

Package ‘volcano3D’

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Type Package

Title Interactive Plotting of Three-Way Differential Expression Analysis

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URL <https://katrionagoldmann.github.io/volcano3D/index.html>,
<https://github.com/KatrionaGoldmann/volcano3D>

BugReports <https://github.com/KatrionaGoldmann/volcano3D/issues>

Description Differential expression (DE) analysis can be used to discover quantitative changes in expression levels between experimental groups. Such results are typically visualised using volcano plots, however in cases where more than two experimental groups are involved, visualising results can become convoluted and it quickly becomes difficult to see the wood for the trees. This package provides easy-to-use functions to extract and visualise outputs from DE between three groups (primarily aimed at 'limma' and 'DESeq2' outputs). We present novel methods to map DE results into polar coordinates to enable users to combine and simultaneously view three sets of results. These graphics also possess optional 'plotly' outputs for interactive and three-dimensional functionality, as seen in Lewis et. al. (2019) <doi:10.1016/j.celrep.2019.07.091>.

Language en-gb

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Encoding UTF-8

LazyData true

Depends R (>= 3.5)

VignetteBuilder knitr

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NeedsCompilation no

Imports plotly, ggplot2, ggpubr, ggrepel, methods

Suggests knitr, rmarkdown, kableExtra, usethis

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boxplot_trio	<i>Boxplot to compare groups</i>
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Description

Plots the expression of a specific row in expression to compare the three groups in a boxplot using either ggplot or plotly.

Usage

```
boxplot_trio(
  polar,
  value,
  box_colours = c("green3", "blue", "red"),
  test = "polar_pvalue",
  levels_order = NULL,
  my_comparisons = NULL,
  text_size = 10,
  stat_colour = "black",
  stat_size = 3,
  step_increase = 0.05,
  plot_method = "ggplot",
  ...
)
```

Arguments

polar	A polar object including expression data from groups of interest. Created by polar_coords .
value	The row name or number in polar@expression to be analysed
box_colours	The fill colours for each box assigned in order of levels_order. Default = c('green3', 'blue', 'red').
test	The statistical test used to compare expression. Allowed values include: <ul style="list-style-type: none"> • polar_pvalue (default) and 'polar_padj' for the pvalues and adjusted pvalues in the polar object. • polar_multi_pvalue and polar_multi_padj for the pvalues and adjusted pvalues across all groups using the polar@multi_group_test columns. • t.test (parametric) and wilcox.test (non-parametric). Perform comparison between groups of samples. • anova (parametric) and kruskal.test (non-parametric). Perform one-way ANOVA test comparing multiple groups.
levels_order	A character vector stating the contrast groups to be plotted, in order. If NULL this defaults to the levels in polar@sampledata[, polar@contrast].
my_comparisons	A list of contrasts to pass to stat_compare_means . If NULL (default) all contrast pvalues are calculated and plotted.
text_size	The font size of text (default = 10)
stat_colour	Colour to print statistics (default="black").
stat_size	The font size of statistical parameter (default = 3).
step_increase	The distance between statistics on the y-axis (default = 0.05).
plot_method	Whether to use 'plotly' or 'ggplot'. Default is 'ggplot'
...	Other parameters for stat_compare_means

Value

Returns a boxplot featuring the differential expression between groups in comparison with annotated pvalues.

References

Lewis, Myles J., et al. (2019). [Molecular portraits of early rheumatoid arthritis identify clinical and treatment response phenotypes](#). *Cell reports*, **28**:9

Examples

```
data(example_data)
syn_polar <- polar_coords(sampledata = syn_example_meta,
                          contrast = "Pathotype",
                          groups = NULL,
                          pvalues = syn_example_p,
                          expression = syn_example_rld,
                          p_col_suffix = "pvalue",
```

```

      padj_col_suffix = "padj",
      non_sig_name = "Not Significant",
      multi_group_prefix = "LRT",
      significance_cutoff = 0.01,
      fc_col_suffix='log2FoldChange',
      fc_cutoff = 0.3)

boxplot_trio(syn_polar, value = "SLAMF6", plot_method="plotly")
boxplot_trio(syn_polar, value = "SLAMF6")

```

grid-class

An S4 class to define the polar grid coordinates system.

