Package 'spatialLIBD'

October 27, 2026

Title spatialLIBD: an R/Bioconductor package to visualize spatially-resolved transcriptomics data

Version 1.24.0

Date 2026-01-09

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.

License Artistic-2.0

Encoding UTF-8

LazyData true

Imports shiny, golem, ggplot2, cowplot, plotly, viridisLite, shinyWidgets, sessioninfo, grid, grDevices, methods, AnnotationHub, utils, png, scater, DT, ExperimentHub, SummarizedExperiment, stats, graphics, S4Vectors, IRanges, benchmarkme, SingleCellExperiment, BiocFileCache, jsonlite, tibble, rtracklayer, Matrix, BiocGenerics, GenomicRanges, magick, paletteer, scuttle, edgeR, limma, statmod, MatrixGenerics, rlang, dplyr, ComplexHeatmap, circlize

RoxygenNote 7.3.3

Roxygen list(markdown = TRUE)

URL https://github.com/LieberInstitute/spatialLIBD

BugReports https://support.bioconductor.org/tag/spatialLIBD

Suggests knitr, RefManageR, rmarkdown, BiocStyle, testthat (>= 2.1.0), covr, here, BiocManager, lobstr, DropletUtils, RColorBrewer

VignetteBuilder knitr

biocViews Homo_sapiens_Data, ExperimentHub, SequencingData, SingleCellData, ExpressionData, Tissue, PackageTypeData, SpatialData

Depends SpatialExperiment (>= 1.3.3), R (>= 4.1)

git_url https://git.bioconductor.org/packages/spatialLIBD

git_branch RELEASE_3_23

git_last_commit c9e01b2

git_last_commit_date 2026-04-28

Repository Bioconductor 3.23

Date/Publication 2026-10-27

Author Leonardo Collado-Torres [aut, cre] (ORCID:
<https://orcid.org/0000-0003-2140-308X>),
Kristen R. Maynard [ctb] (ORCID:
<https://orcid.org/0000-0003-0031-8468>),
Andrew E. Jaffe [ctb] (ORCID: <https://orcid.org/0000-0001-6886-1454>),
Brenda Pardo [ctb] (ORCID: <https://orcid.org/0000-0001-8103-7136>),
Abby Spangler [ctb] (ORCID: <https://orcid.org/0000-0002-0028-9348>),
Jesús Vélez Santiago [ctb] (ORCID:
<https://orcid.org/0000-0001-5128-3838>),
Lukas M. Weber [ctb] (ORCID: <https://orcid.org/0000-0002-3282-1730>),
Louise Huuki-Myers [ctb] (ORCID:
<https://orcid.org/0000-0001-5148-3602>),
Nicholas Eagles [ctb] (ORCID: <https://orcid.org/0000-0002-9808-5254>)

Maintainer Leonardo Collado-Torres <lcolladotor@gmail.com>

Contents

spatialLIBD-package . . . 3
add10xVisiumAnalysis . . . 4
add_images . . . 5
add_key . . . 7
add_qc_metrics . . . 8
annotate_registered_clusters . . . 10
check_modeling_results . . . 11
check_sce . . . 12
check_sce_layer . . . 13
check_spe . . . 14
cluster_export . . . 15
cluster_import . . . 16
enough_ram . . . 17
fetch_data . . . 17
frame_limits . . . 21
gene_set_enrichment . . . 22
gene_set_enrichment_plot . . . 24
geom_spatial . . . 27
get_colors . . . 29
img_edit . . . 30
img_update . . . 32
img_update_all . . . 33
layer_boxplot . . . 34

layer_stat_cor . . . 37
layer_stat_cor_plot . . . 39
libd_layer_colors . . . 41
locate_images . . . 42
multi_gene_pca . . . 43
multi_gene_sparsity . . . 43
multi_gene_z_score . . . 44
prep_stitched_data . . . 45
read10xVisiumAnalysis . . . 45
read10xVisiumWrapper . . . 46
registration_block_cor . . . 48
registration_model . . . 49
registration_pseudobulk . . . 50
registration_stats_anova . . . 51
registration_stats_enrichment . . . 53
registration_stats_pairwise . . . 55
registration_wrapper . . . 56
run_app . . . 58
sce_to_spe . . . 63
sig_genes_extract . . . 64
sig_genes_extract_all . . . 66
sort_clusters . . . 67
tstats_Human_DLPFC_snRNAseq_Nguyen_topLayer . . . 68
vis_clus . . . 69
vis_clus_p . . . 72
vis_gene . . . 74
vis_gene_p . . . 78
vis_grid_clus . . . 80
vis_grid_gene . . . 83
vis_image . . . 85

Index **88**

`spatialLIBD-package` *spatialLIBD: spatialLIBD: an R/Bioconductor package to visualize spatially-resolved transcriptomics data*

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.

Author(s)

Maintainer: Leonardo Collado-Torres <lcolladotor@gmail.com> (ORCID)

Other contributors:

- Kristen R. Maynard <Kristen.Maynard@libd.org> (ORCID) [contributor]
- Andrew E. Jaffe <andrew.jaffe@libd.org> (ORCID) [contributor]
- Brenda Pardo <bpardo@lcgej.unam.mx> (ORCID) [contributor]
- Abby Spangler <aspangle@gmail.com> (ORCID) [contributor]
- Jesús Vélez Santiago <jvelezmagic@gmail.com> (ORCID) [contributor]
- Lukas M. Weber <lukas.weber.edu@gmail.com> (ORCID) [contributor]
- Louise Huuki-Myers <lahuuki@gmail.com> (ORCID) [contributor]
- Nicholas Eagles <nickeagles77@gmail.com> (ORCID) [contributor]

See Also

Useful links:

- https://github.com/LieberInstitute/spatialLIBD
- Report bugs at https://support.bioconductor.org/tag/spatialLIBD

`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 *Add non-standard images with the same dimensions as current ones*

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	A SpatialExperiment-class object. See `fetch_data()` for how to download some example objects or `read10xVisiumWrapper()` to read in `spaceranger --count` output files and build your own spe object.
image_dir	A `character(1)` specifying a path to a directory containing image files with the pattern `sampleID_pattern.png`.
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 spe 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$key_original <- spe$key
    spe$key <- NULL

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

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

    ## Below we restore the original 'key'
    spe$key <- spe$key_original
    spe$key_original <- NULL
    head(spe$key)
}
```

add_qc_metrics *Quality Control for Spatial Data*

Description

This function identify spots in a SpatialExperiment-class (SPE) with outlier quality control values: low sum_umi or sum_gene, or high expr_chrM_ratio, utilizing scuttle::isOutlier. Also identifies in-tissue edge spots and distance to the edge for each spot.

Usage

```
add_qc_metrics(spe, overwrite = FALSE)
```

Arguments

spe	a SpatialExperiment object that has sum_umi, sum_gene, expr_chrM_ratio, and in_tissue variables in the colData(spe). Note that these are automatically created when you build your spe object with spatialLIBD::read10xVisiumWrapper().
overwrite	a logical(1) specifying whether to overwrite the 7 colData(spe) columns that this function creates. If set to FALSE and any of them are present, the function will return an error.

Details

The initial version of this function lives at https://github.com/LieberInstitute/Visium_SPG_AD/blob/master/code/07_spot_qc/01_qc_metrics_and_segmentation.R.

Value

A SpatialExperiment with added quality control information added to the colData().

scran_low_lib_size shows spots that have a low library size.

scran_low_n_features spots with a low number of expressed genes.

scran_high_Mito_percent spots with a high percent of mitochondrial gene expression.

scran_discard spots belonging to either scran_low_lib_size, scran_low_n_feature, or scran_high_Mito_percent.

edge_spot spots that are automatically detected as the edge spots of the in_tissue section.

edge_distance closest distance in number of spots to either the vertical or horizontal edge.

scran_low_lib_size_edge spots that have a low library size and are an edge spot.

Author(s)

Louise A. Huuki-Myers

Examples

```
## Obtain the necessary data
spe_pre_qc <- fetch_data("spatialDLPFC_Visium_example_subset")

## For now, we fake out tissue spots in example data
spe_qc <- spe_pre_qc
spe_qc$in_tissue[spe_qc$array_col < 10] <- FALSE

## adds QC metrics to colData of the spe
spe_qc <- add_qc_metrics(spe_qc, overwrite = TRUE)
vars <- colnames(colData(spe_qc))
vars[grep("^(scran|edge)", vars)]

## visualize edge spots
vis_clus(spe_qc, sampleid = "Br6432_ant", clustervar = "edge_spot")

## specify your own colors
vis_clus(
    spe_qc,
    sampleid = "Br6432_ant",
    clustervar = "edge_spot",
    colors = c(
        "TRUE" = "lightgreen",
        "FALSE" = "pink",
        "NA" = "red"
    )
)
vis_gene(spe_qc, sampleid = "Br6432_ant", geneid = "edge_distance", minCount = -1)

## Visualize scran QC flags

## Check the spots with low library size as detected by scran::isOutlier()
vis_clus(spe_qc, sample_id = "Br6432_ant", clustervar = "scran_low_lib_size")

## Violin plot of library size with low library size highlighted in a
## different color.
scater::plotColData(spe_qc[, spe_qc$in_tissue], x = "sample_id", y = "sum_umi", colour_by = "scran_low_lib_size")

## Check any spots that scran::isOutlier() flagged
vis_clus(spe_qc, sampleid = "Br6432_ant", clustervar = "scran_discard")

## Low library spots that are on the edge of the tissue
vis_clus(spe_qc, sampleid = "Br6432_ant", clustervar = "scran_low_lib_size_edge")

## Use `low_library_size` (or other variables) and `edge_distance` as you
## please.
spe_qc$our_low_lib_edge <- spe_qc$scran_low_lib_size & spe_qc$edge_distance < 5

vis_clus(spe_qc, sample_id = "Br6432_ant", clustervar = "our_low_lib_edge")

## Clean up
rm(spe_qc, spe_pre_qc, vars)
```

annotate_registered_clusters *Annotated spatially-registered clusters*

Description

Once you have computed the enrichment t-statistics for your sc/snRNA-seq data using `registration_wrapper()` and related functions, you can then use `layer_stat_cor()` and `layer_stat_cor_plot()` to perform the spatial registartion of your sc/snRNA-seq data. This function helps interpret that matrix and assign layer labels to your clusters.

Usage

```
annotate_registered_clusters(
  cor_stats_layer,
  confidence_threshold = 0.25,
  cutoff_merge_ratio = 0.25
)
```

Arguments

`cor_stats_layer`
: The output of `layer_stat_cor()`.

`confidence_threshold`
: A `numeric(1)` specifying the minimum correlation that a given cluster must have against any of the layers (by default) to be considered as having a 'good' assignment. Otherwise, the confidence will be 'poor' and the final label will have an asterisk.

`cutoff_merge_ratio`
: A `numeric(1)` specifying the threshold for merging or not layer assignments (by default). This is a proportion of the difference between the current correlation and the next highest given the units of the next highest correlation. Defaults to a difference of 25% of the next highest correlation: if the observed difference is lower than this threshold, then we keep merging. Higher values will lead to more layers (by default) being merged.

Details

If you change the input `modeling_results` to `layer_stat_cor()` then the interpretation of this function could change. For example, maybe you have your own spatially-resolved transcriptomics data that doesn't have to be about DLPFC layers.

Value

A `data.frame` with 3 columns. Your `clusters`, the `layer_confidence` which depends on `confidence_threshold`, and the `layer_label`.

See Also

Other Layer correlation functions: layer_stat_cor(), 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"
)

## Obtain labels
annotate_registered_clusters(cor_stats_layer)

## More relaxed merging threshold
annotate_registered_clusters(cor_stats_layer, cutoff_merge_ratio = 1)
```

---

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. Typically this is the set of reference statistics used in layer_stat_cor().

Value

The input object if all checks are passed.

