

Package ‘volcano3D’

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Type Package

Title 3D Volcano Plots and Polar Plots for Three-Class Data

Version 2.0.0

Maintainer Katriona Goldmann <k.goldmann@qmul.ac.uk>

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

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

Description Generates interactive plots for analysing and visualising three-class high dimensional data. It is particularly suited to visualising differences in continuous attributes such as gene/protein/biomarker expression levels between three groups. Differential gene/biomarker expression analysis between two classes is typically shown as a volcano plot. However, with three groups this type of visualisation is particularly difficult to interpret. This package generates 3D volcano plots and 3-way polar plots for easier interpretation of three-class data.

Language en-gb

License GPL-2

Encoding UTF-8

LazyData true

biocViews

Depends R (>= 3.5)

VignetteBuilder knitr

RoxygenNote 7.2.0

NeedsCompilation no

Imports plotly, ggplot2, ggpubr, htmlwidgets, magrittr, methods,
Rfast, matrixTests

Suggests DESeq2, edgeR, limma, qvalue, SummarizedExperiment, knitr,
rmarkdown, kableExtra, usethis

Author Katriona Goldmann [aut, cre] (<<https://orcid.org/0000-0002-9073-6323>>),
Myles Lewis [aut] (<<https://orcid.org/0000-0001-9365-5345>>)

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| add_animation | <i>Add mode bar button to rotate the plot</i> |
|---------------|---|

Description

Add mode bar button to rotate the plot

Usage

```
add_animation(
  p,
  rotate_icon_path = NULL,
  stop_icon_path = NULL,
  rotate_colour = "#c7c7c7",
  stop_colour = "#ff6347",
  scale = "scale(0.4) translate(-4, -4)",
  speed = 320,
  shiny_event_names = c()
)
```

Arguments

| | |
|-------------------|--|
| p | The volcano3D plot |
| rotate_icon_path | The svg icon path for rotation. If NULL a play button is used |
| stop_icon_path | The svg icon path for stop button. If NULL a pause button is used |
| rotate_colour | The colour for the rotate button (default="#c7c7c7") |
| stop_colour | The colour for the stop button (default='ff6347', a.k.a 'tomato') |
| scale | Scaling for rotation button |
| speed | The rotation speed |
| shiny_event_names | If using shiny, pass in any shiny event names which should stop rotation when triggered (e.g. shiny_event_names = c('replot')) |

Value

Returns a rotating cylindrical 3D plotly plot featuring variables on a tri-axis radial graph with the $-\log_{10}(\text{multi-group test p-value})$ 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(outcome = syn_example_meta$Pathotype,
                          data = t(syn_example_r1d))

p <- volcano3D(syn_polar,
               label_rows = c("COBL", "TRES2"))
add_animation(p)
```

boxplot_trio

Boxplot to compare groups

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

| | |
|-----------------------------|---|
| <code>polar</code> | A 'volc3d' object including expression data from groups of interest. Created by polar_coords . |
| <code>value</code> | The column name or number in <code>polar@data</code> to be analysed |
| <code>box_colours</code> | The fill colours for each box assigned in order of <code>levels_order</code> . Default = <code>c('green3', 'blue', 'red')</code> . |
| <code>test</code> | The statistical test used to compare expression. Allowed values include: <ul style="list-style-type: none"> • <code>polar_pvalue</code> (default) and <code>'polar_padj'</code> for the pvalues and adjusted pvalues in the polar object. • <code>polar_multi_pvalue</code> and <code>polar_multi_padj</code> for the pvalues and adjusted pvalues across all groups using the <code>polar@multi_group_test</code> columns. • <code>t.test</code> (parametric) and <code>wilcox.test</code> (non-parametric). Perform comparison between groups of samples. • <code>anova</code> (parametric) and <code>kruskal.test</code> (non-parametric). Perform one-way ANOVA test comparing multiple groups. |
| <code>levels_order</code> | A character vector stating the contrast groups to be plotted, in order. If 'NULL' this defaults to the levels in <code>'polar@outcome'</code> . |
| <code>my_comparisons</code> | A list of contrasts to pass to stat_compare_means . If 'NULL' (default) all contrast pvalues are calculated and plotted. |
| <code>text_size</code> | The font size of text (default = 10) |
| <code>stat_colour</code> | Colour to print statistics (default="black"). |
| <code>stat_size</code> | The font size of statistical parameter (default = 3). |
| <code>step_increase</code> | The distance between statistics on the y-axis (default = 0.05). |
| <code>plot_method</code> | Whether to use 'plotly' or 'ggplot'. Default is 'ggplot' |
| <code>...</code> | 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(outcome = syn_example_meta$Pathotype,
                          data = t(syn_example_rld))

boxplot_trio(syn_polar, value = "COBL", plot_method="plotly")
boxplot_trio(syn_polar, value = "COBL")
```

 calc_pvals

Calculate one-way test and pairwise tests

Description

Internal function for calculating 3-class group test (either one-way ANOVA or Kruskal-Wallis test) and pairwise tests (either t-test or Wilcoxon test) on multi-column data against an outcome parameter with 3 levels.