Description

An S4 class to define the polar grid coordinates system.

Slots

polar_grid The coordinates for the cylindrical grid segments with x,y,z coordinates

axes The axes features for 'plotly'

axis_labs The axis labels

r The grid radius

z The grid height

text_coords data frame for axis label cartesian coordinates (x, y, z)

n_r_breaks The number of ticks on the r axis

n_z_breaks The number of ticks on the z axis

r_breaks The r axis ticks as a numeric

z_breaks The z axis ticks as a numeric

polar-class

An S4 class to define the polar coordinates and pvalues for polar differential expression plots

Description

An S4 class to define the polar coordinates and pvalues for polar differential expression plots

Slots

- `sampledata` Sample data with ID and contrast column.
- `contrast` The column name in 'sampledata' which contains the three-group contrast factor used for comparisons.
- `pvalues` A data frame containing the p-values, and adjusted p-values, for all three comparisons between groups in the contrast factor, as well as optional fold changes and multi-group tests.
- `multi_group_test` Column name prefix for statistical tests between all three groups
- `expression` A data frame or matrix containing the expression data. This is used to calculate z-score and fold change, therefore it should be a normalised expression object such as log transformed or variance stabilised.
- `polar` A data frame containing:
- The axis score or mean expression for each of the three groups in comparison
 - The z-score polar coordinates: 'y_zscore', 'x_zscore' and 'r_zscore'
 - The fold-change polar coordinates: 'y_fc', 'x_fc' and 'r_fc'
 - 'angle': The angle in radians for polar coordinates
 - 'angle_degrees': The angle in degrees
 - 'maxExp': The group with the highest expression
 - 'sig': The significance category
- `non_sig_name` The category name for variables which are not significant

polar_coords

Coordinates for Three Way Polar Plot

Description

This function creates a polar object of S4 class for downstream plots containing the p-values from a three-way group comparison, expression data sample data and polar coordinates.

Usage

```
polar_coords(
  sampledata,
  contrast,
  pvalues,
  expression,
  groups = NULL,
  p_col_suffix = "pvalues",
  padj_col_suffix = "padj",
  fc_col_suffix = NULL,
  padjust_method = "BH",
  multi_group_prefix = NULL,
  non_sig_name = "Not Significant",
  significance_cutoff = 0.01,
  fc_cutoff = 0.3,
  label_column = NULL
)
```

Arguments

sampledata	A data frame containing the sample information. This must contain: an ID column containing the sample IDs which can be matched to the 'expression' data and a contrast column containing the three-level factor used for contrasts.
contrast	The column name in 'sampledata' which contains the three-level factor used for contrast.
pvalues	A data frame containing: <ul style="list-style-type: none"> • three 'p_col_suffix' columns: one for the pvalue for each comparison between groups; • three optional 'fc_col_suffix' columns for the fold change between each comparison (if NULL, no Fold Change columns are included); • three optional 'padj_col_suffix' columns (if NULL adjusted p values are calculated using 'padjust_method'); • and optional 'p', 'padj' and 'fc' columns for a three-way test, such as ANOVA or likelihood ratio test, defined by 'multi_group_prefix'.
expression	An optional data frame containing expression data for downstream analysis and visualisation. The rows must contain probes which match the rows in pvalues and the columns must contain samples which match sampledata\$ID.
groups	The groups to be compared (if NULL this defaults to levels(sampledata[, 'contrasts'])).
p_col_suffix	The suffix word to define columns containing p values (default = 'pvalues'). These must not contain underscores;
padj_col_suffix	The suffix word to define columns containing adjusted p values (default = 'padj'). These must not contain underscores. If NULL these will be calculated using padjust_method
fc_col_suffix	The optional suffix word to define columns containing log fold change values (default = 'logFC'). These must not contain underscores.
padjust_method	The method used to calculate adjusted p values if padj_col_suffix is NULL (default = 'BH'). See p.adjust .
multi_group_prefix	Optional column prefix for statistics (p, padj, and fold change) across all three groups (typically ANOVA or likelihood ratio tests). default = NULL. These must not contain underscores
non_sig_name	Category name to assign to non-significant points
significance_cutoff	Value defining the significance cut-off (points with pvalues below this point will be classed as non_sig_name)
fc_cutoff	The cut-off for fold change, below which markers will be classed as non_sig_name' (default = 0.3).
label_column	Optional column name in pvalues for markers to be labelled with at plotting stage. If NULL the rownames of pvalues are used.

