

# Package ‘SpatialExperiment’

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**Title** S4 Class for Spatially Resolved -omics Data

**Description** Defines an S4 class for storing data from spatial -omics experiments. The class extends SingleCellExperiment to support storage and retrieval of additional information from spot-based and molecule-based platforms, including spatial coordinates, images, and image metadata. A specialized constructor function is included for data from the 10x Genomics Visium platform.

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imgData-methods	<i>Methods for handling image-related data</i>
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## Description

The set of functions described below is designed to handle the image-related data stored inside a SpatialExperiment's imgData int\_metadata field. These include:

- getImg, addImg, rmvImg to retrieve/add/remove an image entry to/from the imgData DataFrame
- imgSource, imgRaster to retrieve the path/URL and raster object, respectively, associated with an image or set of images

## Usage

```
## S4 method for signature 'SpatialExperiment'
getImg(x, sample_id = NULL, image_id = NULL)
```

```
## S4 method for signature 'SpatialExperiment'
addImg(x, imageSource, scaleFactor, sample_id, image_id, load = TRUE)
```

```
## S4 method for signature 'SpatialExperiment'
```

```

rmvImg(x, sample_id = NULL, image_id = NULL)

## S4 method for signature 'SpatialExperiment'
imgSource(x, sample_id = NULL, image_id = NULL, path = FALSE)

## S4 method for signature 'SpatialExperiment'
imgRaster(x, sample_id = NULL, image_id = NULL)

## S4 method for signature 'SpatialExperiment'
rotateImg(x, sample_id = NULL, image_id = NULL, degrees = 90)

## S4 method for signature 'SpatialExperiment'
mirrorImg(x, sample_id = NULL, image_id = NULL, axis = c("h", "v"))

```

### Arguments

x	a <a href="#">SpatialExperiment</a>
sample_id	character string, TRUE or NULL specifying sample/image identifier(s); here, TRUE is equivalent to all samples/images and NULL specifies the first available entry (see details)
image_id	see sample_id
imageSource	a character string specifying an image file name (.png, .jpg or .tif) or URL to source the image from
scaleFactor	single numeric scale factor used to rescale spatial coordinates according to the image's resolution
load	logical; should the image(s) be loaded into memory as a raster object? if FALSE, will store the path/URL instead
path	logical; for <a href="#">RemoteSpatialImages</a> , TRUE returns the path to the image's cached file, and FALSE its URL. For <a href="#">Stored/LoadedSpatialImages</a> , a path/NA is returned, irrespective of path.
degrees	single numeric in +/-[0,90,...,360] specifying how many degrees to rotate. A negative/positive value corresponds to counter-/clockwise rotation
axis	character string specifying whether to mirror horizontally ("h") or vertically ("v")

### Value

`getImg()` returns a single or list of [SpatialImage\(s\)](#).

`add/rmvImg()` return a [SpatialExperiment](#) with modified `imgData`; specifically, they create/remove an image entry (row) in the `imgData` `DataFrame`.

`imgRaster/Source()` access relevant data in the [SpatialImage\(s\)](#) stored inside the `imgData`'s data field. Depending on whether or not multiple entries are accessed, a character string or vector is returned by `imgSource()`, and a single or list of raster object(s) is returned by `imgRaster()`.

`rotate/mirrorImg()` return a [LoadedSpatialImage](#) with modified a raster matrix.

**Author(s)**

Helena L. Crowell

**Examples**

```
example(read10xVisium)

# 'SpatialImage' accession
(spi <- getImg(spe))
plot(imgRaster(spi))

# remove an image
imgData(spe)
spe <- rmvImg(spe,
  sample_id = "section1",
  image_id = "lowres")
imgData(spe)

# add an image
url <- "https://i.redd.it/3pw5uah7xo041.jpg"
spe <- addImg(spe,
  sample_id = "section1",
  image_id = "pomeranian",
  imageSource = url,
  scaleFactor = NA_real_,
  load = FALSE)

# extract image
img <- imgRaster(spe,
  sample_id = "section1",
  image_id = "pomeranian")
plot(img)

#####
# transformations #
#####

# clockwise rotation
spe1 <- rotateImg(spe,
  degrees = 90) # first image

spe2 <- rotateImg(spe,
  sample_id = TRUE,
  image_id = TRUE,
  degrees = 90) # all images

par(mfrow = c(1, 3))
plot(imgRaster(spe))
plot(imgRaster(spe1))
plot(imgRaster(spe2))

# horizontal/vertical mirroring
```

```
spe1 <- mirrorImg(spe, axis = "h")
spe2 <- mirrorImg(spe, axis = "v")

par(mfrow = c(1, 3))
plot(imgRaster(spe))
plot(imgRaster(spe1))
plot(imgRaster(spe2))
```

---

read10xVisium

*Load data from a 10x Genomics Visium experiment*


---

### Description

Creates a [SpatialExperiment](#) from the Space Ranger output directories for 10x Genomics Visium spatial gene expression data.