Usage

```
calc_pvals(
  outcome,
  data,
  pcutoff = 0.05,
  padj.method = "BH",
  group_test = c("anova", "kruskal.test"),
  pairwise_test = c("t.test", "wilcoxon"),
  exact = FALSE,
  filter_pairwise = TRUE
)
```

Arguments

| | |
|---------|--|
| outcome | Outcome vector with 3 groups, ideally as a factor. If it is not a factor, this will be coerced to a factor. This must have exactly 3 levels. |
| data | Dataframe or matrix with variables in columns |
| pcutoff | Cut-off for p-value significance |

| | |
|-----------------|--|
| padj.method | Can be any method available in 'p.adjust' or "qvalue". The option "none" is a pass-through. |
| group_test | Specifies statistical test for 3-class group comparison. "anova" means one-way ANOVA, "kruskal.test" means Kruskal-Wallis test. |
| pairwise_test | Specifies statistical test for pairwise comparisons |
| exact | Logical which is only used with 'pairwise_test = "wilcoxon"' |
| filter_pairwise | Logical. If 'TRUE' (the default) p-value adjustment on pairwise statistical tests is only conducted on attributes which reached the threshold for significance after p-value adjustment on the group statistical test. |

deseq_polar

Convert DESeq2 objects to a volcano3d object

Description

This function takes 2 'DESeqDataSet' objects and converts the results to a 'volc3d' object.

Usage

```
deseq_polar(
  object,
  objectLRT,
  contrast,
  data = NULL,
  pcutoff = 0.05,
  padj.method = "BH",
  filter_pairwise = TRUE,
  ...
)
```

Arguments

| | |
|-----------|---|
| object | An object of class 'DESeqDataSet' with the full design formula. The function 'DESeq' needs to have been run. |
| objectLRT | An object of class 'DESeqDataSet' with the reduced design formula. The function 'DESeq' needs to have been run on this object with argument 'test="LRT"'. |
| contrast | Character value specifying column within the metadata stored in the DESeq2 dataset objects is the outcome variable. This column must contain a factor with 3 levels. |
| data | Optional matrix containing gene expression data. If not supplied, the function will pull the expression data from within the DESeq2 object using the DESeq2 function 'assay()'. NOTE: for consistency with gene expression datasets, genes are in rows. |
| pcutoff | Cut-off for p-value significance |

padj.method Can be any method available in 'p.adjust' or "qvalue". The option "none" is a pass-through.

filter_pairwise Logical whether adjusted p-value pairwise statistical tests are only conducted on genes which reach significant adjusted p-value cut-off on the group likelihood ratio test

... Optional arguments passed to [polar_coords()]

Examples

```
## Not run:
library(DESeq2)

counts <- matrix(rnbinom(n=1500, mu=100, size=1/0.5), ncol=15)
cond <- factor(rep(1:3, each=5), labels = c('A', 'B', 'C'))

# object construction
dds <- DESeqDataSetFromMatrix(counts, DataFrame(cond), ~ cond)

# standard analysis
dds <- DESeq(dds)

# Likelihood ratio test
ddsLRT <- DESeq(dds, test="LRT", reduced= ~ 1)

polar <- deseq_polar(dds, ddsLRT, "cond")
volcano3D(polar)
radial_ggplot(polar)

## End(Not run)
```

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_coords

Coordinates for Three Way Polar Plot

Description

This function creates a 'volc3d' object of S3 class for downstream plots containing the p-values from a three-way group comparison, expression data sample data and polar coordinates.