Value

Returns an S4 polar object containing:

- 'polar' A data.frame containing:
 - The mean expression for each of the three groups in comparison
 - The z-score polar coordinates: 'y_zscore', 'x_zscore' and 'r_zscore'
 - The fold-change polar coordinates: 'y_fc', 'x_fc' and 'r_fc'
 - 'angle': The angle in radians for polar coordinates
 - 'angle_degrees': The angle in degrees
 - 'maxExp': The maximally expressed group
 - 'sig': The significance group
- 'pvalues' A data frame containing the p-values, adjusted p-values, and optional log(fold changes) for all three comparisons between groups in the contrast factor, as well as optional multi-group tests.
- 'sampledata' Sample data with column ID and contrast
- 'contrast' The column name in 'sampledata' which contains the three-group contrast factor used for comparisons.
- 'multi_group_test' Column name prefix for statistical tests between all three groups
- 'expression' An optional data frame or matrix containing the expression data
- 'non_sig_name' The category name for variables which are classed as not significant

References

Lewis, Myles J., et al. (2019). [Molecular portraits of early rheumatoid arthritis identify clinical and treatment response phenotypes](#). *Cell reports*, **28**:9

Examples

```
data(example_data)
syn_polar <- polar_coords(sampledata=syn_example_meta,
  contrast="Pathotype",
  groups = NULL,
  pvalues = syn_example_p,
  expression = syn_example_rld,
  p_col_suffix = "pvalue",
  padj_col_suffix = "padj",
  fc_col_suffix = NULL,
  padjust_method = "BH",
  multi_group_prefix = NULL,
  non_sig_name = "Not Significant",
  significance_cutoff = 0.01,
  fc_cutoff=0.3,
  label_column = NULL)
table(syn_polar@polar$sig)
```

polar_grid

Grid required for 3D volcano plot and 2D radial plots

Description

Generates a cylindrical grid of the appropriate dimensions for a 3D volcano plot

Usage

```
polar_grid(
  r_vector = NULL,
  z_vector = NULL,
  r_axis_ticks = NULL,
  z_axis_ticks = NULL,
  axis_angle = 5/6,
  n_spokes = 12,
  ...
)
```

Arguments

r_vector	An optional numerical vector for the radial coordinates. This is used to calculate breaks on the r axis using pretty . If this is NULL the r_axis_ticks are used as breaks.
z_vector	An optional numerical vector for the z coordinates. This is used to calculate breaks on the z axis using pretty . If this is NULL the z_axis_ticks are used as breaks.
r_axis_ticks	A numerical vector of breaks for the radial axis (used if r_vector is NULL).
z_axis_ticks	A numerical vector of breaks for the z axis (used if z_vector is NULL).
axis_angle	angle in radians to position the radial axis (default = 5/6)
n_spokes	the number of outward spokes to be plotted (default = 12)
...	optional parameters for pretty on the r axis

Value

Returns an S4 grid object containing:

