

Package ‘spatialLIBD’

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Description Inspect interactively the spatially-resolved transcriptomics data from the 10x Genomics Visium platform as well as data from the Maynard, Collado-Torres et al, Nature Neuroscience, 2021 project analyzed by Lieber Institute for Brain Development (LIBD) researchers and collaborators.

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`add10xVisiumAnalysis` *Add analysis data from a 10x Genomics Visium experiment to a SPE object*

Description

This function adds to a SPE ([SpatialExperiment-class](#)) object the output from `read10xVisiumAnalysis()`.

Usage

```
add10xVisiumAnalysis(spe, visium_analysis)
```

Arguments

`spe` A [SpatialExperiment-class](#) object.
`visium_analysis` The output from `read10xVisiumAnalysis()`.

Details

You might want to use `read10xVisiumWrapper()` instead of using this function directly.

Value

A [SpatialExperiment-class](#) object with the clustering results from SpaceRanger added to `colData(spe)` and the dimension reduction results added to `reducedDims(spe)`. Added data starts with the `10x_` prefix to make them easy to differentiate.

See Also

Other Utility functions for reading data from SpaceRanger output by 10x Genomics: [read10xVisiumAnalysis\(\)](#), [read10xVisiumWrapper\(\)](#)

Examples

```
## See 'Using spatialLIBD with 10x Genomics public datasets' for
## a full example using this function.
if (interactive()) {
  browseVignettes(package = "spatialLIBD")
}

## Note that ?SpatialExperiment::read10xVisium doesn't include all the files
## we need to illustrate read10xVisiumWrapper().
```

| | |
|------------|---|
| add_images | <i>Add non-standard images with the same dimensions as current ones</i> |
|------------|---|

Description

This function re-uses the `SpatialExperiment::scaleFactors()` from current images when adding new images. This is useful if you take for example a multi-channel VisiumIF image and break into several single-channel images that all have the same dimensions. So you could have a set of images such as `channel_01_lowres` and `channel_02_lowres` that have the same dimensions and viewing area as the lowres image produced by SpaceRanger, each with only one channel. Similarly, you might have done some image manipulation for a given image and generated one or more images with the same dimensions as existing images.

Usage

```
add_images(
  spe,
  image_dir,
  image_pattern,
  image_id_current = "lowres",
  image_id = image_pattern,
  image_paths = locate_images(spe, image_dir, image_pattern)
)
```

Arguments

| | |
|------------------|---|
| spe | Defaults to the output of <code>fetch_data(type = 'spe')</code> . This is a SpatialExperiment-class object with the spot-level Visium data and information required for visualizing the histology. See fetch_data() for more details. |
| image_dir | A character(1) specifying a path to a directory containing image files with the pattern <code>sampleID_pattern.png</code> . |
| image_pattern | A character(1) specifying the pattern for the image files. |
| image_id_current | A character(1) specifying the name of the current existing image in <code>spe</code> that has the same scaling factor that to be used with the additional images. |

| | |
|-------------|--|
| image_id | A character(1) specifying the name to use in the new images. It cannot be the same as one used for existing images in spe for a given sample. It equals image_pattern by default. |
| image_paths | A named character() vector with the paths to the images. The names have to match the spe\$sample_id and cannot be repeated. By default locate_images() is used but you can alternatively specify image_paths and ignore image_dir and image_pattern. |

Value

A [SpatialExperiment-class](#) object with the additional image data in imgData(spe).

See Also

Other Functions for adding non-standard images: [locate_images\(\)](#)

Examples

```
if (enough_ram()) {
  ## Obtain the necessary data
  if (!exists("spe")) spe <- fetch_data("spe")

  ## Add an image
  SpatialExperiment::imgData(add_images(
    spe,
    image_id_current = "lowres",
    image_id = "lowres_aws",
    image_paths = c("151507" = "https://spatial-dlpfc.s3.us-east-2.amazonaws.com/images/151507_tissue_lowres_i
  ))
}
```

add_key

Create a unique spot identifier

Description

This function adds spe\$key to a [SpatialExperiment-class](#) object which is unique across all spots.

Usage

```
add_key(spe, overwrite = TRUE)
```

Arguments

| | |
|-----------|--|
| spe | A SpatialExperiment-class object. |
| overwrite | A logical(1) indicating whether to overwrite the spe\$key. |

Value

A [SpatialExperiment-class](#) object with key added to the colData(spe) that is unique across all spots.

Examples

```
if (enough_ram()) {
  ## Obtain the necessary data
  if (!exists("spe")) spe <- fetch_data("spe")

  ## This object already has a 'key'
  head(spe$key)

  ## We can clean it
  spe$key <- NULL

  ## and then add it back
  head(add_key(spe)$key)

  ## Note that the original 'key' order was 'sample_id'_'barcode' and we'
  ## have since changed it to 'barcode'_'sample_id'.
}
```

check_modeling_results

Check input modeling_results

Description

This function checks that the modeling_results object has the appropriate structure. For more details please check the vignette documentation.

Usage

```
check_modeling_results(modeling_results)
```

Arguments

modeling_results

Defaults to the output of `fetch_data(type = 'modeling_results')`. This is a list of tables with the columns `f_stat_*` or `t_stat_*` as well as `p_value_*` and `fdr_*` plus `ensembl`. The column name is used to extract the statistic results, the p-values, and the FDR adjusted p-values. Then the `ensembl` column is used for matching in some cases. See [fetch_data\(\)](#) for more details.

Value

The input object if all checks are passed.

See Also

Other Check input functions: [check_sce_layer\(\)](#), [check_sce\(\)](#), [check_spe\(\)](#)

Examples

```
if (!exists("modeling_results")) {
  modeling_results <- fetch_data(type = "modeling_results")
}

## Check the object
xx <- check_modeling_results(modeling_results)
```

 check_sce

Check input sce

Description

This function checks that the sce object has the appropriate structure. This is a legacy function and we highly encourage you to use [SpatialExperiment-class](#) objects and check them with [check_spe\(\)](#).

Usage

```
check_sce(
  sce,
  variables = c("GraphBased", "ManualAnnotation", "Maynard", "Martinowich",
    paste0("SNN_k50_k", 4:28), "spatialLIBD", "cell_count", "sum_umi", "sum_gene",
    "expr_chrM", "expr_chrM_ratio", "SpatialDE_PCA", "SpatialDE_pool_PCA", "HVG_PCA",
    "pseudobulk_PCA", "markers_PCA", "SpatialDE_UMAP", "SpatialDE_pool_UMAP", "HVG_UMAP",
    "pseudobulk_UMAP", "markers_UMAP", "SpatialDE_PCA_spatial",
    "SpatialDE_pool_PCA_spatial", "HVG_PCA_spatial", "pseudobulk_PCA_spatial",
    "markers_PCA_spatial", "SpatialDE_UMAP_spatial", "SpatialDE_pool_UMAP_spatial",
    "HVG_UMAP_spatial", "pseudobulk_UMAP_spatial", "markers_UMAP_spatial")
)
```

Arguments

| | |
|-----------|--|
| sce | Defaults to the output of <code>fetch_data(type = 'sce')</code> . This is a SingleCellExperiment object with the spot-level Visium data and information required for visualizing the histology. See fetch_data() for more details. |
| variables | A <code>character()</code> vector of variable names expected to be present in <code>colData(sce)</code> . |

Value

The input object if all checks are passed.

See Also

Other Check input functions: [check_modeling_results\(\)](#), [check_sce_layer\(\)](#), [check_spe\(\)](#)

Examples

```
if (enough_ram()) {
  ## Obtain the necessary data
  if (!exists("sce_example")) sce_example <- fetch_data("sce_example")

  ## Check the object
  check_sce(sce_example)
}
```

| | |
|-----------------|------------------------------|
| check_sce_layer | <i>Check input sce_layer</i> |
|-----------------|------------------------------|

Description

This function checks that the `sce_layer` object has the appropriate structure. For more details please check the vignette documentation.

Usage

```
check_sce_layer(sce_layer, variables = "spatialLIBD")
```

Arguments

| | |
|------------------------|--|
| <code>sce_layer</code> | Defaults to the output of <code>fetch_data(type = 'sce_layer')</code> . This is a Single-CellExperiment object with the spot-level Visium data compressed via pseudo-bulking to the layer-level (group-level) resolution. See fetch_data() for more details. |
| <code>variables</code> | A <code>character()</code> vector of variable names expected to be present in <code>colData(sce_layer)</code> . |

Value

The input object if all checks are passed.

See Also

Other Check input functions: [check_modeling_results\(\)](#), [check_sce\(\)](#), [check_spe\(\)](#)

Examples

```
## Obtain the necessary data
if (!exists("sce_layer")) sce_layer <- fetch_data("sce_layer")

## Check the object
check_sce_layer(sce_layer)
```

| | |
|-----------|------------------------|
| check_spe | <i>Check input spe</i> |
|-----------|------------------------|

Description

This function checks that the spe object has the appropriate structure. For more details please check the vignette documentation.

Usage

```
check_spe(  
  spe,  
  variables = c("sum_umi", "sum_gene", "expr_chrM", "expr_chrM_ratio")  
)
```

Arguments

| | |
|-----------|---|
| spe | Defaults to the output of <code>fetch_data(type = 'spe')</code> . This is a SpatialExperiment-class object with the spot-level Visium data and information required for visualizing the histology. See fetch_data() for more details. |
| variables | A <code>character()</code> vector of variable names expected to be present in <code>colData(spe)</code> . |

Value

The input object if all checks are passed.

Author(s)

Brenda Pardo, Leonardo Collado-Torres

See Also

Other Check input functions: [check_modeling_results\(\)](#), [check_sce_layer\(\)](#), [check_sce\(\)](#)

Examples

```
if (enough_ram()) {  
  ## Obtain the necessary data  
  if (!exists("spe")) spe <- fetch_data("spe")  
  
  ## Check the object  
  check_spe(spe)  
}
```

| | |
|----------------|---|
| cluster_export | <i>Export a column with cluster results</i> |
|----------------|---|

Description

This function creates a `clusters.csv` file similar to the ones created by SpaceRanger at `outs/analysis/clustering` but with the key column that combines the barcode and the `sample_id`, which is needed when the `spe` object contains data from multiple samples given that the barcodes are duplicated.

