# Package 'tidytof'

January 2, 2025

```
Principles
Version 1.1.0
Description This package implements an interactive, scientific analysis
      pipeline for high-dimensional cytometry data built using tidy data principles.
      It is specifically designed to play well with both the tidyverse and
      Bioconductor software ecosystems, with functionality for reading/writing
      data files, data cleaning, preprocessing, clustering,
      visualization, modeling, and other quality-of-life functions. tidytof
      implements a ``grammar" of high-dimensional cytometry data analysis.
License MIT + file LICENSE
Depends R (>= 4.3)
Imports doParallel, dplyr, flowCore, foreach, ggplot2, ggraph, glmnet,
      methods, parallel, purrr, readr, recipes, rlang, stringr,
      survival, tidygraph, tidyr, tidyselect, yardstick, Rcpp,
      tibble, stats, utils, RcppHNSW
Suggests ConsensusClusterPlus, Biobase, broom, covr, diffcyt, emdist,
      FlowSOM, forcats, ggrepel, HDCytoData, knitr, markdown,
      philentropy, rmarkdown, Rtsne, statmod, SummarizedExperiment,
```

Title Analyze High-dimensional Cytometry Data Using Tidy Data

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BugReports https://github.com/keyes-timothy/tidytof/issues

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# Description

Coerce an object into a flowFrame Coerce a tof\_tbl into a flowFrame

# Usage

```
as_flowFrame(x, ...)
## S3 method for class 'tof_tbl'
as_flowFrame(x, ...)
```

# Arguments

A tof\_tbl. Unused. . . .

# Value

A flowFrame

A flowFrame. Note that all non-numeric columns in 'x' will be removed.

# Examples

NULL

NULL

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as\_flowSet

Coerce an object into a flowSet

# Description

```
Coerce an object into a flowSet
Coerce a tof_tbl into a flowSet
```

# Usage

```
as_flowSet(x, ...)
## S3 method for class 'tof_tbl'
as_flowSet(x, group_cols, ...)
```

# **Arguments**

x A tof\_tbl.
... Unused.

group\_cols Unc

Unquoted names of the columns in 'x' that should be used to group cells into separate flowFrames. Supports tidyselect helpers. Defaults to NULL (all cells are written into a single flowFrame).

### Value

A flowSet

A flowSet. Note that all non-numeric columns in 'x' will be removed.

# **Examples**

NULL

NULL

as\_seurat

Coerce an object into a SeuratObject

# Description

Coerce an object into a SeuratObject

Coerce a tof\_tbl into a SeuratObject

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# Usage

```
as_seurat(x, ...)
## S3 method for class 'tof_tbl'
as_seurat(
    x,
    channel_cols = where(tof_is_numeric),
    reduced_dimensions_cols,
    metadata_cols = where(function(.x) !tof_is_numeric(.x)),
    split_reduced_dimensions = FALSE,
    ...
)
```

#### **Arguments**

x A tof\_tbl

... Unused.

channel\_cols Unquoted column names representing columns that contain single-cell protein measurements. Supports tidyselect helpers. If nothing is specified, the default is all numeric columns.

reduced\_dimensions\_cols

Unquoted column names representing columns that contain dimensionality reduction embeddings, such as tSNE or UMAP embeddings. Supports tidyselect helpers.

metadata\_cols

Unquoted column names representing columns that contain metadata about the samples from which each cell was collected. If nothing is specified, the default is all non-numeric columns.

split\_reduced\_dimensions

A boolean value indicating whether the dimensionality results in x should be split into separate slots in the resulting SingleCellExperiment. If FALSE (the default), the split will not be performed and the reducedDims slot in the result will have a single entry ("tidytof\_reduced\_dimensions"). If TRUE, the split will be performed and the reducedDims slot in the result will have 1-4 entries depending on which dimensionality reduction results are present in x ("tidytof\_pca", "tidytof\_tsne", "tidytof\_umap", and "tidytof\_reduced\_dimensions"). Note that "tidytof\_reduced\_dimensions" will include all dimensionality reduction results that are not named according to tidytof's pca, umap, and tsne conventions.

### Value

A SeuratObject A SeuratObject.

### **Examples**

NULL

NULL

```
as_SingleCellExperiment
```

Coerce an object into a SingleCellExperiment

### **Description**

Coerce an object into a SingleCellExperiment Coerce a tof\_tbl into a SingleCellExperiment

### Usage

```
as_SingleCellExperiment(x, ...)
## S3 method for class 'tof_tbl'
as_SingleCellExperiment(
    x,
    channel_cols = where(tof_is_numeric),
    reduced_dimensions_cols,
    metadata_cols = where(function(.x) !tof_is_numeric(.x)),
    split_reduced_dimensions = FALSE,
    ...
)
```

### Arguments

x A tof\_tbl
... Unused.

channel\_cols Unquoted column names representing columns that contain single-cell protein measurements. Supports tidyselect helpers. If nothing is specified, the default is all numeric columns.

reduced\_dimensions\_cols

Unquoted column names representing columns that contain dimensionality reduction embeddings, such as tSNE or UMAP embeddings. Supports tidyselect helpers.

metadata\_cols Unquoted column names representing columns that contain metadata about the samples from which each cell was collected. If nothing is specified, the default is all non-numeric columns.

split\_reduced\_dimensions

A boolean value indicating whether the dimensionality results in x should be split into separate slots in the resulting SingleCellExperiment. If FALSE (the default), the split will not be performed and the reducedDims slot in the result will have a single entry ("tidytof\_reduced\_dimensions"). If TRUE, the

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split will be performed and the reducedDims slot in the result will have 1-4 entries depending on which dimensionality reduction results are present in x ("tidytof\_pca", "tidytof\_tsne", "tidytof\_umap", and "tidytof\_reduced\_dimensions"). Note that "tidytof\_reduced\_dimensions" will include all dimensionality reduction results that are not named according to tidytof's pca, umap, and tsne conventions.

### Value

```
A SingleCellExperiment A SingleCellExperiment.
```

# **Examples**

NULL

NULL

as\_tof\_tbl

Coerce flowFrames or flowSets into tof\_tbl's.

### **Description**

Coerce flowFrames or flowSets into tof\_tbl's.

#### Usage

```
as_tof_tbl(flow_data, sep = "|")
```

# **Arguments**

flow\_data A flowFrame or flowSet

sep A string indicating which symbol should be used to separate antigen names and

metal names in the columns of the output tof\_tbl.

# Value

A tof\_tbl.

# **Examples**

```
input_file <- dir(tidytof_example_data("aml"), full.names = TRUE)[[1]]
input_flowframe <- flowCore::read.FCS(input_file)
tof_tibble <- as_tof_tbl(input_flowframe)</pre>
```

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as\_tof\_tbl.flowSet

Convert an object into a tof\_tbl

# Description

Convert an object into a tof\_tbl

### Usage

```
## S3 method for class 'flowSet'
as_tof_tbl(flow_data, sep = "|")
```

# Arguments

flow\_data A FlowSet

sep A string to use to separate the antigen name and its associated metal in the

column names of the output tibble. Defaults to "I".

#### Value

```
a 'tof_tbl'
```

cosine\_similarity

Find the cosine similarity between two vectors

# **Description**

Find the cosine similarity between two vectors

### Usage

```
cosine\_similarity(x, y)
```

# **Arguments**

x a numeric vector y a numeric vector

# Value

a scalar value representing the cosine similarity between x and y

ddpr\_data 11

ddpr_data	CyTOF data from two samples: 5,000 B-cell lineage cells from a healthy patient and 5,000 B-cell lineage cells from a B-cell precur-
	sor Acute Lymphoblastic Leukemia (BCP-ALL) patient.

# **Description**

A dataset containing CyTOF measurements from immune cells originally studied in the following paper:

Good Z, Sarno J, et al. Single-cell developmental classification of B cell precursor acute lymphoblastic leukemia at diagnosis reveals predictors of relapse. Nat Med. 2018 May;24(4):474-483. doi: 10.1038/nm.4505. Epub 2018 Mar 5. PMID: 29505032; PMCID: PMC5953207.

# Usage

```
data(ddpr_data)
```

#### **Format**

A data frame with 10000 rows and 24 variables:

sample\_name name of the sample from which the data was read

cd45 A CyTOF measurement in raw ion counts

cd19 A CyTOF measurement in raw ion counts

cd22 A CyTOF measurement in raw ion counts

cd79b A CyTOF measurement in raw ion counts

cd20 A CyTOF measurement in raw ion counts

cd34 A CyTOF measurement in raw ion counts

cd123 A CyTOF measurement in raw ion counts

cd10 A CyTOF measurement in raw ion counts

cd24 A CyTOF measurement in raw ion counts

cd127 A CyTOF measurement in raw ion counts

cd43 A CyTOF measurement in raw ion counts

cd38 A CyTOF measurement in raw ion counts

cd58 A CyTOF measurement in raw ion counts

psyk A CyTOF measurement in raw ion counts

p4ebp1 A CyTOF measurement in raw ion counts

pstat5 A CyTOF measurement in raw ion counts

pakt A CyTOF measurement in raw ion counts

**ps6** A CyTOF measurement in raw ion counts

perk A CyTOF measurement in raw ion counts

pcreb A CyTOF measurement in raw ion counts

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#### Value

A data.frame

#### **Source**

https://github.com/kara-davis-lab/DDPR

ddpr\_metadata Clinical metadata for each patient sample in Good & Sarno et al. (2018).

# **Description**

A dataset containing patient-level clinical metadata for samples originally studied in the following paper:

Good Z, Sarno J, et al. Single-cell developmental classification of B cell precursor acute lymphoblastic leukemia at diagnosis reveals predictors of relapse. Nat Med. 2018 May;24(4):474-483. doi: 10.1038/nm.4505. Epub 2018 Mar 5. PMID: 29505032; PMCID: PMC5953207.

#### Usage

data(ddpr\_metadata)

#### **Format**

A data frame with 10000 rows and 12 variables:

patient id Name of the sample from which the data was read

gender Gender of the patient from which each sample was collected

age\_at\_diagnosis Age (in years) of the patient from which each sample was collected

wbc\_count The diagnostic White Blood Cell (WBC) count of the patient from which each sample was collected

mrd\_risk Risk stratification category for each patient using minimal residual disease (MRD) criteria

nci\_rome\_risk Risk stratification category for each patient using National Cancer Institute (NCI) criteria

relapse\_status A string representing whether or not a patient relapsed

time\_to\_relapse The time (in days) it took each patient to relapse. Patients who did not relapse will have the value of NA

**type\_of\_relapse** A string representing the timing of relapse for each patient. "Very early" relapses occurred less than 18 months after diagnosis; "Early" relapses occurred between 18 months and 32 months after diagnosis; "Late" relapses occurred later than 32 months after diagnosis.

**ccr** The number of documented days of continuous complete remission (CCR) for patients who did not relapse. All patients who relapsed will have a value of NA.

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**cohort** A string representing if each sample was used in the "Training" or "Validation" cohort in the original study

**ddpr\_risk** The risk category ("Low" or "High") assigned to each sample using the original paper's risk-stratification algorithm

### Value

A data.frame

#### **Source**

Good Z, Sarno J, et al. Single-cell developmental classification of B cell precursor acute lymphoblastic leukemia at diagnosis reveals predictors of relapse. Nat Med. 2018 May;24(4):474-483. doi: 10.1038/nm.4505. Epub 2018 Mar 5. PMID: 29505032; PMCID: PMC5953207. Supplementary Table 1.

dot

Find the dot product between two vectors.

# **Description**

Find the dot product between two vectors.

# Usage

dot(x, y)

# **Arguments**

x A numeric vector.

y A numeric vector.

# Value

The dot product between x and y.

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get\_extension

Find the extension for a file

# Description

Find the extension for a file

# Usage

```
get_extension(filename)
```

# **Arguments**

filename

A string representing the name of a file in its local directory

### Value

The the file extension of 'filename'

12\_normalize

L2 normalize an input vector x to a length of 1

# Description

L2 normalize an input vector x to a length of 1

# Usage

```
12_normalize(x)
```

# Arguments

Х

a numeric vector

# Value

a vector of length length(x) with a magnitude of 1

magnitude 15

magnitude

Find the magnitude of a vector.

# Description

Find the magnitude of a vector.

# Usage

```
magnitude(x)
```

# Arguments

Х

A numeric vector.