- 'polar_grid' The coordinates for a radial grid
- 'axes' The axes features for 'plotly'
- 'axis_labels' The axis labels
- 'r' The grid radius
- 'z' The grid height
- 'text_coords' The coordinates for text labels
- 'n_r_breaks' The number of ticks on the r axis
- 'n_z_breaks' The number of ticks on the z axis

References

Lewis, Myles J., et al. (2019). **Molecular portraits of early rheumatoid arthritis identify clinical and treatment response phenotypes.** *Cell reports*, **28**:9

Examples

```
data(example_data)
syn_polar <- polar_coords(sampled_data = syn_example_meta,
  contrast = "Pathotype",
  groups = NULL,
  pvalues = syn_example_p,
  expression = syn_example_rld,
  p_col_suffix = "pvalue",
  padj_col_suffix = "padj",
  non_sig_name = "Not Significant",
  multi_group_prefix = "LRT",
  significance_cutoff = 0.01,
  fc_col_suffix = 'log2FoldChange',
  fc_cutoff = 0.3)

grid <- polar_grid(r_vector = syn_polar@polar$r_zscore,
  z_vector = NULL,
  r_axis_ticks = NULL,
  z_axis_ticks = c(0, 8, 16, 32),
  n_spokes = 4)
```

radial_ggplot

'Ggplot' for Three Way Polar Plot

Description

This function creates a radar plot using 'ggplot' for a three-way comparison

Usage

```
radial_ggplot(
  polar,
  colours = c("green3", "cyan", "blue", "purple", "red", "gold2"),
  non_sig_colour = "grey60",
  colour_scale = "discrete",
  continuous_shift = 1.33,
  label_rows = NULL,
  arrow_length = 1,
  grid = NULL,
  fc_or_zscore = "zscore",
  label_size = 5,
  colour_code_labels = TRUE,
  label_colour = NULL,
```

```

grid_colour = "grey80",
axis_colour = "black",
axis_title_size = 5,
axis_label_size = 3,
marker_alpha = 0.7,
marker_size = 3,
marker_outline_colour = "white",
marker_outline_width = 0.5,
axis_angle = 1/6,
legend_size = 20,
...
)

```

Arguments

polar	A polar object with the pvalues between groups of interest and polar coordinates. Created by polar_coords .
colours	A vector of colour names or hex triplets for each of the six groups. Default = c("green3", "cyan", "blue", "purple", "red", "gold2"). Colours are assigned in order: group1+, group1+group2+, group2+, group2+group3+, group3+, group1+group3+.
non_sig_colour	The colour for non-significant markers (default = "grey60").
colour_scale	whether to use a "discrete" or "continuous" colour scale (default = "discrete").
continuous_shift	the number of radians (between 0 and 2) corresponding to the angle to offset the continuous colour scale by. The continuous colour scale is calculated by converting the angle to hue where 0 corresponds to red and 2 to magenta (default = 1.33).
label_rows	A vector of row names or numbers to label.
arrow_length	length of label arrow (default = 1).
grid	An optional grid object. If NULL this will be calculated using default values of polar_grid .
fc_or_zscore	Whether to use the z-score or fold change as magnitude. Options are 'zscore' (default) or 'fc'.
label_size	Font size of labels/annotations (default = 5).
colour_code_labels	Logical whether label annotations should be colour coded. If FALSE label_colour is used.
label_colour	Colour of annotation labels if not colour coded.
grid_colour	The colour of the grid (default="grey80").
axis_colour	The colour of the grid axes and labels (default="black").
axis_title_size	Font size for axis titles (default = 5)
axis_label_size	Font size for axis labels (default = 3)

marker_alpha The alpha parameter for markers (default = 0.7).
marker_size Size of the markers (default = 3).
marker_outline_colour Colour for marker outline (default = white)
marker_outline_width Width for marker outline (default = 0.5)
axis_angle Angle for the radial axis labels in pi radians (default = 1/6).
legend_size Size for the legend text (default = 20).
... Optional grid parameters to pass to [polar_grid](#).

