# Requirements

### Reqs for addImageOmeTiff:

- The user input overlay, a SpatialOverlay object
- The user input ometiff file path (default NULL)
- The user input the res(olution) image layer to extract from the OMETIFF (default NULL)
- The user can input extra variables for imageExtraction function
- The function outputs a SpatialOverlay object
  - The function outputs extracted RGB image in object
  - The function outputs calculates and scales coordinates if initial image or at a different resolution

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-addimageometiff$ 

### Reqs for addImageFile:

- The user input overlay, a SpatialOverlay object
- The user input imageFile path for non-OMETIFF image (default NULL)
- The user input the res(olution) image layer extracted from the OMETIFF (default NULL)
- The function outputs a SpatialOverlay object
  - The function outputs image in object
  - The function outputs calculates and scales coordinates if initial image or at a different resolution

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-addimagefile$ 

#### Regs for add4ChannelImage:

- The user input overlay, a SpatialOverlay object
- The user input ometiff file path (default NULL)
- The user input the res(olution) image layer to extract from the OMETIFF (default NULL)
- The user can input extra variables for imageExtraction function
- The function outputs a Spatial Overlay object
  - The function outputs extracted 4-channel image in object
  - The function outputs calculates and scales coordinates if initial image or at a different resolution

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-add4channelimage$ 

### Reqs for addPlottingFactor:

- The user input overlay, a SpatialOverlay object
- The user input annots, a NanoStringGeoMxSet, matrix, data.frame, or vector
- The user input the plotting Factor: column or row name from annots
- If using NanoStringGeoMxSet, the user input countMatrix in object to pull counts from (default "exprs")
- The function outputs a SpatialOverlay object
  - The function outputs plottingFactor in plotFactors
  - The function matches on SampleID or if unnamed vector assumes correct order

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-addplottingfactor$ 

### Regs for createCoordFile:

- The user input overlay, a SpatialOverlay object
- The user input outline, only outline or all coordinates boolean (default = TRUE)
- The function outputs a SpatialOverlay object
  - The function outputs coordinates in coords
  - The function specifies workflow@scaled and workflow@outline
  - The function only returns outline coords on Geometric datasets
  - The function wraps all coordinate generating functions together to add to SpatialOverlay object

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-createcoordfile$ 

### Reqs for createMask:

- The user input b64string, a base64 encoded string containing AOI position
- The user input metadata, metadata of AOI including height and width
- The user input outline, only outline or all coordinates boolean (default = TRUE)
- The function outputs a binary mask matrix
  - The function outputs binary matrix containing AOI position
  - The function outputs matrix in metadata specified height and width
  - The function outputs only outline coordinates if specified, else all coordinates are output

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-createmask$ 

#### Regs for coordsFromMask:

- The user input mask, binary mask matrix
- The user input metadata, metadata of AOI including X,Y in full image
- The user input outline, only outline or all coordinates boolean (default = TRUE)
- The function outputs a data frame of coordinate values
  - The function outputs data.frame containing X,Y coordinates of all AOIs in slide
  - The function changes coordinates from 1 base to 0 base
  - The function sorts coordinates if returning outline coords

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-coordsfrommask$ 

### Reqs for pencilCoordSorting:

- The user input coords for an AOI
- The user input rangeWidth, distance to look for closest coord (default = 100)
- The function outputs an ordered data.frame of coordinate values
  - The function outputs data.frame containing X,Y coordinates of all AOIs in slide ordered by proximity to the previous coordinate
  - The function does not repeat coordinates

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-pencilsorting coords$ 

### Reqs for boundary:

- The user input mat, binary mask matrix
- The function outputs an vector
  - The function outputs vector of number of neighbors each point has

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-boundary$ 

### Reqs for scaleCoords:

- The user input overlay, a SpatialOverlay object
- The function outputs a SpatialOverlay object
  - The function outputs SpatialOverlay object with coordinates scaled to image size
  - The function does not scale coordinates again if already scaled
  - The function does not scale coordinates if no image in object
  - The function only returns unique coordinates

