If reading segmentations, the cell or nuclei segmentation will be saved to 'cell\_boundaries\_sf.parquet' and 'nucleus\_boundaries\_sf.parquet' respectively in 'data.dir' so next time the boundaries can be read much more quickly. If reading transcript spots ('add\_molecules = TRUE'), then the reformatted transcript spots are saved to file specified in the 'file\_out' argument, which is by default 'tx\_spots.parquet' in the same directory as the rest of the data. If images are present, then the images will be of the BioFormatsImage class and not loaded into memory until necessary in later operations.

#### Note

Sometimes when reading images, you will see this error the first time: 'java.lang.NullPointerException: Cannot invoke "loci.formats.DimensionSwapper.setMetadataFiltered(boolean)" because "RBioFormats.reader" is null'. See this issue https://github.com/aoles/RBioFormats/issues/42 Rerun the code and it should work the second time.

## **Examples**

```
library(SFEData)
library(RBioFormats)
fp <- tempfile()
dir_use <- XeniumOutput("v2", file_path = fp)
# RBioFormats issue
try(sfe <- readXenium(dir_use, add_molecules = TRUE))
sfe <- readXenium(dir_use, add_molecules = TRUE)
unlink(dir_use, recursive = TRUE)</pre>
```

reexports

Functions re-exported from other packages

## **Description**

These are some commonly used getters and setters of classes that SFE inherits so you don't have to separately attach those packages to use these functions.

```
colData(x, ...)
rowData(x, use.names = TRUE, ...)
colData(x, ...) <- value
spatialCoords(x, ...)
spatialCoords(x) <- value</pre>
```

removeEmptySpace

```
spatialCoordsNames(x)
getImg(x, ...)
imgData(x)
rmvImg(x, ...)
counts(object, ...)
logcounts(object, ...)
reducedDim(x, type, ...)
```

#### **Arguments**

x A SummarizedExperiment object.

... For assay, arguments in ... are forwarded to assays.

For rbind, cbind, ... contains SummarizedExperiment objects to be com-

bined.

For other accessors, ignored.

use.names For rowData: Like mcols(x), by default rowData(x) propagates the rownames

of x to the returned DataFrame object (note that for a SummarizedExperiment object, the rownames are also the names i.e. rownames(x) is always the same as names(x)). Setting use.names=FALSE suppresses this propagation i.e. it returns a DataFrame object with no rownames. Use this when rowData(x) fails, which can happen when the rownames contain NAs (because the rownames of a SummarizedExperiment object can contain NAs, but the rownames of a DataFrame

object cannot).

For combineRows and combineCols: See Combining section below.

value An object of a class specified in the S4 method signature or as outlined in 'De-

tails'.

object A SingleCellExperiment object, which includes SFE.

type Name or numeric index to indicate which reducedDim to get, such as "PCA".

By default the first item in reducedDims.

removeEmptySpace Remove empty space

## **Description**

For each sample independently, all geometries and spatialCoords are translated so the origin is at the minimum coordinates of the bounding box of all geometries of the sample. This way coordinates of different samples will be more comparable. This removes empty space in the images if present.

rotateImg 65

## Usage

```
removeEmptySpace(sfe, sample_id = "all")
```

## **Arguments**

sfe An SFE object.

sample\_id Sample to remove empty space.

## Value

An SFE object with empty space removed.

## Note

Unlike other functions in this package, this function operates on all samples by default.

## **Examples**

```
library(SFEData)
library(SingleCellExperiment)
sfe <- McKellarMuscleData("full")
# Only keep spots on tissue
sfe <- sfe[, colData(sfe)$in_tissue]
# Move the coordinates of the tissue
sfe <- removeEmptySpace(sfe)</pre>
```

rotateImg

Rotate image

# **Description**

As in SpatialExperiment, rotation here must be a multiple of 90 degrees.

```
## $4 method for signature 'SpatRasterImage'
rotateImg(x, degrees, maxcell = 1e+07, ...)
## $4 method for signature 'BioFormatsImage'
rotateImg(x, degrees, ...)
## $4 method for signature 'ExtImage'
rotateImg(x, degrees, ...)
```

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## **Arguments**

x	An object of class *Image as implemented in this package.
degrees	How many degrees to rotate. Positive number means clockwise and negative number means counterclockwise.
maxcell	Max number of pixels to load SpatRasterImage into memory. The default 1e7 is chosen because this is the approximate number of pixels in the medium resolution image at resolution = 4L in Xenium OME-TIFF to make different methods of this function consistent.
	Ignored. It's there so different methods can all be passed to the same lapply in the method for SFE objects. Some methods have extra arguments.

## Value

SpatRasterImage will be loaded into memory and converted to ExtImage. Otherwise \*Image object of the same class.

## See Also

```
Other image methods: SFE-image, affineImg(), cropImg(), dim,BioFormatsImage-method, ext(), imgRaster(), imgSource(), mirrorImg(), scaleImg(), translateImg(), transposeImg()
```

rowGeometries

Row geometry getters and setters

## **Description**

rowGeometries are geometries that corresponding to rows of the gene count matrix, such as sm-FISH transcript spots. The txSpots() function is a convenience wrapper for transcript spots, although this entirely depends on the rowGeometry being named txSpots.

```
rowGeometry(x, type = 1L, sample_id = 1L, withDimnames = TRUE)
rowGeometry(
    x,
    type = 1L,
    sample_id = 1L,
    withDimnames = TRUE,
    partial = FALSE,
    translate = TRUE
) <- value
rowGeometries(x, sample_id = "all", withDimnames = TRUE)
rowGeometries(</pre>
```