See Also

Other Check input functions: check_sce(), check_sce_layer(), 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 fetch_data(type = 'sce'). 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 character() vector of variable names expected to be present in colData(sce).

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 *Check input sce_layer*

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

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

Value

The input object if all checks are passed.

See Also

Other Check input functions: check_modeling_results(), check_sce(), check_spe()

Examples

```
## Obtain example data from the HumanPilot project
## (Maynard, Collado-Torres, et al, 2021)
if (!exists("sce_layer")) sce_layer <- fetch_data("sce_layer")

## Check the pseudo-bulked data
check_sce_layer(sce_layer)
```

check_spe *Check input spe*

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	A SpatialExperiment-class object. See `fetch_data()` for how to download some example objects or `read10xVisiumWrapper()` to read in `spaceranger --count` output files and build your own spe object.
variables	A `character()` vector of variable names expected to be present in `colData(spe)`.

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()`, `check_sce_layer()`

Examples

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

    ## Check the object
    check_spe(spe)
}
```

`cluster_export` *Export a column with cluster results*

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

`spe`	A SpatialExperiment-class object. See `fetch_data()` for how to download some example objects or `read10xVisiumWrapper()` to read in `spaceranger --count` output files and build your own `spe` object.
`cluster_var`	A `character(1)` with the name of the variable you wish to export.
`cluster_dir`	A `character(1)` specifying the output directory, similar to the `outs/analysis/clustering` produced by SpaceRanger.
`overwrite`	A `logical(1)` indicating whether to overwrite the `spe$key`.

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` *Import cluster results*

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`	A SpatialExperiment-class object. See `fetch_data()` for how to download some example objects or `read10xVisiumWrapper()` to read in `spaceranger --count` output files and build your own `spe` object.
`cluster_dir`	A `character(1)` specifying the output directory, similar to the `outs/analysis/clustering` 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 `spe$key`.

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` Visium, Visium Spatial Proteogenomics (Visium-SPG), or single nucleus RNA-seq (snRNA-seq) data and results analyzed by LIBD from multiple projects. If `ExperimentHub` is not available, this function will download the files from Dropbox using `BiocFileCache::bfcrpath()` unless the files are present already at `destdir`. Note that `ExperimentHub` and `BiocFileCache` 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",
    "spatialDLPFC_Visium", "spatialDLPFC_Visium_example_subset",
    "spatialDLPFC_Visium_pseudobulk", "spatialDLPFC_Visium_modeling_results",
    "spatialDLPFC_Visium_SPG", "spatialDLPFC_snRNAseq",
   "Visium_SPG_AD_Visium_wholegenome_spe", "Visium_SPG_AD_Visium_targeted_spe",
    "Visium_SPG_AD_Visium_wholegenome_pseudobulk_spe",
   "Visium_SPG_AD_Visium_wholegenome_modeling_results", "visiumStitched_brain_spe",
    "visiumStitched_brain_spaceranger", "visiumStitched_brain_Fiji_out",

    "LFF_spatial_ERC_SRT", "LFF_spatial_ERC_SRT_pseudobulk",
    "LFF_spatial_ERC_SRT_modeling_results", "LFF_spatial_ERC_snRNAseq",
    "LFF_spatial_ERC_snRNAseq_pseudobulk_broad",
    "LFF_spatial_ERC_snRNAseq_pseudobulk_subcluster",
    "LFF_spatial_ERC_snRNAseq_modeling_results_broad",
    "LFF_spatial_ERC_snRNAseq_modeling_results_subcluster"),
  destdir = tempdir(),
  eh = ExperimentHub::ExperimentHub(),
  bfc = BiocFileCache::BiocFileCache()
)
```

Arguments

type	A `character(1)` specifying which file you want to download. It can either be: `sce` for the SingleCellExperiment object containing the spot-level data that includes the information for visualizing the clusters/genes on top of the Visium histology, `sce_layer` for the SingleCellExperiment object containing the layer-level data (pseudo-bulked from the spot-level), or `modeling_results` for the list of tables with the `enrichment`, `pairwise`, and `anova` model results from the layer-level data. It can also be `sce_example` which is a reduced version of `sce` just for example purposes. The initial version of `spatialLIBD` downloaded data only from https://github.com/LieberInstitute/HumanPilot. As of BioC version 3.13 `spe` downloads a SpatialExperiment-class object. As of version 1.11.6, this function also allows downloading data from the http://research.libd.org/spatialDLPFC/ and https://github.com/LieberInstitute/Human_DLPFC_Deconvolution projects. As of version 1.11.12, data from https://github.com/LieberInstitute/Visium_SPG_AD can be downloaded. As of version 1.17.3, data from https://research.libd.org/visiumStitched_brain/ can be downloaded. As of version 1.23.1, data from https://research.libd.org/LFF_spatial_ERC/ can be downloaded.
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`.

References

Please always cite the `spatialLIBD` publication whenever you use `fetch_data()` to download data.

Pardo B, Spangler A, Weber LM, Page SC, Hicks SC, Jaffe AE, Martinowich K, Maynard KR, Collado-Torres L. spatialLIBD: an R/Bioconductor package to visualize spatially-resolved transcriptomics data. BMC Genomics. 2022 Jun 10;23(1):434. doi: 10.1186/s12864-022-08601-w. PubMed PMID: 35689177; PubMed Central PMCID: PMC9188087.

Additionally, please cite the relevant publication describing the data generation and initial data analysis for the dataset you are using.

- For "sce", "sce_layer", "modeling_results", "sce_example", and "spe" which are files from the `HumanPilot` study https://github.com/LieberInstitute/HumanPilot please cite: Maynard KR, Collado-Torres L, Weber LM, Uytingco C, Barry BK, Williams SR, Catallini JL 2nd, Tran MN, Besich Z, Tippani M, Chew J, Yin Y, Kleinman JE, Hyde TM, Rao N, Hicks SC, Martinowich K, Jaffe AE. Transcriptome-scale spatial gene expression in the human dorsolateral prefrontal cortex. Nat Neurosci. 2021 Mar;24(3):425-436. doi: 10.1038/s41593-020-00787-0. Epub 2021 Feb 8. PubMed PMID: 33558695; PubMed Central PMCID: PMC8095368.
- For `spatialDLPFC` files http://research.libd.org/spatialDLPFC/ please cite: Huuki-Myers LA, Spangler A, Eagles NJ, Montgomery KD, Kwon SH, Guo B, Grant-Peters M, Divecha HR, Tippani M, Sriworarat C, Nguyen AB, Ravichandran P, Tran MN, Seyedian A, Hyde TM, Kleinman JE, Battle A, Page SC, Ryten M, Hicks SC, Martinowich K, Collado-Torres L, Maynard KR. A data-driven single-cell and spatial transcriptomic map of the human prefrontal cortex. Science. 2024 May 24;384(6698):eadh1938. doi: 10.1126/science.adh1938. Epub 2024 May 24. PubMed PMID: 38781370; PubMed Central PMCID: PMC11398705.
- Note that `spatialDLPFC_snRNAseq` from https://github.com/LieberInstitute/Human_DLPFC_Deconvolution was also described in the following publication: Huuki-Myers LA, Montgomery KD, Kwon SH, Cinquemani S, Eagles NJ, Gonzalez-Padilla D, Maden SK, Kleinman JE, Hyde TM, Hicks SC, Maynard KR, Collado-Torres L. Benchmark of cellular deconvolution methods using a multi-assay dataset from postmortem human prefrontal cortex. Genome Biol. 2025 Apr 7;26(1):88. doi: 10.1186/s13059-025-03552-3. PubMed PMID: 40197307; PubMed Central PMCID: PMC11978107.
- For `Visium_SPG_AD` files https://research.libd.org/Visium_SPG_AD please cite: Kwon SH, Parthiban S, Tippani M, Divecha HR, Eagles NJ, Lobana JS, Williams SR, Mak M, Bharadwaj RA, Kleinman JE, Hyde TM, Page SC, Hicks SC, Martinowich K, Maynard KR, Collado-Torres L. Influence of Alzheimer's disease related neuropathology on local microenvironment gene expression in the human inferior temporal cortex. GEN Biotechnol. 2023

Oct;2(5):399-417. doi: 10.1089/genbio.2023.0019. Epub 2023 Oct 16. PubMed PMID: 39329069; PubMed Central PMCID: PMC11426291.

- For `visiumStitched_brain` files https://research.libd.org/visiumStitched_brain/ please cite: Eagles NJ, Bach SV, Tippani M, Ravichandran P, Du Y, Miller RA, Hyde TM, Page SC, Martinowich K, Collado-Torres L. Integrating gene expression and imaging data across Visium capture areas with visiumStitched. BMC Genomics. 2024 Nov 13;25(1):1077. doi: 10.1186/s12864-024-10991-y. PubMed PMID: 39533203; PubMed Central PMCID: PMC11559125.
- For `LFF_spatial_ERC` files https://research.libd.org/LFF_spatial_ERC/ please cite: Huuki-Myers LA, Divecha HR, Bach SV, Valentine MR, Eagles NJ, Mulvey B, Bharadwaj RA, Zhang R, Evans JR, Grant-Peters M, Miller RA, Kleinman JE, Han S, Hyde TM, Page SC, Weinberger DR, Martinowich K, Ryten M, Maynard KR, Collado-Torres L. APOE E4 Alzheimer's Risk Converges on an Oligodendrocyte Subtype in the Human Entorhinal Cortex. bioRxiv. 2025 Nov 20;. doi: 10.1101/2025.11.20.689483. PubMed PMID: 41332786; PubMed Central PMCID: PMC12667772.

Examples

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

## Explore the data
sce_layer

## How to download and load "spatialDLPFC_snRNAseq"
## A similar process is needed for downloading and loading other
## HDF5SummarizedExperiment files:
## * "LFF_spatial_ERC_SRT"
## * LFF_spatial_ERC_snRNAseq"

## Not run:
sce_path_zip <- fetch_data("spatialDLPFC_snRNAseq")
sce_path <- unzip(sce_path_zip, exdir = tempdir())
sce <- HDF5Array::loadHDF5SummarizedExperiment(
    file.path(tempdir(), "sce_DLPFC_annotated")
)
sce
#> class: SingleCellExperiment
#> dim: 36601 77604
#> metadata(3): Samples cell_type_colors cell_type_colors_broad
#> assays(2): counts logcounts
#> rownames(36601): MIR1302-2HG FAM138A ... AC007325.4 AC007325.2
#> rowData names(7): source type ... gene_type binomial_deviance
#> colnames(77604): 1_AAACCCAAGTTCTCTT-1 1_AAACCCACAAGGTCTT-1 ... 19_TTTGTTGTCTCATTGT-1 19_TTTGTTGTCTTAAGGC-1
#> colData names(32): Sample Barcode ... cellType_layer layer_annotation
#> reducedDimNames(4): GLMPCA_approx TSNE UMAP HARMONY
#> mainExpName: NULL
#> altExpNames(0):
lobstr::obj_size(sce)
#> 172.28 MB
```

```
## End(Not run)
```

`frame_limits` *Identify the image limits*

Description

This function is useful for automatically cropping the images. It finds the edge points (min and max on both the X and Y axis) in pixels based on a particular image. This function takes advantage of the known design of Visium slides as documented at https://support.10xgenomics.com/spatial-gene-expression/software/pipelines/latest/output/spatial and https://kb.10xgenomics.com/hc/en-us/articles/360041426992. That is, that for a regular Visium slide, the array row has a range from 0 to 77, the array col from 0 to 127, the capture area has a 6.5 mm edge length, the the fiducial frame area has an edge of 8 mm, and spot centers are about 100 um from each other.

Usage

```
frame_limits(
  spe,
  sampleid,
  image_id = "lowres",
  visium_grid = list(row_min = 0, row_max = 77, col_min = 0, col_max = 127,
    fiducial_vs_capture_edge = (8 - 6.5) * 1000/2/100)
)
```

Arguments

`spe`	A SpatialExperiment-class object. See `fetch_data()` for how to download some example objects or `read10xVisiumWrapper()` to read in `spaceranger --count` output files and build your own `spe` object.
`sampleid`	A `character(1)` specifying which sample to plot from `colData(spe)$sample_id` (formerly `colData(spe)$sample_name`).
`image_id`	A `character(1)` with the name of the image ID you want to use in the background.
`visium_grid`	A named list with the parameters known about the Visium grid. This can change for Visium HD vs regular Visium for example.

Value

A named list with `y_min`, `y_max`, `x_min`, and `x_max` pixels from the selected image that can be used for cropping the image.

Author(s)

Louise Huuki-Myers and Leonardo Collado-Torres

See Also

Other Spatial cluster visualization functions: vis_clus(), vis_clus_p(), vis_grid_clus(), vis_image()

Examples

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

    ## Obtain the frame limits for one sample
    frame_limits(spe, sampleid = "151673")
}
```

---

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. Test the alternative hypothesis that OR > 1, i.e. that gene set is over-represented in the set of enriched genes. 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 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. Typically this is the set of reference statistics used in layer_stat_cor().

model_type
: A named element of the modeling_results list. By default that is either enrichment for the model that tests one human brain layer against the rest (one group vs the rest), pairwise which compares two layers (groups) denoted by layerA-layerB such that layerA is greater than layerB, and anova which determines if any layer (group) is different from the rest adjusting for the mean expression level. The statistics for enrichment and pairwise are t-statistics while the anova 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.

