

Package ‘SpatialOmicsOverlay’

April 18, 2024

Title Spatial Overlay for Omic Data from Nanostring GeoMx Data

Description Tools for NanoString Technologies GeoMx Technology. Package to easily graph on top of an OME-TIFF image. Plotting annotations can range from tissue segment to gene expression.

Version 1.3.1

Encoding UTF-8

Depends R (>= 4.1.0)

Imports S4Vectors, Biobase, base64enc, EBImage, ggplot2, XML, scattermore, dplyr, pbapply, data.table, readxl, magick, grDevices, stringr, plotrix, GeomxTools, BiocFileCache, stats, utils, methods, ggtext, tools, RBioFormats

Suggests knitr, rmarkdown, testthat (>= 3.0.0), stringi, qpdf, pheatmap, viridis, cowplot, vdiff, sf

License MIT

Collate addImage.R addPlottingFactor.R coordinateGeneration.R imageManipulation.R omeExtraction.R plottingBasics.R readSpatialOverlay.R removeSamples.R SpatialPosition-class.R SpatialOverlay-class.R utils.R xmlParsing.R

biocViews GeneExpression, Transcription, CellBasedAssays, DataImport, Transcriptomics, Proteomics, ProprietaryPlatforms, RNASeq, Spatial, DataRepresentation, Visualization

VignetteEngine knitr::rmarkdown

VignetteBuilder knitr

RoxygenNote 7.2.3

Config/testthat/edition 3

git_url <https://git.bioconductor.org/packages/SpatialOmicsOverlay>

git_branch devel

git_last_commit a233949

git_last_commit_date 2023-10-25

Repository Bioconductor 3.19

Date/Publication 2024-04-17

Author Maddy Griswold [cre, aut],
Megan Vandenberg [ctb],
Stephanie Zimmerman [ctb]

Maintainer Maddy Griswold <mgriswold@nanostring.com>

Contents

add4ChannelImage	2
addImageFile	3
addImageOmeTiff	4
addPlottingFactor	5
bookendStr	6
changeColoringIntensity	7
changeImageColoring	8
checkValidRes	9
createCoordFile	9
createMask	10
cropSamples	11
cropTissue	12
downloadMouseBrainImage	12
flipX	13
flipY	14
fluorLegend	14
moveCoords	15
parseOverlayAttrs	16
parseScanMetadata	17
plotSpatialOverlay	17
readLabWorksheet	19
readSpatialOverlay	20
recolor	21
removeSample	22
SpatialOverlay-class	22
SpatialPosition-class	24
xmlExtraction	25
Index	26

add4ChannelImage	<i>Add 4-channel image to SpatialOverlay from OME-TIFF. Allows for recoloring of image</i>
------------------	--

Description

Add 4-channel image to SpatialOverlay from OME-TIFF. Allows for recoloring of image

Usage

```
add4ChannelImage(overlay, ometiff = NULL, res = NULL, ...)
```

Arguments

overlay	SpatialOverlay object
ometiff	File path to OME-TIFF. NULL indicates pull info from overlay
res	resolution layer, 1 = largest & higher values = smaller. The images increase in resolution and memory. The largest image your environment can hold is recommended. NULL indicates pull info from overlay
...	Extra variables

Value

SpatialOverlay object with image

Examples

```
muBrain <- readRDS(unzip(system.file("extdata", "muBrainSubset_SpatialOverlay.zip",
                                   package = "SpatialOmicsOverlay")))

image <- downloadMouseBrainImage()

muBrain <- add4ChannelImage(overlay = muBrain,
                           ometiff = image, res = 8)

dim(EBImage::imageData(showImage(muBrain)))
```

addImageFile	<i>Add image to SpatialOverlay from disk</i>
--------------	--

Description

Add image to SpatialOverlay from disk

Usage

```
addImageFile(overlay, imageFile = NULL, res = NULL)
```

Arguments

overlay	SpatialOverlay object
imageFile	path to image
res	what resolution is the image given? 1 = largest, higher number = smaller This value will affect the coordinates of the overlays. res = 2, resolution is 1/2 the size as the raw image res = 3, resolution is 1/4 the size as the raw image res = 4, resolution is 1/8 the size as the raw image resolution = 1/2^(res-1)