Usage

```
polar_coords(
  outcome,
  data,
  pvals = NULL,
  padj = pvals,
  pcutoff = 0.05,
  scheme = c("grey60", "red", "gold2", "green3", "cyan", "blue", "purple"),
  labs = NULL,
  ...
)
```

Arguments

| | |
|---------|---|
| outcome | Outcome vector with 3 groups, ideally as a factor. If it is not a factor, this will be coerced to a factor. This must have exactly 3 levels. NOTE: if 'pvals' is given, the order of the levels in 'outcome' must correspond to the order of columns in 'pvals'. |
| data | Dataframe or matrix with variables in columns |
| pvals | Matrix or dataframe with p-values. The first column represents a test across all 3 categories such as one-way ANOVA or likelihood ratio test. Columns 2-4 represent pairwise tests comparing groups A vs B, A vs C and B vs C, where A, B, C represent levels 1, 2, 3 in 'outcome'. Columns 2-4 must be provided in the correct order. If 'pvals' is not given, it is calculated using the function [calc_pvals]. |
| padj | Matrix or dataframe with adjusted p-values. If not supplied, defaults to use nominal p-values from 'pvals'. |
| pcutoff | Cut-off for p-value significance |
| scheme | Vector of colours starting with non-significant variables |

labs Optional character vector for labelling groups. Default 'NULL' leads to abbreviated labels based on levels in 'outcome' using [abbreviate]. A vector of length 3 with custom abbreviated names for the outcome levels can be supplied. Otherwise a vector length 7 is expected, of the form "ns", "B+", "B+C+", "C+", "A+C+", "A+", "A+B+", where "ns" means non-significant and A, B, C refer to levels 1, 2, 3 in 'outcome', and must be in the correct order.

... Optional arguments passed to [calc_pvals]

Value

Returns an S4 'volc3d' object containing:

- 'df' A list of 2 dataframes. Each dataframe contains both x,y,z coordinates as well as polar coordinates r, angle. The first dataframe has coordinates on scaled data. The 2nd dataframe has unscaled data (e.g. log₂ fold change for gene expression).
- 'outcome' The three-group contrast factor used for comparisons
- 'data' Dataframe or matrix containing the expression data
- 'pvals' A dataframe containing p-values. First column is the 3-way comparison (LRT or ANOVA). Columns 2-4 are pairwise comparisons between groups A vs B, A vs C and B vs C, where A, B, C are the 3 levels in the outcome factor.
- 'padj' A dataframe containing p-values adjusted for multiple testing

Examples

```
data(example_data)
syn_polar <- polar_coords(outcome = syn_example_meta$Pathotype,
                          data = t(syn_example_r1d))
```

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,
  axes_from_origin = TRUE,
  ...
)
```

Arguments

| | |
|------------------|--|
| r_vector | An optional numerical vector for the radial coordinates. This is used to calculate breaks on the r axis using <code>pretty</code> . 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 <code>pretty</code> . 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) |
| axes_from_origin | Whether the axis should start at 0 or the first break (default = TRUE) |
| ... | optional parameters for <code>pretty</code> 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(outcome = syn_example_meta$Pathotype,
                          data = t(syn_example_r1d))

grid <- polar_grid(r_vector=syn_polar@df[[1]]$r,
                  z_vector=NULL,
                  r_axis_ticks = NULL,
                  z_axis_ticks = c(0, 8, 16, 32),
                  n_spokes = 4)
```

| | |
|---------------|--|
| radial_ggplot | <i>'Ggplot' for Three Way Polar Plot</i> |
|---------------|--|

Description

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

Usage

```
radial_ggplot(  
  polar,  
  type = 1,  
  colours = NULL,  
  label_rows = NULL,  
  arrow_length = 1,  
  label_size = 5,  
  colour_code_labels = FALSE,  
  label_colour = "black",  
  grid_colour = "grey80",  
  grid_width = 0.7,  
  axis_colour = "black",  
  axis_width = 1,  
  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 'volc3d' object with the p-values between groups of interest and polar coordinates. Created by polar_coords . |
| type | Numeric value whether to use scaled (z-score) or unscaled (fold change) as magnitude. Options are 1 = z-score (default) or 2 = unscaled/fold change. |
| colours | A vector of colours for the non-significant points and each of the six groups. |
| label_rows | A vector of row names or indices to label |
| arrow_length | The length of label arrows |
| 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") |
| grid_width | The width of the axis lines (default=0.6) |
| axis_colour | The colour of the grid axes and labels (default="black") |
| axis_width | The width of the axis lines (default=1) |
| 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(outcome = syn_example_meta$Pathotype,
                          data = t(syn_example_rld))

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

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,
  type = 1,
  colours = polar@scheme,
  label_rows = NULL,
  arrow_length = 50,
  label_size = 14,
  colour_code_labels = FALSE,
  label_colour = "black",
  grid_colour = "grey80",
  grid_width = 1,
  marker_size = 7,
  marker_alpha = 0.8,
  marker_outline_colour = "white",
  marker_outline_width = 0.5,
  axis_title_size = 16,
  axis_label_size = 10,
  axis_colour = "black",
  axis_width = 2,
  axis_ticks = NULL,
  axis_angle = 5/6,
  ...
)
```