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```
Χ,
  sample_id = "all",
 withDimnames = TRUE,
 partial = FALSE,
  translate = TRUE
) <- value
rowGeometryNames(x)
rowGeometryNames(x) <- value</pre>
txSpots(x, sample_id = 1L, withDimnames = TRUE)
txSpots(
  х,
  sample_id = 1L,
 withDimnames = TRUE,
 partial = FALSE,
  translate = TRUE
) <- value
```

## **Arguments**

x A SpatialFeatureExperiment object.

type An integer specifying the index or string specifying the name of the \*Geometry

to query or replace. If missing, then the first item in the \*Geometries will be

returned or replaced.

sample\_id Sample ID to get or set geometries.

withDimnames Logical. If TRUE, then the dimnames (colnames or rownames) of the gene count

matrix should correspond to row names of the sf data frames of interest.

partial In setters, if a rowGeometry of the same name exists, whether to only replace

the rows present in value.

translate Logical. Only used if removeEmptySpace has been run of the SFE object. If

that's the case, this argument indicates whether the new value to be assigned to the geometry is in the coordinates prior to removal of empty space so it should be translated to match the new coordinates after removing empty space. Default

to TRUE.

value Value to set. For dimGeometry, must be a sf data frame with the same number

of rows as size in the dimension of interest, or an ordinary data frame that can be converted to such a sf data frame (see df2sf). For dimGeometries, must be

a list of such sf or ordinary data frames.

#### **Details**

When there are multiple samples in the SFE object, rowGeometries for each sample has the sample\_id appended to the name of the geometry. For example, if the name is txSpots and the

68 sampleIDs

sample ID is sample01, then the actual name of the rowGeometry is txSpots\_sample01. In the getter, one can still specify rowGeometry(sfe, "txSpots", sample\_id = "sample01").

Appending the sample\_id is unnecessary when there is only one sample, but sample\_id will be appended when to SFE objects are combined with cbind. It is necessary to distinguish bewteen different samples because they can have overlapping coordinate values.

## See Also

```
[dimGeometries()], [colGeometries()]
```

## **Examples**

```
library(SFEData)
library(RBioFormats)
fp <- tempfile()
dir_use <- XeniumOutput("v2", file_path = fp)
# RBioFormats issue
try(sfe <- readXenium(dir_use, add_molecules = TRUE))
sfe <- readXenium(dir_use, add_molecules = TRUE)
rowGeometries(sfe)
rowGeometryNames(sfe)
tx <- rowGeometry(sfe, "txSpots")
txSpots(sfe)
unlink(dir_use, recursive = TRUE)</pre>
```

sampleIDs

Get all unique sample IDs

## **Description**

The title is self-explanatory.

#### Usage

```
sampleIDs(sfe)
```

## **Arguments**

sfe

A SpatialFeatureExperiment object.

#### Value

A character vector of all unique entries of the sample\_id column in colData(x).

## **Examples**

```
library(SFEData)
sfe <- McKellarMuscleData(dataset = "small")
sampleIDs(sfe)</pre>
```

```
save {\tt RDS}, {\tt Spatial Feature Experiment-method} \\ Save {\tt Spatial Feature Experiment\ as\ RDS\ file}
```

## **Description**

Saving SFE objects as RDS files is complicated by the SpatRaster class of the images. If present, the images need to be wrapped with the wrap function in terra before serializing the SFE object. Otherwise the images will be invalid pointers when the RDS is reloaded. If the image does not fit in memory and its file source is unknown, then it will be written to a temporary file, which is reloaded when the RDS file is loaded. When an SFE object with images is read from an RDS file, the images will not be unwrapped until necessary.

## Usage

```
## S4 method for signature 'SpatialFeatureExperiment'
saveRDS(
  object,
  file = "",
  ascii = FALSE,
  version = NULL,
  compress = TRUE,
  refhook = NULL
)
```

# Arguments

object	:	A SpatialFeatureExperiment object.
file		a connection or the name of the file where the ${\sf R}$ object is saved to or read from.
ascii		a logical. If TRUE or NA, an ASCII representation is written; otherwise (default), a binary one is used. See the comments in the help for save.
versio	on	the workspace format version to use. NULL specifies the current default version (3). The only other supported value is 2, the default from R $1.4.0$ to R $3.5.0$ .
compre	ess	a logical specifying whether saving to a named file is to use "gzip" compression, or one of "gzip", "bzip2" or "xz" to indicate the type of compression to be used. Ignored if file is a connection.
refhoo	ok	a hook function for handling reference objects.

## Value

Invisibly NULL.

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## **Examples**

```
outdir <- system.file("extdata", package = "SpatialFeatureExperiment")
samples <- file.path(outdir, paste0("sample0", 1:2))
sfe <- read10xVisiumSFE(samples, type = "sparse", data = "filtered")
saveRDS(sfe, "foo.rds")
# Clean up
file.remove("foo.rds")</pre>
```

scaleImg

Scale image

## **Description**

This function scales the image about its center. After scaling, the center of the image is not shifted.

## Usage

```
## S4 method for signature 'AlignedSpatialImage'
scaleImg(x, factor, ...)
```

## **Arguments**

An object of class \*Image as implemented in this package.
 Numeric, scaling factor.
 Ignored. It's there so different methods can all be passed to the same lapply in the method for SFE objects. Some methods have extra arguments.

## Value

A \*Image object of the same class that has been scaled. Behind the scene, it's only the extent that has been changed and the images are not changed. The center of the image is unchanged.

#### See Also

```
Other image methods: SFE-image, affineImg(), cropImg(), dim,BioFormatsImage-method, ext(), imgRaster(), imgSource(), mirrorImg(), rotateImg(), translateImg(), transposeImg()
```

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SFE-image

Methods for handling image-related data

## **Description**

Generics of these functions are defined in SpatialExperiment, except for transposeImg. These SFE methods cater to the new image-related classes in SFE. The SPE method for getImg, rmvImg, and imgRaster don't need to be modified for SFE and are hence not implemented here, but are simply re-exported.

