

# Package ‘SpatialOmicsOverlay’

December 27, 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** 1.6.0

**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** 7.3.1

**Config/testthat/edition** 3

**git\_url** <https://git.bioconductor.org/packages/SpatialOmicsOverlay>

**git\_branch** RELEASE\_3\_20

**git\_last\_commit** f16323e

**git\_last\_commit\_date** 2024-10-29

**Repository** Bioconductor 3.20

**Date/Publication** 2024-12-26

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

### 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 = "SpatialOmicOverlay")))

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 = "SpatialOmicOverlay")))

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	<a href="#">SpatialOverlay</a> 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 = "SpatialOmicsOverlay")))

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

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 = "ROINum")

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 = "ROINum")

head(plotFactors(muBrain))

```

---

annotMatching	<i>Match ROIs in annotation file to xml</i>
---------------	---------------------------------------------

---

### Description

Match ROIs in annotation file to xml

### Usage

```
annotMatching(annots, ROIum, maskNum, maskText, segCol = NULL)
```

### Arguments

annots	df of annotations
ROIum	ROI number from xml file
maskNum	number of masks for ROI, used for AOI matching in software <= v2.4
maskText	segment name, used for AOI matching in software v2.4+
segCol	column containing segment name, if NULL function will determine automatically

### Value

df with ROI unique identifiers

---

bookendStr	<i>Print long string in more managable fashion</i>
------------	----------------------------------------------------

---

**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	<i>Update color intensities for changing to RGB image</i>
-------------------------	-----------------------------------------------------------

---

**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 = "SpatialOmicsOverlay")))

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 = "SpatialOmicsOverlay")))

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                      *Determine lowest resolution image in OME-TIFF*

---

### 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                      *Create coordinate file for entire scan*

---

### 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

*Create a binary mask from a base 64 string*

---

**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 = "SpatialOmicOverlay")))

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	<i>Crop to remove black boundary around tissue.</i>
------------	-----------------------------------------------------

---

**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 = "SpatialOmicsOverlay")))

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://nanosttring.com/products/geomx-digital-spatial-profiler/spatial-organ-atlas/mouse-brain/>

**Value**

mouse brain OME-TIFF

**Examples**

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

---

flipX

*Flip x axis in image and overlay points*

---

**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 = "SpatialOmicsOverlay")))

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
...	segCol in annotMatching, if auto detection doesn't work.

### 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, roiCol = NULL, slideCol = NULL)
```

**Arguments**

lw	lab worksheet file path
slideName	name of slide
roiCol	column containing ROI information, if NULL function will determine automatically
slideCol	column containing slide name, if NULL function will determine automatically

**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,
  ...,
  segCol = NULL
)
```

**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
...	additional parameters for 'readLabWorksheet' like 'roiCol' and 'slideCol'
segCol	additional parameter for 'annotMatching' if default search doesn't work. For default search, set to NULL

**Value**

[SpatialOverlay](#) of slide

**See Also**

[SpatialOverlay-class](#)

**Examples**

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

image <- downloadMouseBrainImage()

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

---

recolor

---

*recolor images after changing colors and/or color intensities*


---

**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 = "SpatialOmicOverlay")))

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    *Class to Contain NanoString Spatial Overlay Images and Data*

---

### 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 <a href="#">SpatialPosition</a> 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 <a href="#">addPlottingFactor</a>
workflow	A list containing meta-data on the processing workflow including "outline", "labWorksheet", & "scaled". These booleans are kept automatically in <a href="#">readSpatialOverlay</a> 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)
```



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