Package 'spatialLIBD'

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

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

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

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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.

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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 automati-
                cally 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 func-
                   tion 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 Single-CellExperiment object with the spot-level Visium data compressed via pseudo-bulking to the layer-level (group-level) resolution. See fetch_data() for more details.

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"),
  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/ project. As of version 1.11.12, data from https://github.com/LieberInstitute/Visium_SPG_AD 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.

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"
## 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 back-
                ground.

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()](#)

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`.

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 Single-CellExperiment object with the spot-level Visium data compressed via pseudo-bulking to the layer-level (group-level) resolution. See `fetch_data()` for more details.
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 col-
                      umn 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 ac-
                      cess 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 re-
                      verse 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 Complex-Heatmap*

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 sam-
                  ple 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
)
```

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.

Value

A pseudo-bulked [SingleCellExperiment-class](#) object.

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

## Pseudo-bulk
sce_pseudo <- registration_pseudobulk(sce, "Cell_Cycle", "sample_id", c("age"), min_ncells = NULL)
colData(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 EN-SEMBL 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 tran-
scriptomics 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 EN-SEMBL 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 tran-
scriptomics 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 [SingleCellEx-periment](#) 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 [Single-CellExperiment](#) object with the spot-level Visium data compressed via pseudo-bulking to the layer-level (group-level) resolution. See [fetch_data()](#) for more details.

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 Single-
                        CellExperiment object with the spot-level Visium data compressed via pseudo-
                        bulking to the layer-level (group-level) resolution. See `fetch_data()` for more
                        details.

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.

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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()](#)

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()`

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/](http://research.libd.org/visiumStitched/reference/build_spe.html) [reference/build_spe.html](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()](#)

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)
}
```

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