### Value

A scalar value (the magnitude of x).

make\_flowcore\_annotated\_data\_frame

Make the AnnotatedDataFrame needed for the flowFrame class

# Description

Make the AnnotatedDataFrame needed for the flowFrame class

# Usage

```
make_flowcore_annotated_data_frame(maxes_and_mins)
```

# Arguments

maxes\_and\_mins a data.frame containing information about the max and min values of each channel to be saved in the flowFrame.

### Value

An AnnotatedDataFrame.

# **Examples**

NULL

new\_tof\_model

metal\_masterlist

A character vector of metal name patterns supported by tidytof.

### **Description**

A character vector used by 'tof\_read\_fcs' and 'tof\_read\_data' to detect and parse which CyTOF metals correspond to each channel in an input .fcs file.

# Usage

```
data(metal_masterlist)
```

#### **Format**

A character vector in which each entry is a pattern that tidytof searches for in every CyTOF channel in input .fcs files. These patterns are an amalgamate of example .fcs files sampled from the studies linked below.

#### Value

A named character vector.

#### Source

https://github.com/kara-davis-lab/DDPR https://cytobank.org/nolanlab/reports/Levine2015.html https://cytobank.org/nolanlab/reports/Spitzer2015.html https://cytobank.org/nolanlab/reports/Spitzer2017.html https://community.cytobank.org/cytobank/projects/609

new\_tof\_model

Constructor for a tof\_model.

# Description

Constructor for a tof\_model.

# Usage

```
new_tof_model(
  model,
  recipe,
  penalty,
  mixture,
  model_type = c("linear", "two-class", "multiclass", "survival"),
  outcome_colnames,
  training_data
)
```

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### **Arguments**

model A glmnet model.

recipe A prepped recipe object.

penalty A double indicating which lambda value should be used within the glmnet path.

mixture A double indicating which alpha value was used to fit the glmnet model.

model\_type A string indicating which type of glmnet model is being fit.

outcome\_colnames

TO DO

training\_data TO DO

### Value

A 'tof\_model', an S3 class that includes a trained glmnet model and the recipe used to perform its associated preprocessing.

new\_tof\_tibble

Constructor for a tof\_tibble.

### **Description**

Constructor for a tof\_tibble.

# Usage

```
new_tof_tibble(x = dplyr::tibble(), panel = dplyr::tibble())
```

# **Arguments**

x A data frame or tibble containing single-cell mass cytometry data such that rows

are cells and columns are CyTOF measurements.

panel A data.frame or tibble containing information about the panel for the mass cy-

tometry data in x.

# Value

A 'tof\_tbl', an tibble extension that tracks a few other attributes that are useful for CyTOF data analysis.

### See Also

```
Other tof_tbl utilities: tof_get_panel(), tof_set_panel()
```

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phenograph\_data

CyTOF data from 6,000 healthy immune cells from a single patient.

#### **Description**

A dataset containing CyTOF measurements from healthy control cells originally studied in the following paper:

Levine JH, Simonds EF, et al. Data-Driven Phenotypic Dissection of AML Reveals Progenitor-like Cells that Correlate with Prognosis. Cell. 2015 Jul 2;162(1):184-97. doi: 10.1016/j.cell.2015.05.047. Epub 2015 Jun 18. PMID: 26095251; PMCID: PMC4508757.

### Usage

data(phenograph\_data)

#### **Format**

A data frame with 6000 rows and 26 variables:

sample\_name Name of the sample from which the data was read

phenograph\_cluster Numeric ID of the cluster assignment of each row

cd19 A CyTOF measurement in raw ion counts

cd11b A CyTOF measurement in raw ion counts

cd34 A CyTOF measurement in raw ion counts

cd45 A CyTOF measurement in raw ion counts

cd123 A CyTOF measurement in raw ion counts

cd33 A CyTOF measurement in raw ion counts

cd47 A CyTOF measurement in raw ion counts

cd7 A CyTOF measurement in raw ion counts

cd44 A CyTOF measurement in raw ion counts

cd38 A CyTOF measurement in raw ion counts

cd3 A CyTOF measurement in raw ion counts

cd117 A CyTOF measurement in raw ion counts

cd64 A CyTOF measurement in raw ion counts

cd41 A CyTOF measurement in raw ion counts

pstat3 A CyTOF measurement in raw ion counts

pstat5 A CyTOF measurement in raw ion counts

pampk A CyTOF measurement in raw ion counts

p4ebp1 A CyTOF measurement in raw ion counts

**ps6** A CyTOF measurement in raw ion counts

reexports 19

```
pcreb A CyTOF measurement in raw ion counts
pzap70-syk A CyTOF measurement in raw ion counts
prb A CyTOF measurement in raw ion counts
perk1-2 A CyTOF measurement in raw ion counts
```

### **Details**

2000 cells from 3 clusters identified in the original paper have been sampled.

### Value

A data.frame

#### **Source**

```
https://cytobank.org/nolanlab/reports/Levine2015.html
```

reexports

Objects exported from other packages

# **Description**

These objects are imported from other packages. Follow the links below to see their documentation.

### Value

See documentation in each object's original package.

# **Examples**

```
\# See examples in each object's original package NULL
```

rev_asinh	Reverses arcsinh transformation with cofactor 'scale_factor' and a shift of 'shift_factor'.

# Description

Reverses arcsinh transformation with cofactor 'scale\_factor' and a shift of 'shift\_factor'.

# Usage

```
rev_asinh(x, shift_factor, scale_factor)
```

# **Arguments**

x	A numeric vector.
shift_factor	The scalar value 'a' in the following equation used to transform high-dimensional cytometry raw data ion counts using the hyperbolic arcsinh function: 'new_x <- $asinh(a + b * x)$ '.
scale_factor	The scalar value 'b' in the following equation used to transform high-dimensional cytometry raw data ion counts using the hyperbolic arcsinh function: 'new_x <- $asinh(a + b * x)$ '.

### Value

A numeric vector after undergoing reverse arcsinh transformation

# **Examples**

```
shift_factor <- 0
scale_factor <- 1 / 5
input_value <- 20
asinh_value <- asinh(shift_factor + input_value * scale_factor)
restored_value <- rev_asinh(asinh_value, shift_factor, scale_factor)</pre>
```

# **Description**

tidytof comes bundled with a number of sample .fcs files in its inst/extdata directory. This function makes them easy to access.

tof\_analyze\_abundance 21

#### Usage

```
tidytof_example_data(dataset_name = NULL)
```

### **Arguments**

(each of which is from a different study) will be listed.

#### Value

A character vector of file paths where the requested .fcs files are located. If 'dataset\_name' is NULL, a character vector of dataset names (that can be used as values for 'dataset\_name') is returned instead.

### **Examples**

```
tidytof_example_data()
tidytof_example_data(dataset_name = "phenograph")
```

 $\begin{tabular}{ll} to f\_analyze\_abundance & \textit{Perform Differential Abundance Analysis (DAA) on high-dimensional} \\ & cytometry & data \end{tabular}$ 

### **Description**

This function performs differential abundance analysis on the cell clusters contained within a 'tof\_tbl' using one of three methods ("diffcyt", "glmm", and "ttest"). It wraps the members of the 'tof\_analyze\_abundance\_\*' function family: tof\_analyze\_abundance\_diffcyt, tof\_analyze\_abundance\_glmm, and tof\_analyze\_abundance\_ttes

### Usage

```
tof_analyze_abundance(tof_tibble, method = c("diffcyt", "glmm", "ttest"), ...)
```

# Arguments

tof\_tibble A 'tof tbl' or a 'tibble'.

method A string indicating which statistical method should be used. Valid values include

"diffcyt", "glmm", and "ttest".

... Additional arguments to pass onto the 'tof\_analyze\_abundance\_\*' function fam-

ily member corresponding to the chosen method.

### Value

A tibble or nested tibble containing the differential abundance results from the chosen method. See tof\_analyze\_abundance\_diffcyt, tof\_analyze\_abundance\_glmm, and tof\_analyze\_abundance\_ttest for details.

### See Also

Other differential abundance analysis functions: tof\_analyze\_abundance\_diffcyt(), tof\_analyze\_abundance\_glmm(), tof\_analyze\_abundance\_ttest()

# **Examples**

```
\mbox{\# For differential discovery examples, please see the package vignettes <math display="inline">\mbox{\scriptsize NULL}
```

```
tof\_analyze\_abundance\_diffcyt\\ Differential\ Abundance\ Analysis\ (DAA)\ with\ diffcyt
```

# **Description**

This function performs differential abundance analysis on the cell clusters contained within a 'tof\_tbl' using one of three methods implemented in the diffcyt package for differential discovery analysis in high-dimensional cytometry data.

### Usage

```
tof_analyze_abundance_diffcyt(
  tof_tibble,
  sample_col,
  cluster_col,
  fixed_effect_cols,
  random_effect_cols,
  diffcyt_method = c("glmm", "edgeR", "voom"),
  include_observation_level_random_effects = FALSE,
  min_cells = 3,
  min_samples = 5,
  alpha = 0.05,
  ...
)
```

### **Arguments**

sample\_col

```
tof_tibble A 'tof_tbl' or a 'tibble'.
```

An unquoted column name indicating which column in 'tof\_tibble' represents the id of the sample from which each cell was collected. 'sample\_col' should serve as a unique identifier for each sample collected during data acquisition - all cells with the same value for 'sample\_col' will be treated as a part of the same observational unit.

cluster\_col

An unquoted column name indicating which column in 'tof\_tibble' stores the cluster ids of the cluster to which each cell belongs. Cluster labels can be produced via any method the user chooses - including manual gating, any of the functions in the 'tof\_cluster\_\*' function family, or any other method.

fixed\_effect\_cols

Unquoted column names representing which columns in 'tof\_tibble' should be used to model fixed effects during the differential abundance analysis. Generally speaking, fixed effects represent the comparisons of biological interest (often the variables manipulated during experiments), such as treated vs. non-treated, before-treatment vs. after-treatment, or healthy vs. non-healthy.

random\_effect\_cols

Optional. Unquoted column names representing which columns in 'tof\_tibble' should be used to model random effects during the differential abundance analysis. Generally speaking, random effects should represent variables that a researcher wants to control/account for, but that are not necessarily of biological interest. Example random effect variables might include batch id, patient id (in a paired design), or patient age.

Note that without multiple samples at each level of each of the random effect variables, it can be easy to overfit mixed models. For most high-dimensional cytometry experiments, 2 or fewer (and often 0) random effect variables are appropriate.

diffcyt\_method

A string indicating which diffcyt method should be used for the differential abundance analysis. Valid methods include "glmm" (the default), "edgeR", and "voom".

include\_observation\_level\_random\_effects

A boolean value indicating if "observation-level random effects" (OLREs) should be included as random effect terms in a "glmm" differential abundance model. For details about what OLREs are, see the diffcyt paper. Only the "glmm" method can model observation-level random effects, and all other values will ignore this argument (and throw a warning if it is set to TRUE). Defaults to FALSE.

min\_cells

An integer value used to filter clusters out of the differential abundance analysis. Clusters are not included in the differential abundance testing if they do not have at least 'min\_cells' in at least 'min\_samples' samples. Defaults to 3.

min\_samples

An integer value used to filter clusters out of the differential abundance analysis. Clusters are not included in the differential abundance testing if they do not have at least 'min\_cells' in at least 'min\_samples' samples. Defaults to 5.

alpha

A numeric value between 0 and 1 indicating which significance level should be applied to multiple-comparison adjusted p-values during the differential abundance analysis. Defaults to 0.05.

. . .

Optional additional arguments to pass to the under-the-hood diffcyt function being used to perform the differential abundance analysis. See testDA\_GLMM, testDA\_edgeR, and testDA\_voom for details.

#### **Details**

The three methods are based on generalized linear mixed models ("glmm"), edgeR ("edgeR"), and voom ("voom"). While both the "glmm" and "voom" methods can model both fixed effects and

random effects, the "edgeR" method can only model fixed effects.

#### Value

A nested tibble with two columns: 'tested\_effect' and 'daa\_results'.