Value

Returns a polar 'ggplot' object featuring variables on a tri-axis radial graph

References

Lewis, Myles J., et al. (2019). [Molecular portraits of early rheumatoid arthritis identify clinical and treatment response phenotypes](#). *Cell reports*, **28**:9

Examples

```
data(example_data)
syn_polar <- polar_coords(sampledata = syn_example_meta,
  contrast = "Pathotype",
  groups = NULL,
  pvalues = syn_example_p,
  expression = syn_example_rld,
  p_col_suffix = "pvalue",
  padj_col_suffix = "padj",
  non_sig_name = "Not Significant",
  multi_group_prefix = "LRT",
  significance_cutoff = 0.01,
  fc_cutoff = 0.3)

radial_ggplot(polar = syn_polar, label_rows = c("SLAMF6"))
```

radial_plotly

Three-way radial comparison Polar Plot (using plotly)

Description

This function creates an interactive plotly object which maps differential expression onto a polar coordinates.

Usage

```
radial_plotly(
  polar,
  colours = c("green3", "cyan", "blue", "purple", "red", "gold2"),
  non_sig_colour = "grey60",
  colour_scale = "discrete",
  continuous_shift = 1.33,
  label_rows = NULL,
  arrow_length = 50,
  grid = NULL,
  fc_or_zscore = "zscore",
  label_size = 14,
  colour_code_labels = TRUE,
  label_colour = NULL,
  grid_colour = "grey80",
  axis_colour = "black",
  marker_size = 6,
  marker_alpha = 0.7,
  marker_outline_colour = "white",
  marker_outline_width = 0.5,
  axis_title_size = 16,
  axis_label_size = 10,
  axis_ticks = NULL,
  axis_angle = 5/6,
  plot_height = 700,
  plot_width = 700,
  ...
)
```

Arguments

polar	A polar object with the pvalues between groups of interest and polar coordinates. Created by polar_coords .
colours	A vector of colour names or hex triplets for each of the six groups. Default = c("green3", "cyan", "blue", "purple", "red", "gold2"). Colours are assigned in order: group1+, group1+group2+, group2+, group2+group3+, group3+, group1+group3+.
non_sig_colour	The colour for non-significant markers (default = "grey60").
colour_scale	whether to use a 'discrete' or 'continuous' colour scale (default = 'discrete').
continuous_shift	The number of radians (between 0 and 6) to offset the continuous colour scale by. This is calculated by converting the angle to a hue using hsv where 0 corresponds to the colour scale starting with red and 360 with magenta (default = 2).
label_rows	A vector of row names or numbers to label.
arrow_length	The length of label arrows (default = 50).
grid	An optional grid object. If NULL this will be calculated using the default values of polar_grid .


```

        multi_group_prefix = "LRT",
        significance_cutoff = 0.01,
        fc_cutoff = 0.3)

radial_plotly(polar = syn_polar, label_rows = c("SLAMF6"))

```

show_grid

Plots grid objects for inspection using plotly

Description

This function creates an interactive grids in polar and cylindrical coordinates

Usage

```
show_grid(grid, plot_height = 700, axis_angle = 0)
```

Arguments

grid	A grid object produced by polar_grid .
plot_height	The plot height in px (default=700),
axis_angle	The angle in radians at which to add axis (default=0).

Value

Returns a list containing a polar and cylindrical coordinate system.

References

Lewis, Myles J., et al. (2019). [Molecular portraits of early rheumatoid arthritis identify clinical and treatment response phenotypes](#). *Cell reports*, **28**:9

Examples

```

data(example_data)
syn_polar <- polar_coords(sampledata = syn_example_meta,
                          contrast = "Pathotype",
                          groups = NULL,
                          pvalues = syn_example_p,
                          expression = syn_example_rld,
                          p_col_suffix = "pvalue",
                          padj_col_suffix = "padj",
                          non_sig_name = "Not Significant",
                          multi_group_prefix = "LRT",
                          significance_cutoff = 0.01,
                          fc_col_suffix='log2FoldChange',
                          fc_cutoff = 0.3)

grid <- polar_grid(r_vector=syn_polar@polar$r_zscore,