Usage

```
cluster_export(  
  spe,  
  cluster_var,  
  cluster_dir = file.path(tempdir(), "exported_clusters"),  
  overwrite = TRUE  
)
```

Arguments

| | |
|--------------------------|---|
| <code>spe</code> | Defaults to the output of <code>fetch_data(type = 'spe')</code> . This is a SpatialExperiment-class object with the spot-level Visium data and information required for visualizing the histology. See fetch_data() for more details. |
| <code>cluster_var</code> | A character(1) with the name of the variable you wish to export. |
| <code>cluster_dir</code> | A character(1) specifying the output directory, similar to the <code>outs/analysis/clustering</code> produced by SpaceRanger. |
| <code>overwrite</code> | A logical(1) indicating whether to overwrite the <code>spe\$key</code> . |

Value

The path to the exported `clusters.csv` file.

See Also

Other cluster export/import utility functions: [cluster_import\(\)](#)

Examples

```
if (enough_ram()) {  
  ## Obtain the necessary data  
  if (!exists("spe")) spe <- fetch_data("spe")  
  
  ## Export two cluster variables  
  cluster_export(spe, "spatialLIBD")  
  cluster_export(spe, "GraphBased")  
}
```

| | |
|----------------|-------------------------------|
| cluster_import | <i>Import cluster results</i> |
|----------------|-------------------------------|

Description

This function imports previously exported clustering results with `cluster_export()` and adds them to the `colData()` slot of your [SpatialExperiment-class](#) object.

Usage

```
cluster_import(  
  spe,  
  cluster_dir = file.path(tempdir(), "exported_clusters"),  
  prefix = "imported_",  
  overwrite = TRUE  
)
```

Arguments

| | |
|-------------|--|
| spe | Defaults to the output of <code>fetch_data(type = 'spe')</code> . This is a SpatialExperiment-class object with the spot-level Visium data and information required for visualizing the histology. See <code>fetch_data()</code> for more details. |
| cluster_dir | A character(1) specifying the output directory, similar to the <code>outs/analysis/clustering</code> produced by SpaceRanger. |
| prefix | A character(1) specifying the prefix to use when naming these new cluster variables. |
| overwrite | A logical(1) indicating whether to overwrite the <code>spe\$key</code> . |

Value

A [SpatialExperiment-class](#) object with the imported clusters appended on the `colData()`.

See Also

Other cluster export/import utility functions: `cluster_export()`

Examples

```
if (enough_ram()) {  
  ## Obtain the necessary data  
  if (!exists("spe")) spe <- fetch_data("spe")  
  
  ## Export two cluster variables  
  cluster_export(spe, "spatialLIBD")  
  cluster_export(spe, "GraphBased")  
  
  ## Re-import them
```

```

        colData(cluster_import(spe))
    }

```

enough_ram *Determine if you have enough RAM memory*

Description

This function determines if you have enough RAM memory on your system.

Usage

```
enough_ram(how_much = 4e+09)
```

Arguments

how_much The number of bytes you want to compare against.

Details

If `benchmarkme::get_ram()` fails, this function will return FALSE as a save bet.

Value

A logical(1) indicating whether your system has enough RAM memory.

Examples

```

## Do you have ~ 4 GB in your system?
enough_ram(4e9)

## Do you have ~ 100 GB in your system
enough_ram(100e9)

```

fetch_data *Download the Human DLPFC Visium data from LIBD*

Description

This function downloads from ExperimentHub the dorsolateral prefrontal cortex (DLPFC) human Visium data and results analyzed by LIBD. If ExperimentHub is not available, it will download the files from Dropbox using `utils::download.file()` unless the files are present already at `destdir`. Note that ExperimentHub will cache the data and automatically detect if you have previously downloaded it, thus making it the preferred way to interact with the data.

Usage

```
fetch_data(
  type = c("sce", "sce_layer", "modeling_results", "sce_example", "spe"),
  destdir = tempdir(),
  eh = ExperimentHub::ExperimentHub(),
  bfc = BiocFileCache::BiocFileCache()
)
```

Arguments

| | |
|---------|--|
| type | A character(1) specifying which file you want to download. It can either be: <code>sce</code> for the SingleCellExperiment object containing the spot-level data that includes the information for visualizing the clusters/genes on top of the Visium histology, <code>sce_layer</code> for the SingleCellExperiment object containing the layer-level data (pseudo-bulked from the spot-level), or <code>modeling_results</code> for the list of tables with the enrichment, pairwise, and anova model results from the layer-level data. It can also be <code>sce_example</code> which is a reduced version of <code>sce</code> just for example purposes. As of BioC version 3.13 <code>spe</code> downloads a SpatialExperiment-class object. |
| destdir | The destination directory to where files will be downloaded to in case the ExperimentHub resource is not available. If you already downloaded the files, you can set this to the current path where the files were previously downloaded to avoid re-downloading them. |
| eh | An ExperimentHub object ExperimentHub-class . |
| bfc | A BiocFileCache object BiocFileCache-class . Used when eh is not available. |

Details

The data was initially prepared by scripts at <https://github.com/LieberInstitute/HumanPilot> and further refined by https://github.com/LieberInstitute/spatialLIBD/blob/master/inst/scripts/make-data_spatialLIBD.R.

Value

The requested object: `sce`, `sce_layer`, `ve` or `modeling_results` that you have to assign to an object. If you didn't you can still avoid re-loading the object by using `.Last.value`.

Examples

```
## Download the SingleCellExperiment object
## at the layer-level
if (!exists("sce_layer")) sce_layer <- fetch_data("sce_layer")

## Explore the data
sce_layer
```

gene_set_enrichment *Evaluate the enrichment for a list of gene sets*

Description

Using the layer-level (group-level) data, this function evaluates whether list of gene sets (Ensembl gene IDs) are enriched among the significant genes (FDR < 0.1 by default) genes for a given model type result. If you want to check depleted genes, change reverse to TRUE.

Usage

```
gene_set_enrichment(
  gene_list,
  fdr_cut = 0.1,
  modeling_results = fetch_data(type = "modeling_results"),
  model_type = names(modeling_results)[1],
  reverse = FALSE
)
```

Arguments

| | |
|------------------|---|
| gene_list | A named list object (could be a data.frame) where each element of the list is a character vector of Ensembl gene IDs. |
| fdr_cut | A numeric(1) specifying the FDR cutoff to use for determining significance among the modeling results genes. |
| modeling_results | Defaults to the output of <code>fetch_data(type = 'modeling_results')</code> . This is a list of tables with the columns <code>f_stat_*</code> or <code>t_stat_*</code> as well as <code>p_value_*</code> and <code>fdr_*</code> plus <code>ensembl</code> . The column name is used to extract the statistic results, the p-values, and the FDR adjusted p-values. Then the <code>ensembl</code> column is used for matching in some cases. See <code>fetch_data()</code> for more details. |
| model_type | A named element of the <code>modeling_results</code> list. By default that is either <code>enrichment</code> for the model that tests one human brain layer against the rest (one group vs the rest), <code>pairwise</code> which compares two layers (groups) denoted by <code>layerA-layerB</code> such that <code>layerA</code> is greater than <code>layerB</code> , and <code>anova</code> which determines if any layer (group) is different from the rest adjusting for the mean expression level. The statistics for <code>enrichment</code> and <code>pairwise</code> are t-statistics while the <code>anova</code> model ones are F-statistics. |
| reverse | A logical(1) indicating whether to multiply by -1 the input statistics and reverse the <code>layerA-layerB</code> column names (using the <code>-</code>) into <code>layerB-layerA</code> . |

Details

Check https://github.com/LieberInstitute/HumanPilot/blob/master/Analysis/Layer_Guesses/check_clinical_gene_sets.R to see a full script from where this family of functions is derived from.

Value

A table in long format with the enrichment results using `stats::fisher.test()`.

Author(s)

Andrew E Jaffe, Leonardo Collado-Torres

See Also

Other Gene set enrichment functions: `gene_set_enrichment_plot()`

Examples

```
## Read in the SFARI gene sets included in the package
asd_sfari <- utils::read.csv(
  system.file(
    "extdata",
    "SFARI-Gene_genes_01-03-2020release_02-04-2020export.csv",
    package = "spatialLIBD"
  ),
  as.is = TRUE
)

## Format them appropriately
asd_sfari_geneList <- list(
  Gene_SFARI_all = asd_sfari$ensembl.id,
  Gene_SFARI_high = asd_sfari$ensembl.id[asd_sfari$gene.score < 3],
  Gene_SFARI_syndromic = asd_sfari$ensembl.id[asd_sfari$syndromic == 1]
)

## Obtain the necessary data
if (!exists("modeling_results")) {
  modeling_results <- fetch_data(type = "modeling_results")
}

## Compute the gene set enrichment results
asd_sfari_enrichment <- gene_set_enrichment(
  gene_list = asd_sfari_geneList,
  modeling_results = modeling_results,
  model_type = "enrichment"
)

## Explore the results
asd_sfari_enrichment
```

gene_set_enrichment_plot

Plot the gene set enrichment results

Description

This function takes the output of `gene_set_enrichment()` and creates a heatmap visualization of the results.

Usage

```
gene_set_enrichment_plot(
  enrichment,
  xlabs = unique(enrichment$ID),
  PThresh = 12,
  ORcut = 3,
  enrichOnly = FALSE,
  layerHeights = c(0, seq_len(length(unique(enrichment$test))) * 15,
  mypal = c("white", (grDevices::colorRampPalette(RColorBrewer::brewer.pal(9,
    "YlOrRd")))(50))),
  cex = 1.2
)
```

Arguments

| | |
|---------------------------|---|
| <code>enrichment</code> | The output of <code>gene_set_enrichment()</code> . |
| <code>xlabs</code> | A vector of names in the same order and length as <code>unique(enrichment\$ID)</code> . Gets passed to <code>layer_matrix_plot()</code> . |
| <code>PThresh</code> | A numeric(1) specifying the P-value threshold for the maximum value in the $-\log_{10}(p)$ scale. |
| <code>ORcut</code> | A numeric(1) specifying the P-value threshold for the minimum value in the $-\log_{10}(p)$ scale for printing the odds ratio values in the cells of the resulting plot. |
| <code>enrichOnly</code> | A logical(1) indicating whether to show only odds ratio values greater than 1. |
| <code>layerHeights</code> | A numeric() vector of length equal to <code>length(unique(enrichment\$test)) + 1</code> that starts at 0 specifying where to plot the y-axis breaks which can be used for re-creating the length of each brain layer. Gets passed to <code>layer_matrix_plot()</code> . |
| <code>mypal</code> | A vector with the color palette to use. Gets passed to <code>layer_matrix_plot()</code> . |
| <code>cex</code> | Passed to <code>layer_matrix_plot()</code> . |

Details

Check https://github.com/LieberInstitute/HumanPilot/blob/master/Analysis/Layer_Guesses/check_clinical_gene_sets.R to see a full script from where this family of functions is derived from.