### Usage

```
read10xVisium(
  samples = "",
  sample_id = paste0("sample", sprintf("%02d", seq_along(samples))),
  type = c("HDF5", "sparse"),
  data = c("filtered", "raw"),
  images = "lowres",
  load = TRUE
)
```

### Arguments

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
sample_id	character string specifying unique sample identifiers, one for each directory specified via samples; ignored if <code>!is.null(names(samples))</code>
type	character string specifying the type of format to read count data from (see <code>read10xCounts</code> )
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"
load	logical; should the image(s) be loaded into memory as a grab? If FALSE, will store the path/URL instead.

## Details

The constructor assumes data from each sample are located in a single output directory as returned by Space Ranger, thus having the following file organization (where "raw/filtered" refers to either "raw" or "filtered" to match the 'data' argument.) The base directory "outs/" from Space Ranger can either be included manually in the paths provided in 'samples', or can be ignored; if ignored, it will be added automatically. The '.h5' files are used if 'type = "HDF5"'. (Note that 'tissue\_positions.csv' was renamed in Space Ranger v2.0.0.)

```
sample
· | — outs
· · | — raw/filtered_feature_bc_matrix.h5
· · | — raw/filtered_feature_bc_matrix
· · · | — barcodes.tsv.gz
· · · | — features.tsv.gz
· · · | — matrix.mtx.gz
· · | — spatial
· · · | — scalefactors_json.json
· · · | — tissue_lowres_image.png
· · · | — tissue_positions.csv
```

## Value

a [SpatialExperiment](#) object

## Author(s)

Helena L. Crowell

## Examples

```
dir <- system.file(
  file.path("extdata", "10xVisium"),
  package = "SpatialExperiment")

sample_ids <- c("section1", "section2")
samples <- file.path(dir, sample_ids, "outs")

list.files(samples[1])
list.files(file.path(samples[1], "spatial"))
file.path(samples[1], "raw_feature_bc_matrix")

(spe <- read10xVisium(samples, sample_ids,
  type = "sparse", data = "raw",
  images = "lowres", load = FALSE))

# base directory 'outs/' from Space Ranger can also be omitted
samples2 <- file.path(dir, sample_ids)
(spe2 <- read10xVisium(samples2, sample_ids,
  type = "sparse", data = "raw",
  images = "lowres", load = FALSE))
```

```
# tabulate number of spots mapped to tissue
cd <- colData(spe)
table(
  in_tissue = cd$in_tissue,
  sample_id = cd$sample_id)

# view available images
imgData(spe)
```

---

readImgData

*Read images & scale factors for 10x Genomics Visium*

---

### Description

Function to read in images and scale factors for 10x Genomics Visium data, and return as a valid [imgData](#) DataFrame.

### Usage

```
readImgData(
  path = ".",
  sample_id = names(path),
  imageSources = file.path(path, "tissue_lowres_image.png"),
  scaleFactors = file.path(path, "scalefactors_json.json"),
  load = TRUE
)
```

### Arguments

path	a path where to find one or more images
sample_id	the sample_id for the <a href="#">SpatialExperiment</a> object
imageSources	the images source path(s)
scaleFactors	the .json file where to find the scale factors
load	logical; should the image(s) be loaded into memory as a grob? If FALSE, will store the path/URL instead.

### Value

a [DataFrame](#)

### Author(s)

Helena L. Crowell

## Examples

```
dir <- system.file(
  file.path("extdata", "10xVisium", "section1", "outs", "spatial"),
  package = "SpatialExperiment")

# base directory contains
# - scale factors (scalefactors_json.json)
# - one image (tissue_lowres_image.png)
list.files(dir)

# read in images & scale factors
# as valid 'imgData' 'DFrame'
readImgData(dir, sample_id = "foo")
```

---

SpatialExperiment-assays

*Methods for named assays*

---

## Description

The `SpatialExperiment` class provides methods for getting or setting named `assays`. For example, `molecules(spe)` will get or set an assay named `molecules` from object `spe`, equivalent to `assay(spe, i = "molecules")`. This provides a convenient interface for users and encourages standardization of assay names across packages.

## Available methods

In the following code, `spe` is a `SpatialExperiment` object, `value` is a `BumpyMatrix`-like object with the same dimensions as `spe`, and `...` are further arguments passed to `assay` (for the getter) or `assay<-` (for the setter).

```
molecules(x, ...), molecules(x, ...) <- value: Get or set an assay named molecules, which is usually assumed to be a BumpyMatrix-formatted object containing spatial coordinates (and any other information) of the individual molecules per gene per cell.
```

## Author(s)

Dario Righelli

## See Also

`assay` and `assay<-`

## Examples

```
example(SpatialExperiment)
molecules(spe_mol)
```



---

 SpatialExperiment-class

*The SpatialExperiment class*


---

## Description

The `SpatialExperiment` class is designed to represent spatially resolved transcriptomics (ST) data. It inherits from the `SingleCellExperiment` class and is used in the same manner. In addition, the class supports storage of spatial information via `spatialCoords` and storage of images via `imgData`.