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-scalecoords$ 

### Reqs for imageColoring:

- The user input omeImage, a 4-channel AnnotatedImage object
- The user input scanMeta, scan metadata including fluorescence info
- The function outputs a AnnotatedImage object
  - The function outputs an RGB AnnotatedImage object
  - The function scales to min and max intensity if given in scanMeta
  - The function adds 4-channel RGB together for final RGB matrix
  - The function normalizes RGB matrix

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-image coloring$ 

#### Regs for changeImageColoring:

- The user input overlay, a SpatialOverlay object with 4-channel image
- The user input color, new color hex or R color name
- The user input dye, Dye or DisplayName to change color of
- The function outputs a SpatialOverlay object
  - The function outputs SpatialOverlay object with fluorescence ColorCode updated
  - The function only works on objects with 4-channel images
  - The function only changes valid dye colors

Specifications: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md#specs-for-changeimagecoloring

### Reqs for changeColoringIntensity:

- The user input overlay, a SpatialOverlay object with 4-channel image
- The user input minInten, new minimum intensity value (default = NULL)
- The user input maxInten, new maximum intensity value (default = NULL)
- The user input dye, Dye or DisplayName to change color of
- The function outputs a SpatialOverlay object
  - The function outputs SpatialOverlay object with fluorescence MinIntensity and/or MaxIntensity updated
  - The function only works on objects with 4-channel images
  - The function only changes valid dye colors

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-change$ coloring intensity

### Reqs for recolor:

- The user input overlay, a SpatialOverlay object with 4-channel image
- The function outputs a SpatialOverlay object
  - The function outputs SpatialOverlay object with RGB image
  - The function only works on objects with 4-channel images
  - The function only scales coordinates if needed
  - The function crops to tissue

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-recolor$ 

# Reqs for flipY:

- The user input overlay, a SpatialOverlay object
- The function outputs a SpatialOverlay object
  - The function outputs SpatialOverlay object with Y axis flipped
  - The function flips image and coordinates

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-flipy$ 

#### Reqs for flipX:

- The user input overlay, a SpatialOverlay object
- The function outputs a SpatialOverlay object
  - The function outputs SpatialOverlay object with X axis flipped
  - The function flips image and coordinates

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-flipx$ 

### Reqs for cropSamples:

- The user input overlay, a SpatialOverlay object
- The user input sampleIDs, sampleIDs of AOIs to include
- The user input buffer, percent of image to add on each edge (default = 0.1)
- The user input sampsOnly, only show given samples (default = TRUE)
- The function outputs a SpatialOverlay object
  - The function outputs Spatial Overlay object with cropped image and coordinates only showing given samples
  - The function only works on valid sampleIDs
  - The function only works on RGB images
  - The function requires coordinates to be generated
  - The function requires image in overlay
  - The function generates min/max coords to crop on given sampleIDs
  - The function filters coordinates to only given sampleIDs if desired

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-cropsamples$ 

### Reqs for cropTissue:

- The user input overlay, a SpatialOverlay object
- The user input buffer, percent of image to add on each edge (default = 0.05)
- The function outputs a SpatialOverlay object
  - The function outputs SpatialOverlay object with cropped image and coordinates minimizing black background around tissue
  - The function can work on 4-channel or RGB image
  - The function requires coordinates to be generated with RGB image
  - The function requires image in overlay
  - The function generates min/max coords to crop on tissue

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-croptissue$ 

### Reqs for xmlExtraction:

- The user input ometiff, file path
- The user input saveFile, boolean (default = FALSE)
- The function outputs a list
  - The function outputs list containing all xml info
  - The function only works on valid paths
  - The function saves file to same folder as ometiff if desired

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-xmlextraction$ 

### Reqs for imageExtraction:

- The user input ometiff, file path
- The user input res(olution), image layer to extract from the OMETIFF (default = 6)