The first column, 'tested\_effect' is a character vector indicating which term in the differential abundance model was used for significance testing. The values in this row are obtained by pasting together the column names for each fixed effect variable and each of its values. For example, a fixed effect column named 'fixed\_effect' with levels "a", "b", and "c" have two terms in 'tested\_effect': "fixed\_effectb" and "fixed\_effectc" (note that level "a" of fixed\_effect is set as the reference level during dummy coding). These values correspond to the terms in the differential abundance model that represent the difference in cluster abundances between samples with fixed\_effect = "b" and fixed\_effect = "a" and between samples with fixed\_effect = "c" and fixed\_effect = "a", respectively. In addition, the first row in 'tested\_effect' will always represent the "omnibus" test, or the test that there were significant differences between any levels of any fixed effect variable in the model.

The second column, 'daa\_results' is a list of tibbles in which each entry gives the differential abundance results for each tested\_effect. Within each entry of 'daa\_results', you will find several columns including the following: \* 'p\_val', the p-value associated with each tested effect in each input cluster \* 'p\_adj', the multiple-comparison adjusted p-value (using the p.adjust function) \* Other values associated with the underlying method used to perform the differential abundance analysis (such as the log-fold change of cluster abundance between the levels being compared). For details, see glmFit, voom, topTable, and testDA\_GLMM.

### See Also

Other differential abundance analysis functions: tof\_analyze\_abundance(), tof\_analyze\_abundance\_glmm(), tof\_analyze\_abundance\_ttest()

### **Examples**

# For differential discovery examples, please see the package vignettes NULL

tof\_analyze\_abundance\_glmm

Differential Abundance Analysis (DAA) with generalized linear mixed-models (GLMMs)

# **Description**

This function performs differential abundance analysis on the cell clusters contained within a 'tof\_tbl' using generalized linear mixed-models. Users specify which columns represent sample, cluster, fixed effect, and random effect information, and a (mixed) binomial regression model is fit using either glmer or glm.

### Usage

```
tof_analyze_abundance_glmm(
  tof_tibble,
  sample_col,
  cluster_col,
  fixed_effect_cols,
  random_effect_cols,
  min_cells = 3,
  min_samples = 5,
  alpha = 0.05
)
```

# **Arguments**

tof\_tibble

A 'tof tbl' or a 'tibble'.

sample\_col

An unquoted column name indicating which column in 'tof\_tibble' represents the id of the sample from which each cell was collected. 'sample\_col' should serve as a unique identifier for each sample collected during data acquisition - all cells with the same value for 'sample\_col' will be treated as a part of the same observational unit.

cluster col

An unquoted column name indicating which column in 'tof\_tibble' stores the cluster ids of the cluster to which each cell belongs. Cluster labels can be produced via any method the user chooses - including manual gating, any of the functions in the 'tof\_cluster\_\*' function family, or any other method.

fixed\_effect\_cols

Unquoted column names representing which columns in 'tof\_tibble' should be used to model fixed effects during the differential abundance analysis. Supports tidyselect helpers.

Generally speaking, fixed effects should represent the comparisons of biological interest (often the the variables manipulated during experiments), such as treated vs. non-treated, before-treatment vs. after-treatment, or healthy vs. non-healthy.

random\_effect\_cols

Unquoted column names representing which columns in 'tof\_tibble' should be used to model random effects during the differential abundance analysis. Supports tidyselection.

Generally speaking, random effects should represent variables that a researcher wants to control/account for, but that are not necessarily of biological interest. Example random effect variables might include batch id, patient id (in a paired design), or patient age.

Note that without many samples at each level of each of the random effect variables, it can be easy to overfit mixed models. For most high-dimensional cytometry experiments, 2 or fewer (and often 0) random effect variables are appropriate.

min\_cells

An integer value used to filter clusters out of the differential abundance analysis. Clusters are not included in the differential abundance testing if they do not have at least 'min\_cells' in at least 'min\_samples' samples. Defaults to 3.

min\_samples An integer value used to filter clusters out of the differential abundance analysis.

Clusters are not included in the differential abundance testing if they do not have

at least 'min\_cells' in at least 'min\_samples' samples. Defaults to 5.

alpha A numeric value between 0 and 1 indicating which significance level should be

applied to multiple-comparison adjusted p-values during the differential abun-

dance analysis. Defaults to 0.05.

#### Value

A nested tibble with two columns: 'tested effect' and 'daa results'.

The first column, 'tested\_effect', is a character vector indicating which term in the differential abundance model was used for significance testing. The values in this row are obtained by pasting together the column names for each fixed effect variable and each of its values. For example, a fixed effect column named fixed\_effect with levels "a", "b", and "c" have two terms in 'tested\_effect': "fixed\_effectb" and "fixed\_effectc" (note that level "a" of fixed\_effect is set as the reference level during dummy coding). These values correspond to the terms in the differential abundance model that represent the difference in cluster abundances between samples with fixed\_effect = "b" and fixed\_effect = "a" and between samples with fixed\_effect = "c" and fixed\_effect = "a", respectively. In addition, note that the first row in 'tested\_effect' will always represent the "omnibus" test, or the test that there were significant differences between any levels of any fixed effect variable in the model.

The second column, 'daa\_results', is a list of tibbles in which each entry gives the differential abundance results for each tested\_effect. Within each entry of 'daa\_results', you will find 'p\_value', the p-value associated with each tested effect in each input cluster; 'p\_adj', the multiple-comparison adjusted p-value (using the p.adjust function), and other values associated with the underlying method used to perform the differential abundance analysis (such as the log-fold change of cluster abundance between the levels being compared).

#### See Also

Other differential abundance analysis functions: tof\_analyze\_abundance(), tof\_analyze\_abundance\_diffcyt(), tof\_analyze\_abundance\_ttest()

#### **Examples**

 $\ensuremath{\text{\#}}\xspace$  For differential discovery examples, please see the package vignettes NULL

tof\_analyze\_abundance\_ttest

Differential Abundance Analysis (DAA) with t-tests

# **Description**

This function performs differential abundance analysis on the cell clusters contained within a 'tof\_tbl' using simple t-tests. Users specify which columns represent sample, cluster, and effect information, and either a paired or unpaired t-test (one per cluster) is used to detect significant differences between sample types.

# Usage

```
tof_analyze_abundance_ttest(
  tof_tibble,
  cluster_col,
  effect_col,
  group_cols,
  test_type = c("unpaired", "paired"),
  min_cells = 3,
  min_samples = 5,
  alpha = 0.05,
  quiet = FALSE
)
```

# **Arguments**

tof_tibble	A 'tof_tbl' or a 'tibble'.
cluster_col	An unquoted column name indicating which column in 'tof_tibble' stores the cluster ids of the cluster to which each cell belongs. Cluster labels can be produced via any method the user chooses - including manual gating, any of the functions in the 'tof_cluster_*' function family, or any other method.
effect_col	Unquoted column name representing which column in 'tof_tibble' should be used to break samples into groups for the t-test. Should only have 2 unique values.
group_cols	Unquoted names of the columns other than 'effect_col' that should be used to group cells into independent observations. Fills a similar role to 'sample_col' in other 'tof_analyze_abundance_*' functions. For example, if an experiment involves analyzing samples taken from multiple patients at two timepoints (with 'effect_col = timepoint'), then group_cols should be the name of the column representing patient IDs.
test_type	A string indicating whether the t-test should be "unpaired" (the default) or "paired"
min_cells	An integer value used to filter clusters out of the differential abundance analysis. Clusters are not included in the differential abundance testing if they do not have at least 'min_cells' in at least 'min_samples' samples. Defaults to 3.
min_samples	An integer value used to filter clusters out of the differential abundance analysis. Clusters are not included in the differential abundance testing if they do not have at least 'min_cells' in at least 'min_samples' samples. Defaults to 5.
alpha	A numeric value between 0 and 1 indicating which significance level should be applied to multiple-comparison adjusted p-values during the differential abun-

dance analysis. Defaults to 0.05.

quiet

A boolean value indicating whether warnings should be printed. Defaults to 'TRUE'.

#### Value

A tibble with 7 columns:

{cluster\_col} The name/ID of the cluster being tested. Each entry in this column will match a unique value in the input {cluster\_col}.

t The t-statistic computed for each cluster.

df The degrees of freedom used for the t-test for each cluster.

**p\_val** The (unadjusted) p-value for the t-test for each cluster.

 $p\_adj$  The p.adjust-adjusted p-value for the t-test for each cluster.

significant A character vector that will be "\*" for clusters for which p\_adj < alpha and "" otherwise.

**mean\_diff** For an unpaired t-test, the difference between the average proportions of each cluster in the two levels of 'effect\_col'. For a paired t-test, the average difference between the proportions of each cluster in the two levels of 'effect\_col' within a given patient.

mean\_fc For an unpaired t-test, the ratio between the average proportions of each cluster in the two levels of 'effect\_col'. For a paired t-test, the average ratio between the proportions of each cluster in the two levels of 'effect\_col' within a given patient. 0.001 is added to the denominator of the ratio to avoid divide-by-zero errors.

The "levels" attribute of the result indicates the order in which the different levels of the 'effect\_col' were considered. The 'mean\_diff' value for each row of the output is computed by subtracting the second level from the first level, and the 'mean\_fc' value for each row is computed by dividing the first level by the second level.

### See Also

Other differential abundance analysis functions: tof\_analyze\_abundance(), tof\_analyze\_abundance\_diffcyt(), tof\_analyze\_abundance\_glmm()

#### **Examples**

# For differential discovery examples, please see the package vignettes NULL

tof\_analyze\_expression

Perform Differential Expression Analysis (DEA) on high-dimensional cytometry data

### **Description**

This function performs differential expression analysis on the cell clusters contained within a 'tof\_tbl' using one of three methods ("diffcyt", "glmm", and "ttest"). It wraps the members of the 'tof\_analyze\_expression\_\* function family: tof\_analyze\_expression\_diffcyt, tof\_analyze\_expression\_lmm, and tof\_analyze\_expression\_tterms.

### Usage

```
tof_analyze_expression(tof_tibble, method = c("diffcyt", "glmm", "ttest"), ...)
```

### **Arguments**

tof\_tibble A 'tof\_tbl' or a 'tibble'.

method A string indicating which statistical method should be used. Valid values include

"diffcyt", "lmm", and "ttest".

... Additional arguments to pass onto the 'tof\_analyze\_expression\_\*' function fam-

ily member corresponding to the chosen method.

#### Value

A tibble or nested tibble containing the differential abundance results from the chosen method. See tof\_analyze\_expression\_diffcyt, tof\_analyze\_expression\_lmm, and tof\_analyze\_expression\_ttest for details.

#### See Also

Other differential expression analysis functions: tof\_analyze\_expression\_diffcyt(), tof\_analyze\_expression\_lmm() tof\_analyze\_expression\_ttest()

### **Examples**

# For differential discovery examples, please see the package vignettes NULL

```
tof_analyze_expression_diffcyt
```

Differential Expression Analysis (DEA) with diffcyt

# **Description**

This function performs differential expression analysis on the cell clusters contained within a 'tof\_tbl' using one of two methods implemented in the diffcyt package for differential discovery analysis in high-dimensional cytometry data.

### Usage

```
tof_analyze_expression_diffcyt(
  tof_tibble,
  sample_col,
  cluster_col,
  marker_cols = where(tof_is_numeric),
  fixed_effect_cols,
  random_effect_cols,
  diffcyt_method = c("lmm", "limma"),
  include_observation_level_random_effects = FALSE,
  min_cells = 3,
  min_samples = 5,
  alpha = 0.05,
  ...
)
```

### **Arguments**

tof\_tibble

A 'tof\_tbl' or a 'tibble'.

sample\_col

An unquoted column name indicating which column in 'tof\_tibble' represents the id of the sample from which each cell was collected. 'sample\_col' should serve as a unique identifier for each sample collected during data acquisition - all cells with the same value for 'sample\_col' will be treated as a part of the same observational unit.

cluster\_col

An unquoted column name indicating which column in 'tof\_tibble' stores the cluster ids of the cluster to which each cell belongs. Cluster labels can be produced via any method the user chooses - including manual gating, any of the functions in the 'tof\_cluster\_\*' function family, or any other method.

marker\_cols

Unquoted column names representing which columns in 'tof\_tibble' (i.e. which high-dimensional cytometry protein measurements) should be tested for differential expression between levels of the 'fixed\_effect\_cols'. Defaults to all numeric (integer or double) columns. Supports tidyselect helpers.

fixed\_effect\_cols

Unquoted column names representing which columns in 'tof\_tibble' should be used to model fixed effects during the differential expression analysis. Generally speaking, fixed effects represent the comparisons of biological interest (often the the variables manipulated during experiments), such as treated vs. non-treated, before-treatment vs. after-treatment, or healthy vs. non-healthy.

random\_effect\_cols

Unquoted column names representing which columns in 'tof\_tibble' should be used to model random effects during the differential expression analysis. Generally speaking, random effects represent variables that a researcher wants to control/account for, but that are not necessarily of biological interest. Example random effect variables might include batch id, patient id (in a paired design), or patient age.