Value

SpatialOverlay object with image

addImageOmeTiff *Add image to SpatialOverlay from OME-TIFF*

Description

Add image to SpatialOverlay from OME-TIFF

Usage

```
addImageOmeTiff(overlay, ometiff = NULL, res = NULL, ...)
```

Arguments

overlay	SpatialOverlay object
ometiff	File path to OME-TIFF. NULL indicates pull info from overlay
res	resolution layer, 1 = largest & higher values = smaller. The images increase in resolution and memory. The largest image your environment can hold is recommended. NULL indicates pull info from overlay
...	Extra variables

Value

SpatialOverlay object with image

Examples

```
muBrain <- readRDS(unzip(system.file("extdata", "muBrainSubset_SpatialOverlay.zip",
                                   package = "SpatialOmicsOverlay")))

image <- downloadMouseBrainImage()

muBrain <- addImageOmeTiff(overlay = muBrain,
                          ometiff = image, res = 8)

showImage(muBrain)
```

addPlottingFactor *Add plotting factor to [SpatialOverlay](#) object*

Description

Add plotting factor to [SpatialOverlay](#) object

Usage

```
addPlottingFactor(overlay, annots, plottingFactor, ...)

## S4 method for signature 'NanoStringGeoMxSet'
addPlottingFactor(overlay, annots, plottingFactor, countMatrix = "exprs")

## S4 method for signature 'matrix'
addPlottingFactor(overlay, annots, plottingFactor)

## S4 method for signature 'tbl_df'
addPlottingFactor(overlay, annots, plottingFactor)

## S4 method for signature 'tbl'
addPlottingFactor(overlay, annots, plottingFactor)

## S4 method for signature 'data.frame'
addPlottingFactor(overlay, annots, plottingFactor)

## S4 method for signature 'character'
addPlottingFactor(overlay, annots, plottingFactor)

## S4 method for signature 'numeric'
addPlottingFactor(overlay, annots, plottingFactor)

## S4 method for signature 'factor'
addPlottingFactor(overlay, annots, plottingFactor)
```

Arguments

overlay	SpatialOverlay object
annots	factor vector with the plottingFactor. if names match sample names in overlay vector will be matched on those, otherwise assumed in the correct order
plottingFactor	name of the new plotting factor
...	if using NanoStringGeoMxSet, name of count matrix to pull counts from
countMatrix	name of count matrix to pull counts from

Value

[SpatialOverlay](#) object with new plotting factor

Examples

```

muBrain <- readRDS(unzip(system.file("extdata", "muBrainSubset_SpatialOverlay.zip",
                                   package = "SpatialOmicOverlay")))

muBrainLW <- system.file("extdata", "muBrain_LabWorksheet.txt",
                        package = "SpatialOmicOverlay")

muBrainLW <- readLabWorksheet(muBrainLW, slideName = "D5761 (3)")

muBrain <- addPlottingFactor(overlay = muBrain,
                             annots = muBrainLW,
                             plottingFactor = "segment")

muBrainGxT <- readRDS(unzip(system.file("extdata", "muBrain_GxT.zip",
                                       package = "SpatialOmicOverlay")))

muBrain <- addPlottingFactor(overlay = muBrain,
                             annots = muBrainGxT,
                             plottingFactor = "Calm1",
                             countMatrix = "exprs")

muBrain <- addPlottingFactor(overlay = muBrain,
                             annots = seq_len(length(sampNames(muBrain))),
                             plottingFactor = "ROI_Num")

head(plotFactors(muBrain))

muBrain <- readRDS(unzip(system.file("extdata", "muBrainSubset_SpatialOverlay.zip",
                                   package = "SpatialOmicOverlay")))

muBrain <- addPlottingFactor(overlay = muBrain,
                             annots = as.factor(seq_len(length(sampNames(muBrain)))),
                             plottingFactor = "ROI_Num")

head(plotFactors(muBrain))

```

bookendStr

Print long string in more manageable fashion

Description

Print first and last n characters of string in this format: "### ... ### (x total char)"

Usage

```
bookendStr(x, bookend = 8)
```

Arguments

x	long string
bookend	number of characters on either side to print

Value

reformatted string

Examples

```
start_string <- stringi::stri_rand_strings(n = 1, length = 250)
bookendStr(start_string, bookend = 6)
```

changeColoringIntensity

Update color intensities for changing to RGB image

Description

Update color intensities for changing to RGB image

Usage

```
changeColoringIntensity(overlay, minInten = NULL, maxInten = NULL, dye)
```