- The user input scanMeta, scan metadata from XML (default = NULL)
- The user input saveFile, boolean (default = FALSE)
- The user input fileType, image type to save as (default = "tiff")
- The user input color, turn into RGB image boolean (default = TRUE)
- The function outputs an image
  - If color == TRUE
    - \* The function outputs magick-image pointer
    - \* The function colors image
    - \* The function saves image to same folder as ometiff if desired
    - \* The function only saves to valid fileTypes
  - If color == FALSE
    - \* The function outputs AnnotatedImage
  - The function only works on valid paths
  - The function only extracts valid res layers
  - The function extracts scanMeta if NULL

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-image extraction$ 

### Regs for checkValidRes:

- The user input ometiff, file path
- The function outputs an numeric
  - The function returns highest valid res(olution) value for ometiff

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-checkvalidres$ 

#### Regs for plotSpatialOverlay:

- The user input overlay, file path
- The user input colorBy, coloring factor (default = "sampleID")
- The user input hiRes(olution) plotting boolean (default = TRUE)
- The user input alpha, opacity value (default = 1)
- The user input legend, show legend boolean (default = TRUE)
- The user input scaleBar, show scale bar boolean (default = TRUE)
- The user input image, show image boolean (default = TRUE)
- The user input fluorLegend, show fluorescence legend (default = FALSE)
- The user input  $\dots$ , extra values for scale bar plotting
- The user input corner, scale bar corner (default = "bottomright")
- The user input scaleBarWidth, scale bar width of image percentage (default = 0.2)
- The user input scaleBarColor, scale bar color (default = "black")
- The user input scaleBarFontSize, scale bar font size value (default = 6)
- The user input scaleBarLineSize, scale bar line size value (default = 1.5)
- The user input textDistance, distance of text from line value (default = 2)
- The function outputs a ggplot object
  - The function recolors image if 4-channel is given
  - The function only works on valid plotFactors unless sampleID is given
  - The function only adds fluorLegend if image = TRUE and desired regardless of plotting call
  - The function adds image to background if image = TRUE

- The function uses image coordinates if image is attached even with image = FALSE
- The function removes legend if desired
- The function adds scalebar if desired
- The function customizes scale bar

Specifications: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md#specs-for-plotspatialoverlay

#### Reqs for scaleBarMath:

- The user input scanMetadata, scan metadata from XML specifically PhysicalX/Y
- The user input pts, coordinate data.frame
- The user input scaleBarWidth, percent of image value (default = 0.2)
- The user input image, image from SpatialOverlay (default = NULL)
- The function outputs a list
  - The function returns list of values to print scale bar
  - The function works with or without image
  - The function calculates scale bar width based on um/pixel ratio
  - The function works on all resolutions
  - The function only creates scale bars with round values

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-scalebarmath$ 

### Reqs for scaleBarCalculation:

- The user input corner, location for scale bar (default = "bottomright")
- The user input scaleBar, list from scaleBarMath
- The user input textDistance, distance of text from line value (default = 2)
- The function outputs a data.frame
  - The function returns data.frame of coordinates for scale bar
  - The function only works with valid corner
  - The function calculates scale bar coordinates

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-scalebarcalculation$ 

### Reqs for scaleBarPrinting:

- The user input gp, ggplot object
- The user input scaleBar, list from scaleBarMath
- The user input corner, distance of text from line value (default = 2)
- The user input scaleBarFontSize, scale bar font size value (default = 6)
- The user input scaleBarLineSize, scale bar line size value (default = 1.5)
- The user input scaleBarColor, scale bar color (default = "red)
- The user input textDistance, distance of text from line value (default = 2)
- The user input image, image from SpatialOverlay (default = NULL)
- The user input ..., extra values for scale bar plotting
- The function outputs a ggplot object
  - The function returns ggplot with added scale bar

- The function works with or without image
- The function customizes scale bar

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-scalebarprinting$ 

#### Regs for fluorLegend:

- The user input overlay, SpatialOverlay object
- The user input nrow, number of rows value (default = 4)
- The user input textSize, font size value (default = 10)
- The user input boxColor, color of background (default = "grey")
- The user input alpha, opacity value (default = 0.25)
- The function outputs a ggplot object
  - The function returns ggplot of visualization marker legend
  - The function only works with valid nrows
  - The function colors Target based on ColorCode
  - The function adds background based on user input