```

```

        z_vector=-log(syn_polar@pvalues$LRT_pvalue),
        r_axis_ticks = NULL,
        z_axis_ticks = NULL)
p <- show_grid(grid)
p$polar
p$cyl

```

syn_example_meta	<i>PEAC synovial sample data</i>
------------------	----------------------------------

Description

A dataset containing sample data for 81 synovial biopsies from the PEAC cohort

Usage

```
syn_example_meta
```

Format

A data frame with 81 rows and 4 variables:

ID IDs which match column names in ‘syn_example_rld‘

Pathotype The sample pathotype

Source

[https://www.cell.com/cell-reports/fulltext/S2211-1247\(19\)31007-1](https://www.cell.com/cell-reports/fulltext/S2211-1247(19)31007-1)

syn_example_p	<i>Synovial differential expression of genes across pathotypes in PEAC cohort</i>
---------------	---

Description

A dataset containing the differential expression parameters between different pathotype groups for 81 synovial biopsies from the PEAC cohort.

Usage

```
syn_example_p
```

Format

A data frame with 100 rows representing the most significant genes/probes and 13 columns for each statistical parameter:

Gene The gene name

Fibroid_Lymphoid_pvalue pvalue from fibroid vs lymphoid comparison

Fibroid_Lymphoid_padj adjusted pvalue from fibroid vs lymphoid comparison

Fibroid_Lymphoid_log2FoldChange logarithmic fold change from fibroid vs lymphoid comparison

Lymphoid_Myeloid_pvalue pvalue from lymphoid vs myeloid comparison

Lymphoid_Myeloid_padj adjusted pvalue from lymphoid vs myeloid comparison

Lymphoid_Myeloid_log2FoldChange logarithmic fold change from lymphoid vs myeloid comparison

Myeloid_Fibroid_pvalue pvalue from myeloid vs fibroid comparison

Myeloid_Fibroid_padj adjusted pvalue from myeloid vs fibroid comparison

Myeloid_Fibroid_log2FoldChange logarithmic fold change from myeloid vs fibroid comparison

LRT_pvalue pvalue from three-way likelihood ratio comparison

LRT_padj adjusted pvalue from three-way likelihood ratio comparison

Source

[https://www.cell.com/cell-reports/fulltext/S2211-1247\(19\)31007-1](https://www.cell.com/cell-reports/fulltext/S2211-1247(19)31007-1)

syn_example_rld

PEAC synovial gene expression data

Description

A dataset containing the gene expression data for 81 synovial biopsies from the PEAC cohort

Usage

syn_example_rld

Format

A data frame with 100 rows representing the most significant genes/probes and 81 columns representing samples.

Source

[https://www.cell.com/cell-reports/fulltext/S2211-1247\(19\)31007-1](https://www.cell.com/cell-reports/fulltext/S2211-1247(19)31007-1)

volcano3D

*Three-Dimensional Volcano Plot***Description**

Plots the pvalues from three-way comparisons in 3D space using plotly.

Usage

```
volcano3D(
  polar,
  colours = c("green3", "cyan", "blue", "purple", "red", "gold2"),
  non_sig_colour = "grey60",
  colour_scale = "discrete",
  continuous_shift = 1.33,
  label_rows = c(),
  grid = NULL,
  fc_or_zscore = "zscore",
  label_size = 14,
  arrow_length = 50,
  colour_code_labels = TRUE,
  label_colour = NULL,
  grid_colour = "grey80",
  axis_colour = "black",
  marker_size = 2.7,
  marker_alpha = 1,
  marker_outline_colour = "white",
  marker_outline_width = 0,
  axis_angle = 0.5,
  z_aspectratio = 1,
  xy_aspectratio = 1,
  plot_height = 700,
  ...
)
```