Value

A plot visualizing the gene set enrichment odds ratio and p-value results.

Author(s)

Andrew E Jaffe, Leonardo Collado-Torres

See Also

layer_matrix_plot

Other Gene set enrichment functions: [gene_set_enrichment\(\)](#)**Examples**

```

## Read in the SFARI gene sets included in the package
asd_sfari <- utils::read.csv(
  system.file(
    "extdata",
    "SFARI-Gene_genes_01-03-2020release_02-04-2020export.csv",
    package = "spatialLIBD"
  ),
  as.is = TRUE
)

## Format them appropriately
asd_sfari_geneList <- list(
  Gene_SFARI_all = asd_sfari$ensembl.id,
  Gene_SFARI_high = asd_sfari$ensembl.id[asd_sfari$gene.score < 3],
  Gene_SFARI_syndromic = asd_sfari$ensembl.id[asd_sfari$syndromic == 1]
)

## Obtain the necessary data
if (!exists("modeling_results")) {
  modeling_results <- fetch_data(type = "modeling_results")
}

## Compute the gene set enrichment results
asd_sfari_enrichment <- gene_set_enrichment(
  gene_list = asd_sfari_geneList,
  modeling_results = modeling_results,
  model_type = "enrichment"
)

## Visualize the gene set enrichment results
## with a custom color palette
gene_set_enrichment_plot(
  asd_sfari_enrichment,
  xlabs = gsub(".*_", "", unique(asd_sfari_enrichment$ID)),
  mypal = c(
    "white",
    grDevices::colorRampPalette(
      RColorBrewer::brewer.pal(9, "BuGn")
    )(50)
  )
)

## Specify the layer heights so it resembles more the length of each
## layer in the brain
gene_set_enrichment_plot(
  asd_sfari_enrichment,

```

```

xlabs = gsub(".*_", "", unique(asd_sfari_enrichment$ID)),
layerHeights = c(0, 40, 55, 75, 85, 110, 120, 135),
)

```

geom_spatial

A ggplot2 layer for visualizing the Visium histology

Description

This function defines a `ggplot2::layer()` for visualizing the histology image from Visium. It can be combined with other `ggplot2` functions for visualizing the clusters as in `vis_clus_p()` or gene-level information as in `vis_gene_p()`.

Usage

```

geom_spatial(
  mapping = NULL,
  data = NULL,
  stat = "identity",
  position = "identity",
  na.rm = FALSE,
  show.legend = NA,
  inherit.aes = FALSE,
  ...
)

```

Arguments

| | |
|--------------------------|--|
| <code>mapping</code> | Passed to <code>ggplot2::layer(mapping)</code> where <code>grob</code> , <code>x</code> and <code>y</code> are required. |
| <code>data</code> | Passed to <code>ggplot2::layer(data)</code> . |
| <code>stat</code> | Passed to <code>ggplot2::layer(stat)</code> . |
| <code>position</code> | Passed to <code>ggplot2::layer(position)</code> . |
| <code>na.rm</code> | Passed to <code>ggplot2::layer(params = list(na.rm))</code> . |
| <code>show.legend</code> | Passed to <code>ggplot2::layer(show.legend)</code> . |
| <code>inherit.aes</code> | Passed to <code>ggplot2::layer(inherit.aes)</code> . |
| <code>...</code> | Other arguments passed to <code>ggplot2::layer(params = list(...))</code> . |

Value

A `ggplot2::layer()` for the histology information.

Author(s)

10x Genomics

Examples

```

if (enough_ram()) {
  ## Obtain the necessary data
  if (!exists("spe")) spe <- fetch_data("spe")

  ## Select the first sample and extract the data
  sample_id <- unique(spe$sample_id)[1]
  spe_sub <- spe[, spe$sample_id == sample_id]
  sample_df <- as.data.frame(colData(spe_sub), optional = TRUE)

  ## Obtain the histology image
  img <- SpatialExperiment::imgRaster(spe_sub)

  ## Transform to a rasterGrob object
  grob <- grid::rasterGrob(img, width = grid::unit(1, "npc"), height = grid::unit(1, "npc"))

  ## Make a plot using geom_spatial
  p <- ggplot2::ggplot(
    sample_df,
    ggplot2::aes(
      x = pxl_col_in_fullres * SpatialExperiment::scaleFactors(spe_sub),
      y = pxl_row_in_fullres * SpatialExperiment::scaleFactors(spe_sub),
    )
  ) +
    geom_spatial(
      data = tibble::tibble(grob = list(grob)),
      ggplot2::aes(grob = grob),
      x = 0.5,
      y = 0.5
    )

  ## Show the plot
  print(p)

  ## Clean up
  rm(spe_sub)
}

```

get_colors

Obtain the colors for a set of cluster names

Description

This function returns a vector of colors based on a vector of cluster names. It can be used to automatically assign colors.

Usage

```
get_colors(colors = NULL, clusters)
```

Arguments

| | |
|----------|---|
| colors | A vector of colors. If NULL then a set of default colors will be used when clusters has less than 12 unique values, otherwise Polychrome::palette36 will be used which can generate up to 36 unique colors. If the number of unique clusters is beyond 36 then this function will fail. |
| clusters | A vector of cluster names. |

Value

A named vector where the values are the colors to use for displaying them different clusters. For some use cases, you might have to either change the names or use [unnname\(\)](#).

Examples

```
## Obtain the necessary data
if (!exists("sce_layer")) sce_layer <- fetch_data("sce_layer")

## Example layer colors with the corresponding names
get_colors(libd_layer_colors, sce_layer$layer_guess)
get_colors(libd_layer_colors, sce_layer$layer_guess_reordered_short)

## Example where colors are assigned automatically
## based on a pre-defined set of colors
get_colors(clusters = sce_layer$kmeans_k7)

## Example where Polychrome::palette36.colors() gets used
get_colors(clusters = letters[seq_len(13)])
```

img_edit

Edit a background image

Description

This function uses the magick package to edit the color and perform other image manipulations on a background image. It can be useful if you want to highlight certain features of these images.

Usage

```
img_edit(
  spe,
  sampleid,
  image_id = "lowres",
  channel = NA,
  brightness = 100,
  saturation = 100,
  hue = 100,
  enhance = FALSE,
  contrast_sharpen = NA,
```

```

    quantize_max = NA,
    quantize_dither = TRUE,
    equalize = FALSE,
    normalize = FALSE,
    transparent_color = NA,
    transparent_fuzz = 0,
    background_color = NA,
    median_radius = NA,
    negate = FALSE
  )

```

Arguments

| | |
|-------------------|--|
| spe | Defaults to the output of <code>fetch_data(type = 'spe')</code> . This is a SpatialExperiment-class object with the spot-level Visium data and information required for visualizing the histology. See <code>fetch_data()</code> for more details. |
| sampleid | A character(1) specifying which sample to plot from <code>colData(spe)\$sample_name</code> . |
| image_id | A character(1) with the name of the image ID you want to use in the background. |
| channel | A character(1) passed to <code>magick::image_channel</code> . If NA this step is skipped. |
| brightness | A numeric(1) passed to <code>magick::image_modulate</code> . |
| saturation | A numeric(1) passed to <code>magick::image_modulate</code> . |
| hue | A numeric(1) passed to <code>magick::image_modulate</code> . |
| enhance | A logical(1) controlling whether to use <code>magick::enhance</code> . |
| contrast_sharpen | A numeric(1) passed to <code>magick::image_contrast</code> . If NA this step is skipped. |
| quantize_max | A numeric(1) passed to <code>magick::image_quantize</code> . If NA this step is skipped. |
| quantize_dither | A logical(1) passed to <code>magick::image_quantize</code> . |
| equalize | A logical(1) controlling whether to use <code>magick::equalize</code> . |
| normalize | A logical(1) controlling whether to use <code>magick::normalize</code> . |
| transparent_color | A character(1) passed to <code>magick::image_transparent</code> . If NA this step is skipped. |
| transparent_fuzz | A numeric(1) passed to <code>magick::image_transparent</code> . |
| background_color | A character(1) passed to <code>magick::image_background</code> . If NA this step is skipped. |
| median_radius | A numeric(1) passed to <code>magick::image_median</code> . If NA this step is skipped. |
| negate | A logical(1) controlling whether to use <code>magick::negate</code> . |

Details

The `magick` functions are used in the sequence represented by the arguments to this function. You can alternatively use this function sequentially. Or directly use the `magick` package.

Value

A magick image object such as the one returned by [magick::image_read](#).

See Also

Other Image editing functions: [img_update_all\(\)](#), [img_update\(\)](#)

Examples

```
if (enough_ram()) {
  ## Obtain the necessary data
  if (!exists("spe")) spe <- fetch_data("spe")

  ## Reduce brightness to 25%
  x <- img_edit(spe, sampleid = "151507", brightness = 25)
  plot(x)
}
```

img_update

Update the image for one sample

Description

Edit the image with [img_edit\(\)](#) then update the [imgData\(\)](#).

Usage

```
img_update(
  spe,
  sampleid,
  image_id = "lowres",
  new_image_id = paste0("edited_", image_id),
  overwrite = FALSE,
  ...
)
```

Arguments

| | |
|--------------|--|
| spe | Defaults to the output of fetch_data(type = 'spe') . This is a SpatialExperiment-class object with the spot-level Visium data and information required for visualizing the histology. See fetch_data() for more details. |
| sampleid | A character(1) specifying which sample to plot from <code>colData(spe)\$sample_name</code> . |
| image_id | A character(1) with the name of the image ID you want to use in the background. |
| new_image_id | A character(1) specifying the new <code>image_id</code> to use. |
| overwrite | A logical(1) specifying whether to overwrite the <code>image_id</code> if it already exists. |
| ... | Parameters passed to img_edit() . |

Value

A [SpatialExperiment-class](#) object with an updated `imgData()` slot.

See Also

Other Image editing functions: [img_edit\(\)](#), [img_update_all\(\)](#)

Examples

```
if (enough_ram()) {
  ## Obtain the necessary data
  if (!exists("spe")) spe <- fetch_data("spe")

  ## Reduce brightness to 25% and update the imgData()
  imgData(img_update(spe, sampleid = "151507", brightness = 25))
}
```

| | |
|-----------------------------|--|
| <code>img_update_all</code> | <i>Update the images for all samples</i> |
|-----------------------------|--|

Description

This function uses `img_update()` for all samples. That is, it loops through every sample and edits the image with `img_edit()` and then updates the `imgData()`.