```
## S4 method for signature 'SpatialFeatureExperiment'
addImg(x, imageSource, sample_id = 1L, image_id, extent = NULL, scale_fct = 1)
## S4 method for signature 'SpatialFeatureExperiment'
transposeImg(
  х,
  sample_id = 1L,
  image_id = NULL,
 maxcell = 1e+07,
  filename = ""
)
## S4 method for signature 'SpatialFeatureExperiment'
mirrorImg(
 Х,
  sample_id = 1L,
  image_id = NULL,
  direction = "vertical",
 maxcell = 1e+07,
  filename = ""
)
## S4 method for signature 'SpatialFeatureExperiment'
rotateImg(x, sample_id = 1L, image_id = NULL, degrees, maxcell = 1e+07)
## S4 method for signature 'SpatialFeatureExperiment'
translateImg(x, sample_id = 1L, image_id = NULL, v)
## S4 method for signature 'SpatialFeatureExperiment'
scaleImg(x, sample_id = 1L, image_id = NULL, factor)
## S4 method for signature 'SpatialFeatureExperiment'
affineImg(x, sample_id = 1L, image_id = NULL, M, v)
```

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## **Arguments**

x	A SFE object.
imageSource	a character string specifying an image file name (.png, .jpg or .tif) or URL to source the image from
sample_id	Which sample the image is associated with. Use sampleIDs to get sample IDs present in the SFE object.
image_id	Image ID, such as "lowres" and "hires" for Visium data and "DAPI" and "PolyT" for Vizgen MERFISH data.
extent	A numeric vector of length 4 with names of the set xmin, ymin, xmax, and ymax, specifying the extent of the image.
scale_fct	Scale factor – multiply pixel coordinates in full resolution image by this scale factor should yield pixel coordinates in a different resolution. extent takes precedence over scale_fct.
maxcell	Max number of pixels to load SpatRasterImage into memory. The default 1e7 is chosen because this is the approximate number of pixels in the medium resolution image at resolution = 4L in Xenium OME-TIFF to make different methods of this function consistent.
filename	character. Output filename
direction	character. Should (partially) match "vertical" to flip by rows, or "horizontal" to flip by columns
degrees	How many degrees to rotate. Positive number means clockwise and negative number means counterclockwise.
V	A numeric vector of length 2 specifying the vector in the xy plane to translate the SFE object.
factor	Numeric, scaling factor.
М	A 2x2 numeric matrix for the linear transformation in the xy plane.

## **Details**

Method of transposeImg, mirrorImg, and rotateImg perform the method on all images within the SFE object that are specified with sample\_id and image\_id. For images that are not loaded into memory, rotateImg will load SpatRasterImage into memory and all image operations except translate will load BioFormatsImage into memory.

## Note

If the image is already a GeoTIFF file that already has an extent, then the extent associated with the file will be honored and the extent and scale\_fct arguments are ignored. Transposing the image is just like transposing a matrix. It's flipped about the line going from the top left to the bottom right.

#### See Also

Other image methods: affineImg(), cropImg(), dim, BioFormatsImage-method, ext(), imgRaster(), imgSource(), mirrorImg(), rotateImg(), scaleImg(), translateImg(), transposeImg()

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#### **Examples**

```
library(SFEData)
sfe <- McKellarMuscleData("small")
img_path <- system.file(file.path("extdata", "sample01", "outs", "spatial",
    "tissue_lowres_image.png"), package = "SpatialFeatureExperiment")
sfe <- addImg(sfe, img_path, sample_id = "Vis5A", image_id = "lowres", scale_fct =
0.023)
img <- getImg(sfe)
# SpatRasterImage method
img_t <- transposeImg(img)
# SFE method
sfe <- transposeImg(sfe, sample_id = "Vis5A", image_id = "lowres")</pre>
```

SFE-transform

Affine transfortaion of SFE object in histological space

## **Description**

These functions perform affine transformations on SFE objects, including all geometries and images. The transformation is performed on each sample as a whole. This differs from functions such as mirrorImg in that mirrorImg and rotateImg transform the image with the center at the center of the image itself. In contrast, the center of transformation here is the center of the bounding box of the entire sample, including images.

## Usage

```
transpose(sfe, sample_id = "all", maxcell = NULL, filename = "")
mirror(
    sfe,
    sample_id = "all",
    direction = c("vertical", "horizontal"),
    maxcell = NULL,
    filename = ""
)

rotate(sfe, sample_id = "all", degrees, maxcell = 1e+07)

translate(sfe, sample_id = "all", v)

scale(sfe, sample_id = "all", factor)

affine(sfe, sample_id = "all", M, v, maxcell = 1e+07)
```

## Arguments

sfe

An SFE object.

sample_id	Sample(s) to transform.
maxcell	Rotating SpatRasterImage will convert it into ExtImage, loading the image into memory. This argument specifies the maximum number of pixels in the image loaded into memory. The image will be down sampled to approximately this number of pixels.
filename	character. Output filename
direction	character. Should (partially) match "vertical" to flip by rows, or "horizontal" to flip by columns
degrees	How many degrees to rotate. Positive number means clockwise and negative number means counterclockwise.
V	Vector to spatially translate the SFE object.
factor	Numeric, scaling factor.
М	A 2x2 numeric matrix for the linear transformation in the xy plane.

#### **Details**

For images that are not loaded into memory, rotateImg will load SpatRasterImage into memory and all image operations except translate will load BioFormatsImage into memory.

#### Value

An SFE object with the sample(s) transformed.

## **Examples**

```
library(SFEData)
sfe <- McKellarMuscleData("small")
sfe2 <- transpose(sfe)
sfe3 <- mirror(sfe)</pre>
```

show, SpatialFeatureExperiment-method

Print method for SpatialFeatureExperiment

## **Description**

Printing summaries of colGeometries, rowGeometries, and annotGeometries in addition to what's shown for SpatialExperiment. Geometry names and types are printed.

## Usage

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

## **Arguments**

object

A SpatialFeatureExperiment object.

#### Value

None (invisible NULL).

#### **Examples**

```
library(SFEData)
sfe <- McKellarMuscleData(dataset = "small")
sfe # The show method is implicitly called</pre>
```

SpatialFeatureExperiment

Constructor of SpatialFeatureExperiment object

## Description

Create a SpatialFeatureExperiment object.

## Usage