Arguments

overlay	SpatialOverlay object
minInten	value to change MinIntensity to; NULL indicates no change
maxInten	value to change MaxIntensity to; NULL indicates no change
dye	which dye to change color, can be from Dye or DisplayName column from fluor(overlay)

Value

SpatialOverlay object with updated fluor data

Examples

```
muBrain <- readRDS(unzip(system.file("extdata", "muBrainSubset_SpatialOverlay.zip",
                                   package = "SpatialOmicOverlay")))

image <- downloadMouseBrainImage()

muBrain <- add4ChannelImage(overlay = muBrain,
                           ometiff = image, res = 8)

fluor(muBrain)

muBrain <- changeColoringIntensity(overlay = muBrain,
                                   minInten = 500,
                                   maxInten = 10000,
```

```
                                dye = "Cy5")  
  
fluor(muBrain)
```

changeImageColoring *Update color scheme for changing to RGB image*

Description

Update color scheme for changing to RGB image

Usage

```
changeImageColoring(overlay, color, dye)
```

Arguments

overlay	SpatialOverlay object, with 4channel image
color	color to change dye to, hex or color name
dye	which dye to change color, can be from Dye or DisplayName column from fluor(overlay)

Value

SpatialOverlay object with updated fluor data

Examples

```
muBrain <- readRDS(unzip(system.file("extdata", "muBrainSubset_SpatialOverlay.zip",  
                                package = "SpatialOmicOverlay")))  
  
muBrain <- add4ChannelImage(overlay = muBrain,  
                           ometiff = downloadMouseBrainImage(), res = 8)  
  
fluor(muBrain)  
  
muBrain <- changeImageColoring(overlay = muBrain, color = "magenta",  
                              dye = "Cy5")  
muBrain <- changeImageColoring(overlay = muBrain, color = "#42f5ef",  
                              dye = "Alexa 488")  
  
fluor(muBrain)
```

checkValidRes	<i>Determine lowest resolution image in OME-TIFF</i>
---------------	--

Description

Determine lowest resolution image in OME-TIFF

Usage

```
checkValidRes(ometiff)
```

Arguments

ometiff path to OME-TIFF

Value

value of lowest res image

Examples

```
image <- downloadMouseBrainImage()
checkValidRes(ometiff = image)
```

createCoordFile	<i>Create coordinate file for entire scan</i>
-----------------	---

Description

Create coordinate file for entire scan

Usage

```
createCoordFile(overlay, outline = TRUE)
```

Arguments

overlay SpatialOverlay object
outline returned coordinates only contain boundaries, will not work for segmented ROIs

Value

df of coordinates for every AOI in the scan

Examples

```
muBrain <- readRDS(unzip(system.file("extdata", "muBrainSubset_SpatialOverlay.zip",
                                   package = "SpatialOmicOverlay")))

muBrain <- createCoordFile(muBrain, outline = FALSE)

head(coords(muBrain))
```

createMask	<i>Create a binary mask from a base 64 string</i>
------------	---

Description

Create a binary mask from a base 64 string

Usage

```
createMask(b64string, metadata, outline = TRUE)
```

Arguments

b64string	base 64 string
metadata	metadata of AOI including: Height, Width of AOI
outline	only the outline points should be returned

Value

binary mask image

Examples

```
muBrain <- readRDS(unzip(system.file("extdata", "muBrainSubset_SpatialOverlay.zip",
                                   package = "SpatialOmicOverlay")))

samp <- which(sampNames(muBrain) == "DSP-1012999073013-A-F12")

ROIMask <- createMask(b64string = position(overlay(muBrain))[samp],
                    metadata = meta(overlay(muBrain))[samp,],
                    outline = TRUE)

pheatmap::pheatmap(ROIMask, cluster_rows = FALSE, cluster_cols = FALSE)
```

cropSamples	<i>Crop to zoom in on given ROIs</i>
-------------	--------------------------------------

Description

Crop to zoom in on given ROIs

Usage

```
cropSamples(overlay, sampleIDs, buffer = 0.1, sampsOnly = TRUE)
```

Arguments

overlay	SpatialOverlay object
sampleIDs	sampleIDs of ROIs to keep in image
buffer	percent of new image size to add to each edge as a buffer
sampsOnly	should only ROIs with given sampleIDs be in image