#### Specifications:

### Reqs for readSpatialOverlay:

- The user input ometiff, file path
- The user input annots, file path or GeomxSet object
- The user input slideName, name of slide
- The user input image, add image boolean (default = FALSE)
- The user input res, image layer to extract from the OMETIFF (default = NULL)
- The user input saveFile, save file boolean (default = FALSE)
- The user input outline, outline coords only boolean (default = TRUE)
- The function outputs a SpatialOverlay object
  - The function returns starting point SpatialOverlay
  - The function annotations can be NanostringGeomxSet, labWorksheet, or DSPDA output
  - The function is a wrapper for extracting XML, add scan metadata, add overlay attrs, determine dataset segmentation, add image, generate coordinates

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-readspatialoverlay$ 

#### Regs for removeSample:

- The user input overlay, SpatialOverlay object
- The user input remove, sampleIDs to remove
- The function outputs a SpatialOverlay object
  - The function returns a SpatialOverlay without sampleIDs to remove
  - The function only works on valid sampleIDs
  - The function removes samples across all parts of the SpatialOverlay object
  - The function determines if dataset segmentation type

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-removes amples$ 

### Reqs for bookendStr:

- The user input x, string
- The user input bookend, length of bookend value (default = 8)
- The function outputs a string
  - The function returns a string small enough to be legible
  - The function returns first bookend ... last bookend (# total char)

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-bookendstr$ 

### Reqs for readLabWorksheet:

- The user input lw, labworksheet file path
- The user input slideName, slide name
- The function outputs a data.frame
  - The function returns a data.frame with AOI annotations
  - The function only works on valid lw
  - The function only works with valid slide name

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-readlabworksheet$ 

### Regs for downloadMouseBrainImage:

- The function outputs a tiff file path
  - The function returns a cached tiff file path for Spatial Organ Atlas Mouse Brain OMETIFF
  - The function downloads and untars file if not already cached

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-downloadmousebrainimage$ 

#### Regs for parseScanMetadata:

- The user input omexml, xml list
- The function outputs a list
  - The function returns a list of scan metadata including panel, physical size and fluorescence data
  - The function can handle omexml being the file path to ometiff

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-downloadmousebrainimage$ 

#### Regs for parseOverlayAttrs:

- The user input omexml, xml list
- The user input annots, data.frame of annotations
- The user input labworksheet, is annot labworksheet boolean
- The function outputs a SpatialPosition

- The function returns a list of AOI metadata including AOI height, width, and location
- The function can handle xml files from multiple software versions
- The function only returns AOIs in both XML and annots

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-parse overlay attrs$ 

### Regs for physicalSizes:

- The user input omexml, xml list
- The function outputs a list
  - The function returns a list of PhysicalSizes from xml

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-physical sizes$ 

### Reqs for fluorData:

- The user input omexml, xml list
- The function outputs a data.frame
  - The function returns a data frame of fluorescence data from xml including: dye, displayname, color, wavelength, target, exposuretime, colorcode, and min/max intensities if available.
  - The function can work if fluorescence data takes 1 or 2 slots in XML

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-fluordata$ 

#### Regs for decodeB64:

- The user input b64string, base 64 location string
- The user input width, width of AOI
- The user input height, height of AOI
- The function outputs a vector
  - The function returns a binary vector of AOI position

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-decodeb64$ 

# Reqs for annotMatching:

- The user input annots, data.frame of annotations
- The user input ROInum, ROI number from xml
- The user input maskNum, number of masks in ROI
- The user input maskText, segment name
- The function outputs a data.frame
  - The function returns a data frame of the matching sampleID
  - The function only works on valid ROInums
  - The function can work on multiple versions of the software

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-annotmatching$ 

### Regs for SpatialOverlay-class:

- The class contains all info pertaining to image
  - The class contains slideName
  - The class contains scanMetadata
    - \* List of panel, physicalsize, and fluorescence
  - The class contains overlayData
    - \* SpatialPosition
  - The class contains coords
  - The class contains plottingFactors
    - \* Added individually
  - The class contains workflow
    - \* Workflow booleans that affect downstream analysis: outline, labworksheet, scaled
  - The class contains image
    - \* list of image related info: filePath, imagePointer, resolution
- The class contains accessors and replacers where necessary