```
SpatialFeatureExperiment(
   assays,
   colData = DataFrame(),
   rowData = NULL,
   sample_id = "sample01",
   spatialCoordsNames = c("x", "y"),
   spatialCoords = NULL,
   colGeometries = NULL,
   rowGeometries = NULL,
   annotGeometries = NULL,
   annotGeometryType = "POLYGON",
   spatialGraphs = NULL,
   unit = c("full_res_image_pixel", "micron"),
   ...
)
```

# **Arguments**

assays

A list or SimpleList of matrix-like elements, or a matrix-like object (e.g. an ordinary matrix, a data frame, a DataFrame object from the **S4Vectors** package, a sparseMatrix derivative from the **Matrix** package, a DelayedMatrix object from the **DelayedArray** package, etc...). All elements of the list must have the same dimensions, and dimension names (if present) must be consistent across elements and with the row names of rowRanges and colData.

colData An optional DataFrame describing the samples. Row names, if present, become

the column names of the RangedSummarizedExperiment.

A DataFrame object describing the rows. Row names, if present, become the rowData row names of the SummarizedExperiment object. The number of rows of the

DataFrame must equal the number of rows of the matrices in assays.

A character sample identifier, which matches the sample\_id in imgData. The sample\_id

sample\_id will also be stored in a new column in colData, if not already

present. Default = sample01.

spatialCoordsNames

A character vector of column names if \*Geometries arguments have ordinary data frames, to identify the columns in the ordinary data frames that specify the spatial coordinates. If colGeometries is not specified, then this argument will behave as in SpatialExperiment, but colGeometries will be given prece-

dence if provided.

spatialCoords A numeric matrix containing columns of spatial coordinates, as in SpatialExperiment.

> The coordinates are centroids of the entities represented by the columns of the gene count matrix. If colGeometries is also specified, then it will be given priority and a warning is issued. Otherwise, the sf representation of the centroids

will be stored in the colGeometry called centroids.

colGeometries Geometry of the entities that correspond to the columns of the gene count matrix, such as cells and Visium spots. It must be a named list of one of the following:

An sf data frame The geometry column specifies the geometry of the entities.

An ordinary data frame specifying centroids Column names for the coordinates are specified in the spatialCoordsNames argument. For Visium and ST, in addition to the centroid coordinate data frame, the spot diameter in the same unit as the coordinates can be specified in the spotDiamter argu-

**An ordinary data frame specifying polygons** Also use spatialCoordsNames. There should an additional column "ID" to specify which vertices belong to which polygon. The coordinates should not be in list columns. Rather, the data frame should look like it is passed to ggplot2::geom\_polygon. If there are holes, then there must also be a column "subID" that differentiates between the outer polygon and the holes.

In all cases, the data frame should specify the same number of geometries as the number of columns in the gene count matrix. If the column "barcode" is present, then it will be matched to column names of the gene count matrix. Otherwise, the geometries are assumed to be in the same order as columns in the gene count matrix. If the geometries are specified in an ordinary data frame, then it will be converted into sf internally. Named list of data frames because each entity can have multiple geometries, such as whole cell and nuclei segmentations. The geometries are assumed to be POINTs for centroids and POLYGONs for segmentations. If polygons are specified in an ordinary data frame, then anything with fewer than 3 vertices will be removed. For anything other than POINTs, attributes of the geometry will be ignored.

Geometry associated with genes or features, which correspond to rows of the gene count matrix.

rowGeometries

annotGeometries

Geometry of entities that do not correspond to columns or rows of the gene count matrix, such as tissue boundary and pathologist annotations of histological regions, and nuclei segmentation in a Visium dataset. Also a named list as in colGeometries. The ordinary data frame may specify POINTs, POLYGONs, or LINESTRINGs, or their MULTI versions. Each data frame can only specify one type of geometry. For MULTI versions, there must be a column "group" to identify each MULTI geometry.

spotDiameter

Spot diameter for technologies with arrays of spots of fixed diameter per slide, such as Visium, ST, DBiT-seq, and slide-seq. The diameter must be in the same unit as the coordinates in the \*Geometry arguments. Ignored for geometries that are not POINT or MULTIPOINT.

annotGeometryType

Character vector specifying geometry type of each element of the list if annotGeometry is specified. Each element of the vector must be one of POINT, LINESTRING, POLYGON, MULTIPOINT, MULTILINESTRING, and MULTIPOLYGON. Must be either length 1 (same for all elements of the list) or the same length as the list. Ignored if the corresponding element is an sf object.

spatialGraphs

A named list of listw objects (see spdep) for spatial neighborhood graphs.

unit

Unit the coordinates are in, either microns or pixels in full resolution image.

.. Additional arguments passed to the SpatialExperiment and SingleCellExperiment

constructors.

#### Value

A SFE object. If neither colGeometries nor spotDiameter is specified, then a colGeometry called "centroids" will be made, which is essentially the spatial coordinates as sf POINTs. If spotDiameter is specified, but not colGeometries, then the spatial coordinates will be buffered by half the diameter to get spots with the desired diameter, and the resulting colGeometry will be called "spotPoly", for which there's a convenience getter and setter, spotPoly.

```
library(Matrix)
data("visium_row_col")
coords1 <- visium_row_col[visium_row_col$col < 6 & visium_row_col$row < 6, ]
coords1$row <- coords1$row * sqrt(3)
cg <- df2sf(coords1[, c("col", "row")], c("col", "row"), spotDiameter = 0.7)
set.seed(29)
col_inds <- sample(seq_len(13), 13)
row_inds <- sample(seq_len(5), 13, replace = TRUE)
values <- sample(seq_len(5), 13, replace = TRUE)
mat <- sparseMatrix(i = row_inds, j = col_inds, x = values)
colnames(mat) <- coords1$barcode
rownames(mat) <- sample(LETTERS, 5)
rownames(cg) <- colnames(mat)
sfe <- SpatialFeatureExperiment(list(counts = mat),</pre>
```

```
colData = coords1,
   spatialCoordsNames = c("col", "row"),
   spotDiameter = 0.7
)
sfe2 <- SpatialFeatureExperiment(list(counts = mat),
   colGeometries = list(foo = cg)
)</pre>
```

SpatialFeatureExperiment-class

The SpatialFeatureExperiment class

## **Description**

This class inherits from the SpatialExperiment (SPE) class, which in turn inherits from SingleCellExperiment (SCE). SpatialFeatureExperiment stores geometries of spots or cells in sf objects which form columns of a DataFrame which is in turn a column of the int\_colData DataFrame of the underlying SCE object, just like reducedDim in SCE. Geometries of the tissue outline, pathologist annotations, and objects (e.g. nuclei segmentation in a Visium dataset) are stored in sf objects in a named list called annotGeometries in int\_metadata.

SpatialFeatureExperiment-coercion

SpatialFeatureExperiment coercion methods

## **Description**

The SpatialFeatureExperiment class inherits from SpatialExperiment, which in turn inherits from SingleCellExperiment. A SpatialExperiment object with geometries in colGeometries in the int\_colData, rowGeometries in the int\_elementMetadata, or annotGeometries in the int\_metadata can be directly converted to SpatialFeatureExperiment with as(spe, "SpatialFeatureExperiment"). A SpatialExperiment object without the geometries can also be converted; the coordinates in the spatialCoords field will be used to make POINT geometries named "centroids" to add to colGeometries. The geometries can also be supplied separately when using toSpatialFeatureExperiment. Images are converted to SpatRaster.

# Usage