## Arguments

...	Arguments passed to the <code>SingleCellExperiment</code> constructor to fill the slots of the base class.
<code>sample_id</code>	A character sample identifier, which matches the <code>sample_id</code> in <code>imgData</code> . The <code>sample_id</code> will also be stored in a new column in <code>colData</code> , if not already present. Default = <code>sample01</code> .
<code>spatialCoordsNames</code>	A character vector of column names from <code>colData</code> containing spatial coordinates, which will be accessible with <code>spatialCoords</code> . Alternatively, the <code>spatialCoords</code> argument may be provided. If both are provided, <code>spatialCoordsNames</code> is given precedence, and a warning is returned. Default = <code>c("x", "y")</code> .
<code>spatialCoords</code>	A numeric matrix containing columns of spatial coordinates, which will be accessible with <code>spatialCoords</code> . Alternatively, <code>spatialCoordsNames</code> may be provided. If both are provided, <code>spatialCoordsNames</code> is given precedence, and a warning is returned.
<code>scaleFactors</code>	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 <code>imageSources</code> . Default = 1.
<code>imgData</code>	Optional <code>DataFrame</code> containing the image data. Alternatively, this can be built from the arguments <code>imageSources</code> and <code>image_id</code> (see Details).
<code>imageSources</code>	Optional file path(s) or URL(s) for one or more image sources.
<code>image_id</code>	Optional character vector (same length as <code>imageSources</code> ) containing unique image identifiers.
<code>loadImage</code>	Logical indicating whether to load image into memory. Default = <code>FALSE</code> .
<code>spatialDataNames</code>	(Deprecated) A character vector of column names from <code>colData</code> to include in <code>spatialData</code> . Alternatively, the <code>spatialData</code> argument may be provided. If both are provided, <code>spatialDataNames</code> is given precedence, and a warning is returned. (Note: <code>spatialData</code> and <code>spatialDataNames</code> have been deprecated; <code>colData</code> and <code>spatialCoords</code> should be used for all columns. The arguments

have been retained for backward compatibility but may be removed in the future.)

`spatialData` (Deprecated) A `DataFrame` containing columns to store in `spatialData`, which must contain at least the columns of spatial coordinates. Alternatively, `spatialDataNames` may be provided. If both are provided, `spatialDataNames` is given precedence, and a warning is returned. (Note: `spatialData` and `spatialDataNames` have been deprecated; `colData` and `spatialCoords` should be used for all columns. The arguments have been retained for backward compatibility but may be removed in the future.)

## Details

In this class, rows represent genes, and columns represent spots (for spot-based ST platforms) or cells (for molecule-based ST platforms). As for `SingleCellExperiment`, counts and logcounts can be stored in the `assays` slot, and row and column metadata in `rowData` and `colData`. For molecule-based ST data, the additional measurements per molecule per cell can be stored in a `BumpyMatrix`-formatted assay named `molecules`.

The additional arguments in the constructor documented above (e.g. `spatialCoords`, `imgData`, and others) represent the extensions to the `SingleCellExperiment` class to store associated spatial and imaging information for ST data.

The constructor expects `colData` to contain a column named `sample_id`. If this is not present, it will assign the value from the `sample_id` argument. If the `imgData` argument is provided, the constructor expects the `imgData DataFrame` to already be built. Otherwise, it will build it from the `imageSources` and (optional) `image_id` arguments. If `image_id` is not provided, this will be assumed from `sample_id` and `imageSources` instead. To combine multiple samples within a single object, see `combine`.

For 10x Genomics Visium datasets, the function `read10xVisium` can be used to load data and create a `SpatialExperiment` object directly from Space Ranger output files.

## Value

A `SpatialExperiment` object.

## Author(s)

Dario Righelli and Helena L. Crowell

## See Also

?`"SpatialExperiment-methods"`, which includes: `spatialCoords`, `spatialCoordsNames`, `imgData`, `scaleFactors`

?`"SpatialExperiment-assays"`, which includes: `molecules`

?`"SpatialExperiment-colData"`

?`"SpatialExperiment-combine"`

?`"SpatialExperiment-subset"`

?`"SpatialExperiment-misc"`

```
readImgData
?"imgData-methods"
SpatialImage
read10xVisium
```