Usage

```
img_update_all(
  spe,
  image_id = "lowres",
  new_image_id = paste0("edited_", image_id),
  overwrite = FALSE,
  ...
)
```

Arguments

| | |
|---------------------------|---|
| <code>spe</code> | Defaults to the output of <code>fetch_data(type = 'spe')</code> . This is a SpatialExperiment-class object with the spot-level Visium data and information required for visualizing the histology. See fetch_data() for more details. |
| <code>image_id</code> | A character(1) with the name of the image ID you want to use in the background. |
| <code>new_image_id</code> | A character(1) specifying the new <code>image_id</code> to use. |
| <code>overwrite</code> | A logical(1) specifying whether to overwrite the <code>image_id</code> if it already exists. |
| <code>...</code> | Parameters passed to <code>img_edit()</code> . |

Value

A [SpatialExperiment-class](#) object with an updated `imgData()` slot.

See Also

Other Image editing functions: [img_edit\(\)](#), [img_update\(\)](#)

Examples

```
if (enough_ram()) {  
  ## Obtain the necessary data  
  if (!exists("spe")) spe <- fetch_data("spe")  
  
  ## Reduce brightness to 25% for the 'lowres' image for all samples and  
  ## update the imgData()  
  imgData(img_update_all(spe, brightness = 25))  
}
```

layer_boxplot

Layer-level (group-level) boxplots

Description

This function uses the output of [sig_genes_extract_all\(\)](#) as well as the logcounts from the layer-level (group-level) data to visualize the expression of a given gene and display the modeling results for the given gene.

Usage

```
layer_boxplot(  
  i = 1,  
  sig_genes = sig_genes_extract(),  
  short_title = TRUE,  
  sce_layer = fetch_data(type = "sce_layer"),  
  col_bkg_box = "grey80",  
  col_bkg_point = "grey40",  
  col_low_box = "violet",  
  col_low_point = "darkviolet",  
  col_high_box = "skyblue",  
  col_high_point = "dodgerblue4",  
  cex = 2,  
  group_var = "layer_guess_reordered_short",  
  assayname = "logcounts"  
)
```


Arguments

| | |
|-----------------------------|---|
| <code>i</code> | A integer(1) indicating which row of <code>sig_genes</code> do you want to plot. |
| <code>sig_genes</code> | The output of <code>sig_genes_extract_all()</code> . |
| <code>short_title</code> | A logical(1) indicating whether to print a short title or not. |
| <code>sce_layer</code> | Defaults to the output of <code>fetch_data(type = 'sce_layer')</code> . This is a Single-CellExperiment object with the spot-level Visium data compressed via pseudo-bulking to the layer-level (group-level) resolution. See <code>fetch_data()</code> for more details. |
| <code>col_bkg_box</code> | Box background color for layers not used when visualizing the pairwise model results. |
| <code>col_bkg_point</code> | Similar to <code>col_bkg_box</code> but for the points. |
| <code>col_low_box</code> | Box background color for layer(s) with the expected lower expression based on the actual test for row <code>i</code> of <code>sig_genes</code> . |
| <code>col_low_point</code> | Similar to <code>col_low_box</code> but for the points. |
| <code>col_high_box</code> | Similar to <code>col_low_box</code> but for the expected layer(s) with higher expression. |
| <code>col_high_point</code> | Similar to <code>col_high_box</code> but for the points. |
| <code>cex</code> | Controls the size of the text, points and axis legends. |
| <code>group_var</code> | A character(1) specifying a <code>colData(sce_layer)</code> column name to use for the x-axis. |
| <code>assayname</code> | A character(1) specifying the default assay to use from <code>assays(sce_layer)</code> . |

Value

This function creates a boxplot of the layer-level data (group-level) separated by layer and colored based on the model type from row `i` of `sig_genes`.

References

Adapted from https://github.com/LieberInstitute/HumanPilot/blob/master/Analysis/Layer_Guesses/layer_specificity.R

See Also

Other Layer modeling functions: `sig_genes_extract_all()`, `sig_genes_extract()`

Examples

```
## Obtain the necessary data
if (!exists("modeling_results")) {
  modeling_results <- fetch_data(type = "modeling_results")
}
if (!exists("sce_layer")) sce_layer <- fetch_data(type = "sce_layer")

## Top 2 genes from the enrichment model
sig_genes <- sig_genes_extract_all(
  n = 2,
  modeling_results = modeling_results,
```

```
    sce_layer = sce_layer
  )

## Example default boxplot
set.seed(20200206)
layer_boxplot(sig_genes = sig_genes, sce_layer = sce_layer)

## Now show the long title version
set.seed(20200206)
layer_boxplot(
  sig_genes = sig_genes,
  short_title = FALSE,
  sce_layer = sce_layer
)

set.seed(20200206)
layer_boxplot(
  i = which(sig_genes$model_type == "anova")[1],
  sig_genes = sig_genes,
  sce_layer = sce_layer
)

set.seed(20200206)
layer_boxplot(
  i = which(sig_genes$model_type == "pairwise")[1],
  sig_genes = sig_genes,
  sce_layer = sce_layer
)

## Viridis colors displayed in the shiny app
library("viridisLite")
set.seed(20200206)
layer_boxplot(
  sig_genes = sig_genes,
  sce_layer = sce_layer,
  col_low_box = viridis(4)[2],
  col_low_point = viridis(4)[1],
  col_high_box = viridis(4)[3],
  col_high_point = viridis(4)[4]
)

## Paper colors displayed in the shiny app
set.seed(20200206)
layer_boxplot(
  sig_genes = sig_genes,
  sce_layer = sce_layer,
  col_low_box = "palegreen3",
  col_low_point = "springgreen2",
  col_high_box = "darkorange2",
  col_high_point = "orange1"
)

## Blue/red colors displayed in the shiny app
```

```

set.seed(20200206)
layer_boxplot(
  i = which(sig_genes$model_type == "pairwise")[1],
  sig_genes = sig_genes,
  sce_layer = sce_layer,
  col_bkg_box = "grey90",
  col_bkg_point = "grey60",
  col_low_box = "skyblue2",
  col_low_point = "royalblue3",
  col_high_box = "tomato2",
  col_high_point = "firebrick4",
  cex = 3
)

```

layer_matrix_plot

Visualize a matrix of values across human brain layers

Description

This function visualizes a numerical matrix where the Y-axis represents the human brain layers and can be adjusted to represent the length of each brain layer. Cells can optionally have text values. This function is used by [gene_set_enrichment_plot\(\)](#) and [layer_stat_cor_plot\(\)](#).

Usage

```

layer_matrix_plot(
  matrix_values,
  matrix_labels = NULL,
  xlabs = NULL,
  layerHeights = NULL,
  mypal = c("white", (grDevices::colorRampPalette(RColorBrewer::brewer.pal(9,
    "YlOrRd")))(50)),
  breaks = NULL,
  axis.args = NULL,
  srt = 45,
  mar = c(8, 4 + (max(nchar(rownames(matrix_values)))/%3) * 0.5, 4, 2) + 0.1,
  cex = 1.2
)

```

Arguments

| | |
|---------------|---|
| matrix_values | A <code>matrix()</code> with one column per set of interest and one row per layer (group) with numeric values. |
| matrix_labels | Optionally a character <code>matrix()</code> with the same dimensions and <code>dimnames()</code> as <code>matrix_values</code> with text labels for the cells. |
| xlabs | A vector of names in the same order and length as <code>colnames(matrix_values)</code> . |

| | |
|--------------|---|
| layerHeights | A numeric() vector of length equal to nrow(matrix_values) + 1 that starts at 0 specifying where to plot the y-axis breaks which can be used for re-creating the length of each brain layer. |
| mypal | A vector with the color palette to use. |
| breaks | Passed to <code>fields::image.plot()</code> . Used by <code>layer_stat_cor_plot()</code> . |
| axis.args | Passed to <code>fields::image.plot()</code> . Used by <code>layer_stat_cor_plot()</code> . |
| srt | The angle for the x-axis labels. Used by <code>layer_stat_cor_plot()</code> . |
| mar | Passed to <code>graphics::par()</code> . |
| cex | Used for the x-axis labels and the text inside the cells. |

Value

A base R plot visualizing the input `matrix_values` with optional text labels for `matrix_labels`.

Author(s)

Andrew E Jaffe, Leonardo Collado-Torres

Examples

```
## Create some random data
set.seed(20200224)
mat <- matrix(runif(7 * 8, min = -1), nrow = 7)
rownames(mat) <- c("WM", paste0("L", rev(seq_len(6))))
colnames(mat) <- paste0("Var", seq_len(8))

## Create some text labels
mat_text <- matrix("", nrow = 7, ncol = 8, dimnames = dimnames(mat))
diag(mat_text) <- as.character(round(diag(mat), 2))

## Make the plot
layer_matrix_plot(mat, mat_text)

## Try to re-create the anatomical proportions of the human brain layers
layer_matrix_plot(
  mat,
  mat_text,
  layerHeights = c(0, 40, 55, 75, 85, 110, 120, 135),
  cex = 2
)
```

layer_stat_cor

Layer modeling correlation of statistics

Description

Layer modeling correlation of statistics

Usage

```
layer_stat_cor(
  stats,
  modeling_results = fetch_data(type = "modeling_results"),
  model_type = names(modeling_results)[1],
  reverse = FALSE,
  top_n = NULL
)
```

Arguments

| | |
|-------------------------------|---|
| <code>stats</code> | A data.frame where the row names are Ensembl gene IDs, the column names are labels for clusters of cells or cell types, and where each cell contains the given statistic for that gene and cell type. These statistics should be computed similarly to the modeling results from the data we provide. For example, like the enrichment t-statistics that are derived from comparing one layer against the rest. The stats will be matched and then correlated with our statistics. |
| <code>modeling_results</code> | Defaults to the output of <code>fetch_data(type = 'modeling_results')</code> . This is a list of tables with the columns <code>f_stat_*</code> or <code>t_stat_*</code> as well as <code>p_value_*</code> and <code>fdr_*</code> plus <code>ensembl</code> . The column name is used to extract the statistic results, the p-values, and the FDR adjusted p-values. Then the <code>ensembl</code> column is used for matching in some cases. See <code>fetch_data()</code> for more details. |
| <code>model_type</code> | A named element of the <code>modeling_results</code> list. By default that is either <code>enrichment</code> for the model that tests one human brain layer against the rest (one group vs the rest), <code>pairwise</code> which compares two layers (groups) denoted by <code>layerA-layerB</code> such that <code>layerA</code> is greater than <code>layerB</code> , and <code>anova</code> which determines if any layer (group) is different from the rest adjusting for the mean expression level. The statistics for <code>enrichment</code> and <code>pairwise</code> are t-statistics while the <code>anova</code> model ones are F-statistics. |
| <code>reverse</code> | A <code>logical(1)</code> indicating whether to multiply by <code>-1</code> the input statistics and reverse the <code>layerA-layerB</code> column names (using the <code>-</code>) into <code>layerB-layerA</code> . |
| <code>top_n</code> | An <code>integer(1)</code> specifying whether to filter to the top <code>n</code> marker genes. The default is <code>NULL</code> in which case no filtering is done. |

Details

Check https://github.com/LieberInstitute/HumanPilot/blob/master/Analysis/Layer_Guesses/dlpfc_snRNAseq_annotation.R for a full analysis from which this family of functions is derived from.

