

# Package ‘volcano3D’

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**Type** Package

**Title** Interactive Plotting of Three-Way Differential Expression Analysis

**Version** 1.1.0

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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>.

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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 <a href="#">polar_coords</a> .
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"> <li>• polar_pvalue (default) and 'polar_padj' for the pvalues and adjusted pvalues in the polar object.</li> <li>• polar_multi_pvalue and polar_multi_padj for the pvalues and adjusted pvalues across all groups using the polar@multi_group_test columns.</li> <li>• <a href="#">t.test</a> (parametric) and <a href="#">wilcox.test</a> (non-parametric). Perform comparison between groups of samples.</li> <li>• <a href="#">anova</a> (parametric) and <a href="#">kruskal.test</a> (non-parametric). Perform one-way ANOVA test comparing multiple groups.</li> </ul>
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 <a href="#">stat_compare_means</a> . 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 <a href="#">stat_compare_means</a>

**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
- ‘max\_exp’: 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,
    cutoff_criteria = "pvalue"
)

```

### 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"> <li>• three 'p_col_suffix' columns: one for the pvalue for each comparison between groups;</li> <li>• three optional 'fc_col_suffix' columns for the fold change between each comparison (if NULL, no Fold Change columns are included);</li> <li>• three optional 'padj_col_suffix' columns (if NULL adjusted p values are calculated using 'padjust_method');</li> <li>• and optional 'p', 'padj' and 'fc' columns for a three-way test, such as ANOVA or likelihood ratio test, defined by 'multi_group_prefix'.</li> </ul>
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 <a href="#">p.adjust</a> . If NULL no adjusted pvalue is calculated.
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.

`cutoff_criteria` Whether to use pvalue or padj for the colour coding significance cutoff.

## 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
  - 'max\_exp': 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 <a href="#">pretty</a> . 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 <a href="#">pretty</a> . 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 <a href="#">pretty</a> 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(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=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

<code>polar</code>	A polar object with the pvalues between groups of interest and polar coordinates. Created by <a href="#">polar_coords</a> .
<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 (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).
<code>label_rows</code>	A vector of row names or numbers to label.
<code>arrow_length</code>	length of label arrow (default = 1).
<code>grid</code>	An optional grid object. If NULL this will be calculated using default values of <a href="#">polar_grid</a> .
<code>fc_or_zscore</code>	Whether to use the z-score or fold change as magnitude. Options are 'zscore' (default) or 'fc'.
<code>label_size</code>	Font size of labels/annotations (default = 5).
<code>colour_code_labels</code>	Logical whether label annotations should be colour coded. If FALSE <code>label_colour</code> is used.
<code>label_colour</code>	Colour of annotation labels if not colour coded.
<code>grid_colour</code>	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 <a href="#">polar_grid</a> .

### 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(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_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,
  hover_text = "label",
  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 <a href="#">polar_coords</a> .
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 <a href="#">hsv</a> 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 <a href="#">polar_grid</a> .
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 = 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.
hover_text	A character string containing the argument for hover text (default="label"). Possible columns include: <ul style="list-style-type: none"> <li>• "Name" or "label": name and label column for each marker</li> <li>• paste0(group, "_axis") the position for each marker on a given axis</li> <li>• "x_zscore", "y_zscore" or "r_zscore": The position according to z-score</li> <li>• "x_fc", "y_fc" or "r_fc": The position according to fold change</li> <li>• "angle", "angle_degrees": Then marker angle</li> <li>• "max_exp" or "sig": The maximally expressed group or significant group</li> <li>• "col" or "hue": The colour or hue of the marker</li> <li>• paste0(group A, "_", group B, "_pvalue"): The pvalue for comparisons</li> <li>• paste0(group A, "_", group B, "_padj"): The pvalue for comparisons</li> <li>• paste0(group A, "_", group B, "_logFC"): The pvalue for comparisons</li> <li>• paste0(multi_group_test, "_pvalue"), paste0(multi_group_test, "_padj"), paste0(multi_group_test, "_logFC"): The stats for all multi-group tests.</li> </ul>
grid_colour	The colour of the grid (default="grey80").
axis_colour	The colour of the grid axes and labels (default="black").
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_ticks	A numerical vector of radial axis tick breaks. If NULL this will be calculated using <a href="#">pretty</a> .
axis_angle	Angle in radians for the radial axis (default = 5/6).
plot_height	Plot height in px (default=700).
plot_width	Plot width in px (default=700).
...	Optional parameters to pass to <a href="#">polar_grid</a> .

**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(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_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
```

---

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 <a href="#">polar_coords</a> .
significance	Which significance factors to subset to. If NULL levels(syn_polar@polar\$sig)[1] is selected.
output	What object to return. Options are "pvalues", "expression", "polar_df" for subset data frames or "polar" for subset polar 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(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)

subset <- significance_subset(syn_polar, "Lymphoid+", "polar_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 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	<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 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	<i>Three-Dimensional Volcano Plot</i>
-----------	---------------------------------------