### Examples

```
#####
# Example 1: Spot-based ST (10x Visium) using constructor
#####

dir <- system.file(
  file.path("extdata", "10xVisium", "section1", "outs"),
  package = "SpatialExperiment")

# read in counts
fnm <- file.path(dir, "raw_feature_bc_matrix")
sce <- DropletUtils::read10xCounts(fnm)

# read in image data
img <- readImgData(
  path = file.path(dir, "spatial"),
  sample_id="foo")

# read in spatial coordinates
fnm <- file.path(dir, "spatial", "tissue_positions_list.csv")
xyz <- read.csv(fnm, header = FALSE,
  col.names = c(
    "barcode", "in_tissue", "array_row", "array_col",
    "pxl_row_in_fullres", "pxl_col_in_fullres"))

# construct observation & feature metadata
rd <- S4Vectors::DataFrame(
  symbol = rowData(sce)$Symbol)

# construct 'SpatialExperiment'
(spe <- SpatialExperiment(
  assays = list(counts = assay(sce)),
  rowData = rd,
  colData = DataFrame(xyz),
  spatialCoordsNames = c("pxl_col_in_fullres", "pxl_row_in_fullres"),
  imgData = img,
  sample_id = "foo"))

#####
# Example 2: Spot-based ST (10x Visium) using 'read10xVisium'
#####

# see ?read10xVisium for details
example(read10xVisium)

#####
```

```

# Example 3: Molecule-based ST
#####

# create simulated data
n <- 1000; ng <- 50; nc <- 20
# sample xy-coordinates in [0,1]
x <- runif(n)
y <- runif(n)
# assign each molecule to some gene-cell pair
gs <- paste0("gene", seq(ng))
cs <- paste0("cell", seq(nc))
gene <- sample(gs, n, TRUE)
cell <- sample(cs, n, TRUE)
# construct data.frame of molecule coordinates
df <- data.frame(gene, cell, x, y)

# (assure gene & cell are factor so that
# missing observations aren't dropped)
df$gene <- factor(df$gene, gs)
df$cell <- factor(df$cell, cs)

# construct BumpyMatrix
mol <- BumpyMatrix::splitAsBumpyMatrix(
  df[, c("x", "y")],
  row = df$gene, column = df$cell)

# get count matrix
y <- with(df, table(gene, cell))
y <- as.matrix(unclass(y))

# construct SpatialExperiment
(spe_mol <- SpatialExperiment(
  assays = list(
    counts = y,
    molecules = mol)))

```

---

SpatialExperiment-coercion

*SpatialExperiment coercion methods*

---

## Description

The `SpatialExperiment` class inherits from the `SingleCellExperiment` class making it necessary to coerce between these classes. To do so, we designed two different methods: the traditional `as` method and the `toSpatialExperiment` function (recommended). The `as` method checks if the `SingleCellExperiment` object has already populated `int_colData` with three elements: `spatialData`, `spatialCoords`, and `imgData`. It also checks if `colData` already contains a `sample_id`. In case these checks pass the new `SpatialExperiment` will have the same values as the `SingleCellExperiment` passed object. Otherwise a `SpatialExperiment` with default values for these slots will be created.

The `toSpatialExperiment` method expects a `SingleCellExperiment` object and additional arguments as explained in the related section of this documentation. In case the `SingleCellExperiment` object has already populated `int_colData` with `spatialData` and/or `spatialCoords` and/or `imgData`, these will be respectively overwritten in case the arguments `spatialData/spatialDataNames` and/or `spatialCoords/spatialCoordsNames` and/or `imgData` are not `NULL`.

## Arguments

<code>sce</code>	A <code>SingleCellExperiment</code> object.
<code>sample_id</code>	A character sample identifier, which matches the <code>sample_id</code> in <code>imgData</code> . The <code>sample_id</code> will also be stored in a new column in <code>colData</code> , if not already present. Default = <code>sample01</code> .
<code>spatialCoordsNames</code>	A character vector of column names from <code>colData</code> containing spatial coordinates, which will be accessible with <code>spatialCoords</code> . Alternatively, the <code>spatialCoords</code> argument may be provided. If both are provided, <code>spatialCoordsNames</code> is given precedence, and a warning is returned. Default = <code>c("x", "y")</code> .
<code>spatialCoords</code>	A numeric matrix containing columns of spatial coordinates, which will be accessible with <code>spatialCoords</code> . Alternatively, <code>spatialCoordsNames</code> may be provided. If both are provided, <code>spatialCoordsNames</code> is given precedence, and a warning is returned.
<code>scaleFactors</code>	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 <code>imageSources</code> . Default = 1.
<code>imgData</code>	Optional <code>DataFrame</code> containing the image data. Alternatively, this can be built from the arguments <code>imageSources</code> and <code>image_id</code> (see Details).
<code>imageSources</code>	Optional file path(s) or URL(s) for one or more image sources.
<code>image_id</code>	Optional character vector (same length as <code>imageSources</code> ) containing unique image identifiers.
<code>loadImage</code>	Logical indicating whether to load image into memory. Default = <code>FALSE</code> .
<code>spatialDataNames</code>	(Deprecated) A character vector of column names from <code>colData</code> to include in <code>spatialData</code> . Alternatively, the <code>spatialData</code> argument may be provided. If both are provided, <code>spatialDataNames</code> is given precedence, and a warning is returned. (Note: <code>spatialData</code> and <code>spatialDataNames</code> have been deprecated; <code>colData</code> and <code>spatialCoords</code> should be used for all columns. The arguments have been retained for backward compatibility but may be removed in the future.)
<code>spatialData</code>	(Deprecated) A <code>DataFrame</code> containing columns to store in <code>spatialData</code> , which must contain at least the columns of spatial coordinates. Alternatively, <code>spatialDataNames</code> may be provided. If both are provided, <code>spatialDataNames</code> is given precedence, and a warning is returned. (Note: <code>spatialData</code> and <code>spatialDataNames</code> have been deprecated; <code>colData</code> and <code>spatialCoords</code> should be used for all columns. The arguments have been retained for backward compatibility but may be removed in the future.)