 $Specifications: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md\#specs-for-spatialoverlay-class$ 

### Reqs for SpatialPosition-class:

- The class contains all info pertaining to position in space
  - The class contains ROILabel
  - The class contains Sample ID
  - The class contains Height
  - The class contains Width
  - The class contains X
  - The class contains Y
  - The class contains Segmentation
  - The class contains Position
    - \* Base64 encoded string
- The class contains accessors and replacers where necessary

# **Specifications**

Specifications: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/main/specs.md#specs-for-spatialposition-class #### Specs for addImageOmeTiff:

1. The function outputs a list in the image slot containing the expected filePath, imagePointer, and resolution.

test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e308/longtests/testthat/test\_addImage.R#L11 2. The imagePointer is a magick-image with the correct dimensions

test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e308/longtests/testthat/test\_addImage.R#L26 3. The function scales the coordinates.

test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e308/longtests/testthat/test\_addImage. R#L42~4. The function produces reproducible results.

 $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e308/longtests/testthat/test\_addImage.R\#L36$ 

### Specs for add4ChannelImage:

- 1. The function outputs a list in the image slot containing the expected filePath, imagePointer, and resolution.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_addImage.R\#L77$
- 2. The imagePointer is an AnnotatedImage with the correct dimensions.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_addImage.R\#L92$
- 3. The function scales the coordinates.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e3081blogtests/testthat/test\_addImage.R\#L101$

#### Specs for addImageFile:

- 1. The function outputs a list in the image slot containing the expected filePath, imagePointer, and resolution.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_addImage.R\#L130$
- $2. \ \,$  The image Pointer is a magick-image with the correct dimensions.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_addImage.R\#L140$
- 3. The function scales the coordinates.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e3081blogtests/testthat/test\_addImage.R\#L151$
- 4. The function produces reproducible results.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_addImage.R\#L146$

#### Specs for addPlottingFactor:

- 1. The function only works on one factor at a time regardless of input type.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_addPlottingFactor.R\#L16$
- 2. The function gives warning for annotation missing for samples in object regardless of input type.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_addPlottingFactor.R\#L30$
- 3. The function works with a data frame input, column name plotting factor.
  - $test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_addPlottingFactor.R\#L18$
- 4. The function works with a data frame input, row name plotting factor.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_addPlottingFactor.R\#L32$
- 5. The function works with a matrix input, column name plotting factor.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_addPlottingFactor.R\#L46$
- 6. The function works with a matrix input, row name plotting factor.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_addPlottingFactor.R\#L59$
- 7. If vectors aren't named they must be the same length as number of samples in object.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e3081blogtests/testthat/test\_addPlottingFactor.R\#L71$

- 8. The function only matches vectors if they are named, otherwise assumed in correct order. test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30 longtests/testthat/test\_addPlottingFactor.R#L77
- 9. The function works with character vectors. test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30 longtests/testthat/test\_addPlottingFactor.R#L79
- 10. The function works with factor vectors.
- 11. The function works with numeric vectors.
- 12. The function works with a NanostringGeomxSet input, column name plotting factor. test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30 longtests/testthat/test\_addPlottingFactor.R#L139
- $13. \label{lem:composition} \begin{tabular}{l} 13. The function works with a NanostringGeomxSet input, row name plotting factor. \\ test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30 longtests/testthat/test_addPlottingFactor.R\#L148 \\ \end{tabular}$

### Specs for decodeB64:

1. The function produces same values as python truth. test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30 longtests/testthat/test\_coordinateGeneration.R#L19

#### **Specs for createMask:** When outline == FALSE,

- 1. The function creates mask in correct dimension.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_coordinateGeneration.R\#L31$
- 2. The function produces same values as python truth.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30610ngtests/testthat/test\_coordinateGeneration. R\#L33$
- 3. The function produces mask of only 0 & 1.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_coordinateGeneration.R\#L35$
- 4. The function creates matrix.