---

**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,
  axis_title_offset = 1.2,
  label_rows = c(),
  grid = NULL,
  fc_or_zscore = "zscore",
  label_size = 14,
  arrow_length = 50,
  colour_code_labels = TRUE,
  label_colour = NULL,
  hover_text = "label",
  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

polar	A polar object with created by <a href="#">polar_coords</a> .
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 to offset the continuous colour scale by. This is calculated by converting the angle to a hue using <a href="#">hsv</a> where 0 corresponds to the colour scale starting with red and 2 with magenta (default = 2).
axis_title_offset	The position scaling between grid and axis titles (default=1.2)
label_rows	A vector of row names or numbers to label.
grid	An optional grid object. If NULL this will be calculated using default values of <a href="#">polar_grid</a> .
fc_or_zscore	whether to use fold change or z-score for the p-values. Options are 'zscore' (default) or 'fc'.
label_size	font size for labels (default = 14).
arrow_length	The length of label arrows (default = 50).
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.
hover_text	A character string containing the argument for hover text (default="label"). Possible columns include: <ul style="list-style-type: none"> <li>"Name" or "label": name and label column for each marker</li> <li>paste(group, "_axis") the position for each marker on a given axis</li> <li>"x_zscore", "y_zscore" or "r_zscore": The position according to z-score</li> <li>"x_fc", "y_fc" or "r_fc": The position according to fold change</li> <li>"angle", "angle_degrees": Then marker angle</li> <li>"max_exp" or "sig": The maximally expressed group or significant group</li> </ul>



```
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",
  cutoff_criteria = "pvalue",
  label_col = "label",
  label_size = 3,
  text_size = 10,
  marker_alpha = 0.7,
  marker_size = 3,
  marker_outline_colour = "white",
  marker_outline_width = 0.5,
  sig_names = NULL,
  colour_col = FALSE,
  colours = c("salmon", "steelblue", "limegreen", "grey60"),
  drop_unused_cols = TRUE,
  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 adjusted 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').
cutoff_criteria	Whether to use pvalue or padj for the colour coding significance cutoff.
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_alpha	The alpha parameter for markers (default = 0.7).
marker_size	The size of markers (default = 3)
marker_outline_colour	Colour for marker outline (default = white)
marker_outline_width	Width for marker outline (default = 0.5)
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 c('Not Significant', paste('Padj <', p_cutoff), paste(' FC  >', fc_cutoff), paste('Padj <', p_cutoff, ' and  FC  >', fc_cutoff)) is used.
colour_col	Logical whether colour coding has been passed in through 'pvalues_df\$col'.
colours	A character vector of colours to be used. This can be of length 3, 4, 7 or 8 depending on the colour coding desired. <ul style="list-style-type: none"> <li>• If length is 3, c(a,b,c): Only the significant wings are highlighted (where <math>p &gt; p\_cutoff</math> and <math>abs(fc) &gt; fc\_cutoff</math>) on the graph: <ul style="list-style-type: none"> <li>– a: <math>padj \leq p\_cutoff \ \&amp; \ fc \leq -1 * fc\_cutoff</math></li> <li>– b: <math>padj \leq p\_cutoff \ \&amp; \ fc \geq fc\_cutoff</math></li> <li>– c: <math>padj &gt; p\_cutoff \   \ abs(fc) &lt; fc\_cutoff</math></li> </ul> </li> <li>• If length is 4, c(a,b,c,d): <ul style="list-style-type: none"> <li>– a: <math>padj \leq p\_cutoff \ \&amp; \ abs(fc) \geq fc\_cutoff</math></li> <li>– b: <math>padj \leq p\_cutoff \ \&amp; \ abs(fc) &lt; fc\_cutoff</math></li> <li>– c: <math>padj &gt; p\_cutoff \ \&amp; \ abs(fc) \geq fc\_cutoff</math></li> <li>– d: <math>padj &lt; p\_cutoff \ \&amp; \ abs(fc) &lt; fc\_cutoff</math></li> </ul> </li> <li>• If length is 8 c(a,b,c,d,e,f,g,h): Each significance group is colour-coded <ul style="list-style-type: none"> <li>– a: <math>padj \leq p\_cutoff \ \&amp; \ fc \leq -1 * fc\_cutoff</math></li> <li>– b: <math>padj \leq p\_cutoff \ \&amp; \ -1 * fc\_cutoff &lt; fc \leq 0</math></li> </ul> </li> </ul>