Note that without many samples at each level of each of the random effect variables, it can be easy to overfit mixed models. For most high-dimensional cytom-

etry experiments, 2 or fewer (and often 0) random effect variables are appropriate

diffcyt\_method A string indicating which diffcyt method should be used for the differential ex-

pression analysis. Valid methods include "lmm" (the default) and "limma".

include\_observation\_level\_random\_effects

A boolean value indicating if "observation-level random effects" (OLREs) should be included as random effect terms in a "lmm" differential expression model. For details about what OLREs are, see the diffcyt paper. Defaults to FALSE.

min\_cells An integer value used to filter clusters out of the differential expression analysis.

Clusters are not included in the differential expression testing if they do not have

at least 'min\_cells' in at least 'min\_samples' samples. Defaults to 3.

min\_samples An integer value used to filter clusters out of the differential expression analysis.

Clusters are not included in the differential expression testing if they do not have

at least 'min\_cells' in at least 'min\_samples' samples. Defaults to 5.

alpha A numeric value between 0 and 1 indicating which significance level should be

applied to multiple-comparison adjusted p-values during the differential abun-

dance analysis. Defaults to 0.05.

... Optional additional arguments to pass to the under-the-hood diffcyt function

being used to perform the differential expression analysis. See  ${\tt testDS\_LMM}$  and

testDS limma for details.

#### **Details**

The two methods are based on linear mixed models ("lmm") and limma ("limma"). Both the "lmm" and "limma" methods can model both fixed effects and random effects.

#### Value

A nested tibble with two columns: 'tested effect' and 'dea results'.

The first column, 'tested\_effect' is a character vector indicating which term in the differential expression model was used for significance testing. The values in this row are obtained by pasting together the column names for each fixed effect variable and each of its values. For example, a fixed effect column named fixed\_effect with levels "a", "b", and "c" have two terms in 'tested\_effect': "fixed\_effectb" and "fixed\_effectc" (note that level "a" of fixed\_effect is set as the reference level during dummy coding). These values correspond to the terms in the differential expression model that represent the difference in cluster median expression values of each marker between samples with fixed\_effect = "b" and fixed\_effect = "a" and between samples with fixed\_effect = "c" and fixed\_effect = "a", respectively. In addition, note that the first row in 'tested\_effect' will always represent the "omnibus" test, or the test that there are significant differences between any levels of any fixed effect variable in the model.

The second column, 'dea\_results' is a list of tibbles in which each entry gives the differential expression results for each tested\_effect. Within each entry of 'dea\_results', you will find 'p\_val', the p-value associated with each tested effect in each input cluster/marker pair; 'p\_adj', the multiple-comparison adjusted p-value (using the p.adjust function), and other values associated with the underlying method used to perform the differential expression analysis (such as the log-fold change of clusters' median marker expression values between the conditions being compared). Each tibble in 'dea\_results' will also have two columns representing the cluster and marker corresponding to the p-value in each row.

### See Also

Other differential expression analysis functions: tof\_analyze\_expression(), tof\_analyze\_expression\_lmm(), tof\_analyze\_expression\_ttest()

# **Examples**

```
\ensuremath{\mathtt{\#}} For differential discovery examples, please see the package vignettes \ensuremath{\mathsf{NULL}}
```

```
\begin{tabular}{ll} to f\_analyze\_expression\_lmm \\ Differential & Expression & Analysis & (DEA) & with & linear & mixed-models \\ & & (LMMs) \end{tabular}
```

# Description

This function performs differential expression analysis on the cell clusters contained within a 'tof\_tbl' using linear mixed-models. Users specify which columns represent sample, cluster, marker, fixed effect, and random effect information, and a (mixed) linear regression model is fit using either lmer or glm.

# Usage

```
tof_analyze_expression_lmm(
  tof_tibble,
  sample_col,
  cluster_col,
  marker_cols = where(tof_is_numeric),
  fixed_effect_cols,
  random_effect_cols,
  central_tendency_function = median,
  min_cells = 3,
  min_samples = 5,
  alpha = 0.05
)
```

# **Arguments**

sample\_col

tof\_tibble A 'tof\_tbl' or a 'tibble'.

An unquoted column name indicating which column in 'tof\_tibble' represents the id of the sample from which each cell was collected. 'sample\_col' should serve as a unique identifier for each sample collected during data acquisition - all cells with the same value for 'sample\_col' will be treated as a part of the same observational unit.

cluster\_col

An unquoted column name indicating which column in 'tof\_tibble' stores the cluster ids of the cluster to which each cell belongs. Cluster labels can be produced via any method the user chooses - including manual gating, any of the functions in the 'tof\_cluster\_\*' function family, or any other method.

marker\_cols

Unquoted column names representing which columns in 'tof\_tibble' (i.e. which high-dimensional cytometry protein measurements) should be included in the differential discovery analysis. Defaults to all numeric (integer or double) columns. Supports tidyselection.

fixed\_effect\_cols

Unquoted column names representing which columns in 'tof\_tibble' should be used to model fixed effects during the differential expression analysis. Supports tidyselection.

Generally speaking, fixed effects should represent the comparisons of biological interest (often the the variables manipulated during experiments), such as treated vs. non-treated, before-treatment vs. after-treatment, or healthy vs. non-healthy.

random\_effect\_cols

Optional. Unquoted column names representing which columns in 'tof\_tibble' should be used to model random effects during the differential expression analysis. Supports tidyselection.

Generally speaking, random effects should represent variables that a researcher wants to control/account for, but that are not necessarily of biological interest. Example random effect variables might include batch id, patient id (in a paired design), or patient age. Most analyses will not include random effects.

central\_tendency\_function

The function that will be used to calculate the measurement of central tendency for each cluster/marker pair (to be used as the dependent variable in the linear model). Defaults to median.

min\_cells

An integer value used to filter clusters out of the differential expression analysis. Clusters are not included in the differential expression testing if they do not have at least 'min\_cells' in at least 'min\_samples' samples. Defaults to 3.

min\_samples

An integer value used to filter clusters out of the differential expression analysis. Clusters are not included in the differential expression testing if they do not have at least 'min\_cells' in at least 'min\_samples' samples. Defaults to 5.

alpha

A numeric value between 0 and 1 indicating which significance level should be applied to multiple-comparison adjusted p-values during the differential abundance analysis. Defaults to 0.05.

#### **Details**

Specifically, one linear model is fit for each cluster/marker pair. For each cluster/marker pair, a user-supplied measurement of central tendency ('central\_tendency\_function'), such as mean or median, is calculated across all cells in the cluster on a sample-by-sample basis. Then, this central tendency value is used as the dependent variable in a linear model with 'fixed\_effect\_cols' as fixed effects predictors and 'random\_effect\_cols' as random effects predictors. Once all models (one per each cluster/marker pair) are fit, p-values for each coefficient in each model are multiple-comparisons adjusted using the p.adjust function.

#### Value

A nested tibble with two columns: 'tested\_effect' and 'dea\_results'.

The first column, 'tested\_effect' is a character vector indicating which term in the differential expression model was used for significance testing. The values in this row are obtained by pasting together the column names for each fixed effect variable and each of its values. For example, a fixed effect column named fixed\_effect with levels "a", "b", and "c" have two terms in 'tested\_effect': "fixed\_effectb" and "fixed\_effectc" (note that level "a" of fixed\_effect is set as the reference level during dummy coding). These values correspond to the terms in the differential expression model that represent the difference in cluster median expression values of each marker between samples with fixed\_effect = "b" and fixed\_effect = "a" and between samples with fixed\_effect = "c" and fixed\_effect = "a", respectively. In addition, note that the first row in 'tested\_effect' will always represent the "omnibus" test, or the test that there were significant differences between any levels of any fixed effect variable in the model.

The second column, 'dea\_results' is a list of tibbles in which each entry gives the differential expression results for each tested\_effect. Within each entry of 'daa\_results', you will find 'p\_val', the p-value associated with each tested effect in each input cluster/marker pair; 'p\_adj', the multiple-comparison adjusted p-value (using the p.adjust function), and other values associated with the underlying method used to perform the differential expression analysis (such as the log-fold change of clusters' median marker expression values between the levels being compared).

### See Also

Other differential expression analysis functions: tof\_analyze\_expression(), tof\_analyze\_expression\_diffcyt(), tof\_analyze\_expression\_ttest()

### **Examples**

 $\ensuremath{\text{\#}}$  For differential discovery examples, please see the package vignettes NULL

tof\_analyze\_expression\_ttest

Differential Expression Analysis (DEA) with t-tests

### **Description**

This function performs differential expression analysis on the cell clusters contained within a 'tof\_tbl' using simple t-tests. Specifically, either an unpaired or paired t-test will compare samples' marker expression distributions (between two conditions) within each cluster using a user-specified summary function (i.e. mean or median). One t-test is conducted per cluster/marker pair and significant differences between sample types are detected after multiple-hypothesis correction.

### Usage

```
tof_analyze_expression_ttest(
  tof_tibble,
  cluster_col,
  marker_cols = where(tof_is_numeric),
  effect_col,
  group_cols,
  test_type = c("unpaired", "paired"),
  summary_function = mean,
  min_cells = 3,
  min_samples = 5,
  alpha = 0.05,
  quiet = FALSE
)
```

### **Arguments**

tof\_tibble

A 'tof\_tbl' or a 'tibble'.

cluster\_col

An unquoted column name indicating which column in 'tof\_tibble' stores the cluster ids of the cluster to which each cell belongs. Cluster labels can be produced via any method the user chooses - including manual gating, any of the functions in the 'tof\_cluster\_\*' function family, or any other method.

marker\_cols

Unquoted column names representing which columns in 'tof\_tibble' (i.e. which high-dimensional cytometry protein measurements) should be tested for differential expression between levels of the 'effect\_col'. Defaults to all numeric (integer or double) columns. Supports tidyselect helpers.

effect\_col

Unquoted column name representing which column in 'tof\_tibble' should be used to break samples into groups for the t-test. Should only have 2 unique values.

group\_cols

Unquoted names of the columns other than 'effect\_col' that should be used to group cells into independent observations. Fills a similar role to 'sample\_col' in other 'tof\_analyze\_abundance\_\*' functions. For example, if an experiment involves analyzing samples taken from multiple patients at two timepoints (with 'effect\_col = timepoint'), then group\_cols should be the name of the column representing patient IDs.

test\_type

A string indicating whether the t-test should be "unpaired" (the default) or "paired".

summary\_function

The vector-valued function that should be used to summarize the distribution of each marker in each cluster (within each sample, as grouped by 'group\_cols'). Defaults to 'mean'.

min\_cells

An integer value used to filter clusters out of the differential abundance analysis. Clusters are not included in the differential abundance testing if they do not have at least 'min\_cells' in at least 'min\_samples' samples. Defaults to 3.

min\_samples

An integer value used to filter clusters out of the differential abundance analysis. Clusters are not included in the differential abundance testing if they do not have at least 'min\_cells' in at least 'min\_samples' samples. Defaults to 5.

alpha A numeric value between 0 and 1 indicating which significance level should be

applied to multiple-comparison adjusted p-values during the differential abun-

dance analysis. Defaults to 0.05.

quiet A boolean value indicating whether warnings should be printed. Defaults to

'TRUE'.

#### Value

A tibble with 7 columns:

{cluster\_col} The name/ID of the cluster in the cluster/marker pair being tested. Each entry in this column will match a unique value in the input {cluster\_col}.

marker The name of the marker in the cluster/marker pair being tested.

t The t-statistic computed for each cluster.

df The degrees of freedom used for the t-test for each cluster.

**p\_val** The (unadjusted) p-value for the t-test for each cluster.

**p\_adj** The p.adjust-adjusted p-value for the t-test for each cluster.

significant A character vector that will be "\*" for clusters for which p\_adj < alpha and "" otherwise.