```
## S4 method for signature 'SpatialExperiment'
toSpatialFeatureExperiment(
    x,
    colGeometries = NULL,
    rowGeometries = NULL,
    annotGeometries = NULL,
    spatialCoordsNames = c("x", "y"),
```

```
annotGeometryType = "POLYGON",
  spatialGraphs = NULL,
  spotDiameter = NA,
  unit = NULL
)
## S4 method for signature 'SingleCellExperiment'
toSpatialFeatureExperiment(
  х,
  sample_id = "sample01",
  spatialCoordsNames = c("x", "y"),
  spatialCoords = NULL,
  colGeometries = NULL,
  rowGeometries = NULL,
  annotGeometries = NULL,
  annotGeometryType = "POLYGON",
  spatialGraphs = NULL,
  spotDiameter = NA,
  scaleFactors = 1,
  imageSources = NULL,
  image_id = NULL,
  loadImage = TRUE,
  imgData = NULL,
  unit = NULL
)
## S4 method for signature 'Seurat'
toSpatialFeatureExperiment(
  add_molecules = TRUE,
  flip = c("geometry", "image", "none"),
  image_scalefactors = c("lowres", "hires"),
  unit = NULL,
 BPPARAM = SerialParam()
```

#### **Arguments**

 $\label{lem:condition} A \ {\tt SpatialExperiment} \ or \ {\tt Seurat} \ object \ to \ be \ coerced \ to \ a \ {\tt SpatialFeatureExperiment} \ object.$ 

colGeometries

Geometry of the entities that correspond to the columns of the gene count matrix, such as cells and Visium spots. It must be a named list of one of the following:

An sf data frame The geometry column specifies the geometry of the entities.

An ordinary data frame specifying centroids Column names for the coordi-

n ordinary data frame specifying centroids Column names for the coordinates are specified in the spatialCoordsNames argument. For Visium and ST, in addition to the centroid coordinate data frame, the spot diameter in the same unit as the coordinates can be specified in the spotDiamter argument.

An ordinary data frame specifying polygons Also use spatialCoordsNames. There should an additional column "ID" to specify which vertices belong to which polygon. The coordinates should not be in list columns. Rather, the data frame should look like it is passed to ggplot2::geom\_polygon. If there are holes, then there must also be a column "subID" that differentiates between the outer polygon and the holes.

In all cases, the data frame should specify the same number of geometries as the number of columns in the gene count matrix. If the column "barcode" is present, then it will be matched to column names of the gene count matrix. Otherwise, the geometries are assumed to be in the same order as columns in the gene count matrix. If the geometries are specified in an ordinary data frame, then it will be converted into sf internally. Named list of data frames because each entity can have multiple geometries, such as whole cell and nuclei segmentations. The geometries are assumed to be POINTs for centroids and POLYGONs for segmentations. If polygons are specified in an ordinary data frame, then anything with fewer than 3 vertices will be removed. For anything other than POINTs, attributes of the geometry will be ignored.

rowGeometries

Geometry associated with genes or features, which correspond to rows of the gene count matrix.

annotGeometries

Geometry of entities that do not correspond to columns or rows of the gene count matrix, such as tissue boundary and pathologist annotations of histological regions, and nuclei segmentation in a Visium dataset. Also a named list as in colGeometries. The ordinary data frame may specify POINTs, POLYGONs, or LINESTRINGs, or their MULTI versions. Each data frame can only specify one type of geometry. For MULTI versions, there must be a column "group" to identify each MULTI geometry.

#### spatialCoordsNames

A character vector of column names if \*Geometries arguments have ordinary data frames, to identify the columns in the ordinary data frames that specify the spatial coordinates. If colGeometries is not specified, then this argument will behave as in SpatialExperiment, but colGeometries will be given precedence if provided.

## annotGeometryType

Character vector specifying geometry type of each element of the list if annotGeometry is specified. Each element of the vector must be one of POINT, LINESTRING, POLYGON, MULTIPOINT, MULTILINESTRING, and MULTIPOLYGON. Must be either length 1 (same for all elements of the list) or the same length as the list. Ignored if the corresponding element is an sf object.

spatialGraphs

A named list of listw objects (see spdep) for spatial neighborhood graphs.

spotDiameter

Spot diameter for technologies with arrays of spots of fixed diameter per slide, such as Visium, ST, DBiT-seq, and slide-seq. The diameter must be in the same unit as the coordinates in the \*Geometry arguments. Ignored for geometries that are not POINT or MULTIPOINT.

unit

# Default unit is "micron". However for Visium one can choose between "micron" or "full\_res\_image\_pixel".

sample\_id A character sample identifier, which matches the sample\_id in imgData. The

 ${\tt sample\_id} \ will \ also \ be \ stored \ in \ a \ new \ column \ in \ colData, \ if \ not \ already$ 

present. Default = sample01.

spatialCoords A numeric matrix containing columns of spatial coordinates, as in SpatialExperiment.

The coordinates are centroids of the entities represented by the columns of the gene count matrix. If colGeometries is also specified, then it will be given priority and a warning is issued. Otherwise, the sf representation of the centroids

will be stored in the colGeometry called centroids.

scaleFactors Optional scale factors associated with the image(s). This can be provided as

a numeric value, numeric vector, list, or file path to a JSON file for the 10x Genomics Visium platform. For 10x Genomics Visium, the correct scale factor will automatically be selected depending on the resolution of the image from

imageSources. Default = 1.

imageSources Optional file path(s) or URL(s) for one or more image sources.

image\_id Optional character vector (same length as imageSources) containing unique

image identifiers.

loadImage Logical indicating whether to load image into memory. Default = FALSE.

imgData Optional DataFrame containing the image data. Alternatively, this can be built

from the arguments imageSources and image\_id (see Details).

add\_molecules Logical, whether to add transcripts coordinates to an object.

flip To flip the image, geometry coordinates, or none. Because the image has the

origin at the top left while the geometry has origin at the bottom left, one of them needs to be flipped for them to match. If one of them is already flipped.

then use "none". The image will not be flipped if it's GeoTIFF.

image\_scalefactors

# A character, choose between "lowres" or "hires". Only for 10X Visium,

image scaling factors are from 'scalefactors\_json.json' file.

BPPARAM Deprecated when coercing from SpatialExperiment, but is used when coerc-

ing from Seurat object.

## Value

A SpatialFeatureExperiment object