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().

- OR odds ratio.
- Pval p-value for fisher.test().
- test group or layer in the modeling_results.
- NumSig Number of genes from the gene set present in modeling_results & with fdr < fdr_cut and t_stat > 0 (unless reverse = TRUE) for test in modeling results.
- SetSize Number of genes from modeling_results present in gene_set.
- ID name of gene set.
- model_type record of input model type from modeling results.
- fdr_cut record of input frd_cut.
- GeneList List of gene names from input set with fdr < fdr_cut and t_stat > 0, possible that some gene_names are missing from modeling data.

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 with ComplexHeatmap*

Description

This function takes the output of `gene_set_enrichment()` and creates a ComplexHeatmap visualization of the results. Fill of the heatmap represents the -log10(p-val), Odds-ratios are printed for test that pass specified significance threshold `ORcut`.

Usage

```
gene_set_enrichment_plot(
  enrichment,
  xlabs = unique(enrichment$ID),
  PThresh = 12,
  ORcut = 3,
  enrichOnly = FALSE,
  mypal = c("white", RColorBrewer::brewer.pal(9, "YlOrRd")),
  plot_SetSize_bar = FALSE,
  gene_list_length = NULL,
  model_sig_length = NULL,
  model_colors = NULL,
  ...
)
```

Arguments

enrichment	The output of gene_set_enrichment().
xlabs	A vector of names in the same order and length as unique(enrichment$ID).
PThresh	A numeric(1) specifying the P-value threshold for the maximum value in the -log10(p) scale.
ORcut	A numeric(1) specifying the P-value threshold for the minimum value in the -log10(p) scale for printing the odds ratio values in the cells of the resulting plot. Defaults to 3 or p-val < 0.001.
enrichOnly	A logical(1) indicating whether to show only odds ratio values greater than 1.
mypal	A character vector with the color palette to use. Colors will be in order from 0 to lowest P-val max(-log(enrichment$Pval)). Defaults to white, yellow, red pallet.
plot_SetSize_bar	A logical(1) indicating whether to plot SetSize from enrichment as an anno_barplot at the top of the heatmap.
gene_list_length	Optional named numeric vector indicating the length of the gene_list used to calculate enrichment, if included and plot_setSize_bar = TRUE then the top anno_barplot will show the SetSize and the difference from the length of the input gene_list.
model_sig_length	Optional named numeric vector indicating the number of significant genes in modeling_results used to calculate enrichment. If included anno_barplot will be added to rows.
model_colors	named character vector of colors. It adds colors to row annotations.
...	Additional parameters passed to ComplexHeatmap::Heatmap().

Details

Includes functionality to plot the size of the input gene sets as barplot annotations.

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 (Heatmap-class) visualizing the gene set enrichment odds ratio and p-value results.

Author(s)

Andrew E Jaffe, Leonardo Collado-Torres, Louise Huuki-Myers

See Also

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_safari_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_safari_geneList,
    modeling_results = modeling_results,
    model_type = "enrichment"
)

## Visualize the gene set enrichment results

## Default plot
gene_set_enrichment_plot(
    enrichment = asd_sfari_enrichment
)

## Use a custom green color palette & use shorter gene set names
## (x-axis labels)
gene_set_enrichment_plot(
    asd_sfari_enrichment,
    xlabs = gsub(".*_", "", unique(asd_sfari_enrichment$ID)),
    mypal = c("white", RColorBrewer::brewer.pal(9, "BuGn"))
)

## Add bar plot annotations for SetSize of model genes in the gene_lists
gene_set_enrichment_plot(
    asd_sfari_enrichment,
    xlabs = gsub(".*_", "", unique(asd_sfari_enrichment$ID)),
    plot_SetSize_bar = TRUE
)
```

```
## Add stacked bar plot annotations showing SetSize and difference from the
## length of the input gene_list
gene_set_enrichment_plot(
    asd_sfari_enrichment,
    xlabs = gsub(".*_", "", unique(asd_sfari_enrichment$ID)),
    plot_SetSize_bar = TRUE,
    gene_list_length = lapply(asd_safari_geneList, length)
)

## add bar plot annotations for number of enriched genes from layers
if (!exists("sce_layer")) sce_layer <- fetch_data(type = "sce_layer")
sig_genes <- sig_genes_extract(
    modeling_results = modeling_results,
    model = "enrichment",
    sce_layer = sce_layer,
    n = nrow(sce_layer)
)

sig_genes <- sig_genes[sig_genes$fdr < 0.1, ]
n_sig_model <- as.list(table(sig_genes$test))

## add barplot with n significant genes from modeling
gene_set_enrichment_plot(
    asd_sfari_enrichment,
    xlabs = gsub(".*_", "", unique(asd_sfari_enrichment$ID)),
    plot_SetSize_bar = TRUE,
    model_sig_length = n_sig_model
)

## add color annotations
gene_set_enrichment_plot(
    asd_sfari_enrichment,
    xlabs = gsub(".*_", "", unique(asd_sfari_enrichment$ID)),
    plot_SetSize_bar = TRUE,
    model_colors = libd_layer_colors
)

## add barplot with n significant genes from modeling filled with model color
gene_set_enrichment_plot(
    asd_sfari_enrichment,
    xlabs = gsub(".*_", "", unique(asd_sfari_enrichment$ID)),
    plot_SetSize_bar = TRUE,
    model_sig_length = n_sig_model,
    model_colors = libd_layer_colors
)
```

`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

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

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 unname().

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)])

## What happens if you have a logical variable with NAs?
set.seed(20240712)
log_var <- sample(c(TRUE, FALSE, NA),
    1000,
    replace = TRUE,
    prob = c(0.3, 0.15, 0.55)
)
log_var_sorted <- sort_clusters(log_var)
get_colors(colors = NULL, clusters = log_var_sorted)
```

---

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	A SpatialExperiment-class object. See `fetch_data()` for how to download some example objects or `read10xVisiumWrapper()` to read in `spaceranger --count` output files and build your own spe object.
sampleid	A `character(1)` specifying which sample to plot from `colData(spe)$sample_id` (formerly `colData(spe)$sample_name`).
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 magick::image_channel. If `NA` this step is skipped.
brightness	A `numeric(1)` passed to magick::image_modulate.
saturation	A `numeric(1)` passed to magick::image_modulate.
hue	A `numeric(1)` passed to magick::image_modulate.
enhance	A `logical(1)` controlling whether to use magick::enhance.
contrast_sharpen	A `numeric(1)` passed to magick::image_contrast. If `NA` this step is skipped.
quantize_max	A `numeric(1)` passed to magick::image_quantize. If `NA` this step is skipped.
quantize_dither	A `logical(1)` passed to magick::image_quantize.
equalize	A `logical(1)` controlling whether to use magick::equalize.
normalize	A `logical(1)` controlling whether to use magick::normalize.
transparent_color	A `character(1)` passed to magick::image_transparent. If `NA` this step is skipped.
transparent_fuzz	A `numeric(1)` passed to magick::image_transparent.
background_color	A `character(1)` passed to magick::image_background. If `NA` this step is skipped.
median_radius	A `numeric(1)` passed to magick::image_median. If `NA` this step is skipped.
negate	A `logical(1)` controlling whether to use magick::negate.

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(), img_update_all()

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	A SpatialExperiment-class object. See fetch_data() for how to download some example objects or read10xVisiumWrapper() to read in spaceranger --count output files and build your own spe object.
sampleid	A character(1) specifying which sample to plot from colData(spe)$sample_id (formerly colData(spe)$sample_name).
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 image_id to use.
overwrite	A logical(1) specifying whether to overwrite the image_id 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))
}
```

---

img_update_all *Update the images for all samples*

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

spe	A SpatialExperiment-class object. See fetch_data() for how to download some example objects or read10xVisiumWrapper() to read in spaceranger --count output files and build your own spe object.
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 image_id to use.
overwrite	A logical(1) specifying whether to overwrite the image_id 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()`

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

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

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(), 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")

## 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_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

`stats`	A query `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 the reference statistics. If using the output of `registration_wrapper()` then use `$enrichment` to access the results from `registration_stats_enrichment()`. This function will automatically extract the statistics and assign the ENSEMBL gene IDs to the row names of the query matrix.
`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. Typically this is the set of reference statistics used in `layer_stat_cor()`.
`model_type`	A named element of the `modeling_results` list. By default that is either `enrichment` for the model that tests one human brain layer against the rest (one group vs the rest), `pairwise` which compares two layers (groups) denoted by `layerA-layerB` such that `layerA` is greater than `layerB`, and `anova` which determines if any layer (group) is different from the rest adjusting for the mean expression level. The statistics for `enrichment` and `pairwise` are t-statistics while the `anova` 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`.
`top_n`	An `integer(1)` specifying whether to filter to the top n marker genes. The default is `NULL` 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 the query `stats` and the reference statistics using only the ENSEMBL gene IDs present in both tables. The columns are sorted using hierarchical clustering.

Author(s)

Andrew E Jaffe, Leonardo Collado-Torres

See Also

Other Layer correlation functions: `annotate_registered_clusters()`, `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 correlation of layer modeling t-statistics with ComplexHeatmap*

Description

This function makes a ComplexHeatmap from the correlation matrix between a reference and query modeling statistics from `layer_stat_cor()`. For example, between the query statistics from a set of cell cluster/types derived from scRNA-seq or snRNA-seq data (among other types) and the reference layer statistics from the Human DLPFC Visium data (when using the default arguments).

Usage