**mean\_diff** For an unpaired t-test, the difference between the average proportions of each cluster in the two levels of 'effect\_col'. For a paired t-test, the average difference between the proportions of each cluster in the two levels of 'effect\_col' within a given patient.

mean\_fc For an unpaired t-test, the ratio between the average proportions of each cluster in the two levels of 'effect\_col'. For a paired t-test, the average ratio between the proportions of each cluster in the two levels of 'effect\_col' within a given patient. 0.001 is added to the denominator of the ratio to avoid divide-by-zero errors.

The "levels" attribute of the result indicates the order in which the different levels of the 'effect\_col' were considered. The 'mean\_diff' value for each row of the output is computed subtracting the second level from the first level, and the 'mean\_fc' value for each row is computed by dividing the first level by the second level.

### See Also

Other differential expression analysis functions: tof\_analyze\_expression(), tof\_analyze\_expression\_diffcyt(), tof\_analyze\_expression\_lmm()

### **Examples**

 $\mbox{\# For differential discovery examples, please see the package vignettes}$  NULL

tof\_annotate\_clusters 37

## Description

This function adds an additional column to a 'tibble' or 'tof\_tbl' to allow users to incorporate manual cell type labels for clusters identified using unsupervised algorithms.

## Usage

```
tof_annotate_clusters(tof_tibble, cluster_col, annotations)
```

## Arguments

tof\_tibble 'tof\_tbl' or 'tibble'.

cluster\_col An unquoted column name indicating which column in 'tof\_tibble' contains the

ids of the unsupervised cluster to which each cell belongs. Cluster labels can be produced via any method the user chooses - including manual gating, any of the

functions in the 'tof\_cluster\_\*' function family, or any other method.

annotations A data structure indicating how to annotate each cluster id in 'cluster\_col'. 'an-

notations' can be provided as a data.frame with two columns (the first should have the same name as 'cluster\_col' and contain each unique cluster id; the second can have any name and should contain a character vector indicating which manual annotation should be matched with each cluster id in the first column). 'annotations' can also be provided as a named character vector; in this case, each entry in 'annotations' should be a unique cluster id, and the names for each entry should be the corresponding manual cluster annotation. See below for

examples.

#### Value

A 'tof\_tbl' with the same number of rows as 'tof\_tibble' and one additional column containing the manual cluster annotations for each cell (as a character vector). If 'annotations' was provided as a data.frame, the new column will have the same name as the column containing the cluster annotations in 'annotations'. If 'annotations' was provided as a named character vector, the new column will be named '{cluster\_col}\_annotation'.

## **Examples**

```
sim_data <-
    dplyr::tibble(
        cd45 = rnorm(n = 1000),
        cd38 = c(rnorm(n = 500), rnorm(n = 500, mean = 2)),
        cd34 = c(rnorm(n = 500), rnorm(n = 500, mean = 4)),
        cd19 = rnorm(n = 1000),
        cluster_id = c(rep("a", 500), rep("b", 500))</pre>
```

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```
)
# using named character vector
sim_data |>
   tof_annotate_clusters(
       cluster_col = cluster_id,
        annotations = c("macrophage" = "a", "dendritic cell" = "b")
# using two-column data.frame
annotation_data_frame <-</pre>
   data.frame(
        cluster_id = c("a", "b"),
        cluster_annotation = c("macrophage", "dendritic cell")
   )
sim_data |>
    tof_annotate_clusters(
       cluster_col = cluster_id,
        annotations = annotation_data_frame
   )
```

## **Description**

Perform developmental clustering on CyTOF data using a pre-fit classifier

## Usage

```
tof_apply_classifier(
  cancer_tibble = NULL,
  classifier_fit = NULL,
  distance_function = c("mahalanobis", "cosine", "pearson"),
  num_cores = 1,
  parallel_vars
)
```

### **Arguments**

cancer\_tibble A 'tibble' or 'tof\_tibble' containing cells to be classified into their nearest healthy subpopulation (generally cancer cells).

classifier\_fit A nested 'tibble' produced by 'tof\_build\_classifier' in which each row represents a healthy cell subpopulation into which the cells in 'cancer\_tibble' should be classified using minimum distance.

tof\_assess\_channels 39

distance\_function

num\_cores

A string indicating which distance function should be used to perform the classification of the classification

sification. Options are "mahalanobis" (the default), "cosine", and "pearson".

An integer indicating the number of CPU cores used to parallelize the classifi-

cation. Defaults to 1 (a single core).

breaking up the data in order to parallelize the classification. Defaults to NULL.

Supports tidyselect helpers.

#### Value

A tibble with 'nrow(cancer\_tibble)' rows and 'nrow(classifier\_fit) + 1' columns. Each row represents a cell from 'cancer\_tibble', and 'nrow(classifier\_fit)' of the columns represent the distance between the cell and each of the healthy subpopulations' cluster centroids. The final column represents the cluster id of the healthy subpopulation with the minimum distance to the cell represented by that row.

## **Examples**

NULL

## **Description**

Detect low-expression (i.e. potentially failed) channels in high-dimensional cytometry data

## Usage

```
tof_assess_channels(
  tof_tibble,
  channel_cols = where(tof_is_numeric),
  negative_threshold = asinh(10/5),
  negative_proportion_flag = 0.95
)
```

#### **Arguments**

tof\_tibble A 'tof\_tbl' or 'tibble'.

channel\_cols A vector of unquoted column names representing columns that contain singlecell protein measurements. Supports tidyselect helpers. If nothing is specified,

the default is to analyze all numeric columns.

```
negative_threshold
```

A scalar indicating the threshold below which a measurement should be considered negative. Defaults to the hyperbolic arcsine transformation of 10 counts.

```
negative_proportion_flag
```

A scalar between 0 and 1 indicating the proportion of cells in tof\_tibble that need to be below 'negative\_threshold' for a given marker in order for that marker to be flagged. Defaults to 0.95.

#### Value

A tibble 3 columns and a number of rows equal to the number of columns in 'tof\_tibble' chosen by 'channel\_cols'. The three columns are "channel", a character vector of channel names, "negative\_proportion", a numeric vector with values between 0 and 1 indicating how many cells in 'tof\_tibble' below 'negative\_threshold' for each channel, and 'flagged\_channel', a boolean vector indicating whether or not a channel has been flagged as potentially failed (TRUE means that the channel had a large number of cells below 'negative\_threshold').

## **Examples**

```
# simulate some data
sim_data <-
    data.frame(
        cd4 = rnorm(n = 100, mean = 5, sd = 0.5),
        cd8 = rnorm(n = 100, mean = 0, sd = 0.1),
        cd33 = rnorm(n = 100, mean = 10, sd = 0.1)
    )

tof_assess_channels(tof_tibble = sim_data)

tof_assess_channels(tof_tibble = sim_data, channel_cols = c(cd4, cd8))

tof_assess_channels(tof_tibble = sim_data, negative_threshold = 2)</pre>
```

tof\_assess\_clusters\_distance

Assess a clustering result by calculating the z-score of each cell's mahalanobis distance to its cluster centroid and flagging outliers.

### **Description**

This function evaluates the result of a clustering procedure by comparing the mahalanobis distance between each cell and the centroid of the cluster to which it was assigned among all cells in a given cluster. All cells with a mahalanobis-distance z-score above a user-specified threshold are flagged as potentially anomalous. Note that the z-score is calculated using a modified formula to minimize the effect of outliers (Z = x - median(x) / mad(x)).

### Usage

```
tof_assess_clusters_distance(
  tof_tibble,
  cluster_col,
  marker_cols = where(tof_is_numeric),
  z_threshold = 3,
  augment = FALSE
)
```

A 'tof tbl' or 'tibble'.

### Arguments

tof\_tibble

z\_threshold

Cluster\_col An unquoted column name indicating which column in 'tof\_tibble' stores the cluster ids for the cluster to which each cell belongs. Cluster labels can be produced via any method the user chooses - including manual gating, any of the functions in the 'tof\_cluster\_\*' function family, or any other method.

Marker\_cols Unquoted column names indicating which column in 'tof\_tibble' should be interpreted as markers to be used in the mahalanobis distance calculation. Defaults to all numeric columns. Supports tidyselection.

A scalar indicating the distance z-score threshold above which a cell should be considered anomalous. Defaults to 3.

A boolean value indicating if the output should column-bind the computed flags for each cell (see below) as new columns in 'tof\_tibble' (TRUE) or if a tibble including only the computed flags should be returned (FALSE, the default).

#### Value

If augment = FALSE (the default), a tibble with 3 columns: ".mahalanobis\_distance" (the mahalanobis distance from each cell to the centroid of tits assigned cluster), "z\_score" (the modified z-score of each cell's mahalanobis distance relative to all other cells in the dataset), and "flagged\_cell" (a boolean indicating whether or not each cell was flagged as having a z-score above z\_threshold). If augment = TRUE, the same 3 columns will be column-bound to tof\_tibble, and the resulting tibble will be returned.

## **Examples**

```
rnorm(n = 100, sd = 0.2, mean = -10),
                rnorm(n = 500, mean = 4),
                rnorm(n = 500, mean = 60)
            ),
        cd19 = c(rnorm(n = 100, sd = 0.3, mean = 10), rnorm(n = 1000)),
        cluster_id = c(rep("a", 100), rep("b", 500), rep("c", 500)),
        dataset = "inner"
   )
sim_data_outer <-
    dplyr::tibble(
        cd45 = c(rnorm(n = 10), rnorm(50, mean = 3), rnorm(n = 50, mean = -12)),
        cd38 =
            c(
                rnorm(n = 10, sd = 0.5),
                rnorm(n = 50, mean = -10),
                rnorm(n = 50, mean = 10)
            ),
        cd34 =
            c(
                rnorm(n = 10, sd = 0.2, mean = -15),
                rnorm(n = 50, mean = 15),
                rnorm(n = 50, mean = 70)
        cd19 = c(rnorm(n = 10, sd = 0.3, mean = 19), rnorm(n = 100)),
        cluster_id = c(rep("a", 10), rep("b", 50), rep("c", 50)),
        dataset = "outer"
    )
sim_data <- rbind(sim_data_inner, sim_data_outer)</pre>
# detect anomalous cells (in this case, the "outer" dataset contains small
# clusters that get lumped into the larger clusters in the "inner" dataset)
z_result <-
    sim_data |>
    tof_assess_clusters_distance(cluster_col = cluster_id, z_threshold = 2.5)
```

tof\_assess\_clusters\_entropy

Assess a clustering result by calculating the shannon entropy of each cell's mahalanobis distance to all cluster centroids and flagging outliers.

### **Description**

This function evaluates the result of a clustering procedure by calculating the mahalanobis distance between each cell and the centroids of all clusters in the dataset and finding the shannon entropy of the resulting vector of distances. All cells with an entropy threshold above a user-specified threshold are flagged as potentially anomalous. Entropy is minimized (to 0) when a cell is close to one (or a

small number) of clusters, but far from the rest of them. If a cell is close to multiple cluster centroids (i.e. has an ambiguous phenotype), its entropy will be large.

### Usage

```
tof_assess_clusters_entropy(
  tof_tibble,
  cluster_col,
  marker_cols = where(tof_is_numeric),
  entropy_threshold,
  entropy_quantile = 0.9,
  num_closest_clusters,
  augment = FALSE
)
```

## **Arguments**

tof\_tibble

A 'tof tbl' or 'tibble'.

cluster\_col

An unquoted column name indicating which column in 'tof\_tibble' stores the cluster ids for the cluster to which each cell belongs. Cluster labels can be produced via any method the user chooses - including manual gating, any of the functions in the 'tof\_cluster\_\*' function family, or any other method.

marker\_cols

Unquoted column names indicating which column in 'tof\_tibble' should be interpreted as markers to be used in the mahalanobis distance calculation. Defaults to all numeric columns. Supports tidyselection.

entropy\_threshold

A scalar indicating the entropy threshold above which a cell should be considered anomalous. If unspecified, a threshold will be computed using 'entropy\_quantile' (see below). (Note: Entropy is often between 0 and 1, but can be larger with many classes/clusters).

entropy\_quantile

A scalar between 0 and 1 indicating the entropy quantile above which a cell should be considered anomalous. Defaults to 0.9, which means that cells with an entropy above the 90th percentile will be flagged. Ignored if entropy\_threshold is specified directly.

num\_closest\_clusters

An integer indicating how many of a cell's closest cluster centroids should have their mahalanobis distance included in the entropy calculation. Playing with this argument will allow you to ignore distances to clusters that are far away from each cell (and thus may distort the result, as many distant centroids with large distances can artificially inflate a cells' entropy value; that being said, this is rarely an issue empirically). Defaults to all clusters in tof tibble.

augment

A boolean value indicating if the output should column-bind the computed flags for each cell (see below) as new columns in 'tof\_tibble' (TRUE) or if a tibble including only the computed flags should be returned (FALSE, the default).

#### Value

If augment = FALSE (the default), a tibble with 2 + NUM\_CLUSTERS columns. where NUM\_CLUSTERS is the number of unique clusters in cluster\_col. Two of the columns will be "entropy" (the entropy value for each cell) and "flagged\_cell" (a boolean value indicating if each cell had an entropy value above entropy\_threshold). The other NUM\_CLUSTERS columns will contain the mahalanobis distances from each cell to each of the clusters in cluster\_col (named ".mahalanobis\_{cluster\_name})"). If augment = TRUE, the same 2 + NUM\_CLUSTERS columns will be column-bound to tof\_tibble, and the resulting tibble will be returned.

## **Examples**