```
library(SpatialExperiment)
example(read10xVisium)
# There can't be duplicate barcodes
colnames(spe) <- make.unique(colnames(spe), sep = "-")
rownames(spatialCoords(spe)) <- colnames(spe)
sfe <- toSpatialFeatureExperiment(spe)
# For coercing Seurat to SFE see this -> ./vignettes/seurat_sfe_coerce.Rmd
```

SpatialFeatureExperiment-subset

Subsetting SpatialFeatureExperiment objects

#### **Description**

The method for SFE reconstructs the spatial graphs when the SFE object is subsetted as the listw objects encodes the nodes with indices which are no longer valid after subsetting as some nodes are no longer present.

## Usage

```
## S4 method for signature 'SpatialFeatureExperiment,ANY,ANY,ANY' x[i, j, ..., drop = FALSE]
```

## **Arguments**

Χ	A SpatialFeatureExperiment object.

i Row indices for subsetting.

j column indices for subsetting.

... Passed to the SingleCellExperiment method of [.

drop Logical. If FALSE, then a warning will be issued that the node indices in the

graphs are no longer valid so the row and col graphs affected by subsetting are dropped. At present, this only works with the wrapper functions in this package that take in SFE objects and records the info required to reconstruct the graphs.

While this argument is ignored for SummarizedExperiment

## Value

A subsetted SpatialFeatureExperiment object.

```
# Just like subsetting matrices and SingleCellExperiment
library(SFEData)
sfe <- McKellarMuscleData(dataset = "small")
sfe_subset <- sfe[seq_len(10), seq_len(10), drop = TRUE]
# Gives warning as graph reconstruction fails
sfe_subset <- sfe[seq_len(10), seq_len(10)]</pre>
```

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spatialGraphs

Spatial graph methods

#### **Description**

Spatial neighborhood graphs as spdep's listw objects are stored in the int\_metadata of the SFE object. The listw class is used because spdep has many useful methods that rely on the neighborhood graph as listw.

## Usage

```
## S4 method for signature 'SpatialFeatureExperiment'
spatialGraphs(x, MARGIN = NULL, sample_id = "all", name = "all")
colGraphs(x, sample_id = "all", name = "all")
rowGraphs(x, sample_id = "all", name = "all")
annotGraphs(x, sample_id = "all", name = "all")
## S4 replacement method for signature 'SpatialFeatureExperiment'
spatialGraphs(x, MARGIN = NULL, sample_id = "all", name = "all") <- value</pre>
colGraphs(x, sample_id = "all", name = "all") <- value</pre>
rowGraphs(x, sample_id = "all", name = "all") <- value</pre>
annotGraphs(x, sample_id = "all", name = "all") <- value</pre>
## S4 method for signature 'SpatialFeatureExperiment, numeric'
spatialGraphNames(x, MARGIN, sample_id = 1L)
## S4 replacement method for signature 'SpatialFeatureExperiment,numeric,ANY,character'
spatialGraphNames(x, MARGIN, sample_id = 1L) <- value</pre>
colGraphNames(x, sample_id = 1L)
rowGraphNames(x, sample_id = 1L)
annotGraphNames(x, sample_id = 1L)
colGraphNames(x, sample_id = 1L) <- value</pre>
rowGraphNames(x, sample_id = 1L) <- value</pre>
annotGraphNames(x, sample_id = 1L) <- value</pre>
```

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```
## S4 method for signature 'SpatialFeatureExperiment'
spatialGraph(x, type = 1L, MARGIN, sample_id = 1L)

colGraph(x, type = 1L, sample_id = 1L)

rowGraph(x, type = 1L, sample_id = 1L)

annotGraph(x, type = 1L, sample_id = 1L)

## S4 replacement method for signature 'SpatialFeatureExperiment'
spatialGraph(x, type = 1L, MARGIN, sample_id = NULL) <- value

colGraph(x, type = 1L, sample_id = 1L) <- value

rowGraph(x, type = 1L, sample_id = 1L) <- value

annotGraph(x, type = 1L, sample_id = 1L) <- value</pre>
```

#### **Arguments**

X	A SpatialFeatureExperiment object
^	11 Spatiali catal CExpel Illelle object

MARGIN As in apply. 1 stands for rows and 2 stands for columns. In addition, 3 stands

for spatial neighborhood graphs that correspond to annotGeometries.

sample\_id Name of the sample the graph is associated with. This is useful when multiple

pieces of tissues are in the same SFE object (say for a joint dimension reduction and clustering) and the spatial neighborhood is only meaningful within the same

piece of tissue. See the sample\_id argument in SpatialExperiment.

name Name of the graphs to add to each sample\_id; used in the spatialGraphs re-

placement method as it must be character while type can be either an integer

index or a name.

value A listw object (\*Graph), or a named list of listw objects (\*Graphs)

where the names of the top level list are sample\_ids when adding graphs for all samples in the margin of interest, or a list of listw objects when adding graphs

for one sample in one margin.

type An integer specifying the index or string specifying the name of the \*Graph to

query or replace. If missing, then the first item in the \*Graph will be returned or

replaced.

#### Value

Getters for multiple graphs return a named list. Getters for names return a character vector of the names. Getters for single graphs return a listw object. Setters return an SFE object.

```
library(SFEData)
sfe <- McKellarMuscleData(dataset = "small")
g1 <- findVisiumGraph(sfe)</pre>
```