Value

SpatialOverlay object

Examples

```
muBrain <- readRDS(unzip(system.file("extdata", "muBrainSubset_SpatialOverlay.zip",
                                   package = "SpatialOmicsOverlay")))

image <- downloadMouseBrainImage()

muBrain <- addImageOmeTiff(overlay = muBrain,
                          ometiff = image, res = 8)

samps <- sampNames(muBrain)

muBrainCrop <- suppressWarnings(cropSamples(overlay = muBrain,
                                           sampleIDs = samps,
                                           sampsOnly = TRUE))

plotSpatialOverlay(overlay = muBrainCrop, scaleBar = FALSE,
                  hiRes = TRUE, legend = FALSE)
```

cropTissue

Crop to remove black boundary around tissue.

Description

Crop to remove black boundary around tissue.

Usage

```
cropTissue(overlay, buffer = 0.05)
```

Arguments

overlay SpatialOverlay object
buffer percent of new image size to add to each edge as a buffer

Value

SpatialOverlay object

Examples

```
muBrain <- readRDS(unzip(system.file("extdata", "muBrainSubset_SpatialOverlay.zip",  
                                   package = "SpatialOmicOverlay")))

image <- downloadMouseBrainImage()

muBrain <- addImageOmeTiff(overlay = muBrain,  
                           ometiff = image, res = 8)

muBrainCrop <- cropTissue(overlay = muBrain)

plotSpatialOverlay(overlay = muBrainCrop, legend = FALSE,  
                   hiRes = FALSE, scaleBar = FALSE)
```

downloadMouseBrainImage

Download Mouse Brain OME-TIFF from NanoString's Spatial Organ Atlas

Description

Download Mouse Brain OME-TIFF from NanoString's Spatial Organ Atlas

Usage

```
downloadMouseBrainImage()
```

Details

<https://nanosting.com/products/geomx-digital-spatial-profiler/spatial-organ-atlas/mouse-brain/>

Value

mouse brain OME-TIFF

Examples

```
image <- downloadMouseBrainImage()
```

flipX	<i>Flip x axis in image and overlay points</i>
-------	--

Description

Flip x axis in image and overlay points

Usage

```
flipX(overlay)
```

Arguments

overlay SpatialOverlay object

Value

SpatialOverlay object with x axis flipped

Examples

```
muBrain <- readRDS(unzip(system.file("extdata", "muBrainSubset_SpatialOverlay.zip",  
                                  package = "SpatialOmicOverlay")))
```

```
image <- downloadMouseBrainImage()
```

```
muBrain <- addImageOmeTiff(overlay = muBrain,  
                           ometiff = image, res = 8)
```

```
showImage(flipX(muBrain))
```

flipY *Flip y axis in image and overlay points*

Description

Flip y axis in image and overlay points

Usage

```
flipY(overlay)
```

Arguments

overlay SpatialOverlay object

Value

SpatialOverlay object with y axis flipped

Examples

```
muBrain <- readRDS(unzip(system.file("extdata", "muBrainSubset_SpatialOverlay.zip",
                                   package = "SpatialOmicOverlay")))

image <- downloadMouseBrainImage()

muBrain <- addImageOmeTiff(overlay = muBrain,
                          ometiff = image, res = 8)

showImage(flipY(muBrain))
```

fluorLegend *Add legend of fluorescence targets that make up image*

Description

Creates legend that can be overlaid on image using cowplot.

Usage

```
fluorLegend(overlay, nrow = 4, textSize = 10, boxColor = "grey", alpha = 0.25)
```

Arguments

overlay	SpatialOverlay
nrow	number of rows in the legend. Most studies have 4 which is where the values came from: 1 = horizontal legend, 4 = vertical legend, 2 = box legend
textSize	font size
boxColor	color of box behind legend
alpha	alpha value of box behind legend

Value

gp of fluorescence legend

Examples

```
muBrain <- readRDS(unzip(system.file("extdata", "muBrainSubset_SpatialOverlay.zip",
                                   package = "SpatialOmicOverlay")))

# image <- downloadMouseBrainImage()

# muBrain <- addImageOmeTiff(overlay = muBrain,
#                             ometiff = image, res = 8)

gp <- plotSpatialOverlay(overlay = muBrain,
                          hiRes = FALSE, scaleBar = FALSE)

legend <- fluorLegend(muBrain, nrow = 2, textSize = 3, boxColor = "red")

cowplot::ggdraw() +
  cowplot::draw_plot(gp) +
  cowplot::draw_plot(legend, scale = 0.12, x = -0.3, y = -0.25)
```

moveCoords

Move coordinates if they don't match image

Description

If generated coordinates do not match the image use this function to move coordinates. Coordinates are only changed 1 pixel at a time.