Value

A correlation matrix between `stats` and our statistics using only the Ensembl gene IDs present in both tables. The columns are sorted using a hierarchical cluster.

Author(s)

Andrew E Jaffe, Leonardo Collado-Torres

See Also

Other Layer correlation functions: [layer_stat_cor_plot\(\)](#)

Examples

```
## Obtain the necessary data
if (!exists("modeling_results")) {
  modeling_results <- fetch_data(type = "modeling_results")
}

## Compute the correlations
cor_stats_layer <- layer_stat_cor(
  tstats_Human_DLPFC_snRNAseq_Nguyen_topLayer,
  modeling_results,
  model_type = "enrichment"
)

## Explore the correlation matrix
head(cor_stats_layer[, seq_len(3)])
summary(cor_stats_layer)

## Repeat with top_n set to 10
summary(layer_stat_cor(
  tstats_Human_DLPFC_snRNAseq_Nguyen_topLayer,
  modeling_results,
  model_type = "enrichment",
  top_n = 10
))
```

`layer_stat_cor_plot` *Visualize the layer modeling correlation of statistics*

Description

This function makes a heatmap from the [layer_stat_cor\(\)](#) correlation matrix between a given set of cell cluster/type statistics derived from scRNA-seq or snRNA-seq data (among other types) and the layer statistics from the Human DLPFC Visium data (when using the default arguments).

Usage

```
layer_stat_cor_plot(
  cor_stats_layer,
  max = 0.81,
  min = -max,
  layerHeights = NULL,
  cex = 1.2
)
```

Arguments

| | |
|-----------------|--|
| cor_stats_layer | The output of <code>layer_stat_cor()</code> . |
| max | A numeric(1) specifying the highest correlation value for the color scale (should be between 0 and 1). |
| min | A numeric(1) specifying the lowest correlation value for the color scale (should be between 0 and -1). |
| layerHeights | A numeric() vector of length equal to <code>ncol(cor_stats_layer) + 1</code> that starts at 0 specifying where to plot the y-axis breaks which can be used for re-creating the length of each brain layer. Gets passed to <code>layer_matrix_plot()</code> . |
| cex | Passed to <code>layer_matrix_plot()</code> . |

Details

Check https://github.com/LieberInstitute/HumanPilot/blob/master/Analysis/Layer_Guesses/dlpfc_snRNAseq_annotation.R for a full analysis from which this family of functions is derived from.

Value

A heatmap for the correlation matrix between statistics.

Author(s)

Andrew E Jaffe, Leonardo Collado-Torres

See Also

`layer_matrix_plot`

Other Layer correlation functions: `layer_stat_cor()`

Examples

```
## Obtain the necessary data
if (!exists("modeling_results")) {
  modeling_results <- fetch_data(type = "modeling_results")
}

## Compute the correlations
cor_stats_layer <- layer_stat_cor(
  tstats_Human_DLPFC_snRNAseq_Nguyen_topLayer,
  modeling_results,
  model_type = "enrichment"
)

## Visualize the correlation matrix
layer_stat_cor_plot(cor_stats_layer, max = max(cor_stats_layer))

## Restrict the range of colors further
layer_stat_cor_plot(cor_stats_layer, max = 0.3)
```

```
## Repeat with just the top 10 layer marker genes
layer_stat_cor_plot(layer_stat_cor(
  tstats_Human_DLPFC_snRNAseq_Nguyen_topLayer,
  modeling_results,
  model_type = "enrichment",
  top_n = 10
), max = 0.3)

## Now with the "pairwise" modeling results and also top_n = 10
layer_stat_cor_plot(layer_stat_cor(
  tstats_Human_DLPFC_snRNAseq_Nguyen_topLayer,
  modeling_results,
  model_type = "pairwise",
  top_n = 10
), max = 0.3)
```

libd_layer_colors *Vector of LIBD layer colors*

Description

A named vector of colors to use for the LIBD layers designed by Lukas M. Weber with feedback from the spatialLIBD collaborators.

Usage

```
libd_layer_colors
```

Format

A vector of length 9 with colors for Layers 1 through 9, WM, NA and a special WM2 that is present in some of the unsupervised clustering results.

locate_images *Locate image files*

Description

Creates a named character() vector that can be helpful for locating image files and used with add_images(). This function is not necessary if the image files don't use the spe\$sample_id.

Usage

```
locate_images(spe, image_dir, image_pattern)
```


Arguments

| | |
|---------------|--|
| spe | Defaults to the output of <code>fetch_data(type = 'spe')</code> . This is a SpatialExperiment-class object with the spot-level Visium data and information required for visualizing the histology. See <code>fetch_data()</code> for more details. |
| image_dir | A character(1) specifying a path to a directory containing image files with the pattern <code>sampleID_pattern.png</code> . |
| image_pattern | A character(1) specifying the pattern for the image files. |

Value

A named character() vector with the path to images.

See Also

Other Functions for adding non-standard images: `add_images()`

Examples

```
## Not run:
locate_images(spe, tempdir(), "testImage")

## End(Not run)
```

`read10xVisiumAnalysis` *Load analysis data from a 10x Genomics Visium experiment*

Description

This function expands `SpatialExperiment::read10xVisium()` by reading analysis outputs from SpaceRanger by 10x Genomics.

Usage

```
read10xVisiumAnalysis(
  samples = "",
  sample_id = paste0("sample", sprintf("%02d", seq_along(samples)))
)
```

Arguments

| | |
|-----------|---|
| samples | Passed to <code>SpatialExperiment::read10xVisium()</code> . |
| sample_id | Passed to <code>SpatialExperiment::read10xVisium()</code> . |

Details

You might want to use `read10xVisiumWrapper()` instead of using this function directly.

Value

A named `list()` with the information about the clustering and the dimension reduction (projections) from the SpaceRanger output by 10x Genomics.

See Also

Other Utility functions for reading data from SpaceRanger output by 10x Genomics: [add10xVisiumAnalysis\(\)](#), [read10xVisiumWrapper\(\)](#)

Examples

```
## See 'Using spatialLIBD with 10x Genomics public datasets' for
## a full example using this function.
if (interactive()) {
  browseVignettes(package = "spatialLIBD")
}

## Note that ?SpatialExperiment::read10xVisium doesn't include all the files
## we need to illustrate read10xVisiumWrapper().
```

| | |
|----------------------|--|
| read10xVisiumWrapper | <i>Load data from a 10x Genomics Visium experiment and make it spatialLIBD-ready</i> |
|----------------------|--|

Description

This function expands `SpatialExperiment::read10xVisium()` to include analysis results from SpaceRanger by 10x Genomics as well as add information needed by `run_app()` to visualize the data with the spatialLIBD shiny web application.

Usage

```
read10xVisiumWrapper(
  samples = "",
  sample_id = paste0("sample", sprintf("%02d", seq_along(samples))),
  type = c("HDF5", "sparse"),
  data = c("filtered", "raw"),
  images = c("lowres", "hires", "detected", "aligned"),
  load = TRUE,
  reference_gtf = NULL,
  chrM = "chrM",
  gtf_cols = c("source", "type", "gene_id", "gene_version", "gene_name", "gene_type"),
  verbose = TRUE
)
```

Arguments

| | |
|---------------|---|
| samples | Passed to <code>SpatialExperiment::read10xVisium()</code> . |
| sample_id | Passed to <code>SpatialExperiment::read10xVisium()</code> . |
| type | Passed to <code>SpatialExperiment::read10xVisium()</code> . |
| data | Passed to <code>SpatialExperiment::read10xVisium()</code> . |
| images | Passed to <code>SpatialExperiment::read10xVisium()</code> . |
| load | Passed to <code>SpatialExperiment::read10xVisium()</code> . |
| reference_gtf | A character(1) specifying the path to the reference genes.gtf file. If not specified, it will be automatically inferred from the web_summary.html file for the first samples. |
| chrM | A character(1) specifying the chromosome name of the mitochondrial chromosome. Defaults to chrM. |
| gtf_cols | A character() specifying which columns to keep from the GTF file. "gene_name" and "gene_id" have to be included in gtf_cols. |
| verbose | A logical(1) specifying whether to show progress updates. |

Value

A `SpatialExperiment` object with the clustering and dimension reduction (projection) results from SpaceRanger by 10x Genomics as well as other information used by `run_app()` for visualizing the gene expression data.

See Also

Other Utility functions for reading data from SpaceRanger output by 10x Genomics: `add10xVisiumAnalysis()`, `read10xVisiumAnalysis()`

Examples

```
## See 'Using spatialLIBD with 10x Genomics public datasets' for
## a full example using this function.
if (interactive()) {
  browseVignettes(package = "spatialLIBD")
}

## Note that ?SpatialExperiment::read10xVisium doesn't include all the files
## we need to illustrate read10xVisiumWrapper().
```

run_app

Run the spatialLIBD Shiny Application

Description

This function runs the shiny application that allows users to interact with the Visium spatial transcriptomics data from LIBD (by default) or any other data that you have shaped according to our object structure.