```
# simulate data
sim data <-
   dplyr::tibble(
     cd45 = c(rnorm(n = 1000, sd = 1.5), rnorm(n = 1000, mean = 2), rnorm(n = 1000, mean = -2)),
     cd38 = c(rnorm(n = 1000, sd = 1.5), rnorm(n = 1000, mean = 2), rnorm(n = 1000, mean = -2)),
     cd34 = c(rnorm(n = 1000, sd = 1.5), rnorm(n = 1000, mean = 2), rnorm(n = 1000, mean = -2)),
     cd19 = c(rnorm(n = 1000, sd = 1.5), rnorm(n = 1000, mean = 2), rnorm(n = 1000, mean = -2)),
       cluster_id = c(rep("a", 1000), rep("b", 1000), rep("c", 1000))
   )
# imagine a "reference" dataset in which "cluster a" isn't present
sim_data_reference <-
   sim_data |>
   dplyr::filter(cluster_id %in% c("b", "c"))
# if we cluster into the reference dataset, we will force all cells in
# cluster a into a population where they don't fit very well
sim_data <-
   sim_data |>
   tof_cluster(
       healthy_tibble = sim_data_reference,
       healthy_label_col = cluster_id,
       method = "ddpr"
   )
# we can evaluate the clustering quality by calculating by the entropy of the
# mahalanobis distance vector for each cell to all cluster centroids
entropy_result <-
   sim_data |>
   tof_assess_clusters_entropy(
       cluster_col = .mahalanobis_cluster,
       marker_cols = starts_with("cd"),
       entropy_quantile = 0.8,
        augment = TRUE
# most cells in "cluster a" are flagged, and few cells in the other clusters are
flagged_cluster_proportions <-
   entropy_result |>
   dplyr::group_by(cluster_id) |>
```

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```
dplyr::summarize(
    prop_flagged = mean(flagged_cell)
)
```

tof\_assess\_clusters\_knn

Assess a clustering result by calculating a cell's cluster assignment to that of its K nearest neighbors.

#### **Description**

This function evaluates the result of a clustering procedure by finding the cell's K nearest neighbors, determining which cluster the majority of them are assigned to, and checking if this matches the cell's own cluster assignment. If the cluster assignment of the majority of a cell's nearest neighbors does not match with the cell's own cluster assignment, the cell is flagged as potentially anomalous.

## Usage

```
tof_assess_clusters_knn(
   tof_tibble,
   cluster_col,
   marker_cols = where(tof_is_numeric),
   num_neighbors = min(10, nrow(tof_tibble)),
   distance_function = c("euclidean", "cosine", "12", "ip"),
   augment = FALSE
)
```

## **Arguments**

tof\_tibble A 'tof\_tbl' or 'tibble'.

cluster\_col An unquoted column name indicating which column in 'tof\_tibble' stores the

cluster ids for the cluster to which each cell belongs. Cluster labels can be produced via any method the user chooses - including manual gating, any of the

functions in the 'tof\_cluster\_\*' function family, or any other method.

marker\_cols Unquoted column names indicating which column in 'tof\_tibble' should be interpreted as markers to be used in the mahalanobis distance calculation. Defaults

to all numeric columns. Supports tidyselection.

num\_neighbors An integer indicating how many neighbors should be found during the nearest

neighbor calculation.

distance\_function

augment

A string indicating which distance function should be used to perform the k

nearest neighbor calculation. Options are "euclidean" (the default) and "cosine".

A boolean value indicating if the output should column-bind the computed flags for each cell (see below) as new columns in 'tof\_tibble' (TRUE) or if a tibble including only the computed flags should be returned (FALSE, the default).

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### Value

If augment = FALSE (the default), a tibble with 2 columns: ".knn\_cluster" (a character vector indicating which cluster received the majority vote of each cell's k nearest neighbors) and "flagged\_cell" (a boolean value indicating if the cell's cluster assignment matched the majority vote (TRUE) or not (FALSE)). If augment = TRUE, the same 2 columns will be column-bound to tof\_tibble, and the resulting tibble will be returned.

### **Examples**

```
sim_data <-
    dplyr::tibble(
    cd45 = c(rnorm(n = 1000, sd = 1.5), rnorm(n = 1000, mean = 2), rnorm(n = 1000, mean = -2)),
    cd38 = c(rnorm(n = 1000, sd = 1.5), rnorm(n = 1000, mean = 2), rnorm(n = 1000, mean = -2)),
    cd34 = c(rnorm(n = 1000, sd = 1.5), rnorm(n = 1000, mean = 2), rnorm(n = 1000, mean = -2)),
    cd19 = c(rnorm(n = 1000, sd = 1.5), rnorm(n = 1000, mean = 2), rnorm(n = 1000, mean = -2)),
    cluster_id = c(rep("a", 1000), rep("b", 1000), rep("c", 1000))
)

knn_result <-
    sim_data |>
    tof_assess_clusters_knn(
        cluster_col = cluster_id,
        num_neighbors = 10
)
```

### **Description**

This function performs a simplified version of flowAI's statistical test to detect time periods with abnormal flow rates over the course of a flow cytometry experiment. Briefly, the relative flow rates for each timestep throughout data acquisition are calculated (see tof\_calculate\_flow\_rate), and outlier timepoints with particularly high or low flow rates (i.e. those beyond extreme values of the t-distribution across timesteps) are flagged.

### Usage

```
tof_assess_flow_rate(
  tof_tibble,
  time_col,
  group_cols,
  num_timesteps = nrow(tof_tibble)/1000,
  alpha_threshold = 0.01,
  visualize = FALSE,
  ...,
  augment = FALSE
)
```

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## Arguments

tof\_tibble A 'tof\_tbl' or 'tibble'.

time\_col An unquoted column name indicating which column in 'tof\_tibble' contains the time at which each cell was collected.

group\_cols Optional. Unquoted column names indicating which columns should be used to group cells before analysis. Flow rate calculation is then performed independently within each group. Supports tidyselect helpers.

num\_timesteps The number of bins into which 'time\_col' should be split. to define "timesteps" of the data collection process. The number of cells analyzed by the cytometer will be counted in each bin separately and will represent the relative average

flow rate for that timestep in data collection.

alpha\_threshold

visualize

augment

A scalar between 0 and 1 indicating the two-tailed significance level at which to draw outlier thresholds in the t-distribution with 'num\_timesteps' - 1 degrees of freedom. Defaults to 0.01.

A boolean value indicating if a plot should be generated to visualize each timestep's relative flow rate (by group) instead of returning the tibble directly. Defaults to FALSE.

.. Optional additional arguments to pass to facet\_wrap. Ignored if visualize = FALSE.

A boolean value indicating if the output should column-bind the computed flags for each cell (see below) as new columns in 'tof\_tibble' (TRUE) or if a tibble including only the computed flags should be returned (FALSE, the default).

#### Value

A tibble with the same number of rows as 'tof\_tibble'. If augment = FALSE (the default), it will have 3 columns: "{time\_col}" (the same column as 'time\_col'), "timestep" (the numeric timestep to which each cell was assigned based on its value for 'time\_col'), and "flagged\_window" (a boolean vector indicating if each cell was collecting during a timestep flagged for having a high or low flow rate). If augment = TRUE, these 3 columns will be column-bound to 'tof\_tibble' to return an augmented version of the input dataset. (Note that in this case, time\_col will not be duplicated). If visualize = TRUE, then a ggplot object is returned instead of a tibble.

## Examples

```
set.seed(1000L)
sim_data <-
    data.frame(
    cd4 = rnorm(n = 1000, mean = 5, sd = 0.5),
    cd8 = rnorm(n = 1000, mean = 0, sd = 0.1),
    cd33 = rnorm(n = 1000, mean = 10, sd = 0.1),
    file_name = c(rep("a", times = 500), rep("b", times = 500)),
    time =
        c(
        sample(1:100, size = 200, replace = TRUE),
        sample(100:400, size = 300, replace = TRUE),</pre>
```

```
sample(1:150, size = 400, replace = TRUE),
                sample(1:500, size = 100, replace = TRUE)
            )
   )
sim_data |>
    tof_assess_flow_rate(
        time_col = time,
        num_timesteps = 20,
        visualize = TRUE
   )
sim_data |>
    tof_assess_flow_rate(
        time_col = time,
        group_cols = file_name,
        num_timesteps = 20,
        visualize = TRUE
    )
```

tof\_assess\_flow\_rate\_tibble

Detect flow rate abnormalities in high-dimensional cytometry data (stored in a single data.frame)

# Description

This function performs a simplified version of flowAI's statistical test to detect time periods with abnormal flow rates over the course of a flow cytometry experiment. Briefly, the relative flow rates for each timestep throughout data acquisition are calculated (see tof\_calculate\_flow\_rate), and outlier timepoints with particularly high or low flow rates (i.e. those beyond extreme values of the t-distribution across timesteps) are flagged.

#### **Usage**

```
tof_assess_flow_rate_tibble(
  tof_tibble,
  time_col,
  num_timesteps = nrow(tof_tibble)/1000,
  alpha_threshold = 0.01,
  augment = FALSE
)
```

### **Arguments**

tof\_tibble A 'tof\_tbl' or 'tibble'.

time\_col An unquoted column name indicating which column in 'tof\_tibble' contains the time at which each cell was collected.

num\_timesteps

The number of bins into which 'time\_col' should be split. to define "timesteps" of the data collection process. The number of cells analyzed by the cytometer will be counted in each bin separately and will represent the relative average flow rate for that timestep in data collection.

alpha\_threshold

A scalar between 0 and 1 indicating the two-tailed significance level at which to draw outlier thresholds in the t-distribution with 'num\_timesteps' - 1 degrees of freedom. Defaults to 0.01.

augment

A boolean value indicating if the output should column-bind the computed flags for each cell (see below) as new columns in 'tof\_tibble' (TRUE) or if a tibble including only the computed flags should be returned (FALSE, the default).

#### Value

A tibble with the same number of rows as 'tof\_tibble'. If augment = FALSE (the default), it will have 3 columns: "{time\_col}" (the same column as 'time\_col'), "timestep" (the numeric timestep to which each cell was assigned based on its value for 'time\_col'), and "flagged\_window" (a boolean vector indicating if each cell was collecting during a timestep flagged for having a high or low flow rate). If augment = TRUE, these 3 columns will be column-bound to 'tof\_tibble' to return an augmented version of the input dataset. (Note that in this case, time\_col will not be duplicated).

## **Examples**

```
set.seed(1000L)
sim_data <-
    data.frame(
        cd4 = rnorm(n = 1000, mean = 5, sd = 0.5),
        cd8 = rnorm(n = 1000, mean = 0, sd = 0.1),
        cd33 = rnorm(n = 1000, mean = 10, sd = 0.1),
        time =
            c(
                sample(1:100, size = 200, replace = TRUE),
                sample(100:400, size = 300, replace = TRUE),
                sample(1:150, size = 400, replace = TRUE),
                sample(1:500, size = 100, replace = TRUE)
            )
    )
sim_data |>
    tof_assess_flow_rate(
        time_col = time,
        num_timesteps = 20,
        visualize = TRUE
   )
```

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tof\_assess\_model

Assess a trained elastic net model

## **Description**

This function assesses a trained 'tof\_model''s performance on new data by computing model typespecific performance measurements. If new data isn't provided, performance metrics for the training data will be provided.