Usage

```
moveCoords(overlay, direction = "right")
```

Arguments

overlay	SpatialOverlay object
direction	which direction should coordinates move: left, right, up, down

Value

SpatialOverlay object

Examples

```
muBrain <- readRDS(unzip(system.file("extdata", "muBrainSubset_SpatialOverlay.zip",
                                   package = "SpatialOmicOverlay")))
head(coords(muBrain), 3)
head(coords(moveCoords(muBrain, direction = "up")), 3)
```

parseOverlayAttrs *Parse the xml file for AOI attributes in GeoMx images*

Description

Parse the xml file for AOI attributes in GeoMx images

Usage

```
parseOverlayAttrs(omexml, annots, labworksheet)
```

Arguments

omexml	xml file from OME-TIFF, can provide path to OME-TIFF and xml will automatically be extracted
annots	df of annotations
labworksheet	annots are from lab worksheet file

Value

SpatialPosition of AOIs containing metadata and base64encoded positions

Examples

```
image <- downloadMouseBrainImage()
xml <- xmlExtraction(ometiff = image)
muBrainLW <- system.file("extdata", "muBrain_LabWorksheet.txt",
                        package = "SpatialOmicOverlay")
muBrainLW <- readLabWorksheet(muBrainLW, slideName = "D5761 (3)")
overlay <- parseOverlayAttrs(omexml = xml,
                             annots = muBrainLW,
                             labworksheet = TRUE)
```

parseScanMetadata *Parse the xml file for the scan metadata of GeoMx images*

Description

Parse the xml file for the scan metadata of GeoMx images

Usage

```
parseScanMetadata(omexml)
```

Arguments

omexml xml file from OME-TIFF, can provide path to OME-TIFF and xml will automatically be extracted

Value

metadata for entire scan

Examples

```
image <- downloadMouseBrainImage()
xml <- xmlExtraction(ometiff = image)
scan_metadata <- parseScanMetadata(omexml = xml)
```

plotSpatialOverlay *overlay plots*

Description

overlay plots

Usage

```
plotSpatialOverlay(
  overlay,
  colorBy = "sampleID",
  hiRes = TRUE,
  alpha = 1,
  legend = TRUE,
  scaleBar = TRUE,
  image = TRUE,
```

```

    fluorLegend = FALSE,
    ...,
    corner = "bottomright",
    scaleBarWidth = 0.2,
    scaleBarMicrons = NULL,
    scaleBarColor = NULL,
    scaleBarFontSize = 6,
    scaleBarLineSize = 1.5,
    textDistance = 2
  )

```

Arguments

overlay	SpatialOverlay object
colorBy	annotation to color by
hiRes	generated figures are either high resolution or print quickly. Note: hiRes and outline ggplots use fill, lowRes uses color
alpha	opacity of overlays
legend	should legend be plotted
scaleBar	should scale bar be plotted
image	should image be plotted, image must be added to SpatialOverlay object
fluorLegend	should viz marker on the image be added to legend
...	additional parameters for scale bar line & text, will affect both
corner	where in the figure should the scale bar be printed. Options: "bottomright" "topright" "bottomleft" "topleft" "bottomcenter" "topcenter"
scaleBarWidth	percent of total figure the scale bar should take up
scaleBarMicrons	specific microns to set scale bar at, overrides scaleBarWidth if set
scaleBarColor	scale bar & text color
scaleBarFontSize	font size
scaleBarLineSize	width of line
textDistance	text's distance from scale bar.

Value

gp

Note

hiRes and outline ggplots use fill, lowRes uses color

Examples

```
muBrain <- readRDS(unzip(system.file("extdata", "muBrainSubset_SpatialOverlay.zip",
                                   package = "SpatialOmicOverlay")))

plotSpatialOverlay(overlay = muBrain, legend = FALSE,
                   hiRes = FALSE, scaleBar = FALSE)
```

readLabWorksheet	<i>Read lab worksheet into dataframe of annotations</i>
------------------	---

Description

Read lab worksheet into dataframe of annotations

Usage

```
readLabWorksheet(lw, slideName)
```

Arguments

lw	lab worksheet file path
slideName	name of slide

Value

df of ROI annotations

Examples

```
muBrainLW <- system.file("extdata", "muBrain_LabWorksheet.txt",
                         package = "SpatialOmicOverlay")

muBrainLW <- readLabWorksheet(muBrainLW, slideName = "D5761 (3)")
```

readSpatialOverlay *Read in [SpatialOverlay](#) from tiff file and annotations*

Description

Create an instance of class [SpatialOverlay](#) by reading data from OME-TIFF and annotation sheet.