Arguments

<code>polar</code>	A polar object with created by <code>polar_coords</code> .
<code>colours</code>	A vector of colour names or hex triplets for each of the six groups. Default = <code>c("green3", "cyan", "blue", "purple", "red", "gold2")</code> . Colours are assigned in order: <code>group1+</code> , <code>group1+group2+</code> , <code>group2+</code> , <code>group2+group3+</code> , <code>group3+</code> , <code>group1+group3+</code> .
<code>non_sig_colour</code>	The colour for non-significant markers (default='grey60').
<code>colour_scale</code>	whether to use a 'discrete' or 'continuous' colour scale (default = 'discrete').
<code>continuous_shift</code>	The number of radians to offset the continuous colour scale by. This is calculated by converting the angle to a hue using <code>hsv</code> where 0 corresponds to the colour scale starting with red and 2 with magenta (default = 2).


```

        p_col_suffix = "pvalue",
        padj_col_suffix = "padj",
        non_sig_name = "Not Significant",
        multi_group_prefix = "LRT",
        significance_cutoff = 0.01,
        fc_col_suffix='log2FoldChange',
        fc_cutoff = 0.3)

volcano3D(syn_polar,
  label_rows = c("FMOD", "LAMP5", "TNNT3"),
  xy_aspectratio = 1,
  label_size = 10,
  z_aspectratio = 0.9)

```

volcano_plot	<i>Creates a single volcano plot</i>
--------------	--------------------------------------

Description

This function creates a volcano plot for one comparison group

Usage

```

volcano_plot(
  pvalues_df,
  comparison,
  p_cutoff = 0.05,
  fc_cutoff = 1,
  label_rows = NULL,
  p_col_suffix = "pvalue",
  padj_col_suffix = "padj",
  fc_col_suffix = "logFC",
  label_col = "label",
  label_size = 3,
  text_size = 10,
  marker_size = 0.7,
  shared_legend_size = 1,
  sig_names = NULL,
  colours = c("grey60", "salmon", "steelblue", "limegreen"),
  fc_line = TRUE,
  p_line = TRUE,
  line_colours = c("black", "black")
)

```

Arguments

`pvalues_df` The pvalues data frame. This must contain a pvalue, padj, and logFC column as well as a label column.

comparison	The comparison (column_prefix) to use.
p_cutoff	The cut-off for pvalue significance (default = 0.05).
fc_cutoff	The cut-off for fold change significance (default = 1).
label_rows	Row numbers or names of values to be annotated/labelled (default = NULL).
p_col_suffix	The suffix word to define columns containing p values (default = 'pvalues').
padj_col_suffix	The suffix word to define columns containing adjusted p values (default = 'padj'). If NULL these will be calculated using padjust_method.
fc_col_suffix	The optional suffix word to define columns containing log fold change values (default = 'logFC').
label_col	Optional column name in 'pvalues_df' for labelling markers. If NULL the row-names of pvalues are used.
label_size	The font size of labels (default = 3)
text_size	The font size of text (default = 10)
marker_size	The size of markers (default = 0.7)
shared_legend_size	The size for the legend (default = 1).
sig_names	A character vector of labels to be used for: non-significant; adjusted $p < p_cutoff$; $ Fold\ Change > fc_cutoff$; and finally adjusted $p < p_cutoff$. If NULL <code>c('Not Significant', paste('Padj <', 'p_cutoff'), paste(' FC >', 'fc_cutoff'), paste('Padj <', 'p_cutoff', 'and FC >', 'fc_cutoff'))</code> is used.
colours	A character vector of colours to be used for non-significant; adjusted $p < p_cutoff$; $ Fold\ Change > fc_cutoff$; and adjusted $p < p_cutoff$. default = <code>p_cutoff & Fold Change > fc_cutoff</code> markers respectively (default = <code>c('grey60', 'salmon', 'steel-blue', 'limegreen')</code>).
fc_line	Logical whether to add vertical dashed line at <code>fc_cutoff</code> (default = TRUE).
p_line	Logical whether to add horizontal dashed line at <code>p_cutoff</code> (default = TRUE).
line_colours	A character vector stating the colour of lines to be used for <code>fc_line</code> and <code>p_line</code> respectively (default = <code>c('black', 'black')</code>).