```
layer_stat_cor_plot(
  cor_stats_layer,
  color_max = max(cor_stats_layer),
  color_min = min(cor_stats_layer),
  color_scale = RColorBrewer::brewer.pal(7, "PRGn"),
  query_colors = NULL,
  reference_colors = NULL,
  annotation = NULL,
  ...
)
```

Arguments

`cor_stats_layer`	The output of `layer_stat_cor()`.
`color_max`	A `numeric(1)` specifying the highest correlation value for the color scale (should be between 0 and 1).
`color_min`	A `numeric(1)` specifying the lowest correlation value for the color scale (should be between 0 and -1).
`color_scale`	A `character` vector with three or more values specifying the color scale for the fill of the heatmap. The first value is used for `color_min`, the middle for zero, and the last for `color_max`. If an even number of colors are supplied, the last color is dropped to center zero.
`query_colors`	named `character` vector of colors, Adds colors to query row annotations.

reference_colors
: named character vector of colors, Adds colors to reference column annotations.

annotation
: annotation data.frame output of annotate_registered_clusters(), adds 'X' for good confidence annotations, '*' for poor confidence.

...
: Additional parameters passed to ComplexHeatmap::Heatmap() such as cluster_rows and cluster_columns.

Details

Includes functionality to add color annotations, (helpful to match to colors in Visium spot plots), and annotations from annotate_registered_clusters().

Value

(Heatmap-class) plot of t-stat correlations

Author(s)

Louise Huuki-Myers

See Also

Other Layer correlation functions: annotate_registered_clusters(), layer_stat_cor()

Examples

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

## query spatialDLPFC modeling results
query_modeling_results <- fetch_data(
    type = "spatialDLPFC_Visium_modeling_results"
)

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

## Visualize the correlation matrix

## Default plot with no annotations and defaults for ComplexHeatmap()
layer_stat_cor_plot(cor_stats_layer)

## add Annotation colors
```

```
## add libd_layer_colors to reference Human Pilot layers
layer_stat_cor_plot(cor_stats_layer, reference_colors = libd_layer_colors)

## obtain colors for the query clusters
cluster_colors <- get_colors(clusters = rownames(cor_stats_layer))
layer_stat_cor_plot(cor_stats_layer,
    query_colors = cluster_colors,
    reference_colors = libd_layer_colors
)

## Apply additional ComplexHeatmap param
layer_stat_cor_plot(cor_stats_layer,
    cluster_rows = FALSE,
    cluster_columns = FALSE
)

## Add annotation
annotation_df <- annotate_registered_clusters(
    cor_stats_layer,
    confidence_threshold = .55
)
layer_stat_cor_plot(cor_stats_layer, annotation = annotation_df)

## change fill color scale
layer_stat_cor_plot(cor_stats_layer,
    color_scale = RColorBrewer::brewer.pal(2, "PiYG")
)

## All together
layer_stat_cor_plot(
    cor_stats_layer,
    color_scale = RColorBrewer::brewer.pal(5, "PiYG"),
    query_colors = cluster_colors,
    reference_colors = libd_layer_colors,
    annotation = annotation_df,
    cluster_rows = FALSE,
    cluster_columns = FALSE
)
```

`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`	A SpatialExperiment-class object. See `fetch_data()` for how to download some example objects or `read10xVisiumWrapper()` to read in `spaceranger --count` output files and build your own `spe` object.
`image_dir`	A `character(1)` specifying a path to a directory containing image files with the pattern `sampleID_pattern.png`.
`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)
```

multi_gene_pca *Combine multiple continuous variables through PCA*

Description

PCA is performed on `cont_mat`, the matrix of multiple continuous features. The first PC is returned, representing the dominant spatial signature of the feature set. Its direction is negated if necessary so that the majority of coefficients across features are positive (when the features are highly correlated, this encourages spots with higher values to represent areas of higher expression of the features).

Usage

```
multi_gene_pca(cont_mat)
```

Arguments

`cont_mat`	A `matrix()` with spots as rows and 2 or more continuous variables as columns.

Value

A `numeric()` vector with one element per spot, summarizing the multiple continuous variables.

Author(s)

Nicholas J. Eagles

See Also

Other functions for summarizing expression of multiple continuous variables simultaneously: `multi_gene_sparsity()`, `multi_gene_z_score()`

multi_gene_sparsity *Combine multiple continuous variables by proportion of positive values*

Description

To summarize multiple features, the proportion of features with positive values for each spot is computed.

Usage

```
multi_gene_sparsity(cont_mat)
```

Arguments

`cont_mat`	A `matrix()` with spots as rows and 2 or more continuous variables as columns.

Value

A numeric() vector with one element per spot, summarizing the multiple continuous variables.

Author(s)

Nicholas J. Eagles

See Also

Other functions for summarizing expression of multiple continuous variables simultaneously: multi_gene_pca(), multi_gene_z_score()

---

multi_gene_z_score *Combine multiple continuous variables by averaging Z scores*

Description

To summarize multiple features, each is normalized to represent a Z-score. Scores are averaged to return a single vector.

Usage

```
multi_gene_z_score(cont_mat)
```

Arguments

cont_mat	A matrix() with spots as rows and 2 or more continuous variables as columns.

Value

A numeric() vector with one element per spot, summarizing the multiple continuous variables.

Author(s)

Nicholas J. Eagles

See Also

Other functions for summarizing expression of multiple continuous variables simultaneously: multi_gene_pca(), multi_gene_sparsity()

prep_stitched_data *Prepare stitched data for plotting*

Description

Given a SpatialExperiment built with visiumStitched::build_spe() http://research.libd.org/visiumStitched/reference/build_spe.html, drop excluded spots (specified by spe$exclude_overlapping) and compute an appropriate spot size for plotting with vis_gene() or vis_clus(), assuming the plot will be written to a PDF of default dimensions (i.e. width = 7 and height = 7).

Usage

```
prep_stitched_data(spe, point_size, image_id)
```

Arguments

spe	A SpatialExperiment built with visiumStitched::build_spe(), containing a logical spe$exclude_overlapping column specifying which spots to display in plots
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.
image_id	A character(1) with the name of the image ID you want to use in the background.

Value

A list with names spe and point_size containing a filtered, ready-to-plot SpatialExperiment and an appropriate spot size (passed to vis_gene() or vis_clus()), respectively

Author(s)

Nicholas J. Eagles

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 SpatialExperiment::read10xVisium().
sample_id	Passed to SpatialExperiment::read10xVisium().

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 *Load data from a 10x Genomics Visium experiment and make it spatialLIBD-ready*

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 SpatialExperiment::read10xVisium().
sample_id	Passed to SpatialExperiment::read10xVisium().
type	Passed to SpatialExperiment::read10xVisium().
data	Passed to SpatialExperiment::read10xVisium().
images	Passed to SpatialExperiment::read10xVisium().
load	Passed to SpatialExperiment::read10xVisium().
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 visualzing 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().
```

registration_block_cor *Spatial registration: block correlation*

Description

This function computes the block correlation using the sample ID as the blocking factor. This takes into account that cells in scRNA-seq data or spots in spatially-resolved transcriptomics data from Visium (or similar) have a sample ID batch effect.

Usage

```
registration_block_cor(
  sce_pseudo,
  registration_model,
  var_sample_id = "registration_sample_id"
)
```

Arguments

sce_pseudo	The output of registration_pseudobulk().
registration_model	The output from registration_model().
var_sample_id	A character(1) specifying the colData(sce_pseudo) variable with the sample ID.

Value

A numeric(1) with the block correlation at the sample ID level.

See Also

Other spatial registration and statistical modeling functions: registration_model(), registration_pseudobulk(), registration_stats_anova(), registration_stats_enrichment(), registration_stats_pairwise(), registration_wrapper()

Examples

```
example("registration_model", package = "spatialLIBD")
block_cor <- registration_block_cor(sce_pseudo, registration_mod)
```

registration_model *Spatial registration: model*

Description

This function defines the statistical model that will be used for computing the block correlation as well as pairwise statistics. It is useful to check it in case your sample-level covariates need to be casted. For example, an `integer()` variable might have to be casted into a `factor()` if you wish to model it as a categorical variable and not a continuous one.

Usage

```
registration_model(
  sce_pseudo,
  covars = NULL,
  var_registration = "registration_variable"
)
```

Arguments

`sce_pseudo`	The output of `registration_pseudobulk()`.
`covars`	A `character()` with names of sample-level covariates.
`var_registration`	A `character(1)` specifying the `colData(sce_pseudo)` variable of interest against which will be used for computing the relevant statistics.

Value

The output of `model.matrix()` which you can inspect to verify that your sample-level covariates are being properly modeled.

See Also

Other spatial registration and statistical modeling functions: `registration_block_cor()`, `registration_pseudobulk()`, `registration_stats_anova()`, `registration_stats_enrichment()`, `registration_stats_pairwise()`, `registration_wrapper()`

Examples

```
example("registration_pseudobulk", package = "spatialLIBD")
registration_mod <- registration_model(sce_pseudo, "age")
head(registration_mod)
```

registration_pseudobulk *Spatial registration: pseudobulk*

Description

Pseudo-bulk the gene expression, filter lowly-expressed genes, and normalize. This is the first step for spatial registration and for statistical modeling.

Usage

```
registration_pseudobulk(
  sce,
  var_registration,
  var_sample_id,
  covars = NULL,
  min_ncells = 10,
  pseudobulk_rds_file = NULL,
  filter_expr = TRUE,
  mito_gene = NULL
)
```

Arguments

sce	A SingleCellExperiment-class object or one that inherits its properties.
var_registration	A `character(1)` specifying the `colData(sce)` variable of interest against which will be used for computing the relevant statistics. This should be a categorical variable, with all categories syntaticly valid (could be used as an R variable, no special characters or leading numbers), ex. 'L1.2', 'celltype2' not 'L1/2' or '2'.
var_sample_id	A `character(1)` specifying the `colData(sce)` variable with the sample ID.
covars	A `character()` with names of sample-level covariates.
min_ncells	An `integer(1)` greater than 0 specifying the minimum number of cells (for scRNA-seq) or spots (for spatial) that are combined when pseudo-bulking. Pseudo-bulked samples with less than `min_ncells` on `sce_pseudo$ncells` will be dropped.
pseudobulk_rds_file	A `character(1)` specifying the path for saving an RDS file with the pseudo-bulked object. It's useful to specify this since pseudo-bulking can take hours to run on large datasets.
filter_expr	A `logical(1)` specifying whether to filter pseudobulked counts with `edgeR::filterByExpr`. Defaults to `TRUE`, filtering is recommended for spatail registratrion workflow.
mito_gene	An optional `logical()` vector indicating which genes are mitochondrial, used to calculate pseudo bulked mitochondrial expression rate `expr_chrM` and `pseudo_expr_chrM`. The length has to match the `nrow(sce)`.

Value

A pseudo-bulked SingleCellExperiment-class object. The `logcounts()` assay are `log2-CPM` values calculated with `edgeR::cpm(log = TRUE)`. See https://github.com/LieberInstitute/spatialLIBD/issues/106 and https://support.bioconductor.org/p/9161754 for more details about the math behind `scuttle::logNormFactors()`, `edgeR::cpm()`, and their differences.

See Also

Other spatial registration and statistical modeling functions: `registration_block_cor()`, `registration_model()`, `registration_stats_anova()`, `registration_stats_enrichment()`, `registration_stats_pairwise()`, `registration_wrapper()`

Examples

```
## Ensure reproducibility of example data
set.seed(20220907)

## Generate example data
sce <- scuttle::mockSCE()

## Add some sample IDs
sce$sample_id <- sample(LETTERS[1:5], ncol(sce), replace = TRUE)

## Add a sample-level covariate: age
ages <- rnorm(5, mean = 20, sd = 4)
names(ages) <- LETTERS[1:5]
sce$age <- ages[sce$sample_id]

## Add gene-level information
rowData(sce)$gene_id <- paste0("ENSG", seq_len(nrow(sce)))
rowData(sce)$gene_name <- paste0("gene", seq_len(nrow(sce)))

## Pseudo-bulk by Cell Cycle
sce_pseudo <- registration_pseudobulk(
    sce,
    var_registration = "Cell_Cycle",
    var_sample_id = "sample_id",
    covars = c("age"),
    min_ncells = NULL
)
colData(sce_pseudo)
rowData(sce_pseudo)
```

`registration_stats_anova`

Spatial registration: compute ANOVA statistics

Description

This function computes the gene ANOVA F-statistics (at least one group is different from the rest). These F-statistics can be used for spatial registration with `layer_stat_cor()` and related functions. Although, they are more typically used for identifying ANOVA-marker genes.