Usage

```
readSpatialOverlay(  
  ometiff,  
  annots,  
  slideName,  
  image = FALSE,  
  res = NULL,  
  saveFile = FALSE,  
  outline = TRUE  
)
```

Arguments

ometiff	path to OME-TIFF
annots	path to annotation file: can be labWorksheet, DA excel file, or delimited file
slideName	name of slide
image	should image be extracted from OME-TIFF
res	resolution of image 1 = largest, higher number = smaller This value will affect the coordinates of the overlays. res = 2, resolution is 1/2 the size as the raw image res = 3, resolution is 1/4 the size as the raw image res = 4, resolution is 1/8 the size as the raw image resolution = 1/2^(res-1)
saveFile	should xml & image be saved, file is saved in working directory with same name as OME-TIFF
outline	returned coordinates only contain outlinearies, will not work for segmented ROIs

Value

[SpatialOverlay](#) of slide

See Also

[SpatialOverlay-class](#)

Examples

```
muBrain_GxT <- readRDS(unzip(system.file("extdata", "muBrain_GxT.zip",
                                       package = "SpatialOmicsOverlay")))

image <- downloadMouseBrainImage()

muBrain <- readSpatialOverlay(ometiff = image, annots = muBrain_GxT[,1:5],
                             slideName = "D5761 (3)", image = TRUE, res = 8,
                             saveFile = FALSE, outline = FALSE)
```

recolor	<i>recolor images after changing colors and/or color intensities</i>
---------	--

Description

recolor images after changing colors and/or color intensities

Usage

```
recolor(overlay)
```

Arguments

overlay SpatialOverlay object

Value

SpatialOverlay object with RGB image

Examples

```
muBrain <- readRDS(unzip(system.file("extdata", "muBrainSubset_SpatialOverlay.zip",
                                       package = "SpatialOmicsOverlay")))

image <- downloadMouseBrainImage()

muBrain <- add4ChannelImage(overlay = muBrain,
                           ometiff = image, res = 8)

muBrain <- changeImageColoring(overlay = muBrain, color = "magenta",
                              dye = "Cy5")

showImage(recolor(muBrain))
```

removeSample	<i>Remove sample(s) from SpatialOverlay</i>
--------------	---

Description

Remove sample(s) from SpatialOverlay

Usage

```
removeSample(overlay, remove)
```

Arguments

overlay	SpatialOverlay object
remove	sampNames of overlay to remove

Value

SpatialOverlay object without samples in remove

Examples

```
muBrain <- readRDS(unzip(system.file("extdata", "muBrainSubset_SpatialOverlay.zip",
                                   package = "SpatialOmicOverlay")))

muBrainLW <- system.file("extdata", "muBrain_LabWorksheet.txt",
                        package = "SpatialOmicOverlay")

muBrainLW <- readLabWorksheet(muBrainLW, slideName = "D5761 (3)")

samps <- muBrainLW$Sample_ID[muBrainLW$segment != "Full ROI"]

muBrainSub <- removeSample(overlay = muBrain, remove = samps)

muBrain
muBrainSub
```

SpatialOverlay-class	<i>Class to Contain NanoString Spatial Overlay Images and Data</i>
----------------------	--

Description

The SpatialOverlay class organizes the pertinent information from the OME-TIFFs allowing for plotting on top of or beside the image

Usage

```
SpatialOverlay(slideName,
               scanMetadata,
               overlayData,
               coords = NULL,
               plottingFactors = NULL,
               workflow = list(outline=FALSE,
                              labWorksheet=TRUE,
                              scaled=FALSE),
               image = list(filePath = NULL,
                             imagePointer = NULL,
                             resolution = NULL))
```