**Examples**

```

dir <- system.file(
  file.path("extdata", "10xVisium", "section1", "outs"),
  package = "SpatialExperiment")

# read in counts
fnm <- file.path(dir, "raw_feature_bc_matrix")
sce <- DropletUtils::read10xCounts(fnm)

# read in spatial coordinates
fnm <- file.path(dir, "spatial", "tissue_positions_list.csv")
xyz <- read.csv(fnm, header = FALSE,
  col.names = c("barcode", "in_tissue", "array_row", "array_col",
  "pxl_row_in_fullres", "pxl_col_in_fullres"))

# read in image data
img <- readImgData(
  path = file.path(dir, "spatial"),
  sample_id = "sample01")

## as method
(spe <- as(sce, "SpatialExperiment"))

colData(sce) <- DataFrame(xyz[,c(1:4)])
int_colData(sce)$spatialCoords <- as.matrix(xyz[,c(5,6)])

## Coercing an sce without imgData
(spe <- as(sce, "SpatialExperiment"))

## Coercing an sce with imgData
int_colData(sce)$imgData <- img
(spe <- as(sce, "SpatialExperiment"))

## toSpatialExperiment method
colData(sce) <- DataFrame(xyz)
(spe <- toSpatialExperiment(sce,
  imgData = img,
  spatialCoordsNames = c("pxl_col_in_fullres", "pxl_row_in_fullres"),
  sample_id = "sample01"))

```

---

SpatialExperiment-colData

*SpatialExperiment colData*

---

**Description**

The `SpatialExperiment` class provides a modified `colData` setter, which ensures that the `SpatialExperiment` object remains valid.

**Usage**

```
## S4 replacement method for signature 'SpatialExperiment,DataFrame'  
colData(x) <- value  
  
## S4 replacement method for signature 'SpatialExperiment,`NULL`'  
colData(x) <- value
```

**Arguments**

```
x          a SpatialExperiment  
value     a DataFrame
```

**Details**

The `colData` setter performs several checks to ensure validity. If the replacement `colData` does not contain a `sample_id` column, the existing `sample_ids` will be retained. If the replacement `colData` contains `sample_ids`, a check is performed to ensure the number of unique `sample_ids` is the same, i.e. a one-to-one mapping is possible. If the replacement is `NULL`, the `sample_ids` are retained. In addition, checks are performed against the `sample_ids` in [imgData](#).

**Value**

a [SpatialExperiment](#) object with updated `colData`

**Examples**

```
example(read10xVisium)  
  
# empty replacement retains sample identifiers  
colData(spe) <- NULL  
names(colData(spe))  
  
# replacement of sample identifiers  
# requires one-to-one mapping  
  
## invalid replacement  
  
tryCatch(  
  spe$sample_id <- seq(ncol(spe)),  
  error = function(e) message(e))  
  
## valid replacement  
  
old <- c("section1", "section2")  
new <- c("sample_A", "sample_B")  
idx <- match(spe$sample_id, old)  
  
tmp <- spe  
tmp$sample_id <- new[idx]  
table(spe$sample_id, tmp$sample_id)
```

---

SpatialExperiment-combine

*Combining SpatialExperiment objects*

---

## Description

The `SpatialExperiment` class provides modified methods to combine multiple `SpatialExperiment` objects by column, for example from multiple samples. These methods ensure that all data fields remain synchronized when samples are added or removed.

## Usage

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

## Arguments

... a list of `SpatialExperiment` objects  
deparse.level refer to `?rbind`

## Value

a combined `SpatialExperiment` object

## Combining

The ... argument is assumed to contain one or more `SpatialExperiment` objects.

`cbind(..., deparse.level=1)`: Returns a `SpatialExperiment` where all objects in ... are combined column-wise, i.e., columns in successive objects are appended to the first object.

Each `SpatialExperiment` object in ... must have the same `colData` (with the same `spatialCoords`). If multiple objects use the same `sample_id`, the method will proceed by assigning unique `sample_ids`.

Additionally, the method combines `imgData` by row using `rbind`.

Refer to `?cbind,SingleCellExperiment-method` for details on how metadata and other inherited attributes are combined in the output object.

Refer to `?cbind` for the interpretation of `deparse.level`.

## Author(s)

Dario Righelli



**Examples**

```

example(read10xVisium, echo = FALSE)

# merging with duplicated 'sample_id's
# will automatically assign unique identifiers
spe1 <- spe2 <- spe
spe3 <- cbind(spe1, spe2)
unique(spe3$sample_id)

# assign unique sample identifiers
spe1 <- spe2 <- spe
spe1$sample_id <- paste(spe1$sample_id, "sample1", sep = ".")
spe2$sample_id <- paste(spe2$sample_id, "sample2", sep = ".")

# combine into single object
spe <- cbind(spe1, spe2)

# view joint 'imgData'
imgData(spe)

# tabulate number of spots mapped to tissue
cd <- colData(spe)
table(
  in_tissue = cd$in_tissue,
  sample_id = cd$sample_id)

```

---

 SpatialExperiment-methods

*Methods for spatial attributes*


---

**Description**

The `SpatialExperiment` class provides a family of methods to get and set spatial data attributes in `SpatialExperiment` objects. Spatial attributes include `spatialCoords`, `imgData`, and `scaleFactors`, as well as methods to rotate and mirror `SpatialExperiment` objects and their spatial coordinates.