SpatRasterImage 85

```
g2 <- findSpatialNeighbors(sfe)</pre>
# Set all graphs of a margin by a named list
spatialGraphs(sfe, MARGIN = 2L, sample_id = "Vis5A") <-</pre>
    list(tri2nb = g2, visium = g1)
# Or equivalently
colGraphs(sfe, sample_id = "Vis5A") <- list(tri2nb = g2, visium = g1)</pre>
# Get all graphs of a margin, returning a named list
gs <- spatialGraphs(sfe, MARGIN = 2L)</pre>
# Or equivalently
gs <- colGraphs(sfe)</pre>
# Set graph of the same name and same margin for multiple samples
# Each sample has a separate graph
sfe2 <- McKellarMuscleData("small2")</pre>
sfe_combined <- cbind(sfe, sfe2)</pre>
colGraphs(sfe_combined, name = "visium", sample_id = "all") <-</pre>
    findVisiumGraph(sfe_combined, sample_id = "all")
# Get graph names
spatialGraphNames(sfe, MARGIN = 2L, sample_id = "Vis5A")
# Or equivalently (sample_id optional as only one sample is present)
colGraphNames(sfe)
# Set graph names
spatialGraphNames(sfe, MARGIN = 2L) <- c("foo", "bar")</pre>
colGraphNames(sfe) <- c("tri2nb", "visium")</pre>
# MARGIN = 1 means rowGraphs; MARGIN = 3 means annotation graphs (annotGraphs)
# for both getters and setters
# Set single graph by
# Spatial graph for myofibers
g_myofiber <- findSpatialNeighbors(sfe,</pre>
    type = "myofiber_simplified",
   MARGIN = 3L
spatialGraph(sfe, type = "myofiber", MARGIN = 3L) <- g_myofiber</pre>
# Or equivalently
annotGraph(sfe, "myofiber") <- g_myofiber</pre>
# Get a specific graph by name
g <- spatialGraph(sfe, "myofiber", MARGIN = 3L)</pre>
g2 <- spatialGraph(sfe, "visium", MARGIN = 2L)</pre>
# Or equivalently
g <- annotGraph(sfe, "myofiber")</pre>
g2 <- colGraph(sfe, "visium")</pre>
```

86 splitByCol

## **Description**

SpatialFeatureExperiment and the Voyager package work with images differently from SpatialExperiment. In SFE and Voyager's, plotting functions for SFE objects, the images can be read with rast and represented as SpatRaster, so the image is not entirely loaded into memory unless necessary. Plotting will not load a large image into memory; rather the image will be downsampled and the downsampled version is plotted. A SpatRasterImage object (as of Bioc 3.19 or SFE version 1.6 and above) is a SpatRaster object but also inheriting from VirtualSpatialImage as required by SpatialExperiment.

## Usage

```
SpatRasterImage(img)
## S4 method for signature 'SpatRasterImage'
show(object)
```

## **Arguments**

img A SpatRaster or PackedSpatRaster object.

object A SpatRasterImage object.

#### Value

A SpatRasterImage object.

## **Examples**

# Example code

splitByCol

Split SFE object with categorical vector or geometry

#### **Description**

The split methods for SFE split an SFE object into multiple SFE objects by geometries (all cells/spots intersecting with each geometry will become a separate SFE object). The splitSamples function splits the SFE object by sample\_id so each sample will become a separate SFE object. The splitContiguity function splits the SFE object by contiguity of an annotGeometry, which by default is "tissueBoundary".

# Usage

```
## S4 method for signature 'SpatialFeatureExperiment,sf'
splitByCol(x, f, sample_id = "all", colGeometryName = 1L, cover = FALSE)
## S4 method for signature 'SpatialFeatureExperiment,sfc'
splitByCol(x, f, sample_id = 1L, colGeometryName = 1L, cover = FALSE)
```

splitByCol 87

```
## S4 method for signature 'SpatialFeatureExperiment,list'
splitByCol(x, f, sample_id = "all", colGeometryName = 1L, cover = FALSE)

splitSamples(x)

splitContiguity(
    x,
    colGeometryName = 1L,
    annotGeometryName = "tissueBoundary",
    min_area = 0,
    cover = FALSE
)
```

## **Arguments**

x An SFE object

f

It can be a sf data frame or sfc to split by geometry. Each row of the sf data frame or each element in the sfc will correspond to a new SFE object. The sf data frame must have a column sample\_id when splitting multiple samples. Can also be a list of sfc whose names correspond to sample\_ids to split.

sample\_id

Which samples to split.

colGeometryName

Which colGeometry to use to determine which cells or spots should belong to which new SFE object when splitting by sf or sfc. Default to the first one.

cover

Logical, whether the geometries in x must be entirely covered by y if op = st\_intersection or whether x must be entirely outside y if op = st\_difference. Only relevant when keep\_whole != "none".

annotGeometryName

Name of annotGeometry to use to split by contiguity.

min\_area

Minimum area in the same unit as the geometry coordinates (squared) for each piece to be considered a separate piece when splitting by contiguity. Only pieces that are large enough are considered.

#### Value

A list of SFE objects.

```
# example code
```

88 st\_any\_pred

st\_any\_pred

Simple geometry predicates

## **Description**

Unlike functions in sf like st\_intersects, this function simply returns a logical vector indicating whether each geometry in x intersects (or returns TRUE from other predicates) anything in y, preferably when y only contains a small number of geometries or is one single MULTI geometry. This is useful when cropping or subsetting an SFE object with a geometry, such as tissue boundary or histological region polygons or a bounding box.

## Usage

```
st_any_pred(x, y, pred)
st_any_intersects(x, y)
st_n_pred(x, y, pred)
st_n_intersects(x, y)
```

#### **Arguments**

x An object of class sf, sfc, or sfg.
 y Another object of class sf, sfc, or sfg.
 pred A geometric binary predicate function, such as st\_intersects. It should return an object of class sgbp, for sparse predicates.

#### Value

For st\_any\_\*, a logical vector indicating whether each geometry in x intersects (or other predicates such as is covered by) anything in y. Simplified from the sgbp results which indicate which item in y each item in x intersects, which might not always be relevant. For st\_n\_\*, an integer vector indicating the number of geometries in y returns TRUE for each geometry in x.

```
library(sf)
pts <- st_sfc(
    st_point(c(.5, .5)), st_point(c(1.5, 1.5)),
    st_point(c(2.5, 2.5))
)
pol <- st_polygon(list(rbind(c(0, 0), c(2, 0), c(2, 2), c(0, 2), c(0, 0))))
st_any_pred(pts, pol, pred = st_disjoint)
st_any_intersects(pts, pol)
st_n_pred(pts, pol, pred = st_disjoint)
st_n_intersects(pts, pol)</pre>
```

toExtImage 89

toExtImage	Convert images to ExtImage	

#### **Description**

The ExtImage class is a thin wrapper around the Image class in ExtImage so it inherits from VirtualSpatialImage as required by SpatialExperiment and has extent as used in Voyager's plotting functions. This function converts SpatRasterImage (thin wrapper around the class in terra) and BioFormatsImage into ExtImage for image operations as implemented in the ExtImage package.