Arguments

slideName	The name of the slide in the SpatialOverlay object
scanMetadata	A list containing the scan metadata: panel(s) used, Physical sizes (x,y) for scale bar, fluorescence data, and segmentation info
overlayData	An SpatialPosition containing individual sample info: SampleID, Height, Width, X&Y coordinates in overall scan, segmentation, and base64 encoded position
coords	An optional data.frame with coordinates derived from the encoded position.
plottingFactors	An optional data.frame with annotations to plot on. These can be added using addPlottingFactor
workflow	A list containing meta-data on the processing workflow including "outline", "labWorksheet", & "scaled". These booleans are kept automatically in readSpatialOverlay and are used for logic gates in downstream processing
image	A list containing location of and pointer to the image: "filePath", "imagePointer", & "resolution"

Value

An S4 class containing image data from a NanoString GeoMx experiment

Accessing

SpatialOverlay objects have the following accessor methods:

sampNames(object) extracts the sample names of each ROI in the slide.

slideName(object) extracts the slide name.

overlay(object) extracts the SpatialPosition information for each ROI.

scanMeta(object) extracts the scan metadata.

scanMeta(object) coords(object): extracts the coordinates for the entire scan.

plotFactors(object) extracts available plotting factors.

labWork(object) extracts the boolean if a lab worksheet was used.

outline(object) extracts the boolean if only the outline points were generated.

seg(object) extracts if there are any segmented ROIs in the slide.

scaleBarRatio(object) extracts the scale bar ratio from scanMeta for the X axis

fluor(object) extracts fluorescence information for the scan.

showImage(object) prints image.

res(object) extracts resolution of image.

workflow(object) extracts workflow data.

scaled(object) extracts if coordinates have been scaled.

imageInfo(object) extracts image data.

See Also

[readSpatialOverlay](#)

SpatialPosition-class *Class to Contain NanoString Spatial Overlay ROI information*

Description

The SpatialPosition class organizes the pertinent ROI specific information from the OME-TIFFs

Usage

```
SpatialPosition(position)
```

Arguments

position	data.frame containing information from the OME-TIFF: "ROIlabel" order of ROIs "Sample_ID" unique identifier "Height" total height of ROI "Width" total width of ROI "X" top left corner (x coordinate) of ROI in total scan "Y" top left corner (y coordinate) of ROI in total scan "Segmentation" part of segmented ROI "Position" base64 encoding of coordinates
----------	--

Value

An S4 class containing image data from a NanoString GeoMx experiment

Accessing

SpatialPosition objects have the following accessor methods:

spatialPos(object) returns SpatialPosition object

meta(object) extracts the metadata for each ROI, does not include the base64 encoding.

position(object) extracts the base64 encoding for each ROI, CAUTION: very long strings.

See Also

[SpatialOverlay-class](#)

xmlExtraction	<i>Extract xml from OME-TIFF</i>
---------------	----------------------------------

Description

Extract xml from OME-TIFF

Usage

```
xmlExtraction(ometiff, saveFile = FALSE, outdir = NULL)
```

Arguments

ometiff	path to OME-TIFF
saveFile	should xml be saved, file is saved in working directory with same name as OME-TIFF
outdir	output directory for saved xml. If NULL, saved in same directory as OME-TIFF

Value

list of xml data

Examples

```
image <- downloadMouseBrainImage()
xml <- xmlExtraction(ometiff = image)
```

Index

- * **classes**
 - SpatialOverlay-class, [22](#)
 - SpatialPosition-class, [24](#)
- * **methods**
 - SpatialOverlay-class, [22](#)
 - SpatialPosition-class, [24](#)

- add4ChannelImage, [2](#)
- addImageFile, [3](#)
- addImageOmeTiff, [4](#)
- addPlottingFactor, [5](#), [23](#)
- addPlottingFactor, character-method
(addPlottingFactor), [5](#)
- addPlottingFactor, data.frame-method
(addPlottingFactor), [5](#)
- addPlottingFactor, factor-method
(addPlottingFactor), [5](#)
- addPlottingFactor, matrix-method
(addPlottingFactor), [5](#)
- addPlottingFactor, NanoStringGeoMxSet-method
(addPlottingFactor), [5](#)
- addPlottingFactor, numeric-method
(addPlottingFactor), [5](#)
- addPlottingFactor, tbl-method
(addPlottingFactor), [5](#)
- addPlottingFactor, tbl_df-method
(addPlottingFactor), [5](#)

- bookendStr, [6](#)