**Usage**

```

## S4 method for signature 'SpatialExperiment'
spatialData(x)

## S4 replacement method for signature 'SpatialExperiment,DFrame'
spatialData(x) <- value

## S4 replacement method for signature 'SpatialExperiment,`NULL`'
spatialData(x) <- value

## S4 method for signature 'SpatialExperiment'

```

```

spatialDataNames(x)

## S4 replacement method for signature 'SpatialExperiment,character'
spatialDataNames(x) <- value

## S4 replacement method for signature 'SpatialExperiment,`NULL`'
spatialDataNames(x) <- value

## S4 method for signature 'SpatialExperiment'
spatialCoords(x)

## S4 replacement method for signature 'SpatialExperiment,matrix'
spatialCoords(x) <- value

## S4 replacement method for signature 'SpatialExperiment,`NULL`'
spatialCoords(x) <- value

## S4 method for signature 'SpatialExperiment'
spatialCoordsNames(x)

## S4 replacement method for signature 'SpatialExperiment,character'
spatialCoordsNames(x) <- value

## S4 replacement method for signature 'SpatialExperiment,`NULL`'
spatialCoordsNames(x) <- value

## S4 method for signature 'SpatialExperiment'
scaleFactors(x, sample_id = TRUE, image_id = TRUE)

## S4 method for signature 'SpatialExperiment'
x$name

## S4 method for signature 'SpatialExperiment'
imgData(x)

## S4 replacement method for signature 'SpatialExperiment,DataFrame'
imgData(x) <- value

## S4 replacement method for signature 'SpatialExperiment,`NULL`'
imgData(x) <- value

```

### Arguments

x	A <a href="#">SpatialExperiment</a> object.
value	Replacement value for replacement methods.
sample_id	Logical value or character vector specifying sample identifier(s) for scaleFactors. Default = TRUE (all samples).

image_id	Logical value or character vector specifying image identifier(s) for scaleFactors. Default = TRUE (all images).
name	The name of the colData column to extract.

### Details

Additional details for each type of data attribute are provided below.

Note: `spatialData` and `spatialDataNames` (previously used to store a subset of columns from `colData`) have been deprecated. All columns should be stored in either `spatialCoords` (numeric matrix containing spatial coordinates) or `colData` (all other columns). The `spatialData/spatialDataNames` functionality has been retained for backward compatibility but may be removed in the future.

See `rotateCoords`, `mirrorCoords`, `rotateObject`, or `mirrorObject` for details on methods to rotate and mirror `SpatialExperiment` objects and their `spatialCoords`.

### Value

Return value varies depending on method, as described below.

#### `spatialData` and `spatialCoords` methods

`spatialData(x) <- value`: The `spatialData` setter expects a `DataFrame`. If the input does not contain an `in_tissue` column, this will be included with a default value of 1.

`spatialCoords(x)`: Getter for numeric matrix of spatial coordinates.

`spatialCoords(x) <- value`: Setter for numeric matrix of spatial coordinates.

#### `spatialDataNames` and `spatialCoordsNames` methods

`spatialDataNames(x)`: Returns the names of the `colData` associated with the spatial information, which are stored in the `int_metadata`.

`spatialDataNames(x) <- value`: Setter to replace column names in the `spatialData` `DataFrame`.

`spatialCoordsNames(x)`: Returns the defined names of the spatial coordinates (e.g. `c("x", "y")`).

`spatialCoordsNames(x) <- value`: Setter to define the names of the spatial coordinate columns.

#### `imgData` methods

`imgData(x)`: Getter to return the `imgData` `DataFrame`.

`imgData(x) <- value`: Setter to provide a `DataFrame` object as `imgData` of the `SpatialExperiment` object.

#### Other methods

`scaleFactors(x, sample_id, image_id)`: Getter to return the scale factors associated with the `sample_id(s)` and `image_id(s)` provided. This is related to the stored image(s) in the `SpatialExperiment` `imgData` structure. See argument descriptions for further details.

**Examples**

```
example(read10xVisium)

# spatialCoords returns a numeric matrix
head(spatialCoords(spe))

# change spatial coordinate names
spatialCoordsNames(spe)
spatialCoordsNames(spe) <- c("x", "y")
head(spatialCoords(spe))

# imgData and scale factors
imgData(spe)
scaleFactors(spe)

# tabulate number of spots mapped to tissue
cd <- colData(spe)
table(
  in_tissue = cd$in_tissue,
  sample_id = cd$sample_id)
```

---

SpatialExperiment-misc

*Miscellaneous SpatialExperiment methods*

---

**Description**

Miscellaneous methods for the [SpatialExperiment](#) class and its descendants that do not fit into any other documentation category such as, for example, show methods.