Usage

```
registration_stats_anova(
  sce_pseudo,
  block_cor,
  covars = NULL,
  var_registration = "registration_variable",
  var_sample_id = "registration_sample_id",
  gene_ensembl = NULL,
  gene_name = NULL,
  suffix = ""
)
```

Arguments

`sce_pseudo`	The output of `registration_pseudobulk()`.
`block_cor`	A `numeric(1)` computed with `registration_block_cor()`.
`covars`	A `character()` with names of sample-level covariates.
`var_registration`	A `character(1)` specifying the `colData(sce_pseudo)` variable of interest against which will be used for computing the relevant statistics.
`var_sample_id`	A `character(1)` specifying the `colData(sce_pseudo)` variable with the sample ID.
`gene_ensembl`	A `character(1)` specifying the `rowData(sce_pseudo)` column with the ENSEMBL gene IDs. This will be used by `layer_stat_cor()`.
`gene_name`	A `character(1)` specifying the `rowData(sce_pseudo)` column with the gene names (symbols).
`suffix`	A `character(1)` specifying the suffix to use for the F-statistics column. This is particularly useful if you will run this function more than once and want to be able to merge the results.

Value

A `data.frame()` with the ANOVA statistical results. This is similar to `fetch_data("modeling_results")$anova`.

See Also

Other spatial registration and statistical modeling functions: `registration_block_cor()`, `registration_model()`, `registration_pseudobulk()`, `registration_stats_enrichment()`, `registration_stats_pairwise()`, `registration_wrapper()`

Examples

```
example("registration_block_cor", package = "spatialLIBD")
results_anova <- registration_stats_anova(sce_pseudo,
    block_cor, "age",
    gene_ensembl = "ensembl", gene_name = "gene_name", suffix = "example"
)
head(results_anova)

## Specifying `block_cor = NaN` then ignores the correlation structure
results_anova_nan <- registration_stats_anova(sce_pseudo,
    block_cor = NaN, "age",
    gene_ensembl = "ensembl", gene_name = "gene_name", suffix = "example"
)
head(results_anova_nan)

## Note that you can merge multiple of these data.frames if you run this
## function for different sets. For example, maybe you drop one group
## before pseudo-bulking if you know that there are many differences between
## that group and others. For example, we have dropped the white matter (WM)
## prior to computing ANOVA F-statistics.

## no covariates
results_anova_nocovar <- registration_stats_anova(sce_pseudo,
    block_cor,
    covars = NULL,
    gene_ensembl = "ensembl", gene_name = "gene_name", suffix = "nocovar"
)
head(results_anova_nocovar)

## Merge both results into a single data.frame, thanks to having different
## 'suffix' values.
results_anova_merged <- merge(results_anova, results_anova_nocovar)
head(results_anova_merged)
```

---

registration_stats_enrichment

Spatial registration: compute enrichment statistics

Description

This function computes the gene enrichment t-statistics (one group > the rest). These t-statistics are the ones typically used for spatial registration with `layer_stat_cor()` and related functions.

Usage

```
registration_stats_enrichment(
  sce_pseudo,
  block_cor,
  covars = NULL,
```

```
  var_registration = "registration_variable",
  var_sample_id = "registration_sample_id",
  gene_ensembl = NULL,
  gene_name = NULL
)
```

Arguments

sce_pseudo	The output of registration_pseudobulk().
block_cor	A numeric(1) computed with registration_block_cor().
covars	A character() with names of sample-level covariates.
var_registration	A character(1) specifying the colData(sce_pseudo) variable of interest against which will be used for computing the relevant statistics.
var_sample_id	A character(1) specifying the colData(sce_pseudo) variable with the sample ID.
gene_ensembl	A character(1) specifying the rowData(sce_pseudo) column with the ENSEMBL gene IDs. This will be used by layer_stat_cor().
gene_name	A character(1) specifying the rowData(sce_pseudo) column with the gene names (symbols).

Value

A data.frame() with the enrichment statistical results. This is similar to fetch_data("modeling_results")$enrichment

See Also

Other spatial registration and statistical modeling functions: registration_block_cor(), registration_model(), registration_pseudobulk(), registration_stats_anova(), registration_stats_pairwise(), registration_wrapper()

Examples

```
example("registration_block_cor", package = "spatialLIBD")
results_enrichment <- registration_stats_enrichment(sce_pseudo,
    block_cor, "age",
    gene_ensembl = "ensembl", gene_name = "gene_name"
)
head(results_enrichment)

## Specifying `block_cor = NaN` then ignores the correlation structure
results_enrichment_nan <- registration_stats_enrichment(sce_pseudo,
    block_cor = NaN, "age",
    gene_ensembl = "ensembl", gene_name = "gene_name"
)
head(results_enrichment_nan)
```

registration_stats_pairwise *Spatial registration: compute pairwise statistics*

Description

This function computes the gene pairwise t-statistics (one group > another, for all combinations). These t-statistics can be used for spatial registration with `layer_stat_cor()` and related functions. Although, they are more typically used for identifying pairwise-marker genes.

Usage

```
registration_stats_pairwise(
  sce_pseudo,
  registration_model,
  block_cor,
  var_registration = "registration_variable",
  var_sample_id = "registration_sample_id",
  gene_ensembl = NULL,
  gene_name = NULL
)
```

Arguments

`sce_pseudo`	The output of `registration_pseudobulk()`.
`registration_model`	The output from `registration_model()`.
`block_cor`	A `numeric(1)` computed with `registration_block_cor()`.
`var_registration`	A `character(1)` specifying the `colData(sce_pseudo)` variable of interest against which will be used for computing the relevant statistics.
`var_sample_id`	A `character(1)` specifying the `colData(sce_pseudo)` variable with the sample ID.
`gene_ensembl`	A `character(1)` specifying the `rowData(sce_pseudo)` column with the ENSEMBL gene IDs. This will be used by `layer_stat_cor()`.
`gene_name`	A `character(1)` specifying the `rowData(sce_pseudo)` column with the gene names (symbols).

Value

A `data.frame()` with the pairwise statistical results. This is similar to `fetch_data("modeling_results")$pairwise`.

See Also

Other spatial registration and statistical modeling functions: `registration_block_cor()`, `registration_model()`, `registration_pseudobulk()`, `registration_stats_anova()`, `registration_stats_enrichment()`, `registration_wrapper()`

Examples

```
example("registration_block_cor", package = "spatialLIBD")
results_pairwise <- registration_stats_pairwise(sce_pseudo,
    registration_mod, block_cor,
    gene_ensembl = "ensembl", gene_name = "gene_name"
)
head(results_pairwise)

## Specifying `block_cor = NaN` then ignores the correlation structure
results_pairwise_nan <- registration_stats_pairwise(sce_pseudo,
    registration_mod,
    block_cor = NaN,
    gene_ensembl = "ensembl", gene_name = "gene_name"
)
head(results_pairwise_nan)
```

---

registration_wrapper *Spatial registration: wrapper function*

Description

This function is provided for convenience. It runs all the functions required for computing the `modeling_results`. This can be useful for finding marker genes on a new spatially-resolved transcriptomics dataset and thus using it for `run_app()`. The results from this function can also be used for performing spatial registration through `layer_stat_cor()` and related functions of sc/snRNA-seq datasets.

Usage

```
registration_wrapper(
  sce,
  var_registration,
  var_sample_id,
  covars = NULL,
  gene_ensembl = NULL,
  gene_name = NULL,
  suffix = "",
  min_ncells = 10,
  pseudobulk_rds_file = NULL
)
```

Arguments

sce	A SingleCellExperiment-class object or one that inherits its properties.
var_registration	A `character(1)` specifying the `colData(sce)` variable of interest against which will be used for computing the relevant statistics. This should be a categorical variable, with all categories syntaticly valid (could be used as an R variable, no special characters or leading numbers), ex. 'L1.2', 'celltype2' not 'L1/2' or '2'.

var_sample_id
: A character(1) specifying the colData(sce) variable with the sample ID.

covars
: A character() with names of sample-level covariates.

gene_ensembl
: A character(1) specifying the rowData(sce_pseudo) column with the ENSEMBL gene IDs. This will be used by layer_stat_cor().

gene_name
: A character(1) specifying the rowData(sce_pseudo) column with the gene names (symbols).

suffix
: A character(1) specifying the suffix to use for the F-statistics column. This is particularly useful if you will run this function more than once and want to be able to merge the results.

min_ncells
: An integer(1) greater than 0 specifying the minimum number of cells (for scRNA-seq) or spots (for spatial) that are combined when pseudo-bulking. Pseudo-bulked samples with less than min_ncells on sce_pseudo$ncells will be dropped.

pseudobulk_rds_file
: A character(1) specifying the path for saving an RDS file with the pseudo-bulked object. It's useful to specify this since pseudo-bulking can take hours to run on large datasets.

Details

We chose a default of min_ncells = 10 based on OSCA from section 4.3 at http://bioconductor.org/books/3.15/OSCA.multisample/multi-sample-comparisons.html. They cite https://doi.org/10.1038/s41467-020-19894-4 as the paper where they came up with the definition of "very low" being 10. You might want to use registration_pseudobulk() and manually explore sce_pseudo$ncells to choose the best cutoff.

Value

A list() of data.frame() with the statistical results. This is similar to fetch_data("modeling_results").

See Also

Other spatial registration and statistical modeling functions: registration_block_cor(), registration_model(), registration_pseudobulk(), registration_stats_anova(), registration_stats_enrichment(), registration_stats_pairwise()

Examples

```
## Ensure reproducibility of example data
set.seed(20220907)

## Generate example data
sce <- scuttle::mockSCE()

## Add some sample IDs
sce$sample_id <- sample(LETTERS[1:5], ncol(sce), replace = TRUE)

## Add a sample-level covariate: age
ages <- rnorm(5, mean = 20, sd = 4)
```

```
names(ages) <- LETTERS[1:5]
sce$age <- ages[sce$sample_id]

## Add gene-level information
rowData(sce)$ensembl <- paste0("ENSG", seq_len(nrow(sce)))
rowData(sce)$gene_name <- paste0("gene", seq_len(nrow(sce)))

## Compute all modeling results
example_modeling_results <- registration_wrapper(
    sce,
    var_registration = "Cell_Cycle",
    var_sample_id = "sample_id",
    covars = c("age"),
    gene_ensembl = "ensembl",
    gene_name = "gene_name",
    suffix = "wrapper"
)

## Explore the results from registration_wrapper()
class(example_modeling_results)
length(example_modeling_results)
names(example_modeling_results)
lapply(example_modeling_results, head)
```

---

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",
  auto_crop_default = TRUE,
  is_stitched = 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.
sce_layer	Defaults to the output of `fetch_data(type = 'sce_layer')`. 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 `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. Typically this is the set of reference statistics used in `layer_stat_cor()`.
sig_genes	The output of `sig_genes_extract_all()` which is a table in long format with the modeling results. You can subset this if the object requires too much memory.
docs_path	A `character(1)` specifying the path to the directory containing the website documentation files. The directory has to contain the files: `documentation_sce_layer.md`, `documentation_spe.md`, `favicon.ico`, `footer.html` and `README.md`.
title	A character(1) specifying the title for the app.
spe_discrete_vars	A `character()` 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 `colData(spe)`.
spe_continuous_vars	A `character()` 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 `colData(sce)`.
default_cluster	A `character(1)` with the name of the main cluster (discrete) variable to use. It will have to be present in both `colData(spe)` and `colData(sce_layer)`.
auto_crop_default	A `logical(1)` specifying the default value for automatically cropping the images. Set this to `FALSE` if your images do not follow the Visium grid size expectations, which are key for enabling auto-cropping.

is_stitched — A logical(1) vector: If TRUE, expects a SpatialExperiment-class built with visiumStitched::build_spe(). http://research.libd.org/visiumStitched/reference/build_spe.html; in particular, expects a logical colData column exclude_overlapping specifying which spots to exclude from the plot. Sets auto_crop = FALSE.