- changeColoringIntensity, [7](#)
- changeImageColoring, [8](#)
- checkValidRes, [9](#)
- class:SpatialOverlay
(SpatialOverlay-class), [22](#)
- class:SpatialPosition
(SpatialPosition-class), [24](#)
- coords (SpatialOverlay-class), [22](#)
- coords, SpatialOverlay-method
(SpatialOverlay-class), [22](#)

- createCoordFile, [9](#)
- createMask, [10](#)
- cropSamples, [11](#)
- cropTissue, [12](#)

- downloadMouseBrainImage, [12](#)

- flipX, [13](#)
- flipY, [14](#)
- fluor (SpatialOverlay-class), [22](#)
- fluor, SpatialOverlay-method
(SpatialOverlay-class), [22](#)
- fluorLegend, [14](#)

- imageInfo (SpatialOverlay-class), [22](#)
- imageInfo, SpatialOverlay-method
(SpatialOverlay-class), [22](#)

- labWork (SpatialOverlay-class), [22](#)
- labWork, SpatialOverlay-method
(SpatialOverlay-class), [22](#)

- meta (SpatialPosition-class), [24](#)
- meta, SpatialPosition-method
(SpatialPosition-class), [24](#)
- moveCoords, [15](#)

- outline (SpatialOverlay-class), [22](#)
- outline, SpatialOverlay-method
(SpatialOverlay-class), [22](#)
- overlay (SpatialOverlay-class), [22](#)
- overlay, SpatialOverlay-method
(SpatialOverlay-class), [22](#)

- parseOverlayAttrs, [16](#)
- parseScanMetadata, [17](#)
- plotFactors (SpatialOverlay-class), [22](#)
- plotFactors, SpatialOverlay-method
(SpatialOverlay-class), [22](#)
- plotSpatialOverlay, [17](#)
- position (SpatialPosition-class), [24](#)

- position, SpatialPosition-method
(SpatialPosition-class), [24](#)

- readLabWorksheet, [19](#)
- readSpatialOverlay, [20](#), [23](#), [24](#)
- recolor, [21](#)
- removeSample, [22](#)
- res (SpatialOverlay-class), [22](#)
- res, SpatialOverlay-method
(SpatialOverlay-class), [22](#)

- sampNames (SpatialOverlay-class), [22](#)
- sampNames, SpatialOverlay-method
(SpatialOverlay-class), [22](#)
- scaleBarRatio (SpatialOverlay-class), [22](#)
- scaleBarRatio, SpatialOverlay-method
(SpatialOverlay-class), [22](#)
- scaled (SpatialOverlay-class), [22](#)
- scaled, SpatialOverlay-method
(SpatialOverlay-class), [22](#)
- scanMeta (SpatialOverlay-class), [22](#)
- scanMeta, SpatialOverlay-method
(SpatialOverlay-class), [22](#)
- seg (SpatialOverlay-class), [22](#)
- seg, SpatialOverlay-method
(SpatialOverlay-class), [22](#)
- show, SpatialOverlay-method
(SpatialOverlay-class), [22](#)
- show, SpatialPosition-method
(SpatialPosition-class), [24](#)
- showImage (SpatialOverlay-class), [22](#)
- showImage, SpatialOverlay-method
(SpatialOverlay-class), [22](#)
- slideName (SpatialOverlay-class), [22](#)
- slideName, SpatialOverlay-method
(SpatialOverlay-class), [22](#)
- SpatialOverlay, [5](#), [20](#)
- SpatialOverlay (SpatialOverlay-class),
[22](#)
- SpatialOverlay, character-method
(SpatialOverlay-class), [22](#)
- SpatialOverlay-class, [22](#)
- spatialPos (SpatialPosition-class), [24](#)
- spatialPos, SpatialPosition-method
(SpatialPosition-class), [24](#)
- SpatialPosition, [23](#)
- SpatialPosition
(SpatialPosition-class), [24](#)

- SpatialPosition, data.frame-method
(SpatialPosition-class), [24](#)
- SpatialPosition, environment-method
(SpatialPosition-class), [24](#)
- SpatialPosition, matrix-method
(SpatialPosition-class), [24](#)
- SpatialPosition, missing-method
(SpatialPosition-class), [24](#)
- SpatialPosition-class, [24](#)

- workflow (SpatialOverlay-class), [22](#)
- workflow, SpatialOverlay-method
(SpatialOverlay-class), [22](#)

- xmlExtraction, [25](#)