#### When outline == TRUE,

- 1. The function creates mask in correct dimension.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_coordinateGeneration.R\#L59$
- 2. The function has fewer matches with the python truth.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_coordinateGeneration.R\#L61$
- 3. The function produces mask of only 0 & 1.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e3081blogtests/testthat/test\_coordinateGeneration. R\#L64$

4. The function creates matrix.

 $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_coordinateGeneration.R\#L66$ 

5. The function create mask with < 0.1% of points with 7 or more neighbors.

 $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_coordinateGeneration.R\#L69$ 

### **Specs for coordsFromMask:** When outline == FALSE,

1. The function creates coordinates for mask = 1 points. Coordinates are put into full image range and changed from base1 to base0.

 $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_coordinateGeneration.R\#L42$ 

When outline == TRUE,

1. The function creates coordinates for mask = 1 points. Coordinates are put into full image range and changed from base1 to base0.

 $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_coordinateGeneration.R\#L74$ 

### Specs for pencilSortingCoords:

1. The function sorts outline coordinates by proximity. >99% of differences between adjacent coordinates is 1

test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30 longtests/testthat/test coordinateGeneration. R#L101

2. The function sorts outline coordinates by proximity. The max difference is <100.

 $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_coordinateGeneration.R\#L104$ 

### Specs for createCoordFile:

1. The function places coordinates in correct location.

 $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_coordinateGeneration.R\#L118$ 

2. The function produces same values as python truth.

 $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_coordinateGeneration.R\#L125$ 

3. The function only returns outline coordinates on Geometric data.

 $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_coordinateGeneration.R\#L136$ 

#### Specs for Boundary:

1. The function returns expected number of neighbors.

 $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_coordinateGeneration.R\#L145$ 

#### Specs for scaleCoords:

- 1. The function scales the coordinates based on the size of the image.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_addImage.R\#L46$
- 2. The coordinates are all smaller than the image size.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_addImage.R\#L54$
- 3. There are no duplicated coordinates.
  - test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30 longtests/testthat/test addImage. R#L61
- 4. Coordinates can't be rescaled.
  - test:  $https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_addImage.R\#L67$
- 5. An image must be in object to scale coordinates.

### Specs for xmlExtraction:

- 1. The function only works with valid ometiff file.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_extraction.R\#L4$
- 2. The function returns a valid list with the expected names.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_extraction.R\#L12$
- 3. The function saves xml file in expected location, if desired.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_extraction.R\#L19$
- 4. The function doesn't save file when not asked.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e3081blogtests/testthat/test\_extraction. R\#L28$

## Specs for imageExtraction:

- 1. The function only extracts valid res layers.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_extraction. R\#L38$
- 2. The function extracts expected res layer.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_extraction. R\#L40$
- 3. The function saves file in expected location and in correct & valid fileType.

#### Specs for checkValidRes:

1. The function returns expected value.

### Specs for cropTissue:

- 1. The function returns smaller image.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_imageManipulation.R\#L10$
- 2. The function returns all original coordinates.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_imageManipulation.R\#L16$
- 3. The function produces reproducible results.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_imageManipulation.R\#L19$

#### **Specs for cropSamples:** When sampsOnly = TRUE,

- 1. The function returns smaller image.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_imageManipulation.R\#L28$
- 2. The function returns all coordinates of only the given samples.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e3081blogtests/testthat/test\_imageManipulation.R\#L34$
- 3. The function produces reproducible results.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30610ngtests/testthat/test\_imageManipulation.R\#L19$

### When sampsOnly = FALSE

- 1. The function returns smaller image.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_imageManipulation.R\#L44$
- 2. The function returns all coordinates of the given samples.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_imageManipulation.R\#L50$
- 3. The function returns coordinates within dimensions of cropped image.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_imageManipulation.R\#L55$
- 4. The function produces reproducible results.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_imageManipulation.R\#L66$
- 5. The function only works with valid sampleIDs.