... — Other arguments passed to the list of golem 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:
    ## lobstr::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.
}
```

```
## How to run locally the spatialDLPFC Sp09 spatialLIBD app. That is,
## from http://research.libd.org/spatialDLPFC/#interactive-websites
## how to run https://libd.shinyapps.io/spatialDLPFC_Visium_Sp09 locally.
if (enough_ram(9e9)) {
    ## Download the 3 main objects needed
    spe <- fetch_data("spatialDLPFC_Visium")
    sce_pseudo <- fetch_data("spatialDLPFC_Visium_pseudobulk")
    modeling_results <- fetch_data("spatialDLPFC_Visium_modeling_results")

    ## These are optional commands to further reduce the memory required.
    #
    ## Keep only the "lowres" images. Reduces the object from 6.97 GB to 4.59 GB
    # imgData(spe) <- imgData(spe)[imgData(spe)$image_id == "lowres", ]
    ## Drop the regular counts (keep only the logcounts). Reduces the object
    ## from 4.59 GB to 2.45 GB.
    # counts(spe) <- NULL

    ## For sig_genes_extract_all() to work
    sce_pseudo$spatialLIBD <- sce_pseudo$BayesSpace
    ## Compute the significant genes
    sig_genes <- sig_genes_extract_all(
        n = nrow(sce_pseudo),
        modeling_results = modeling_results,
        sce_layer = sce_pseudo
    )
    ## Reduce the memory from 423.73 MB to 78.88 MB
    lobstr::obj_size(sig_genes)
    sig_genes$in_rows <- NULL
    sig_genes$in_rows_top20 <- NULL
    lobstr::obj_size(sig_genes)

    ## Specify the default variable
    spe$BayesSpace <- spe$BayesSpace_harmony_09
    ## Get all variables
    vars <- colnames(colData(spe))

    ## Set default cluster colors
    colors_BayesSpace <- Polychrome::palette36.colors(28)
    names(colors_BayesSpace) <- c(1:28)
    m <- match(as.character(spe$BayesSpace_harmony_09), names(colors_BayesSpace))
    stopifnot(all(!is.na(m)))
    spe$BayesSpace_colors <- spe$BayesSpace_harmony_09_colors <- colors_BayesSpace[m]

    ## Download documentation files we use
    temp_www <- file.path(tempdir(), "www")
    dir.create(temp_www)
    download.file(
        "https://raw.githubusercontent.com/LieberInstitute/spatialDLPFC/main/README.md",
        file.path(temp_www, "README.md")
    )
    download.file(
     "https://raw.githubusercontent.com/LieberInstitute/spatialDLPFC/main/code/deploy_app_k09/www/documentatio
        file.path(temp_www, "documentation_sce_layer.md")
```

```
    )
    download.file(
     "https://raw.githubusercontent.com/LieberInstitute/spatialDLPFC/main/code/deploy_app_k09/www/documentatio
        file.path(temp_www, "documentation_spe.md")
    )
    download.file(
     "https://raw.githubusercontent.com/LieberInstitute/spatialDLPFC/main/img/favicon.ico",
        file.path(temp_www, "favicon.ico")
    )
    download.file(
     "https://raw.githubusercontent.com/LieberInstitute/spatialDLPFC/main/code/deploy_app_k09/www/footer.html"
        file.path(temp_www, "footer.html")
    )
    list.files(temp_www)

    ## Run the app locally
    run_app(
        spe,
        sce_layer = sce_pseudo,
        modeling_results = modeling_results,
        sig_genes = sig_genes,
        title = "spatialDLPFC, Visium, Sp09",
      spe_discrete_vars = c( # this is the variables for the spe object not the sce_pseudo object
            "BayesSpace",
            "ManualAnnotation",
            vars[grep("^SpaceRanger_|^scran_", vars)],
            vars[grep("^BayesSpace_harmony", vars)],
            vars[grep("^BayesSpace_pca", vars)],
            "graph_based_PCA_within",
            "PCA_SNN_k10_k7",
            "Harmony_SNN_k10_k7",
            "manual_layer_label",
            "wrinkle_type",
            "BayesSpace_colors"
        ),
        spe_continuous_vars = c(
            "sum_umi",
            "sum_gene",
            "expr_chrM",
            "expr_chrM_ratio",
            vars[grep("^VistoSeg_", vars)],
            vars[grep("^layer_", vars)],
            vars[grep("^broad_", vars)]
        ),
        default_cluster = "BayesSpace",
        docs_path = temp_www
    )
}
## See also:
## * https://github.com/LieberInstitute/spatialDLPFC/tree/main/code/deploy_app_k09
## * https://github.com/LieberInstitute/spatialDLPFC/tree/main/code/deploy_app_k09_position
## * https://github.com/LieberInstitute/spatialDLPFC/tree/main/code/deploy_app_k09_position_noWM
## * https://github.com/LieberInstitute/spatialDLPFC/tree/main/code/deploy_app_k16
```

```
## * https://github.com/LieberInstitute/spatialDLPFC/tree/main/code/analysis_IF/03_spatialLIBD_app


## Example for an object with multiple capture areas stitched together with
## <http://research.libd.org/visiumStitched/>.
spe_stitched <- fetch_data("visiumStitched_brain_spe")

## Inspect this object
spe_stitched

## Notice the use of "exclude_overlapping"
table(spe_stitched$exclude_overlapping, useNA = "ifany")

## Run the app with this stitched data
run_app(
    spe = spe_stitched,
    sce_layer = NULL, modeling_results = NULL, sig_genes = NULL,
    title = "visiumStitched example data",
    spe_discrete_vars = c("capture_area", "scran_quick_cluster", "ManualAnnotation"),
    spe_continuous_vars = c("sum_umi", "sum_gene", "expr_chrM", "expr_chrM_ratio"),
    default_cluster = "scran_quick_cluster",
    is_stitched = TRUE
)

## 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 `fetch_data(type = 'sce')`. This is a SingleCellExperiment object with the spot-level Visium data and information required for visualizing the histology. See `fetch_data()` for more details.
`imageData`	A `DataFrame()` 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 `spatialLIBD::fetch_data("sce")`.

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"),
  gene_name = "gene_name"
)
```

Arguments

n	The number of the top ranked genes to extract.

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. Typically this is the set of reference statistics used in `layer_stat_cor()`.

model_type
: A named element of the `modeling_results` list. By default that is either `enrichment` for the model that tests one human brain layer against the rest (one group vs the rest), `pairwise` which compares two layers (groups) denoted by `layerA-layerB` such that `layerA` is greater than `layerB`, and `anova` which determines if any layer (group) is different from the rest adjusting for the mean expression level. The statistics for `enrichment` and `pairwise` are t-statistics while the `anova` 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 `fetch_data(type = 'sce_layer')`. This is a SingleCellExperiment object with the spot-level Visium data compressed via pseudo-bulking to the layer-level (group-level) resolution. See `fetch_data()` for more details.

gene_name
: A `character(1)` specifying the `rowData(sce_layer)` column with the gene names that match the `rownames(modeling_results)`. Defaults to `"gene_name"`.

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_functio

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"),
  gene_name = "gene_name"
)
```

Arguments

`n`	The number of the top ranked genes to extract.
`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. Typically this is the set of reference statistics used in `layer_stat_cor()`.
`sce_layer`	Defaults to the output of `fetch_data(type = 'sce_layer')`. This is a SingleCellExperiment object with the spot-level Visium data compressed via pseudo-bulking to the layer-level (group-level) resolution. See `fetch_data()` for more details.
`gene_name`	A `character(1)` specifying the `rowData(sce_layer)` column with the gene names that match the `rownames(modeling_results)`. Defaults to `"gene_name"`.

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, recasts it as a `factor()`, and sorts the `factor()` levels by frequency such that the most frequent cluster is the first level 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()` version of `clusters` where the levels are ordered by frequency.

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)

## We see that we have 10 elements in this vector, which is
```

```
## an unnamed character vector
clus

## letter 'd' is the most frequent
table(clus)

## Sort them and obtain a factor. Notice that it's a named
## factor, and the names correspond to the original values
## in the character vector.
sort_clusters(clus)

## Since 'd' was the most frequent, it gets assigned to the first level
## in the factor variable.
table(sort_clusters(clus))

## If we skip the first 3 values of clus (which are all 'd'), we can
## change the most frequent cluster. And thus the ordering of the
## factor levels.
sort_clusters(clus, map_subset = seq_len(length(clus)) > 3)

## Let's try with a factor variable
clus_factor <- factor(clus)
## sort_clusters() returns an identical result in this case
stopifnot(identical(sort_clusters(clus), sort_clusters(clus_factor)))

## What happens if you have a logical variable with NAs?
set.seed(20240712)
log_var <- sample(c(TRUE, FALSE, NA),
    1000,
    replace = TRUE,
    prob = c(0.3, 0.15, 0.55)
)
## Here, the NAs are the most frequent group.
table(log_var, useNA = "ifany")

## The NAs are not used for sorting. Since we have more 'TRUE' than 'FALSE'
## then, 'TRUE' becomes the first level.
table(sort_clusters(log_var), useNA = "ifany")
```

`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 https://doi.org/10.1016/j.neuron.2021.09.001 we computed enrichment t-statistics for the cell clusters. The Tran et al data has been subset to the top 100 DLPFC layer markers found in Maynard, Collado-Torres, et al 2021. This data is used in examples such as in `layer_stat_cor_plot()`. The Tran et al data is from the pre-print version of that project.

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 = unique(spe$sample_id)[1],
  clustervar,
 colors = c("#b2df8a", "#e41a1c", "#377eb8", "#4daf4a", "#ff7f00", "gold", "#a65628",
    "#999999", "black", "grey", "white", "purple"),
  spatial = TRUE,
  image_id = "lowres",
  alpha = NA,
  point_size = 2,
  auto_crop = TRUE,
  na_color = "#CCCCCC40",
  is_stitched = FALSE,
  guide_point_size = point_size,
  ...
)
```

Arguments

spe	A SpatialExperiment-class object. See `fetch_data()` for how to download some example objects or `read10xVisiumWrapper()` to read in `spaceranger --count` output files and build your own spe object.
sampleid	A `character(1)` specifying which sample to plot from `colData(spe)$sample_id` (formerly `colData(spe)$sample_name`).
clustervar	A `character(1)` with the name of the `colData(spe)` column that has the cluster values.
colors	A vector of colors to use for visualizing the clusters from `clustervar`. If the vector has names, then those should match the values of `clustervar`.
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`.
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.
auto_crop	A `logical(1)` indicating whether to automatically crop the image / plotting area, which is useful if the Visium capture area is not centered on the image and if the image is not a square.
na_color	A `character(1)` specifying a color for the NA values. If you set `alpha = NA` then it's best to set `na_color` to a color that has alpha blending already, which will make non-NA values pop up more and the NA values will show with a lighter color. This behavior is lost when `alpha` is set to a non-`NA` value.
is_stitched	A `logical(1)` vector: If `TRUE`, expects a SpatialExperiment-class built with `visiumStitched::build_spe()`. http://research.libd.org/visiumStitched/reference/build_spe.html; in particular, expects a logical colData column `exclude_overlapping` specifying which spots to exclude from the plot. Sets `auto_crop = FALSE`.
guide_point_size	A `numeric(1)` specifying the size of the points in guide. Defaults to `point_size`. Increase to improve visability.
...	Passed to paste0() for making the title of the plot following the `sampleid`.