## Usage

```
## S4 method for signature 'BioFormatsImage'
toExtImage(x, resolution = 4L, channel = NULL)
## S4 method for signature 'SpatRasterImage'
toExtImage(x, maxcell = 1e+07, channel = NULL)
```

#### **Arguments**

x Either a BioFormatsImage or SpatRasterImage object.

resolution Integer, which resolution in the BioFormatsImage to read and convert. Defaults

to 4, which is a lower resolution. Ignored if only 1 resolution is present.

channel Integer vector to indicate channel(s) to read. If NULL, then all channels will be

read.

maxcell Maximum number of pixels when SpatRasterImage is read into memory.

## Value

A ExtImage object. The image is loaded into memory.

#### See Also

toSpatRasterImage

|--|--|

## **Description**

The resolution specified from the OME-TIFF file will be read into memory and written to disk as a GeoTIFF file that has the extent. The output file will have the same file name as the input file except without the ome in the extension.

90 translateImg

## Usage

```
## S4 method for signature 'ExtImage'
toSpatRasterImage(
    x,
    save_geotiff = TRUE,
    file_out = "img.tiff",
    overwrite = FALSE
)

## S4 method for signature 'BioFormatsImage'
toSpatRasterImage(
    x,
    save_geotiff = TRUE,
    resolution = 4L,
    channel = NULL,
    overwrite = FALSE
)
```

# Arguments

X	Either a BioFormatsImage or EBIImage object.
save_geotiff	Logical, whether to save the image to GeoTIFF file.
file_out	File to save the non-OME TIFF file for SpatRaster.
overwrite	Logical, whether to overwrite existing file of the same name.
resolution	Integer, which resolution in the BioFormatsImage to read and convert. Defaults to 4, which is a lower resolution. Ignored if only 1 resolution is present.
channel	Integer vector to indicate channel(s) to read. If NULL, then all channels will be read.

# Value

A SpatRasterImage object

# See Also

toExtImage

# Description

This function shifts the spatial extent of the image in the x-y plane.

transposeImg 91

#### Usage

```
## S4 method for signature 'SpatRasterImage'
translateImg(x, v, ...)
## S4 method for signature 'BioFormatsImage'
translateImg(x, v, ...)
## S4 method for signature 'ExtImage'
translateImg(x, v, ...)
```

## **Arguments**

x An object of class \*Image as implemented in this package.

v Numeric vector of length 2 to shift the image in the x-y plane.

Ignored. It's there so different methods can all be passed to the same lapply in the method for SFE objects. Some methods have extra arguments.

#### Value

A \*Image object of the same class that has been shifted in space.

#### See Also

```
Other image methods: SFE-image, affineImg(), cropImg(), dim,BioFormatsImage-method, ext(), imgRaster(), imgSource(), mirrorImg(), rotateImg(), scaleImg(), transposeImg()
```

transposeImg

Transpose images

## **Description**

Swap rows and columns of images. In effect, this will flip the image around the diagonal running from top left to bottom right.

# Usage

```
## S4 method for signature 'SpatRasterImage'
transposeImg(x, filename = "", maxcell = NULL, ...)
## S4 method for signature 'BioFormatsImage'
transposeImg(x, ...)
## S4 method for signature 'ExtImage'
transposeImg(x, ...)
```

#### **Arguments**

Χ	An object of class *Image as implemented in this package.
filename	Output file name for transformed SpatRaster.

maxcell Max number of pixels to load SpatRasterImage into memory. The default

1e7 is chosen because this is the approximate number of pixels in the medium resolution image at resolution = 4L in Xenium OME-TIFF to make different

methods of this function consistent.

... Ignored. It's there so different methods can all be passed to the same lapply in

the method for SFE objects. Some methods have extra arguments.

#### Value

For SpatRasterImage and ExtImage, object of the same class. For BioFormatsImage, the image of the specified resolution is read into memory and then the ExtImage method is called, returning ExtImage. For the extent: xmin and xmax are switched with ymin and ymax.

#### See Also

```
Other image methods: SFE-image, affineImg(), cropImg(), dim,BioFormatsImage-method, ext(), imgRaster(), imgSource(), mirrorImg(), rotateImg(), scaleImg(), translateImg()
```

```
unit, SpatialFeatureExperiment-method
```

Get unit of a SpatialFeatureExperiment

#### **Description**

Length units can be microns or pixels in full resolution image in SFE objects.

#### Usage

```
## S4 method for signature 'SpatialFeatureExperiment'
unit(x)
```

#### **Arguments**

x A SpatialFeatureExperiment object.

#### Value

A string for the name of the unit. At present it's merely a string and udunits is not used.

```
library(SFEData)
sfe <- McKellarMuscleData(dataset = "small")
SpatialFeatureExperiment::unit(sfe)</pre>
```

updateObject 93

updateObject	Update a SpatialFeatureExperiment object
--------------	--

## **Description**

Update a SpatialFeatureExperiment to the latest version of object structure. This is usually called by internal functions.

#### Usage

```
## S4 method for signature 'SpatialFeatureExperiment'
updateObject(object, ..., verbose = FALSE)
SFEVersion(object)
```

#### **Arguments**

object An old SpatialFeatureExperiment object.

Additional arguments that are ignored.

Verbose Logical scalar indicating whether a message should be emitted as the object is updated.

#### **Details**

Version 1.1.4 adds package version to the SFE object. We are considering an overhaul of the spatialGraphs slot in a future version using the sfdep package to decouple the adjacency graph from the edge weights.

#### Value

An updated version of object.

#### See Also

objectVersion, which is used to determine if the object is up-to-date.

```
library(SFEData)
sfe <- McKellarMuscleData("small")
# First version of SFE object doesn't log SFE package version, so should be NULL
SFEVersion(sfe)
sfe <- updateObject(sfe)
# See current version
SFEVersion(sfe)</pre>
```

94 visium\_row\_col

visium\_row\_col

Row and columns of Visium barcodes on the slide

# Description

From Space Ranger 1.3.1.

# Usage

visium\_row\_col

# **Format**

A data frame with 4992 rows with columns barcode, col, and row.

# Source

Space Ranger 1.3.1

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