Arguments

| | |
|--------------------|--|
| polar | A polar object with the pvalues between groups of interest and polar coordinates. Created by polar_coords . |
| type | Numeric value whether to use scaled (z-score) or unscaled (fold change) as magnitude. Options are 1 = z-score (default) or 2 = unscaled/fold change. |
| colours | A vector of colour names or hex triplets for the non-significant points and each of the six groups. |
| label_rows | A vector of row names or numbers to label. |
| arrow_length | The length of label arrows (default = 50). |
| label_size | Font size of labels/annotations (default = 14) |
| colour_code_labels | Logical whether label annotations should be colour coded. If FALSE label_colour is used. |
| label_colour | HTML colour of annotation labels if not colour coded. |
| grid_colour | The colour of the grid (default="grey80") |
| grid_width | The width of the grid lines (default=1) |
| marker_size | Size of the markers (default = 6) |
| marker_alpha | Opacity for the markers (default = 0.7) |

| | |
|-----------------------|---|
| marker_outline_colour | Colour for marker outline (default = white) |
| marker_outline_width | Width for marker outline (default = 0.5) |
| axis_title_size | Font size for axis titles (default = 16) |
| axis_label_size | Font size for axis labels (default = 10) |
| axis_colour | The colour of the grid axes and labels (default="black") |
| axis_width | The width of the axis lines (default=2) |
| axis_ticks | A numerical vector of radial axis tick breaks. If NULL this will be calculated using pretty . |
| axis_angle | Angle in radians for the radial axis (default = 5/6). |
| ... | Optional parameters to pass to polar_grid . |

Value

Returns a plotly plot 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(outcome = syn_example_meta$Pathotype,
                          data = t(syn_example_rld))

radial_plotly(polar = syn_polar, label_rows = c("COBL"))
```

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, z_axis_title_offset = 1.2)
```

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).
z_axis_title_offset Offset for z axis title (default=1.2).

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(outcome = syn_example_meta$Pathotype,
                          data = t(syn_example_rld))

grid <- polar_grid(r_vector=syn_polar@df[[1]]$r,
                  z_vector=syn_polar@df[[1]]$z,
                  r_axis_ticks = NULL,
                  z_axis_ticks = NULL)
p <- show_grid(grid)
p$polar
p$cyl
  
```

significance_subset *Extract a subset population*

Description

Subsets data according to the significance groups.

Usage

```
significance_subset(polar, significance = NULL, output = "pvalues")
```

Arguments

polar A polar object including expression data from groups of interest. Created by [polar_coords](#).
significance Which significance factors to subset to. If NULL levels(syn_polar@polar\$sig)[1] is selected.
output What object to return. Options are "pvals", "padj", "data", "df" for subset dataframes, or "polar" to subset the entire 'volc3d' class object.

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(outcome = syn_example_meta$Pathotype,
                          data = t(syn_example_rld))

subset <- significance_subset(syn_polar, "L+", "df")
```

| | |
|------------------|----------------------------------|
| 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 1 variables:

Pathotype The synovial biopsy histological pathotype

Source

<https://pubmed.ncbi.nlm.nih.gov/31461658/>

| | |
|-----------------|---|
| syn_example_rld | <i>PEAC synovial gene expression data</i> |
|-----------------|---|

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 500 rows representing the most significant genes/probes and 81 columns representing samples.

Source

<https://pubmed.ncbi.nlm.nih.gov/31461658/>

volcano3D

Three-Dimensional Volcano Plot

Description

Plots the three-way comparisons of variables such as gene expression data in 3D space using plotly. x, y position represents polar position on 3 axes representing the amount each variable or gene tends to each of the 3 categories. The z axis represents $-\log_{10}$ P value for the one-way test comparing each variable across the 3 groups.