### Usage

```
tof_assess_model(tof_model, new_data)
```

# **Arguments**

tof\_model

A 'tof\_model' trained using tof\_train\_model

new\_data

A tibble of new observations that should be used to evaluate the 'tof model''s performance. If new\_data isn't provided, model evaluation will will be performed using the training data used to fit the model. Alternatively, the string "tuning" can be provided to access the model's performance metrics during the

(resampled) model tuning process.

#### Value

A list of performance metrics whose components depend on the model type:

- "model\_metrics" A tibble with two columns ("metric" and "value") containing standard performance metrics for each model type. For linear models, the "mse" (the mean squared error of the predictions) and "mae" (the mean absolute error of the predictions). For two-class models, "roc auc" (the area under the Receiver-Operating Curve for the classification), "misclassification error" (the proportion of misclassified observations), "binomial\_deviance" (see deviance.glmnet), "mse" (the mean squared error of the logit function), and "mae" (the mean absolute error of the logit function). For multiclass models, "roc auc" (the area under the Receiver-Operating Curve for the classification using the Hand-Till generalization of the ROC AUC for multiclass models in roc\_auc), "misclassification error" (the proportion of misclassified observations), "multinomial\_deviance" (see deviance.glmnet), and "mse" and "mae" as above. For survival models, "concordance\_index" (Harrel's C index; see deviance.glmnet) and "partial\_likelihood\_deviance" (see deviance.glmnet).
- "roc curve" Reported only for "two-class" and "multiclass" models. For both, a tibble is provided reporting the true-positive rate (tpr) and false-positive rate (fpr) at each threshold for classification for use in plotting a receiver-operating curve. For "multiclass" models, the ".level" column allows for separating the values in roc\_curve such that one ROC can be plotted for each class.
- "confusion matrix" Reported only for "two-class" and "multiclass" models. For both, a tibble is provided reporting the "confusion matrix" of the classification in long-format.

"survival\_curves" Reported only for "survival" models. A tibble indicating each patient's probability of survival (1 - probability(event)) at each timepoint in the dataset and whether each sample was placed in the "high" or "low" risk group according to its predicted relative risk (and the tof\_model's optimal relative\_risk cutoff in the training dataset).

### See Also

Other modeling functions: tof\_create\_grid(), tof\_predict(), tof\_split\_data(), tof\_train\_model()

## **Examples**

```
feature_tibble <-
    dplyr::tibble(
        sample = as.character(1:100),
        cd45 = runif(n = 100),
        pstat5 = runif(n = 100),
        cd34 = runif(n = 100),
        outcome = (3 * cd45) + (4 * pstat5) + rnorm(100)
   )
new_tibble <-
   dplyr::tibble(
       sample = as.character(1:20),
        cd45 = runif(n = 20),
        pstat5 = runif(n = 20),
        cd34 = runif(n = 20),
        outcome = (3 * cd45) + (4 * pstat5) + rnorm(20)
   )
split_data <- tof_split_data(feature_tibble, split_method = "simple")</pre>
# train a regression model
regression_model <-
    tof_train_model(
        split_data = split_data,
        predictor_cols = c(cd45, pstat5, cd34),
        response_col = outcome,
        model_type = "linear"
   )
# assess the model on new data
tof_assess_model(tof_model = regression_model, new_data = new_tibble)
```

tof\_assess\_model\_new\_data

Compute a trained elastic net model's performance metrics using new\_data.

## **Description**

Compute a trained elastic net model's performance metrics using new\_data.

# Usage

```
tof_assess_model_new_data(tof_model, new_data)
```

## Arguments

tof\_model A 'tof\_model' trained using tof\_train\_model

new\_data A tibble of new observations that should be used to evaluate the 'tof\_model''s

performance.

## Value

A list of performance metrics whose components depend on the model type.

```
tof_assess_model_tuning
```

Access a trained elastic net model's performance metrics using its tuning data.

# Description

Access a trained elastic net model's performance metrics using its tuning data.

## Usage

```
tof_assess_model_tuning(tof_model)
```

## **Arguments**

```
tof_model A 'tof_model' trained using tof_train_model
```

## Value

A list of performance metrics whose components depend on the model type.

tof\_batch\_correct 53

tof_batch_correct	Perform groupwise linear rescaling of high-dimensional cytometry
	measurements

## **Description**

This function performs quantile normalization on high-dimensional cytometry data in tidy format using either linear rescaling or quantile normalization. Each channel specified by 'channel\_cols' is batch corrected, and 'group\_cols' can be used to break cells into groups for which the batch correction should be performed separately.

## Usage

```
tof_batch_correct(
  tof_tibble,
  channel_cols,
  group_cols,
  augment = TRUE,
  method = c("rescale", "quantile")
)
```

## Arguments

tof_tibble	A 'tof_tbl' or a 'tibble'.
channel_cols	Unquoted column names representing columns that contain single-cell protein measurements. Supports tidyselect helpers.
group_cols	Optional. Unquoted column names indicating which columns should be used to group cells before batch correction. Batch correction is then performed independently within each group. Supports tidyselect helpers.
augment	A boolean value indicating if the output should replace the 'channel_cols' in 'tof_tibble' with the new, batch corrected columns (TRUE, the default) or if it should only return the batch-corrected columns (FALSE) with all other columns omitted.
method	A string indicating which batch correction method should be used. Valid options are "rescale" for linear scaling (the default) and "quantile" for quantile normalization using normalize.quantiles.

### Value

If augment = TRUE, a tibble with the same number of rows and columns as tof\_tibble, with the columns specified by 'channel\_cols' batch-corrected. If augment = FALSE, a tibble containing only the batch-corrected 'channel\_cols'.

### **Examples**

NULL

```
tof_batch_correct_quantile
```

Batch-correct a tibble of high-dimensional cytometry data using quantile normalization.

## **Description**

This function performs quantile normalization on high-dimensional cytometry data in tidy format using normalize.quantiles. Optionally, groups can be specified and normalized separately.

## Usage

```
tof_batch_correct_quantile(
  tof_tibble,
  channel_cols,
  group_cols,
  augment = TRUE
)
```

## **Arguments**

tof\_tibble A 'tof\_tbl' or a 'tibble'.

measurements. Supports tidyselect helpers.

group\_cols Optional. Unquoted column names indicating which columns should be used

to group cells before batch correction. Batch correction is then performed inde-

pendently within each group. Supports tidyselect helpers.

augment A boolean value indicating if the output should replace the 'channel\_cols' in

'tof\_tibble' with the new, batch corrected columns (TRUE, the default) or if it should only return the batch-corrected columns (FALSE) with all other columns

omitted.

#### Value

If augment = TRUE, a tibble with the same number of rows and columns as tof\_tibble, with the columns specified by 'channel\_cols' batch-corrected. If augment = FALSE, a tibble containing only the batch-corrected 'channel\_cols'.

## **Examples**

NULL

tof\_batch\_correct\_quantile\_tibble

Batch-correct a tibble of high-dimensional cytometry data using quantile normalization.

## **Description**

This function performs quantile normalization on high-dimensional cytometry data in tidy format using normalize.quantiles.

## Usage

```
tof_batch_correct_quantile_tibble(tof_tibble, channel_cols, augment = TRUE)
```

## **Arguments**

tof\_tibble A 'tof\_tbl' or a 'tibble'.

channel\_cols Unquoted column names representing columns that contain single-cell protein

measurements. Supports tidyselect helpers.

augment A boolean value indicating if the output should replace the 'channel\_cols' in

'tof\_tibble' with the new, batch corrected columns (TRUE, the default) or if it should only return the batch-corrected columns (FALSE) with all other columns

omitted.

### Value

If augment = TRUE, a tibble with the same number of rows and columns as tof\_tibble, with the columns specified by 'channel\_cols' batch-corrected. If augment = FALSE, a tibble containing only the batch-corrected 'channel\_cols'.

## **Examples**

NULL

tof\_batch\_correct\_rescale

Perform groupwise linear rescaling of high-dimensional cytometry measurements

### **Description**

This function performs quantile normalization on high-dimensional cytometry data in tidy format using linear rescaling. Each channel specified by 'channel\_cols' is rescaled such that the maximum value is 1 and the minimum value is 0. 'group\_cols' specifies the columns that should be used to break cells into groups in which the rescaling should be performed separately.

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### Usage

```
tof_batch_correct_rescale(tof_tibble, channel_cols, group_cols, augment = TRUE)
```

## **Arguments**

tof\_tibble A 'tof\_tbl' or a 'tibble'.

measurements. Supports tidyselect helpers.

group\_cols Optional. Unquoted column names indicating which columns should be used

to group cells before batch correction. Batch correction is then performed inde-

pendently within each group. Supports tidyselect helpers.

augment A boolean value indicating if the output should replace the 'channel\_cols' in

'tof\_tibble' with the new, batch corrected columns (TRUE, the default) or if it should only return the batch-corrected columns (FALSE) with all other columns

omitted.

#### Value

If augment = TRUE, a tibble with the same number of rows and columns as tof\_tibble, with the columns specified by 'channel\_cols' batch-corrected. If augment = FALSE, a tibble containing only the batch-corrected 'channel\_cols'.

## **Examples**

NULL

### **Description**

This function takes a 'tibble' or 'tof\_tibble' storing healthy cell measurements in each of its rows and a vector ('healthy\_cell\_labels') representing the cell subpopulation to which each cell belongs. It uses these values to calculate several values required to perform "developmental classification" as described in this paper.

## Usage

```
tof_build_classifier(
  healthy_tibble = NULL,
  healthy_cell_labels = NULL,
  classifier_markers = where(tof_is_numeric),
  verbose = FALSE
)
```

#### **Arguments**

healthy\_tibble A 'tibble' or 'tof\_tibble' containing cells from only healthy control samples (i.e. not disease samples).

healthy\_cell\_labels

A character or integer vector of length 'nrow(healthy\_tibble)'. Each entry in this vector should represent the cell subpopulation label (or cluster id) for the corresponding row in 'healthy\_tibble'.

classifier\_markers

Unquoted column names indicating which columns in 'healthy\_tibble' to use in the developmental classification. Defaults to all numeric columns in 'healthy\_tibble'. Supports tidyselect helpers.

verbose

A boolean value indicating if updates should be printed to the console during classification. Defaults to FALSE.

#### Value

A tibble with three columns: **population** (id of the healthy cell population), **centroid** (the centroid vector for that cell population), and **covariance\_matrix** (the covariance matrix for that cell population)

```
tof_calculate_flow_rate
```

Calculate the relative flow rates of different timepoints throughout a flow or mass cytometry run.

## **Description**

Calculate the relative flow rates of different timepoints throughout a flow or mass cytometry run.

### Usage

```
tof_calculate_flow_rate(
  tof_tibble,
  time_col,
  num_timesteps = nrow(tof_tibble)/1000
)
```

# Arguments

tof\_tibble A 'tof\_tbl' or 'tibble'.

time\_col An unquoted column name indicating which column in 'tof\_tibble' contains the

time at which each cell was collected.

num\_timesteps The number of bins into which 'time\_col' should be split. to define "timesteps"

of the data collection process. The number of cells analyzed by the cytometer will be counted in each bin separately and will represent the relative average

flow rate for that timestep in data collection.

#### Value

A tibble with 3 columns and num\_timesteps rows. Each row will represent a single timestep (and an error will be thrown if 'num\_timesteps' is larger than the number of rows in 'tof\_tibble'). The three columns are as follows: "timestep", a numeric vector indicating which timestep is represented by a given row; "time\_window", a factor showing the interval in 'time\_col' over which "timestep" is defined; and "num\_cells", the number of cells that were collected during each timestep.

#### **Examples**

```
# simulate some data
sim_data <-
    data.frame(
        cd4 = rnorm(n = 100, mean = 5, sd = 0.5),
        cd8 = rnorm(n = 100, mean = 0, sd = 0.1),
        cd33 = rnorm(n = 100, mean = 10, sd = 0.1),
        time = sample(1:300, size = 100)
    )

tof_calculate_flow_rate(tof_tibble = sim_data, time_col = time, num_timesteps = 20L)</pre>
```

### Description

Check argument specifications for a glmnet model.