- c:  $\text{padj} \leq \text{p\_cutoff} \ \& \ 0 < \text{fc} < \text{fc\_cutoff}$
- d:  $\text{padj} \leq \text{p\_cutoff} \ \& \ \text{fc} \geq \text{fc\_cutoff}$
- e:  $\text{padj} > \text{p\_cutoff} \ \& \ \text{fc} \leq -1 * \text{fc\_cutoff}$
- f:  $\text{padj} > \text{p\_cutoff} \ \& \ -1 * \text{fc\_cutoff} < \text{fc} \leq 0$
- g:  $\text{padj} > \text{p\_cutoff} \ \& \ 0 < \text{fc} < \text{fc\_cutoff}$
- h:  $\text{padj} > \text{p\_cutoff} \ \& \ \text{fc} \geq \text{fc\_cutoff}$

drop\_unused\_cols Logical whether to drop colours not used from legend (default=T).

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')).

### 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,
  cutoff_criteria = "pvalue",
  fc_cutoff = 1,
  label_rows = NULL,
```

```

label_size = 3,
text_size = 10,
marker_alpha = 0.7,
marker_size = 3,
marker_outline_colour = "white",
marker_outline_width = 0.5,
shared_legend_size = 1,
sig_names = NULL,
colour_scheme = "none",
colours = c("salmon", "steelblue", "limegreen", "grey60"),
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 <a href="#">polar_coords</a> .
p_cutoff	The cut-off for adjusted pvalue significance (default = 0.05).
cutoff_criteria	Whether to use pvalue or padj for the colour coding significance cutoff.
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_alpha	The alpha parameter for markers (default = 0.7).
marker_size	The size of markers (default = 3)
marker_outline_colour	Colour for marker outline (default = white)
marker_outline_width	Width for marker outline (default = 0.5)
shared_legend_size	The size for the legend (default = 1).
sig_names	A character vector of labels to be used for colour coding. If NULL c('Not Significant', paste('Padj <', `p_cutoff`), paste(' FC  >', `fc_cutoff`), paste('Padj <', `p_cutoff`, 'and  FC  >', `fc_cutoff`)) is used.
colour_scheme	How to factor the colour scheme. Colour by "none" for significance group (by p and fold change cut-off), "upregulated" colour-coded according to the upregulated groups, or "polar" for the significance group.
colours	A character vector of colours to be used. This can be of length 3, 4, 7 or 8 depending on the colour coding desired. <ul style="list-style-type: none"> <li>If length is 3, c(a,b,c): Only the significant wings are highlighted (where <math>p &gt; p\_cutoff</math> and <math>abs(fc) &gt; fc\_cutoff</math>) on the graph: <ul style="list-style-type: none"> <li>a: <math>padj \leq p\_cutoff \ \&amp; \ fc \leq -1 * fc\_cutoff</math></li> </ul> </li> </ul>



- b:  $\text{padj} \leq \text{p\_cutoff} \ \& \ \text{fc} \geq \text{fc\_cutoff}$
- c:  $\text{padj} > \text{p\_cutoff} \ \vee \ \text{abs}(\text{fc}) < \text{fc\_cutoff}$
- If length is 4, c(a,b,c,d):
  - a:  $\text{padj} \leq \text{p\_cutoff} \ \& \ \text{abs}(\text{fc}) \geq \text{fc\_cutoff}$
  - b:  $\text{padj} \leq \text{p\_cutoff} \ \& \ \text{abs}(\text{fc}) < \text{fc\_cutoff}$
  - c:  $\text{padj} > \text{p\_cutoff} \ \& \ \text{abs}(\text{fc}) \geq \text{fc\_cutoff}$
  - d:  $\text{padj} < \text{p\_cutoff} \ \& \ \text{abs}(\text{fc}) < \text{fc\_cutoff}$
- If length is 7 the 'polar@polar\$sig' is used to colour code the probes. 'colour\_scheme' must be set to 'polar'
- If length is 8 c(a,b,c,d,e,f,g,h): Each significance group is colour-coded
  - a:  $\text{padj} \leq \text{p\_cutoff} \ \& \ \text{fc} \leq -1 * \text{fc\_cutoff}$
  - b:  $\text{padj} \leq \text{p\_cutoff} \ \& \ -1 * \text{fc\_cutoff} < \text{fc} \leq 0$
  - c:  $\text{padj} \leq \text{p\_cutoff} \ \& \ 0 < \text{fc} < \text{fc\_cutoff}$
  - d:  $\text{padj} \leq \text{p\_cutoff} \ \& \ \text{fc} \geq \text{fc\_cutoff}$
  - e:  $\text{padj} > \text{p\_cutoff} \ \& \ \text{fc} \leq -1 * \text{fc\_cutoff}$
  - f:  $\text{padj} > \text{p\_cutoff} \ \& \ -1 * \text{fc\_cutoff} < \text{fc} \leq 0$
  - g:  $\text{padj} > \text{p\_cutoff} \ \& \ 0 < \text{fc} < \text{fc\_cutoff}$
  - h:  $\text{padj} > \text{p\_cutoff} \ \& \ \text{fc} \geq \text{fc\_cutoff}$

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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