Usage

```
run_app(
  spe = fetch_data(type = "spe"),
  sce_layer = fetch_data(type = "sce_layer"),
  modeling_results = fetch_data(type = "modeling_results"),
  sig_genes = sig_genes_extract_all(n = nrow(sce_layer), modeling_results =
    modeling_results, sce_layer = sce_layer),
  docs_path = system.file("app", "www", package = "spatialLIBD"),
  title = "spatialLIBD",
  spe_discrete_vars = c("spatialLIBD", "GraphBased", "ManualAnnotation", "Maynard",
    "Martinowich", paste0("SNN_k50_k", 4:28), "SpatialDE_PCA", "SpatialDE_pool_PCA",
    "HVG_PCA", "pseudobulk_PCA", "markers_PCA", "SpatialDE_UMAP", "SpatialDE_pool_UMAP",
    "HVG_UMAP", "pseudobulk_UMAP", "markers_UMAP", "SpatialDE_PCA_spatial",
    "SpatialDE_pool_PCA_spatial", "HVG_PCA_spatial", "pseudobulk_PCA_spatial",
    "markers_PCA_spatial", "SpatialDE_UMAP_spatial", "SpatialDE_pool_UMAP_spatial",
    "HVG_UMAP_spatial", "pseudobulk_UMAP_spatial",
    "markers_UMAP_spatial"),
  spe_continuous_vars = c("cell_count", "sum_umi", "sum_gene", "expr_chrM",
    "expr_chrM_ratio"),
  default_cluster = "spatialLIBD",
  ...
)
```

Arguments

| | |
|------------------|--|
| spe | Defaults to the output of <code>fetch_data(type = 'spe')</code> . This is a SpatialExperiment-class object with the spot-level Visium data and information required for visualizing the histology. See fetch_data() for more details. |
| sce_layer | Defaults to the output of <code>fetch_data(type = 'sce_layer')</code> . This is a SingleCellExperiment object with the spot-level Visium data compressed via pseudobulking to the layer-level (group-level) resolution. See fetch_data() for more details. |
| modeling_results | Defaults to the output of <code>fetch_data(type = 'modeling_results')</code> . This is a list of tables with the columns <code>f_stat_*</code> or <code>t_stat_*</code> as well as <code>p_value_*</code> and <code>fdr_*</code> plus <code>ensembl</code> . The column name is used to extract the statistic results, |

| | |
|----------------------------------|---|
| | the p-values, and the FDR adjusted p-values. Then the <code>ensembl</code> column is used for matching in some cases. See <code>fetch_data()</code> for more details. |
| <code>sig_genes</code> | The output of <code>sig_genes_extract_all()</code> which is a table in long format with the modeling results. You can subset this if the object requires too much memory. |
| <code>docs_path</code> | A <code>character(1)</code> specifying the path to the directory containing the website documentation files. The directory has to contain the files: <code>documentation_sce_layer.md</code> , <code>documentation_spe.md</code> , <code>favicon.ico</code> , <code>footer.html</code> and <code>README.md</code> . |
| <code>title</code> | A <code>character(1)</code> specifying the title for the app. |
| <code>spe_discrete_vars</code> | A <code>character()</code> vector of discrete variables that will be available to visualize in the app. Basically, the set of variables with spot-level groups. They will have to be present in <code>colData(spe)</code> . |
| <code>spe_continuous_vars</code> | A <code>character()</code> vector of continuous variables that will be available to visualize in the app using the same scale as genes. They will have to be present in <code>colData(sce)</code> . |
| <code>default_cluster</code> | A <code>character(1)</code> with the name of the main cluster (discrete) variable to use. It will have to be present in both <code>colData(spe)</code> and <code>colData(sce_layer)</code> . |
| <code>...</code> | Other arguments passed to the list of <code>golem</code> options for running the application. |

Details

If you don't have the pseudo-bulked analysis results like we computed them in our project <https://doi.org/10.1038/s41593-020-00787-0> you can set `sce_layer`, `modeling_results` and `sig_genes` to `NULL`. Doing so will disable the pseudo-bulked portion of the web application. See the examples for one such case as well as the vignette that describes how you can use `spatialLIBD` with public data sets provided by 10x Genomics. That vignette is available at http://research.libd.org/spatialLIBD/articles/TenX_data_download.html.

Value

A `shiny.appobj` that contains the input data.

Examples

```
## Not run:
## The default arguments will download the data from the web
## using fetch_data(). If this is the first time you have run this,
## the files will need to be cached by ExperimentHub. Otherwise it
## will re-use the files you have previously downloaded.
if (enough_ram(4e9)) {
  ## Obtain the necessary data
  if (!exists("spe")) spe <- fetch_data("spe")

  ## Create the interactive website
  run_app(spe)
```

```

## You can also run a custom version without the pseudo-bulked
## layer information. This is useful if you are only interested
## in the spatial transcriptomics features.
run_app(spe,
        sce_layer = NULL, modeling_results = NULL, sig_genes = NULL,
        title = "spatialLIBD without layer info"
)

## When using shinyapps.io aim for less than 3 GB of RAM with your
## objects. Check each input object with:
## lobster::obj_size(x)
## Do not create the large input objects on the app.R script before
## subsetting them. Do this outside app.R since the app.R script is
## run at shinyapps.io, so subsetting on that script to reduce the
## memory load is pointless. You have to do it outside of app.R.
}

## End(Not run)

```

sce_to_spe

Convert a SCE object to a SPE one

Description

This function converts a spot-level [SingleCellExperiment-class](#) (SCE) object as generated by `fetch_data()` to a [SpatialExperiment-class](#) (SPE) object.

Usage

```
sce_to_spe(sce = fetch_data("sce"), imageData = NULL)
```

Arguments

| | |
|-----------|---|
| sce | Defaults to the output of <code>fetch_data(type = 'sce')</code> . This is a SingleCellExperiment object with the spot-level Visium data and information required for visualizing the histology. See <code>fetch_data()</code> for more details. |
| imageData | A <code>DataFrame()</code> with image data. Will be used with SpatialExperiment::imgData . If NULL, then this will be constructed for you assuming that you are working with the original data from <code>spatialLIBD::fetch_data("sce")</code> . |

Details

Note that the resulting object is a bit more complex than a regular SPE because it contains the data from the spatialLIBD project which you might otherwise have to generate for your own data.

Value

A a [SpatialExperiment-class](#) object.

Author(s)

Brenda Pardo, Leonardo Collado-Torres

Examples

```
if (enough_ram()) {  
  ## Download the sce data  
  sce <- fetch_data("sce")  
  ## Transform it to a SpatialExperiment object  
  spe <- sce_to_spe(sce)  
}
```

sig_genes_extract *Extract significant genes*

Description

From the layer-level modeling results, this function extracts the top n significant genes. This is the workhorse function used by [sig_genes_extract_all\(\)](#) through which we obtain the information that can then be used by functions such as [layer_boxplot\(\)](#) for constructing informative titles.

Usage

```
sig_genes_extract(  
  n = 10,  
  modeling_results = fetch_data(type = "modeling_results"),  
  model_type = names(modeling_results)[1],  
  reverse = FALSE,  
  sce_layer = fetch_data(type = "sce_layer")  
)
```

Arguments

| | |
|------------------|---|
| n | The number of the top ranked genes to extract. |
| modeling_results | Defaults to the output of <code>fetch_data(type = 'modeling_results')</code> . This is a list of tables with the columns <code>f_stat_*</code> or <code>t_stat_*</code> as well as <code>p_value_*</code> and <code>fdr_*</code> plus <code>ensembl</code> . The column name is used to extract the statistic results, the p-values, and the FDR adjusted p-values. Then the <code>ensembl</code> column is used for matching in some cases. See fetch_data() for more details. |
| model_type | A named element of the <code>modeling_results</code> list. By default that is either <code>enrichment</code> for the model that tests one human brain layer against the rest (one group vs the rest), <code>pairwise</code> which compares two layers (groups) denoted by <code>layerA-layerB</code> such that <code>layerA</code> is greater than <code>layerB</code> , and <code>anova</code> which determines if any layer (group) is different from the rest adjusting for the mean expression level. The statistics for <code>enrichment</code> and <code>pairwise</code> are t-statistics while the <code>anova</code> model ones are F-statistics. |

| | |
|-----------|---|
| reverse | A logical(1) indicating whether to multiply by -1 the input statistics and reverse the layerA-layerB column names (using the -) into layerB-layerA. |
| sce_layer | Defaults to the output of <code>fetch_data(type = 'sce_layer')</code> . This is a Single-CellExperiment object with the spot-level Visium data compressed via pseudo-bulking to the layer-level (group-level) resolution. See <code>fetch_data()</code> for more details. |

Value

A `data.frame()` with the top `n` significant genes (as ordered by their statistics in decreasing order) in long format. The specific columns are described further in the vignette.

References

Adapted from https://github.com/LieberInstitute/HumanPilot/blob/master/Analysis/Layer_Guesses/layer_specificity_function

See Also

Other Layer modeling functions: [layer_boxplot\(\)](#), [sig_genes_extract_all\(\)](#)

Examples

```
## Obtain the necessary data
if (!exists("modeling_results")) {
  modeling_results <- fetch_data(type = "modeling_results")
}
if (!exists("sce_layer")) sce_layer <- fetch_data(type = "sce_layer")

## anova top 10 genes
sig_genes_extract(
  modeling_results = modeling_results,
  sce_layer = sce_layer
)

## Extract all genes
sig_genes_extract(
  modeling_results = modeling_results,
  sce_layer = sce_layer,
  n = nrow(sce_layer)
)
```

`sig_genes_extract_all` *Extract significant genes for all modeling results*

Description

This function combines the output of `sig_genes_extract()` from all the layer-level (group-level) modeling results and builds the data required for functions such as `layer_boxplot()`.

Usage

```
sig_genes_extract_all(  
  n = 10,  
  modeling_results = fetch_data(type = "modeling_results"),  
  sce_layer = fetch_data(type = "sce_layer")  
)
```

Arguments

| | |
|------------------|---|
| n | The number of the top ranked genes to extract. |
| modeling_results | Defaults to the output of <code>fetch_data(type = 'modeling_results')</code> . This is a list of tables with the columns <code>f_stat_*</code> or <code>t_stat_*</code> as well as <code>p_value_*</code> and <code>fdr_*</code> plus <code>ensembl</code> . The column name is used to extract the statistic results, the p-values, and the FDR adjusted p-values. Then the <code>ensembl</code> column is used for matching in some cases. See fetch_data() for more details. |
| sce_layer | Defaults to the output of <code>fetch_data(type = 'sce_layer')</code> . This is a Single-CellExperiment object with the spot-level Visium data compressed via pseudo-bulking to the layer-level (group-level) resolution. See fetch_data() for more details. |

Value

A [DataFrame-class](#) with the extracted statistics in long format. The specific columns are described further in the vignette.

See Also

Other Layer modeling functions: [layer_boxplot\(\)](#), [sig_genes_extract\(\)](#)

Examples

```
## Obtain the necessary data  
if (!exists("modeling_results")) {  
  modeling_results <- fetch_data(type = "modeling_results")  
}  
if (!exists("sce_layer")) sce_layer <- fetch_data(type = "sce_layer")  
  
## top 10 genes for all models  
sig_genes_extract_all(  
  modeling_results = modeling_results,  
  sce_layer = sce_layer  
)
```

sort_clusters *Sort clusters by frequency*

Description

This function takes a vector with cluster labels and sorts it by frequency such that the most frequent cluster is the first one and so on.