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: frame_limits(), vis_clus_p(), vis_grid_clus(), vis_image()

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
    p1 <- vis_clus(
        spe = spe,
        clustervar = "layer_guess_reordered",
        sampleid = "151673",
        colors = libd_layer_colors,
        ... = " LIBD Layers"
    )
    print(p1)

    ## Without auto-cropping the image
    p2 <- vis_clus(
        spe = spe,
        clustervar = "layer_guess_reordered",
        sampleid = "151673",
        colors = libd_layer_colors,
        auto_crop = FALSE,
        ... = " LIBD Layers"
    )
    print(p2)

    ## Without histology
    p3 <- vis_clus(
        spe = spe,
        clustervar = "layer_guess_reordered",
        sampleid = "151673",
        colors = libd_layer_colors,
        ... = " LIBD Layers",
        spatial = FALSE
    )
    print(p3)

    ## With some NA values
    spe$tmp <- spe$layer_guess_reordered
    spe$tmp[spe$sample_id == "151673"][seq_len(500)] <- NA
    p4 <- vis_clus(
        spe = spe,
        clustervar = "tmp",
        sampleid = "151673",
```

```
        colors = libd_layer_colors,
        na_color = "white",
        ... = " LIBD Layers"
    )
    print(p4)

    ## edit plot point size but keep guide size larger
    p5 <- vis_clus(
        spe = spe,
        clustervar = "layer_guess_reordered",
        sampleid = "151673",
        colors = libd_layer_colors,
        na_color = "white",
        point_size = 1,
        guide_point_size = 3,
        ... = " LIBD Layers"
    )
    print(p5)

}
```

---

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 = unique(spe$sample_id)[1],
  colors,
  spatial,
  title,
  image_id = "lowres",
  alpha = NA,
  point_size = 2,
  auto_crop = TRUE,
  na_color = "#CCCCCC40"
)
```

Arguments

spe	A SpatialExperiment-class object. See fetch_data() for how to download some example objects or read10xVisiumWrapper() to read in spaceranger --count output files and build your own spe object.
d	A data.frame() with the sample-level information. This is typically obtained using cbind(colData(spe), spatialCoords(spe)).
clustervar	A character(1) with the name of the colData(spe) column that has the cluster values.
sampleid	A character(1) specifying which sample to plot from colData(spe)$sample_id (formerly colData(spe)$sample_name).
colors	A vector of colors to use for visualizing the clusters from clustervar. If the vector has names, then those should match the values of clustervar.
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 [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.
auto_crop	A logical(1) indicating whether to automatically crop the image / plotting area, which is useful if the Visium capture area is not centered on the image and if the image is not a square.
na_color	A character(1) specifying a color for the NA values. If you set alpha = NA then it's best to set na_color to a color that has alpha blending already, which will make non-NA values pop up more and the NA values will show with a lighter color. This behavior is lost when alpha is set to a non-NA value.

Value

A ggplot2 object.

See Also

Other Spatial cluster visualization functions: frame_limits(), vis_clus(), vis_grid_clus(), vis_image()

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
    p <- 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
    )
    print(p)

    ## 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 = unique(spe$sample_id)[1],
  geneid = rowData(spe)$gene_search[1],
  spatial = TRUE,
  assayname = "logcounts",
  minCount = 0,
  viridis = TRUE,
  image_id = "lowres",
  alpha = NA,
  cont_colors = if (viridis) viridisLite::viridis(21) else c("aquamarine4",
    "springgreen", "goldenrod", "red"),
  point_size = 2,
  auto_crop = TRUE,
  na_color = "#CCCCCC40",
  multi_gene_method = c("z_score", "pca", "sparsity"),
  is_stitched = FALSE,
  cap_percentile = 1,
  ...
)
```

Arguments

spe	A SpatialExperiment-class object. See `fetch_data()` for how to download some example objects or `read10xVisiumWrapper()` to read in `spaceranger --count` output files and build your own spe object.
sampleid	A `character(1)` specifying which sample to plot from `colData(spe)$sample_id` (formerly `colData(spe)$sample_name`).
geneid	A `character()` specifying the gene ID(s) stored in `rowData(spe)$gene_search` or a continuous variable(s) stored in `colData(spe)` to visualize. For each ID, if `rowData(spe)$gene_search` is missing, then `rownames(spe)` is used to search for the gene ID. When a vector of length > 1 is supplied, the continuous variables are combined according to `multi_gene_method`, producing a single value for each spot.
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`.
assayname	The name of the `assays(spe)` to use for extracting the gene expression data. Defaults to `logcounts`.
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 `viridis` 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.
auto_crop	A `logical(1)` indicating whether to automatically crop the image / plotting area, which is useful if the Visium capture area is not centered on the image and if the image is not a square.
na_color	A `character(1)` specifying a color for the NA values. If you set `alpha = NA` then it's best to set `na_color` to a color that has alpha blending already, which will make non-NA values pop up more and the NA values will show with a lighter color. This behavior is lost when `alpha` is set to a non-`NA` value.
multi_gene_method	A `character(1)`: either `"pca"`, `"sparsity"`, or `"z_score"`. This parameter controls how multiple continuous variables are combined for visualization, and only applies when `geneid` has length great than 1. `z_score`: to summarize multiple continuous variables, each is normalized to represent a Z-score. The multiple scores are then averaged. `pca`: PCA dimension reduction is conducted on

	the matrix formed by the continuous variables, and the first PC is then used and multiplied by -1 if needed to have the majority of the values for PC1 to be positive. `sparsity`: the proportion of continuous variables with positive values for each spot is computed. For more details, check the multi gene vignette at https://research.libd.org/spatialLIBD/articles/multi_gene_plots.html.
`is_stitched`	A `logical(1)` vector: If `TRUE`, expects a SpatialExperiment-class built with `visiumStitched::build_spe()`. http://research.libd.org/visiumStitched/reference/build_spe.html; in particular, expects a logical colData column `exclude_overlapping` specifying which spots to exclude from the plot. Sets `auto_crop = FALSE`.
`cap_percentile`	A `numeric(1)` in (0, 1] determining the maximum percentile (as a proportion) at which to cap expression. For example, a value of 0.95 sets the top 5% of expression values to the 95th percentile value. This can help make the color scale more dynamic in the presence of high outliers. Defaults to 1, which effectively performs no capping.
`...`	Passed to paste0() for making the title of the plot following the `sampleid`.

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-viridis scale
    p1 <- vis_gene(
        spe = spe,
        sampleid = "151507",
        viridis = FALSE
    )
    print(p1)

    ## Use a custom set of colors in the reverse order than usual
```

```
p2 <- vis_gene(
    spe = spe,
    sampleid = "151507",
    cont_colors = rev(viridisLite::viridis(21, option = "magma"))
)
print(p2)

## Turn the alpha to 1, which makes the NA values have a full alpha
p2b <- vis_gene(
    spe = spe,
    sampleid = "151507",
    cont_colors = rev(viridisLite::viridis(21, option = "magma")),
    alpha = 1
)
print(p2b)

## Turn the alpha to NA, and use an alpha-blended "forestgreen" for
## the NA values
# https://gist.githubusercontent.com/mages/5339689/raw/2aaa482dfbbecbfcb726525a3d81661f9d802a8e/add.alpha.R
# add.alpha("forestgreen", 0.5)
p2c <- vis_gene(
    spe = spe,
    sampleid = "151507",
    cont_colors = rev(viridisLite::viridis(21, option = "magma")),
    alpha = NA,
    na_color = "#228B2280"
)
print(p2c)

## Visualize a continuous variable, in this case, the ratio of chrM
## gene expression compared to the total expression at the spot-level
p3 <- vis_gene(
    spe = spe,
    sampleid = "151507",
    geneid = "expr_chrM_ratio"
)
print(p3)

## Visualize a gene using the rownames(spe)
p4 <- vis_gene(
    spe = spe,
    sampleid = "151507",
    geneid = rownames(spe)[which(rowData(spe)$gene_name == "MOBP")]
)
print(p4)

## Repeat without auto-cropping the image
p5 <- vis_gene(
    spe = spe,
    sampleid = "151507",
    geneid = rownames(spe)[which(rowData(spe)$gene_name == "MOBP")],
    auto_crop = FALSE
)
```

```
    print(p5)

    #    Define several markers for white matter
    white_matter_genes <- c(
        "ENSG00000197971", "ENSG00000131095", "ENSG00000123560",
        "ENSG00000171885"
    )

    ## Plot all white matter markers at once using the Z-score combination
    ## method. Flatten this quantity at the top 5% of values for plotting
    p6 <- vis_gene(
        spe = spe,
        sampleid = "151507",
        geneid = white_matter_genes,
        multi_gene_method = "z_score",
        cap_percentile = 0.95
    )
    print(p6)

    ## Plot all white matter markers at once using the sparsity combination
    ## method
    p7 <- vis_gene(
        spe = spe,
        sampleid = "151507",
        geneid = white_matter_genes,
        multi_gene_method = "sparsity"
    )
    print(p7)

    ## Plot all white matter markers at once using the PCA combination
    ## method
    p8 <- vis_gene(
        spe = spe,
        sampleid = "151507",
        geneid = white_matter_genes,
        multi_gene_method = "pca"
    )
    print(p8)
}
```

`vis_gene_p` *Sample spatial gene visualization workhorse function*

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 = unique(spe$sample_id)[1],
  spatial,
  title,
  viridis = TRUE,
  image_id = "lowres",
  alpha = NA,
  cont_colors = if (viridis) viridisLite::viridis(21) else c("aquamarine4",
    "springgreen", "goldenrod", "red"),
  point_size = 2,
  auto_crop = TRUE,
  na_color = "#CCCCCC40",
  legend_title = ""
)
```

Arguments

spe	A SpatialExperiment-class object. See `fetch_data()` for how to download some example objects or `read10xVisiumWrapper()` to read in `spaceranger --count` output files and build your own spe object.
d	A `data.frame()` with the sample-level information. This is typically obtained using `cbind(colData(spe), spatialCoords(spe))`. The `data.frame` has to contain a column with the continuous variable data to plot stored under `d$COUNT`.
sampleid	A `character(1)` specifying which sample to plot from `colData(spe)$sample_id` (formerly `colData(spe)$sample_name`).
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.
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 `viridis` 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.
auto_crop	A `logical(1)` indicating whether to automatically crop the image / plotting area, which is useful if the Visium capture area is not centered on the image and if the image is not a square.

na_color	A character(1) specifying a color for the NA values. If you set alpha = NA then it's best to set na_color to a color that has alpha blending already, which will make non-NA values pop up more and the NA values will show with a lighter color. This behavior is lost when alpha is set to a non-NA value.
legend_title	A character(1) specifying the legend title.

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

    ## Don't plot the histology information
    p <- vis_gene_p(
        spe = spe_sub,
        d = df,
        sampleid = "151673",
        title = "151673 chrM expr ratio",
        spatial = FALSE
    )
    print(p)

    ## 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 = NA,
  sample_order = unique(spe$sample_id),
  point_size = 2,
  auto_crop = TRUE,
  na_color = "#CCCCCC40",
  is_stitched = FALSE,
  guide_point_size = point_size,
  ...
)
```

Arguments

spe	A SpatialExperiment-class object. See `fetch_data()` for how to download some example objects or `read10xVisiumWrapper()` to read in `spaceranger --count` output files and build your own spe object.
clustervar	A `character(1)` with the name of the `colData(spe)` 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 `sort_clusters()`.
colors	A vector of colors to use for visualizing the clusters from `clustervar`. If the vector has names, then those should match the values of `clustervar`.
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 plot_grid.
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`.
height	A `numeric(1)` passed to pdf.
width	A `numeric(1)` passed to pdf.
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.
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.

auto_crop — A logical(1) indicating whether to automatically crop the image / plotting area, which is useful if the Visium capture area is not centered on the image and if the image is not a square.

na_color — A character(1) specifying a color for the NA values. If you set alpha = NA then it's best to set na_color to a color that has alpha blending already, which will make non-NA values pop up more and the NA values will show with a lighter color. This behavior is lost when alpha is set to a non-NA value.

is_stitched — A logical(1) vector: If TRUE, expects a SpatialExperiment-class built with visiumStitched::build_spe(). http://research.libd.org/visiumStitched/reference/build_spe.html; in particular, expects a logical colData column exclude_overlapping specifying which spots to exclude from the plot. Sets auto_crop = FALSE.

guide_point_size — A numeric(1) specifying the size of the points in guide. Defaults to point_size. Increase to improve visability.