**Usage**

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

**Arguments**

object            a [SpatialExperiment](#) object

**Value**

Returns NULL

**Author(s)**

Dario Righelli and Helena L. Crowell

**Examples**

```
example(read10xVisium)
spe
```

---

SpatialExperiment-rotate-mirror  
*Methods for spatial attributes*

---

**Description**

The `SpatialExperiment` class provides methods to rotate and mirror `SpatialExperiment` objects and their `spatialCoords`.

**Usage**

```
## S4 method for signature 'SpatialExperiment'
rotateCoords(x, sample_id = NULL, degrees = 90, warn = TRUE)

## S4 method for signature 'SpatialExperiment'
mirrorCoords(x, sample_id = NULL, axis = c("h", "v"), warn = TRUE)

## S4 method for signature 'SpatialExperiment'
rotateObject(x, sample_id = NULL, image_id = NULL, degrees = 90)

## S4 method for signature 'SpatialExperiment'
mirrorObject(x, sample_id = NULL, image_id = NULL, axis = c("h", "v"))
```

**Arguments**

<code>x</code>	A <code>SpatialExperiment</code> object.
<code>sample_id</code>	Logical value or character vector specifying sample identifier(s) for <code>scaleFactors</code> . Default = TRUE (all samples).
<code>degrees</code>	single numeric in +/-[0,90,...,360] specifying how many degrees to rotate. A negative/positive value corresponds to counter-/clockwise rotation. Applicable for <code>rotateCoords</code> and <code>rotateObject</code> methods.
<code>warn</code>	Logical value indicating whether to print a warning about mismatches between coordinates and images, possible with the <code>spatialCoords</code> transformation methods <code>rotateCoords</code> and <code>mirrorCoords</code> .
<code>axis</code>	character string specifying whether to mirror horizontally ("h") or vertically ("v"). Applicable for <code>mirrorCoords</code> and <code>mirrorObject</code> methods.
<code>image_id</code>	Logical value or character vector specifying image identifier(s) for <code>scaleFactors</code> . Default = TRUE (all images).

**Details**

Additional details for each type of data attribute are provided below.

**Value**

Return value varies depending on method, as described below.

**spatialCoords transformation methods**

`rotateCoords(x, sample_id, degrees, warn)`: Apply a rotation to the `spatialCoords` of `x`, potentially subsetted to sample `sample_id` (or without subsetting if `sample_id` is `NULL`), by the specified number of degrees clockwise. Warn about mismatches with images if `warn`.

`mirrorCoords(x, sample_id, axis, warn)`: Reflect the `spatialCoords` of `x` across either the horizontal or vertical axis, specified by supplying "h" or "v" to the `axis` argument, respectively. Subset `x` to just the sample `sample_id`, if not `NULL`. Warn about mismatches with images if `warn`.

**SpatialExperiment transformation wrapper methods**

`rotateObject(x, sample_id, image_id, degrees)`: Apply a rotation to the `spatialCoords` and `imgData` of `x`, potentially subsetted to sample `sample_id` (or without subsetting if `sample_id` is `NULL`), by the specified number of degrees clockwise. Wrapper around `rotateCoords` and `rotateImg`.

`mirrorObject(x, sample_id, image_id, axis)`: Reflect the `spatialCoords` and `imgData` of `x` across either the horizontal or vertical axis, specified by supplying "h" or "v" to the `axis` argument, respectively. Subset `x` to just the sample `sample_id`, if not `NULL`. Wrapper around `mirrorCoords` and `mirrorImg`.

**Author(s)**

Nicholas J. Eagles

**Examples**

```
example(read10xVisium)

# rotateCoords(), mirrorCoords(), rotateObject(), and mirrorObject() return a
# SpatialExperiment, potentially subsetted by sample.

# Subset to just "section1"; rotate coordinates 90 degrees clockwise followed
# by a reflection across the vertical axis
spe_coords <- rotateCoords(spe, sample_id = "section1", degrees = 90)
spe_coords <- mirrorCoords(spe_coords, axis = "v")

# Subset to just "section2"; transform both the imgData() and spatialCoords()
# by a 180-degree rotation then reflection across the vertical axis
spe_wrapper <- rotateObject(spe, sample_id = "section2", degrees = 180)
spe_wrapper <- mirrorObject(spe_wrapper, axis = "v")
```

---

SpatialExperiment-subset

*Subsetting SpatialExperiment objects*

---

### Description

The subsetting method for `SpatialExperiment` objects ensures that spatial data attributes (`spatialCoords` and `imgData`) are subsetted correctly to match rows and columns with the remainder of the object.