Usage

```
sort_clusters(clusters, map_subset = NULL)
```

Arguments

`clusters` A vector with cluster labels.
`map_subset` A logical vector of length equal to `clusters` specifying which elements of `clusters` to use to determine the ranking of the clusters.

Value

A factor of length equal to `clusters` where the levels are the new ordered clusters and the names of the factor are the original values from `clusters`.

Examples

```
## Build an initial set of cluster labels  
clus <- letters[unlist(lapply(4:1, function(x) rep(x, x)))]  
  
## In this case, it's a character vector  
class(clus)  
  
## Sort them and obtain a factor  
sort_clusters(clus)
```

tstats_Human_DLPFC_snRNAseq_Nguyen_topLayer
Cell cluster t-statistics from Tran et al

Description

Using the DLPFC snRNA-seq data from Matthew N Tran et al we computed enrichment t-statistics for the cell clusters. This is a subset of them used in examples such as in [layer_stat_cor_plot\(\)](#).

Usage

```
tstats_Human_DLPFC_snRNAseq_Nguyen_topLayer
```

Format

A matrix with 692 rows and 31 variables where each column is a given cell cluster from Tran et al and each row is one gene. The row names are Ensembl gene IDs which are used by `layer_stat_cor()` to match to our modeling results.

Source

https://github.com/LieberInstitute/HumanPilot/blob/master/Analysis/Layer_Guesses/dlpfc_snRNAseq_annotation.R and https://github.com/LieberInstitute/spatialLIBD/blob/master/dev/02_dev.R#L107-L194.

vis_clus

*Sample spatial cluster visualization***Description**

This function visualizes the clusters for one given sample at the spot-level using (by default) the histology information on the background. To visualize gene-level (or any continuous variable) use `vis_gene()`.

Usage

```
vis_clus(
  spe,
  sampleid,
  clustervar,
  colors = c("#b2df8a", "#e41a1c", "#377eb8", "#4daf4a", "#ff7f00", "gold", "#a65628",
    "#999999", "black", "grey", "white", "purple"),
  spatial = TRUE,
  image_id = "lowres",
  alpha = 1,
  point_size = 2,
  ...
)
```

Arguments

| | |
|------------|---|
| spe | Defaults to the output of <code>fetch_data(type = 'spe')</code> . This is a <code>SpatialExperiment-class</code> object with the spot-level Visium data and information required for visualizing the histology. See <code>fetch_data()</code> for more details. |
| sampleid | A character(1) specifying which sample to plot from <code>colData(spe)\$sample_name</code> . |
| clustervar | A character(1) with the name of the <code>colData(spe)</code> column that has the cluster values. |
| colors | A vector of colors to use for visualizing the clusters from <code>clustervar</code> . If the vector has names, then those should match the values of <code>clustervar</code> . |

| | |
|------------|---|
| spatial | A logical(1) indicating whether to include the histology layer from <code>geom_spatial()</code> . If you plan to use <code>ggplotly()</code> then it's best to set this to FALSE. |
| image_id | A character(1) with the name of the image ID you want to use in the background. |
| alpha | A numeric(1) in the $[0, 1]$ range that specifies the transparency level of the data on the spots. |
| point_size | A numeric(1) specifying the size of the points. Defaults to 1.25. Some colors look better if you use 2 for instance. |
| ... | Passed to <code>paste0()</code> for making the title of the plot following the <code>sampleid</code> . |

Details

This function subsets `spe` to the given sample and prepares the data and title for `vis_clus_p()`.

Value

A `ggplot2` object.

See Also

Other Spatial cluster visualization functions: `vis_clus_p()`, `vis_grid_clus()`

Examples

```
if (enough_ram()) {
  ## Obtain the necessary data
  if (!exists("spe")) spe <- fetch_data("spe")

  ## Check the colors defined by Lukas M Weber
  libd_layer_colors

  ## Use the manual color palette by Lukas M Weber
  vis_clus(
    spe = spe,
    clustervar = "layer_guess_reordered",
    sampleid = "151673",
    colors = libd_layer_colors,
    ... = " LIBD Layers"
  )

  ## Without histology
  vis_clus(
    spe = spe,
    clustervar = "layer_guess_reordered",
    sampleid = "151673",
    colors = libd_layer_colors,
    ... = " LIBD Layers",
    spatial = FALSE
  )
}
```

vis_clus_p

*Sample spatial cluster visualization workhorse function***Description**

This function visualizes the clusters for one given sample at the spot-level using (by default) the histology information on the background. This is the function that does all the plotting behind [vis_clus\(\)](#). To visualize gene-level (or any continuous variable) use [vis_gene_p\(\)](#).

Usage

```
vis_clus_p(
  spe,
  d,
  clustervar,
  sampleid,
  colors,
  spatial,
  title,
  image_id = "lowres",
  alpha = 1,
  point_size = 2
)
```

Arguments

| | |
|------------|---|
| spe | Defaults to the output of <code>fetch_data(type = 'spe')</code> . This is a SpatialExperiment-class object with the spot-level Visium data and information required for visualizing the histology. See fetch_data() for more details. |
| d | A data.frame with the sample-level information. This is typically obtained using <code>cbind(colData(spe), spatialCoords(spe))</code> . |
| clustervar | A character(1) with the name of the <code>colData(spe)</code> column that has the cluster values. |
| sampleid | A character(1) specifying which sample to plot from <code>colData(spe)\$sample_name</code> . |
| colors | A vector of colors to use for visualizing the clusters from <code>clustervar</code> . If the vector has names, then those should match the values of <code>clustervar</code> . |
| spatial | A logical(1) indicating whether to include the histology layer from geom_spatial() . If you plan to use ggplotly() then it's best to set this to FALSE. |
| title | The title for the plot. |
| image_id | A character(1) with the name of the image ID you want to use in the background. |
| alpha | A numeric(1) in the <code>[0, 1]</code> range that specifies the transparency level of the data on the spots. |
| point_size | A numeric(1) specifying the size of the points. Defaults to 1.25. Some colors look better if you use 2 for instance. |

Value

A [ggplot2](#) object.

See Also

Other Spatial cluster visualization functions: [vis_clus\(\)](#), [vis_grid_clus\(\)](#)

Examples

```
if (enough_ram()) {
  ## Obtain the necessary data
  if (!exists("spe")) spe <- fetch_data("spe")
  spe_sub <- spe[, spe$sample_id == "151673"]

  ## Use the manual color palette by Lukas M Weber
  ## Don't plot the histology information
  vis_clus_p(
    spe = spe_sub,
    d = as.data.frame(cbind(colData(spe_sub), SpatialExperiment::spatialCoords(spe_sub)), optional = TRUE),
    clustervar = "layer_guess_reordered",
    sampleid = "151673",
    colors = libd_layer_colors,
    title = "151673 LIBD Layers",
    spatial = FALSE
  )

  ## Clean up
  rm(spe_sub)
}
```

vis_gene

Sample spatial gene visualization

Description

This function visualizes the gene expression stored in `assays(spe)` or any continuous variable stored in `colData(spe)` for one given sample at the spot-level using (by default) the histology information on the background. To visualize clusters (or any discrete variable) use [vis_clus\(\)](#).

Usage

```
vis_gene(
  spe,
  sampleid,
  geneid = "SCGB2A2; ENSG00000110484",
  spatial = TRUE,
  assayname = "logcounts",
  minCount = 0,
```

```

viridis = TRUE,
image_id = "lowres",
alpha = 1,
cont_colors = if (viridis) viridisLite::viridis(21) else c("aquamarine4",
  "springgreen", "goldenrod", "red"),
point_size = 2,
...
)

```

Arguments

| | |
|-------------|--|
| spe | Defaults to the output of <code>fetch_data(type = 'spe')</code> . This is a SpatialExperiment-class object with the spot-level Visium data and information required for visualizing the histology. See <code>fetch_data()</code> for more details. |
| sampleid | A character(1) specifying which sample to plot from <code>colData(spe)\$sample_name</code> . |
| geneid | A character(1) specifying the gene ID stored in <code>rowData(spe)\$gene_search</code> or a continuous variable stored in <code>colData(spe)</code> to visualize. If <code>rowData(spe)\$gene_search</code> is missing, then <code>rownames(spe)</code> is used to search for the gene ID. |
| spatial | A logical(1) indicating whether to include the histology layer from <code>geom_spatial()</code> . If you plan to use <code>ggplotly()</code> then it's best to set this to FALSE. |
| assayname | The name of the assays(spe) to use for extracting the gene expression data. Defaults to <code>logcounts</code> . |
| minCount | A numeric(1) specifying the minimum gene expression (or value in the continuous variable) to visualize. Values at or below this threshold will be set to NA. Defaults to 0. |
| viridis | A logical(1) whether to use the color-blind friendly palette from viridis or the color palette used in the paper that was chosen for contrast when visualizing the data on top of the histology image. One issue is being able to differentiate low values from NA ones due to the purple-ish histology information that is dependent on cell density. |
| image_id | A character(1) with the name of the image ID you want to use in the background. |
| alpha | A numeric(1) in the $[\ 0, \ 1]$ range that specifies the transparency level of the data on the spots. |
| cont_colors | A character() vector of colors that supersedes the <code>viridis</code> argument. |
| point_size | A numeric(1) specifying the size of the points. Defaults to 1.25. Some colors look better if you use 2 for instance. |
| ... | Passed to <code>paste0()</code> for making the title of the plot following the <code>sampleid</code> . |

Details

This function subsets `spe` to the given sample and prepares the data and title for `vis_gene_p()`. It also adds a caption to the plot.

Value

A [ggplot2](#) object.

See Also

Other Spatial gene visualization functions: [vis_gene_p\(\)](#), [vis_grid_gene\(\)](#)

Examples

```

if (enough_ram()) {
  ## Obtain the necessary data
  if (!exists("spe")) spe <- fetch_data("spe")

  ## Valid `geneid` values are those in
  head(rowData(spe)$gene_search)
  ## or continuous variables stored in colData(spe)
  ## or rownames(spe)

  ## Visualize a default gene on the non-iridis scale
  vis_gene(
    spe = spe,
    sampleid = "151507",
    viridis = FALSE
  )

  ## Use a custom set of colors in the reverse order than usual
  vis_gene(
    spe = spe,
    sampleid = "151507",
    cont_colors = rev(viridisLite::viridis(21, option = "magma"))
  )

  ## Visualize a continuous variable, in this case, the ratio of chrM
  ## gene expression compared to the total expression at the spot-level
  vis_gene(
    spe = spe,
    sampleid = "151507",
    geneid = "expr_chrM_ratio"
  )

  ## Visualize a gene using the rownames(spe)
  vis_gene(
    spe = spe,
    sampleid = "151507",
    geneid = rownames(spe)[which(rowData(spe)$gene_name == "MOBP")]
  )
}

```


Description

This function visualizes the gene expression stored in `assays(spe)` or any continuous variable stored in `colData(spe)` for one given sample at the spot-level using (by default) the histology information on the background. This is the function that does all the plotting behind `vis_gene()`. To visualize clusters (or any discrete variable) use `vis_clus_p()`.