# Specs for flipX:

- 1. The function returns expected coordinates.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_imageManipulation.R\#L80$
- 2. The function produces reproducible results.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e3081blogtests/testthat/test\_imageManipulation.R\#L85$

### Specs for flipY:

- 1. The function returns expected coordinates.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_imageManipulation.R\#L90$
- 2. The function produces reproducible results.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_imageManipulation.R\#L95$

### Specs for changeColoringIntensity:

- 1. The function only works on 4-channel images.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_imageManipulation.R\#L100$
- 2. The function changes min/max intensity values of only correct fluor.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_imageManipulation.R\#L110$

### Specs for changeImageColoring:

- 1. The function only works on 4-channel images.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_imageManipulation.R\#L103$
- 2. The function changes ColorCode values of only correct fluor.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e3081blogtests/testthat/test\_imageManipulation.R\#L132$

### Specs for imageColoring:

- 1. The function creates RGB image arrays.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_imageManipulation.R\#L157$
- 2. The function produces reproducible results.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_imageManipulation.R\#L162$

## Specs for recolor:

- 1. The function scales coordinates.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_imageManipulation.R\#L170$
- 2. The function creates RGB image arrays.
- 3. The function produces reproducible results.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e3061blogtests/testthat/test\_imageManipulation.R\#L184$

#### Specs for parseScanMetadata:

- 1. The function works on ometiff variable instead of expected xml.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_parsing.R\#L12$
- 2. The function returns a list with all of the expected names.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e3081blogtests/testthat/test\_parsing. R\#L18$

#### Specs for fluorData:

- 1. The function works on xmls where fluor data takes up 1 or 2 lines.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_parsing.R\#L44$
- 2. The function returns a data frame with all of the expected values.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_parsing.R\#L72$

#### Specs for physicalSizes:

1. The function works returns list with expected names & values.

 $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_parsing.R\#L91$ 

### Specs for parseOverlayAttrs:

- 1. The function requires correct labworksheet boolean.
  - $test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_parsing.R\#L105$
- 2. The function only works with valid sample names.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_parsing.R\#L112$
- 3. The function returns SpatialPosition with correct column names.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_parsing.R\#L122$

### Specs for annotMatching:

1. The function matches sampleIDs correctly between xml and annots.

 $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_parsing.R\#L138$ 

#### Specs for plotSpatialOverlay:

- 1. The function requires valid colorBy variable.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_plotting. R\#L22$
- 2. The function returns a ggplot object for high resolution, low resolution and outline graphing.

- 3. The function returns a ggplot object without legend if desired. test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30 longtests/testthat/test\_plotting.R#L51
- 5. The function works on with both 4-channel and RGB images. test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30 longtests/testthat/test\_plotting.R#L250
- $6. \ \, \text{The function produces reproducible figures.} \quad test: \quad https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e308/longtests/testthat/test_plotting.R\#L37$

### Specs for scaleBarMath:

1. The function expects size to be between 0-1. test:https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e308 longtests/testthat/test\_plotting.R#L113

### Without image

- 2. The function returns a list with the expected names and values. test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30 longtests/testthat/test\_plotting.R#L117
- 3. The function returns a um value in valid sizes. test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30 longtests/testthat/test\_plotting.R#L135
- $4. The function calculates the number of pixels for scale bar correctly. \\ test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30 \\ longtests/testthat/test_plotting.R\#L141$

## With image

- 2. The function returns a list with the expected names and values. test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30 longtests/testthat/test\_plotting.R#L288
- 3. The function returns a um value in valid sizes. test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30 longtests/testthat/test\_plotting.R#L306
- 4. The function calculates the number of pixels for scale bar correctly. test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30clongtests/testthat/test\_plotting.R#L312

#### Specs for scaleBarCalculation:

1. The function only works with valid corner value. test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30 longtests/testthat/test\_plotting.R#L147

### Without image

- 2. The function returns a list of numeric values. test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30 longtests/testthat/test\_plotting.R#L150
- $3. The function calculates the scale bar points the same across the different corner options. \\ test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30 longtests/testthat/test_plotting.R#L154$

### With image

- 2. The function returns a list of numeric values. test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30 longtests/testthat/test\_plotting.R#L321
- 3. The function calculates the scale bar points the same across the different corner options. test:  $https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30clongtests/testthat/test\_plotting.R\#L325$