... — Passed to paste0() for making the title of the plot following the sampleid.

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: frame_limits(), vis_clus(), vis_clus_p(), vis_image()

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 = rowData(spe)$gene_search[1],
  pdf_file,
  assayname = "logcounts",
  minCount = 0,
  return_plots = FALSE,
  spatial = TRUE,
  viridis = TRUE,
  height = 24,
  width = 36,
  image_id = "lowres",
  alpha = NA,
  cont_colors = if (viridis) viridisLite::viridis(21) else c("aquamarine4",
    "springgreen", "goldenrod", "red"),
  sample_order = unique(spe$sample_id),
  point_size = 2,
  auto_crop = TRUE,
  na_color = "#CCCCCC40",
  is_stitched = FALSE,
  cap_percentile = 1,
  ...
)
```

Arguments

spe	A SpatialExperiment-class object. See `fetch_data()` for how to download some example objects or `read10xVisiumWrapper()` to read in `spaceranger --count` output files and build your own spe object.

geneid	A character() specifying the gene ID(s) stored in rowData(spe)$gene_search or a continuous variable(s) stored in colData(spe) to visualize. For each ID, if rowData(spe)$gene_search is missing, then rownames(spe) is used to search for the gene ID. When a vector of length > 1 is supplied, the continuous variables are combined according to multi_gene_method, producing a single value for each spot.
pdf_file	A character(1) specifying the path for the resulting PDF.
assayname	The name of the assays(spe) to use for extracting the gene expression data. Defaults to logcounts.
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.
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 plot_grid.
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.
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.
height	A numeric(1) passed to pdf.
width	A numeric(1) passed to pdf.
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 viridis argument.
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.
auto_crop	A logical(1) indicating whether to automatically crop the image / plotting area, which is useful if the Visium capture area is not centered on the image and if the image is not a square.
na_color	A character(1) specifying a color for the NA values. If you set alpha = NA then it's best to set na_color to a color that has alpha blending already, which will make non-NA values pop up more and the NA values will show with a lighter color. This behavior is lost when alpha is set to a non-NA value.
is_stitched	A logical(1) vector: If TRUE, expects a SpatialExperiment-class built with visiumStitched::build_spe(). http://research.libd.org/visiumStitched/reference/build_spe.html; in particular, expects a logical colData column exclude_overlapping specifying which spots to exclude from the plot. Sets auto_crop = FALSE.

cap_percentile A numeric(1) in (0, 1] determining the maximum percentile (as a proportion) at which to cap expression. For example, a value of 0.95 sets the top 5% of expression values to the 95th percentile value. This can help make the color scale more dynamic in the presence of high outliers. Defaults to 1, which effectively performs no capping.

... Passed to paste0() for making the title of the plot following the sampleid.

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(), vis_gene_p()

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)
}
```

---

vis_image *Sample image visualization*

Description

This function visualizes the histology image for selected sample. Matches crop and settings of vis_clus() and vis_gene().

Usage

```
vis_image(
  spe,
  sampleid = unique(spe$sample_id)[1],
  image_id = "lowres",
  auto_crop = TRUE,
  is_stitched = FALSE,
  title_suffix = NULL
)
```

Arguments

spe	A SpatialExperiment-class object. See fetch_data() for how to download some example objects or read10xVisiumWrapper() to read in spaceranger --count output files and build your own spe object.
sampleid	A character(1) specifying which sample to plot from colData(spe)$sample_id (formerly colData(spe)$sample_name).
image_id	A character(1) with the name of the image ID you want to use in the background.
auto_crop	A logical(1) indicating whether to automatically crop the image / plotting area, which is useful if the Visium capture area is not centered on the image and if the image is not a square.
is_stitched	A logical(1) vector: If TRUE, expects a SpatialExperiment-class built with visiumStitched::build_spe(). http://research.libd.org/visiumStitched/reference/build_spe.html; in particular, expects a logical colData column exclude_overlapping specifying which spots to exclude from the plot. Sets auto_crop = FALSE.
title_suffix	A character(1) passed to paste() to modify the title of the plot following the sampleid.

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: frame_limits(), vis_clus(), vis_clus_p(), vis_grid_clus()

Examples

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

```
  ## Print the "lowres" image for sample 151673
  p1 <- vis_image(
    spe = spe,
    sampleid = "151673"
  )
  print(p1)

  ## Without auto-cropping the image
  p2 <- vis_image(
    spe = spe,
    sampleid = "151673",
    auto_crop = FALSE
  )
  print(p2)
}
```

Index

∗ **Check input functions**
check_modeling_results, 11
check_sce, 12
check_sce_layer, 13
check_spe, 14
∗ **Functions for adding non-standard images**
add_images, 5
locate_images, 42
∗ **Gene set enrichment functions**
gene_set_enrichment, 22
gene_set_enrichment_plot, 24
∗ **Genomics**
add10xVisiumAnalysis, 4
read10xVisiumAnalysis, 45
read10xVisiumWrapper, 46
∗ **Image editing functions**
img_edit, 30
img_update, 32
img_update_all, 33
∗ **Layer correlation functions**
annotate_registered_clusters, 10
layer_stat_cor, 37
layer_stat_cor_plot, 39
∗ **Layer modeling functions**
layer_boxplot, 34
sig_genes_extract, 64
sig_genes_extract_all, 66
∗ **Spatial cluster visualization functions**
frame_limits, 21
vis_clus, 69
vis_clus_p, 72
vis_grid_clus, 80
vis_image, 85
∗ **Spatial gene visualization functions**
vis_gene, 74
vis_gene_p, 78
vis_grid_gene, 83
∗ **SpatialExperiment-related functions**
sce_to_spe, 63
∗ **Utility functions for reading data from SpaceRanger output by 10x**
add10xVisiumAnalysis, 4
read10xVisiumAnalysis, 45
read10xVisiumWrapper, 46
∗ **cluster export/import utility functions**
cluster_export, 15
cluster_import, 16
∗ **datasets**
libd_layer_colors, 41
tstats_Human_DLPFC_snRNAseq_Nguyen_topLayer, 68
∗ **functions for summarizing expression of multiple continuous variables simultaneously**
multi_gene_pca, 43
multi_gene_sparsity, 43
multi_gene_z_score, 44
∗ **internal**
multi_gene_pca, 43
multi_gene_sparsity, 43
multi_gene_z_score, 44
prep_stitched_data, 45
spatialLIBD-package, 3
∗ **spatial registration and statistical modeling functions**
registration_block_cor, 48
registration_model, 49
registration_pseudobulk, 50
registration_stats_anova, 51
registration_stats_enrichment, 53
registration_stats_pairwise, 55
registration_wrapper, 56

add10xVisiumAnalysis, 4, *46, 47*
add_images, 5, *42*
add_key, 7
add_qc_metrics, 8
annotate_registered_clusters, 10, *38*, *40*
annotate_registered_clusters(), *40*

BiocFileCache-class, *18*
BiocFileCache::bfcrpath(), *17*

check_modeling_results, 11, *13, 14*
check_sce, *12*, 12, *13, 14*
check_sce_layer, *12, 13*, 13, *14*
check_spe, *12, 13*, 14
cluster_export, 15, *16*
cluster_import, *15*, 16
ComplexHeatmap::Heatmap(), *25*, *40*

DataFrame-class, *66*

enough_ram, 17
ExperimentHub-class, *18*

fetch_data, 17
fetch_data(), *6*, *11–16*, *21, 22*, *31–33*, *35*, *38*, *42*, *59*, *63*, *65, 66*, *70*, *73*, *75*, *79*, *81*, *83*, *86*
frame_limits, 21, *71*, *73*, *82*, *86*

gene_set_enrichment, 22, *25*
gene_set_enrichment(), *24, 25*
gene_set_enrichment_plot, *23*, 24
geom_spatial, 27
geom_spatial(), *70*, *73*, *75*, *79*, *81*, *84*
get_colors, 29
ggplot2, *70*, *73*, *76*, *80*, *82*, *85, 86*
ggplot2::layer(), *28*
ggplotly(), *70*, *73*, *75*, *79*, *81*, *84*

Heatmap-class, *25*, *40*

img_edit, 30, *33, 34*
img_update, *32*, 32, *34*
img_update_all, *32, 33*, 33

layer_boxplot, 34, *65*, *67*
layer_boxplot(), *64*, *66*
layer_stat_cor, *11*, 37, *40*
layer_stat_cor(), *10*, *39*, *69*
layer_stat_cor_plot, *11*, *38*, 39
layer_stat_cor_plot(), *68*
libd_layer_colors, 41
locate_images, *6*, 42

magick::enhance, *31*
magick::equalize, *31*
magick::image_background, *31*
magick::image_channel, *31*
magick::image_contrast, *31*
magick::image_median, *31*
magick::image_modulate, *31*
magick::image_quantize, *31*
magick::image_read, *32*
magick::image_transparent, *31*
magick::negate, *31*
magick::normalize, *31*
multi_gene_pca, 43, *44*
multi_gene_sparsity, *43*, 43, *44*
multi_gene_z_score, *43, 44*, 44

paste(), *86*
paste0(), *70*, *76*, *82*, *85*
pdf, *81*, *84*
plot_grid, *81*, *84*
prep_stitched_data, 45

read10xVisiumAnalysis, *5*, 45, *47*
read10xVisiumWrapper, *5*, *46*, 46
read10xVisiumWrapper(), *6*, *14–16*, *21*, *31–33*, *42*, *70*, *73*, *75*, *79*, *81*, *83*, *86*
registration_block_cor, 48, *49*, *51, 52*, *54, 55*, *57*
registration_model, *48*, 49, *51, 52*, *54, 55*, *57*
registration_pseudobulk, *48, 49*, 50, *52*, *54, 55*, *57*
registration_stats_anova, *48, 49*, *51*, 51, *54, 55*, *57*
registration_stats_enrichment, *48, 49*, *51, 52*, 53, *55*, *57*
registration_stats_pairwise, *48, 49*, *51, 52*, *54*, 55, *57*
registration_wrapper, *48, 49*, *51, 52*, *54, 55*, 56
run_app, 58

sce_to_spe, 63
scuttle::isOutlier, *8*
shiny.appobj, *60*
sig_genes_extract, *35*, 64, *67*
sig_genes_extract(), *66*
sig_genes_extract_all, *35*, *65*, 66
sig_genes_extract_all(), *34, 35*, *59*, *64*
SingleCellExperiment, *12, 13*, *18*, *35*, *59*, *63*, *65, 66*

SingleCellExperiment-class, *50*, *51*, *56*, *63*
sort_clusters, 67
sort_clusters(), *81*
SpatialExperiment, *8*, *47*
SpatialExperiment-class, *4–8*, *12*, *14–16*, *18*, *21*, *31–34*, *42*, *59*, *60*, *63*, *64*, *70*, *73*, *75*, *76*, *79*, *81–84*, *86*
SpatialExperiment::imgData, *63*
SpatialExperiment::read10xVisium(), *45–47*
spatialLIBD *(*spatialLIBD-package*)*, 3
spatialLIBD-package, 3
stats::fisher.test(), *23*

tstats_Human_DLPFC_snRNAseq_Nguyen_topLayer, 68

unname(), *30*

viridis, *75*, *79*, *84*
vis_clus, *22*, 69, *73*, *82*, *86*
vis_clus(), *72*, *74*, *82*, *85*
vis_clus_p, *22*, *71*, 72, *82*, *86*
vis_clus_p(), *28*, *70*, *78*, *86*
vis_gene, 74, *80*, *85*
vis_gene(), *69*, *78*, *85*
vis_gene_p, *76*, 78, *85*
vis_gene_p(), *28*, *72*, *76*
vis_grid_clus, *22*, *71*, *73*, 80, *86*
vis_grid_clus(), *83*
vis_grid_gene, *76*, *80*, 83
vis_grid_gene(), *80*
vis_image, *22*, *71*, *73*, *82*, 85