Usage

```
vis_gene_p(
  spe,
  d,
  sampleid,
  spatial,
  title,
  viridis = TRUE,
  image_id = "lowres",
  alpha = 1,
  cont_colors = if (viridis) viridisLite::viridis(21) else c("aquamarine4",
    "springgreen", "goldenrod", "red"),
  point_size = 2
)
```

Arguments

| | |
|--------------------------|--|
| <code>spe</code> | Defaults to the output of <code>fetch_data(type = 'spe')</code> . This is a SpatialExperiment-class object with the spot-level Visium data and information required for visualizing the histology. See <code>fetch_data()</code> for more details. |
| <code>d</code> | A data.frame with the sample-level information. This is typically obtained using <code>cbind(colData(spe), spatialCoords(spe))</code> . The data.frame has to contain a column with the continuous variable data to plot stored under <code>d\$COUNT</code> . |
| <code>sampleid</code> | A character(1) specifying which sample to plot from <code>colData(spe)\$sample_name</code> . |
| <code>spatial</code> | A logical(1) indicating whether to include the histology layer from <code>geom_spatial()</code> . If you plan to use <code>ggplotly()</code> then it's best to set this to FALSE. |
| <code>title</code> | The title for the plot. |
| <code>viridis</code> | A logical(1) whether to use the color-blind friendly palette from viridis or the color palette used in the paper that was chosen for contrast when visualizing the data on top of the histology image. One issue is being able to differentiate low values from NA ones due to the purple-ish histology information that is dependent on cell density. |
| <code>image_id</code> | A character(1) with the name of the image ID you want to use in the background. |
| <code>alpha</code> | A numeric(1) in the <code>[0, 1]</code> range that specifies the transparency level of the data on the spots. |
| <code>cont_colors</code> | A character() vector of colors that supersedes the <code>viridis</code> argument. |
| <code>point_size</code> | A numeric(1) specifying the size of the points. Defaults to 1.25. Some colors look better if you use 2 for instance. |

Value

A [ggplot2](#) object.

See Also

Other Spatial gene visualization functions: [vis_gene\(\)](#), [vis_grid_gene\(\)](#)

Examples

```
if (enough_ram()) {
  ## Obtain the necessary data
  if (!exists("spe")) spe <- fetch_data("spe")

  ## Prepare the data for the plotting function
  spe_sub <- spe[, spe$sample_id == "151673"]
  df <- as.data.frame(cbind(colData(spe_sub), SpatialExperiment::spatialCoords(spe_sub)), optional = TRUE)
  df$COUNT <- df$expr_chrM_ratio

  ## Use the manual color palette by Lukas M Weber
  ## Don't plot the histology information
  vis_gene_p(
    spe = spe_sub,
    d = df,
    sampleid = "151673",
    title = "151673 chrM expr ratio",
    spatial = FALSE
  )

  ## Clean up
  rm(spe_sub)
}
```

vis_grid_clus

Sample spatial cluster visualization grid

Description

This function visualizes the clusters for a set of samples at the spot-level using (by default) the histology information on the background. To visualize gene-level (or any continuous variable) use [vis_grid_gene\(\)](#).

Usage

```
vis_grid_clus(
  spe,
  clustervar,
  pdf_file,
  sort_clust = TRUE,
  colors = NULL,
```

```

    return_plots = FALSE,
    spatial = TRUE,
    height = 24,
    width = 36,
    image_id = "lowres",
    alpha = 1,
    sample_order = unique(spe$sample_id),
    point_size = 2,
    ...
  )

```

Arguments

| | |
|--------------|--|
| spe | Defaults to the output of <code>fetch_data(type = 'spe')</code> . This is a SpatialExperiment-class object with the spot-level Visium data and information required for visualizing the histology. See <code>fetch_data()</code> for more details. |
| clustervar | A character(1) with the name of the <code>colData(spe)</code> column that has the cluster values. |
| pdf_file | A character(1) specifying the path for the resulting PDF. |
| sort_clust | A logical(1) indicating whether you want to sort the clusters by frequency using <code>sort_clusters()</code> . |
| colors | A vector of colors to use for visualizing the clusters from <code>clustervar</code> . If the vector has names, then those should match the values of <code>clustervar</code> . |
| return_plots | A logical(1) indicating whether to print the plots to a PDF or to return the list of plots that you can then print using <code>plot_grid</code> . |
| spatial | A logical(1) indicating whether to include the histology layer from <code>geom_spatial()</code> . If you plan to use <code>ggplotly()</code> then it's best to set this to FALSE. |
| height | A numeric(1) passed to <code>pdf</code> . |
| width | A numeric(1) passed to <code>pdf</code> . |
| image_id | A character(1) with the name of the image ID you want to use in the background. |
| alpha | A numeric(1) in the $[\emptyset, 1]$ range that specifies the transparency level of the data on the spots. |
| sample_order | A character() with the names of the samples to use and their order. |
| point_size | A numeric(1) specifying the size of the points. Defaults to 1.25. Some colors look better if you use 2 for instance. |
| ... | Passed to <code>paste0()</code> for making the title of the plot following the <code>sampleid</code> . |

Details

This function prepares the data and then loops through `vis_clus()` for computing the list of `ggplot2` objects.

Value

A list of `ggplot2` objects.

See Also

Other Spatial cluster visualization functions: [vis_clus_p\(\)](#), [vis_clus\(\)](#)

Examples

```
if (enough_ram()) {
  ## Obtain the necessary data
  if (!exists("spe")) spe <- fetch_data("spe")

  ## Subset to two samples of interest and obtain the plot list
  p_list <-
    vis_grid_clus(
      spe[, spe$sample_id %in% c("151673", "151674")],
      "layer_guess_reordered",
      spatial = FALSE,
      return_plots = TRUE,
      sort_clust = FALSE,
      colors = libd_layer_colors
    )

  ## Visualize the spatial adjacent replicates for position = 0 micro meters
  ## for subject 3
  cowplot::plot_grid(plotlist = p_list, ncol = 2)
}
```

 vis_grid_gene

Sample spatial gene visualization grid

Description

This function visualizes the gene expression stored in `assays(spe)` or any continuous variable stored in `colData(spe)` for a set of samples at the spot-level using (by default) the histology information on the background. To visualize clusters (or any discrete variable) use [vis_grid_clus\(\)](#).

Usage

```
vis_grid_gene(
  spe,
  geneid = "SCGB2A2; ENSG00000110484",
  pdf_file,
  assayname = "logcounts",
  minCount = 0,
  return_plots = FALSE,
  spatial = TRUE,
  viridis = TRUE,
  height = 24,
  width = 36,
  image_id = "lowres",
```

```

alpha = 1,
cont_colors = if (viridis) viridisLite::viridis(21) else c("aquamarine4",
  "springgreen", "goldenrod", "red"),
sample_order = unique(spe$sample_id),
point_size = 2,
...
)

```

Arguments

| | |
|--------------|---|
| spe | Defaults to the output of <code>fetch_data(type = 'spe')</code> . This is a SpatialExperiment-class object with the spot-level Visium data and information required for visualizing the histology. See fetch_data() for more details. |
| geneid | A <code>character(1)</code> specifying the gene ID stored in <code>rowData(spe)\$gene_search</code> or a continuous variable stored in <code>colData(spe)</code> to visualize. If <code>rowData(spe)\$gene_search</code> is missing, then <code>rownames(spe)</code> is used to search for the gene ID. |
| pdf_file | A <code>character(1)</code> specifying the path for the resulting PDF. |
| assayname | The name of the assays(spe) to use for extracting the gene expression data. Defaults to <code>logcounts</code> . |
| minCount | A <code>numeric(1)</code> specifying the minimum gene expression (or value in the continuous variable) to visualize. Values at or below this threshold will be set to NA. Defaults to 0. |
| return_plots | A <code>logical(1)</code> indicating whether to print the plots to a PDF or to return the list of plots that you can then print using plot_grid . |
| spatial | A <code>logical(1)</code> indicating whether to include the histology layer from geom_spatial() . If you plan to use ggplotly() then it's best to set this to FALSE. |
| viridis | A <code>logical(1)</code> whether to use the color-blind friendly palette from viridis or the color palette used in the paper that was chosen for contrast when visualizing the data on top of the histology image. One issue is being able to differentiate low values from NA ones due to the purple-ish histology information that is dependent on cell density. |
| height | A <code>numeric(1)</code> passed to pdf . |
| width | A <code>numeric(1)</code> passed to pdf . |
| image_id | A <code>character(1)</code> with the name of the image ID you want to use in the background. |
| alpha | A <code>numeric(1)</code> in the <code>[0, 1]</code> range that specifies the transparency level of the data on the spots. |
| cont_colors | A <code>character()</code> vector of colors that supersedes the <code>viridis</code> argument. |
| sample_order | A <code>character()</code> with the names of the samples to use and their order. |
| point_size | A <code>numeric(1)</code> specifying the size of the points. Defaults to 1.25. Some colors look better if you use 2 for instance. |
| ... | Passed to paste0() for making the title of the plot following the <code>sampleid</code> . |

Details

This function prepares the data and then loops through `vis_gene()` for computing the list of `ggplot2` objects.

Value

A list of `ggplot2` objects.

See Also

Other Spatial gene visualization functions: `vis_gene_p()`, `vis_gene()`

Examples

```
if (enough_ram()) {
  ## Obtain the necessary data
  if (!exists("spe")) spe <- fetch_data("spe")

  ## Subset to two samples of interest and obtain the plot list
  p_list <-
    vis_grid_gene(
      spe[, spe$sample_id %in% c("151673", "151674")],
      spatial = FALSE,
      return_plots = TRUE
    )

  ## Visualize the spatial adjacent replicates for position = 0 micro meters
  ## for subject 3
  cowplot::plot_grid(plotlist = p_list, ncol = 2)
}
```

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