### Specs for scaleBarPrinting: Without image,

- 1. The function only works with valid corner value. test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30 longtests/testthat/test\_plotting.R#L224
- 2. The function produces a ggplot object.
  test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30
  longtests/testthat/test\_plotting.R#L228

  3. The function produces reproducible former test: https://github.com/Nanostring-Biostats/
- 3. The function produces reproducible figures. test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e308/longtests/testthat/test\_plotting. R#L232

### With image

- 2. The function produces a ggplot object. test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30 longtests/testthat/test\_plotting.R#L395
- 3. The function produces reproducible figures. test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e308/longtests/testthat/test\_plotting.R#L399

#### Specs for fluorLegend:

- 1. The function only works on valid nrow values. test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30 longtests/testthat/test\_plotting.R#L405
- 2. The function produces reproducible legends. test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e308/longtests/testthat/test\_plotting. R#L408

#### Specs for readSpatialOverlay:

2. The function only returns samples in both xml and annotation. test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30 longtests/testthat/test\_readSpatialOverlay.R#L25

### With all points

- 1. The function returns a SpatialOverlay object.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_readSpatialOverlay.R\#L32$
- $2. The function returns a Spatial Overlay object with the expected values in the correct locations. \\ test: https://github.com/Nanostring-Biostats/Spatial Omics Overlay/blob/c427265120cc9835f1443541d68a22901195e30 longtests/testthat/test_read Spatial Overlay. R#L35$

#### With image

- 1. The function returns a SpatialOverlay object.
- 2. The function returns a SpatialOverlay object with the expected values in the correct locations. test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30 longtests/testthat/test readSpatialOverlay.R#L65
- 3. The function returns a SpatialOverlay object with image in expected location. test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30 longtests/testthat/test\_readSpatialOverlay.R#L82
- $4. The function returns a Spatial Overlay object with scaled coordinates to image. \\ test: https://github.com/Nanostring-Biostats/Spatial Omics Overlay/blob/c427265120cc9835f1443541d68a22901195e30 longtests/testthat/test_readSpatial Overlay. R\#L88$

# With boundary points

- 1. The function returns a SpatialOverlay object.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30610ngtests/testthat/test\_readSpatialOverlay.R\#L97$
- 2. The function returns a SpatialOverlay object with the expected values in the correct locations. test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30 longtests/testthat/test readSpatialOverlay.R#L100

#### Specs for removeSamples:

- 1. The function only works on valid sample names.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_removeSamples. R\#L25$
- 2. The function works before adding coordinates and plotting factors.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_removeSamples.R\#L31$
- 3. The function works after adding coordinates and before plotting factors.
  - test: https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30 longtests/testthat/test removeSamples. R#L49
- 4. The function works after adding coordinates and plotting factors.

#### Specs for SpatialOverlay-class:

- 1. The class is formatted correctly.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_SpatialOverlay.R\#L17$
- 2. The class accessors work as expected.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_SpatialOverlay.R\#L45$
- 3. The class replacers work as expected.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_SpatialOverlay.R\#L89$

#### Specs for SpatialPosition-class:

- 1. The class is formatted correctly.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_SpatialPosition.R\#L11$
- 2. The class accessors work as expected.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e3081blogtests/testthat/test\_SpatialPosition. R\#L32$

### Specs for bookendStr:

1. The function returns a string in the expected format.

 $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30610ngtests/testthat/test\_utils.R\#L3$ 

### Specs for readLabWorksheet:

- 1. The function only works on correct file paths.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_utils.R\#L24$
- 2. The function only works on correct slide names.
  - $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_utils.R\#L26$
- 3. The function only returns annotations from the specified slide.

#### Specs for downloadMouseBrainImage:

 $1. \ \,$  The function downloads the mouse brain tiff and returns a valid file path.

 $test: \ https://github.com/Nanostring-Biostats/SpatialOmicsOverlay/blob/c427265120cc9835f1443541d68a22901195e30longtests/testthat/test\_utils.R\#L49$