Value

Returns a single volcano plot.

References

Lewis, Myles J., et al. (2019). [Molecular portraits of early rheumatoid arthritis identify clinical and treatment response phenotypes](#). *Cell reports*, **28**:9

Examples

```
data("example_data")
volcano_plot(syn_example_p,
             "Fibroid_Lymphoid",
             label_col = "Gene",
             label_rows=c("SLAMF6"),
             fc_col_suffix="log2FoldChange")
```

volcano_trio

*Volcano Plots for a three-way comparison***Description**

This function creates a volcano plot for all combinations of groups in a factor.

Usage

```
volcano_trio(
  polar,
  p_cutoff = 0.05,
  fc_cutoff = 1,
  label_rows = NULL,
  label_size = 3,
  text_size = 10,
  marker_size = 0.7,
  shared_legend_size = 1,
  sig_names = NULL,
  colours = c("grey60", "salmon", "steelblue", "limegreen"),
  fc_line = TRUE,
  p_line = TRUE,
  line_colours = c("black", "black"),
  share_axes = TRUE
)
```

Arguments

polar	A polar object with the pvalues between groups of interest. Created by polar_coords .
p_cutoff	The cut-off for pvalue significance (default = 0.05).
fc_cutoff	The cut-off for fold change significance (default = 1).
label_rows	Row numbers or names of values to be annotated/labelled (default = NULL).
label_size	The font size of labels (default = 3)
text_size	The font size of text (default = 10)
marker_size	The size of markers (default = 0.7)
shared_legend_size	The size for the legend (default = 1).
sig_names	A character vector of labels to be used for: non-significant; adjusted $p < p_cutoff$; $ Fold\ Change > fc_cutoff$; and finally adjusted $p < p_cutoff$. If NULL <code>c('Not Significant', paste('Padj <', `p_cutoff`), paste(' FC >', `fc_cutoff`), paste('Padj <', `p_cutoff`, 'and FC >', `fc_cutoff`))</code> is used.
colours	A character vector of colours to be used for non-significant; adjusted $p < p_cutoff$; $ Fold\ Change > fc_cutoff$; and adjusted $p < p_cutoff$. default = <code>p_cutoff & Fold Change > fc_cutoff</code> markers respectively (default = <code>c('grey60', 'salmon', 'steelblue', 'limegreen')</code>).

fc_line	Logical whether to add vertical dashed line at fc_cutoff (default = TRUE).
p_line	Logical whether to add horizontal dashed line at p_cutoff (default = TRUE).
line_colours	A character vector stating the colour of lines to be used for fc_line and p_line respectively (default = c('black', 'black')).
share_axes	Logical whether plots should share axes when plotted together.

Value

Returns a list of ggplot volcano plots. The first three elements contain comparisons between all contrasts. The last element in the list is a combined figure for all three plots.

References

Lewis, Myles J., et al. (2019). [Molecular portraits of early rheumatoid arthritis identify clinical and treatment response phenotypes](#). *Cell reports*, **28**:9

Examples

```
data(example_data)
syn_polar <- polar_coords(sampledata = syn_example_meta,
  contrast = "Pathotype",
  groups = NULL,
  pvalues = syn_example_p,
  expression = syn_example_rld,
  p_col_suffix = "pvalue",
  padj_col_suffix = "padj",
  non_sig_name = "Not Significant",
  multi_group_prefix = "LRT",
  significance_cutoff = 0.01,
  fc_col_suffix='log2FoldChange',
  fc_cutoff = 0.3)
syn_volcano_plots <- volcano_trio(polar=syn_polar)
syn_volcano_plots$All
```

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