### Arguments

<code>x</code>	a <code>SpatialExperiment</code> object
<code>i</code>	row indices for subsetting
<code>j</code>	column indices for subsetting

### Value

a `SpatialExperiment` object

### subset

[`:`]: subsetting method

### Examples

```
example(read10xVisium)

dim(spe)

set.seed(123)
idx <- sample(ncol(spe), 10)
sub <- spe[, idx]
dim(sub)
colData(sub)
spatialCoords(sub)
```

---

SpatialImage-class

*The SpatialImage class*

---

### Description

The `SpatialImage` class hierarchy provides representations of images from a variety of sources. It is used by the `SpatialExperiment` class to manage the loading of images across multiple studies.

### Constructor

`SpatialImage(x, is.url)` will return a `SpatialImage` object. The class of the object depends on the type of `x`:

- If `x` is a raster object, a `LoadedSpatialImage` is returned. This represents an image that is fully realized into memory, where the raster representation is stored inside the output object.
- If `x` is a string and `is.url=TRUE` or it starts with `"http://"`, `"https://"` or `"ftp://"`, a `RemoteSpatialImage` is returned. This represents an image that is remotely hosted and retrieved only on request.
- If `x` is a string and `is.url=TRUE` or it does not start with a URL-like prefix, a `StoredSpatialImage` is returned. This represents an image that is stored in a local file and is loaded into memory only on request.

### Getting the raster image

For a `SpatialImage` object `x`, `imgRaster(x, ...)` will return a raster object (see `?as.raster`). This is effectively a matrix of RGB colors for each pixel in the image.

For a `StoredSpatialImage` object `x`, additional arguments in `...` are passed to `image_read`. This controls how the image is read into memory.

For a `RemoteSpatialImage` object `x`, the image file is first downloaded before the raster is returned. Here, `...` may contain an extra cache argument, which should be a `BiocFileCache` object (from the **BiocFileCache** package) specifying the file cache location. The default location is determined by `options("SpatialExperiment.remote.cache.path")`, otherwise it defaults to a subdirectory in the R temporary directory. Any further named arguments in `...` are passed to `image_read`.

`as.raster(x, ...)` is the same as `imgRaster(x, ...)`.

### In-memory caching

For `StoredSpatialImage` and `RemoteSpatialImage` objects, loading the image with `imgRaster` will automatically store the loaded raster object in an in-memory cache. Any subsequent `imgRaster` call will retrieve the raster from the cache, avoiding costly retrieval from the file system.

The cache policy is to evict the least recently used images when a new image would be added that exceeds the maximum cache size. If the new image by itself exceeds the maximum cache size, all images are evicted from the cache to trigger garbage collection and free up memory.

By default, the maximum size of the cache is 4 GB. This can be modified by setting `options("SpatialExperiment.cache.size")` to some number of bytes, e.g., `2^32`.

### Transformations

Two basic image transformations are currently supported for any `SpatialImage` `x`, namely, `rotateImg(x, degrees)` for clockwise (`degrees > 0`) and counter-clockwise (`degrees < 0`) rotation, and `mirrorImg(x, axis)` for horizontal (`axis = "h"`) and vertical (`axis = "v"`) mirroring.

Note that, both `rotateImg()` and `mirrorImg()` operate on the raster matrix of the input `SpatialImage`. Thus, any `SpatialImage` will automatically be coerced into a `LoadedSpatialImage` upon rotation/mirroring.



**Other methods**

`dim(x)` will return an integer vector of length 2, containing the width and height of the image in pixels. Note that this calls `imgRaster` under the hood and thus may interact with the file and memory caches as described above.

For any `SpatialImage` `x`, `as(x, "LoadedSpatialImage")` will create a `LoadedSpatialImage` containing an in-memory raster object.

For a `RemoteSpatialImage` `x`, `as(x, "StoredSpatialImage")` will create a `StoredSpatialImage` pointing to the file cache location.

**Author(s)**

Aaron Lun

**Examples**

```
path <- system.file(
  "extdata", "10xVisium", "section1", "outs", "spatial",
  "tissue_lowres_image.png", package="SpatialExperiment")

spi <- SpatialImage(path)
plot(imgRaster(spi))

# the following operations all use the cache
# so there is no need to reload the image
nrow(spi)
ncol(spi)
plot(as.raster(spi))

# coercing to an explicitly in-memory raster
spi <- as(spi, "LoadedSpatialImage")
plot(as.raster(spi))

#####
# transformations #
#####

# (counter-)clockwise rotation
spi1 <- rotateImg(spi, degrees = +90)
spi2 <- rotateImg(spi, degrees = -90)

par(mfrow = c(1, 3))
plot(as.raster(spi))
plot(as.raster(spi1))
plot(as.raster(spi2))

# horizontal/vertical mirroring
spi1 <- mirrorImg(spi, axis = "h")
spi2 <- mirrorImg(spi, axis = "v")

par(mfrow = c(1, 3))
plot(as.raster(spi))
```

```
plot(as.raster(spi1))  
plot(as.raster(spi2))
```

---

SpatialImage-misc      *Miscellaneous SpatialImage methods*

---

**Description**

Miscellaneous methods for the [SpatialImage](#) class that do not fit into any other documentation category such as, for example, show methods.

**Usage**

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

**Arguments**

object            a SpatialImage object

**Value**

none

**Author(s)**

Helena L. Crowell

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