Usage

```
volcano3D(  
  polar,  
  type = 1,  
  label_rows = c(),  
  label_size = 14,  
  arrow_length = 100,  
  colour_code_labels = FALSE,  
  label_colour = "black",  
  grid_colour = "grey80",  
  grid_width = 2,  
  grid_options = NULL,  
  axis_colour = "black",  
  axis_width = 2,  
  marker_size = 3,  
  marker_outline_width = 0,  
  marker_outline_colour = "white",  
  z_axis_title_offset = 1.2,  
  z_axis_title_size = 12,  
  z_axis_angle = 0.5,  
  radial_axis_title_size = 14,  
  radial_axis_title_offset = 1.2,  
  xy_aspectratio = 1,  
  z_aspectratio = 0.8,  
  camera_eye = list(x = 0.9, y = 0.9, z = 0.9),  
  ...  
)
```

Arguments

| | |
|--------------------------|--|
| polar | Object of S4 class 'volc3d' following call to either 'polar_coords()' or 'DESeq-ToVolc()' |
| type | Either '1' or '2' specifying type of polar coordinates: '1' = Z-scaled, '2' = unscaled (equivalent to log2 fold change for gene expression). |
| label_rows | A vector of row names or numbers to label |
| label_size | font size for labels (default 14). |
| arrow_length | The length of label arrows (default 100) |
| colour_code_labels | Logical whether label annotations should be colour coded. If 'FALSE' 'label_colour' is used. |
| label_colour | HTML colour of annotation labels if not colour coded. |
| grid_colour | The colour of the cylindrical grid (default "grey80") |
| grid_width | The width of the grid lines (default 2) |
| grid_options | Optional list of additional arguments to pass to 'polar_grid()', eg. 'z_axis_ticks' and 'r_axis_ticks' |
| axis_colour | The colour of the grid axes and labels (default "black") |
| axis_width | The width of axis lines (default 2) |
| marker_size | Size of the markers (default 3) |
| marker_outline_width | Width for marker outline (default 0 means no outline) |
| marker_outline_colour | Colour for marker outline (default white) |
| z_axis_title_offset | The position scaling between grid and z axis title (default=1.2) |
| z_axis_title_size | The font size for the z axis title (default=12) |
| z_axis_angle | Angle in radians for the position of z axis (default 0.5) |
| radial_axis_title_size | The font size for the radial (default=15) |
| radial_axis_title_offset | The position scaling between grid and radial axis title (default=1.2) |
| xy_aspectratio | The aspect ratio for the xy axis compared to z (default 1). Increasing this makes the grid wider in the plot window. |
| z_aspectratio | The aspect ratio for the z axis compared to x and y (default 0.8). Decreasing this makes the plot appear more squat. |
| camera_eye | The (x,y,z) components of the start 'eye' camera vector. This vector determines the view point about the origin of this scene. |
| ... | Optional arguments passed to 'plot_ly' |

Value

Returns a cylindrical 3D plotly plot featuring variables on a tri-axis radial graph with the $-\log_{10}(\text{multi-group test p-value})$ 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(outcome = syn_example_meta$Pathotype,
                          data = t(syn_example_rld))
volcano3D(syn_polar)
```

| | |
|------------|--|
| voom_polar | <i>Convert RNA-Seq count data to a volcano3d object using 'limma voom'</i> |
|------------|--|

Description

This function takes a design formula, metadata and raw count data and uses 'limma voom' to analyse the data. The results are converted to a 'volc3d' object ready for plotting a 3d volcano plot or polar plot.

Usage

```
voom_polar(
  formula,
  metadata,
  counts,
  pcutoff = 0.05,
  padj.method = "BH",
  filter_pairwise = TRUE,
  ...
)
```

Arguments

| | |
|-------------|--|
| formula | Design formula which must be of the form '~ 0 + outcome + ...'. The 3-way outcome variable must be the first variable after the '0', and this variable must be a factor with exactly 3 levels. |
| metadata | Matrix or dataframe containing metadata as referenced by 'formula' |
| counts | Matrix containing raw gene expression count data |
| pcutoff | Cut-off for p-value significance |
| padj.method | Can be any method available in 'p.adjust' or "qvalue". The option "none" is a pass-through. |

```
filter_pairwise      Logical whether adjusted p-value pairwise statistical tests are only conducted on
                     genes which reach significant adjusted p-value cut-off on the group likelihood
                     ratio test
...                  Optional arguments passed to [polar_coords()]
```

Examples

```
if (requireNamespace("limma", quietly = TRUE) &
    requireNamespace("edgeR", quietly = TRUE)) {
  library(limma)
  library(edgeR)

  counts <- matrix(rnbinom(n=1500, mu=100, size=1/0.5), ncol=15)
  cond <- factor(rep(1:3, each=5), labels = c('A', 'B', 'C'))
  cond <- data.frame(cond)

  polar <- voom_polar(~0 + cond, cond, counts)

  volcano3D(polar)
  radial_ggplot(polar)
}
```

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