#### Usage

```
tof_check_model_args(
   split_data,
   model_type = c("linear", "two-class", "multiclass", "survival"),
   best_model_type = c("best", "best with sparsity"),
   response_col,
   time_col,
   event_col
)
```

## **Arguments**

split\_data

An 'rsplit' or 'rset' object from the <code>rsample</code> package containing the sample-level data to use for modeling. Alternatively, an unsplit tbl\_df can be provided, though this is not recommended.

model\_type

A string indicating which kind of elastic net model to build. If a continuous response is being predicted, use "linear" for linear regression; if a categorical response with only 2 classes is being predicted, use "two-class" for logistic regression; if a categorical response with more than 2 levels is being predicted,

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use "multiclass" for multinomial regression; and if a time-to-event outcome is being predicted, use "survival" for Cox regression.

best\_model\_type

Currently unused.

response\_col

Unquoted column name indicating which column in the data contained in 'split\_data' should be used as the outcome in a "two-class", "multiclass", or "linear" elastic net model. Must be a factor for "two-class" and "multiclass" models and must be a numeric for "linear" models. Ignored if 'model\_type' is "survival".

time\_col

Unquoted column name indicating which column in the data contained in 'split\_data' represents the time-to-event outcome in a "survival" elastic net model. Must be numeric. Ignored if 'model\_type' is "two-class", "multiclass", or "linear".

event\_col

Unquoted column name indicating which column in the data contained in 'split\_data' represents the time-to-event outcome in a "survival" elastic net model. Must be a binary column - all values should be either 0 or 1 (with 1 indicating the adverse event) or FALSE and TRUE (with TRUE indicating the adverse event). Ignored if 'model\_type' is "two-class", "multiclass", or "linear".

#### Value

A tibble. If arguments are specified correctly, this tibble can be used to create a recipe for preprocessing.

tof\_classify\_cells

Classify each cell (i.e. each row) in a matrix of cancer cells into its most similar healthy developmental subpopulation.

### **Description**

This function uses a specified distance metric to classify each cell in a data.frame or matrix ('cancer\_data') into one of 'nrow(classifier\_fit)' subpopulations based on minimum distance, as described in this paper.

#### **Usage**

```
tof_classify_cells(
  classifier_fit,
  cancer_data,
  distance_function = c("mahalanobis", "cosine", "pearson")
)
```

## **Arguments**

classifier\_fit A tibble produced by tof\_build\_classifier.

cancer\_data

A matrix in which each row corresponds to a cell and each column corresponds to a measured CyTOF antigen.

tof\_cluster

distance\_function

A string indicating which of three distance functions should be used to calculate the distances between each row of 'cancer\_data' and the healthy developmental subpopulations corresponding to each row of 'classifier\_fit'.

#### Value

A data frame in which each column represents the distance between a cell in the input data and each healthy subpopulation cells are being classified into.

tof\_clean\_metric\_names

Rename glmnet's default model evaluation metrics to make them more interpretable

## **Description**

Rename glmnet's default model evaluation metrics to make them more interpretable

## Usage

```
tof_clean_metric_names(metric_tibble, model_type)
```

### **Arguments**

metric\_tibble A tibble in which each column represents a glmnet model evaluation metric with

its default name.

model\_type A string indicating which type of glmnet model was trained.

#### Value

A tibble in which each column represents a glmnet model evaluation metric with its "cleaned" name.

tof\_cluster

Cluster high-dimensional cytometry data.

## **Description**

This function is a wrapper around tidytof's tof\_cluster\_\* function family. It performs clustering on high-dimensional cytometry data using a user-specified method (of 5 choices) and each method's corresponding input parameters.

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## Usage

```
tof_cluster(
  tof_tibble,
  cluster_cols = where(tof_is_numeric),
  group_cols = NULL,
  ...,
  augment = TRUE,
  method
)
```

## **Arguments**

tof_tibble	A 'tof_tbl' or 'tibble'.
cluster_cols	Unquoted column names indicating which columns in 'tof_tibble' to use in computing the clusters. Defaults to all numeric columns in 'tof_tibble'. Supports tidyselect helpers.
group_cols	Optional. Unquoted column names indicating which columns should be used to group cells before clustering. Clustering is then performed on each group independently. Supports tidyselect helpers.
•••	Additional arguments to pass to the 'tof_cluster_*' function family member corresponding to the chosen method.
augment	A boolean value indicating if the output should column-bind the cluster ids of each cell as a new column in 'tof_tibble' (TRUE, the default) or if a single-column tibble including only the cluster ids should be returned (FALSE).
method	A string indicating which clustering methods should be used. Valid values include "flowsom", "phenograph", "kmeans", "ddpr", and "xshift".

### Value

A 'tof\_tbl' or 'tibble' If augment = FALSE, it will have a single column encoding the cluster ids for each cell in 'tof\_tibble'. If augment = TRUE, it will have ncol(tof\_tibble) + 1 columns: each of the (unaltered) columns in 'tof\_tibble' plus an additional column encoding the cluster ids.

## See Also

```
Other clustering functions: tof_cluster_ddpr(), tof_cluster_flowsom(), tof_cluster_kmeans(), tof_cluster_phenograph()
```

## **Examples**

```
sim_data <-
    dplyr::tibble(
        cd45 = rnorm(n = 500),
        cd38 = rnorm(n = 500),
        cd34 = rnorm(n = 500),
        cd19 = rnorm(n = 500)
)</pre>
```

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```
tof_cluster(tof_tibble = sim_data, method = "kmeans")
tof_cluster(tof_tibble = sim_data, method = "phenograph")
```

tof\_cluster\_ddpr

Perform developmental clustering on high-dimensional cytometry data.

## **Description**

This function performs distance-based clustering on high-dimensional cytometry data by sorting cancer cells (passed into the function as 'tof\_tibble') into their most phenotypically similar healthy cell subpopulation (passed into the function using 'healthy\_tibble'). For details about the algorithm used to perform the clustering, see this paper.

#### **Usage**

```
tof_cluster_ddpr(
  tof_tibble,
  healthy_tibble,
  healthy_label_col,
  cluster_cols = where(tof_is_numeric),
  distance_function = c("mahalanobis", "cosine", "pearson"),
  num\_cores = 1L,
  parallel_cols,
  return_distances = FALSE,
  verbose = FALSE
)
```

# **Arguments**

tof\_tibble

A 'tibble' or 'tof\_tbl' containing cells to be classified into their nearest healthy subpopulation (generally cancer cells).

healthy\_tibble A 'tibble' or 'tof\_tibble' containing cells from only healthy control samples (i.e. not disease samples).

healthy\_label\_col

An unquoted column name indicating which column in 'healthy\_tibble' contains the subpopulation label (or cluster id) for each cell in 'healthy\_tibble'.

cluster\_cols

Unquoted column names indicating which columns in 'tof\_tibble' to use in computing the DDPR clusters. Defaults to all numeric columns in 'tof\_tibble'. Supports tidyselect helpers.

distance\_function

A string indicating which distance function should be used to perform the classification. Options are "mahalanobis" (the default), "cosine", and "pearson".

num\_cores

An integer indicating the number of CPU cores used to parallelize the classification. Defaults to 1 (a single core).

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parallel\_cols

Optional. Unquoted column names indicating which columns in 'tof\_tibble' to use for breaking up the data in order to parallelize the classification using 'foreach' on a 'doParallel' backend. Supports tidyselect helpers.

return\_distances

A boolean value indicating whether or not the returned result should include only one column, the cluster ids corresponding to each row of 'tof\_tibble' (return\_distances = FALSE, the default), or if the returned result should include additional columns representing the distance between each row of 'tof\_tibble' and each of the healthy subpopulation centroids (return\_distances = TRUE).

verbose

A boolean value indicating whether progress updates should be printed during developmental classification. Default is FALSE.

### Value

If 'return\_distances = FALSE', a tibble with one column named '.{distance\_function}\_cluster', a character vector of length 'nrow(tof\_tibble)' indicating the id of the developmental cluster to which each cell (i.e. each row) in 'tof\_tibble' was assigned.

If 'return\_distances = TRUE', a tibble with 'nrow(tof\_tibble)' rows and 'nrow(classifier\_fit) + 1' columns. Each row represents a cell from 'tof\_tibble', and 'nrow(classifier\_fit)' of the columns represent the distance between the cell and each of the healthy subpopulations' cluster centroids. The final column represents the cluster id of the healthy subpopulation with the minimum distance to the cell represented by that row.

If 'return\_distances = FALSE', a tibble with one column named '.{distance\_function}\_cluster'. This column will contain an integer vector of length 'nrow(tof\_tibble)' indicating the id of the developmental cluster to which each cell (i.e. each row) in 'tof\_tibble' was assigned.

## See Also

```
Other clustering functions: tof_cluster(), tof_cluster_flowsom(), tof_cluster_kmeans(), tof_cluster_phenograph()
```

## **Examples**

```
sim_data <-
    dplyr::tibble(
        cd45 = rnorm(n = 1000),
        cd38 = rnorm(n = 1000),
        cd34 = rnorm(n = 1000),
        cd19 = rnorm(n = 1000)
)

healthy_data <-
    dplyr::tibble(
        cd45 = rnorm(n = 200),
        cd38 = rnorm(n = 200),
        cd34 = rnorm(n = 200),
        cd19 = rnorm(n = 200),
        cd19 = rnorm(n = 200),
        cd19 = rnorm(n = 200),
        cluster_id = c(rep("a", times = 100), rep("b", times = 100))
)</pre>
```

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```
tof_cluster_ddpr(
   tof_tibble = sim_data,
   healthy_tibble = healthy_data,
   healthy_label_col = cluster_id
)
```

tof\_cluster\_flowsom

Perform FlowSOM clustering on high-dimensional cytometry data

## Description

This function performs FlowSOM clustering on high-dimensional cytometry data using a user-specified selection of input variables/high-dimensional cytometry measurements. It is mostly a convenient wrapper around SOM and MetaClustering.

## Usage

```
tof_cluster_flowsom(
  tof_tibble = NULL,
  cluster_cols = where(tof_is_numeric),
  som_xdim = 10,
  som_ydim = 10,
  som_distance_function = c("euclidean", "manhattan", "chebyshev", "cosine"),
  perform_metaclustering = TRUE,
  num_metaclusters = 20,
  ...
)
```

#### **Arguments**

som\_distance\_function

tof\_tibble A 'tof\_tbl' or 'tibble'.

cluster\_cols Unquoted column names indicating which columns in 'tof\_tibble' to use in computing the flowSOM clusters. Defaults to all numeric columns in 'tof\_tibble'. Supports tidyselect helpers.

som\_xdim The width of the grid used by the self-organizing map. The total number of clusters returned by FlowSOM will be som\_xdim \* som\_ydim, so adjust this value to affect the final number of clusters. Defaults to 10.

som\_ydim The height of the grid used by the self-organizing map. The total number of clusters returned by FlowSOM will be som\_xdim \* som\_ydim, so adjust this value to affect the final number of clusters. Defaults to 10.

The distance function used during self-organizing map calculations. Options are "euclidean" (the default), "manhattan", "chebyshev", and "cosine".

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```
perform_metaclustering
```

A boolean value indicating if metaclustering should be performed on the initial clustering result returned by FlowSOM. Defaults to TRUE.

num\_metaclusters

An integer indicating the maximum number of metaclusters that should be returned after metaclustering. Defaults to 20.

.. Optional additional parameters that can be passed to the BuildSOM function.

#### **Details**

For additional details about the FlowSOM algorithm, see this paper.

#### Value

A tibble with one column named '.flowsom\_cluster' or '.flowsom\_metacluster' depending on the value of 'perform\_metaclustering'. The column will contain an integer vector of length 'nrow(tof\_tibble)' indicating the id of the flowSOM cluster to which each cell (i.e. each row) in 'tof\_tibble' was assigned.

### See Also

Other clustering functions: tof\_cluster(), tof\_cluster\_ddpr(), tof\_cluster\_kmeans(), tof\_cluster\_phenograph()

## **Examples**

```
sim_data <-
    dplyr::tibble(
        cd45 = rnorm(n = 200),
        cd38 = rnorm(n = 200),
        cd34 = rnorm(n = 200),
        cd19 = rnorm(n = 200)
    )

tof_cluster_flowsom(tof_tibble = sim_data, cluster_cols = c(cd45, cd19))</pre>
```

tof\_cluster\_grouped

Cluster (grouped) high-dimensional cytometry data.

### **Description**

This function is a wrapper around tidytof's tof\_cluster\_\* function family and provides a low-level API for clustering grouped data frames. It is a subroutine of tof\_cluster and shouldn't be called directly by users.

## Usage

```
tof_cluster_grouped(tof_tibble, group_cols, ..., augment = TRUE, method)
```

tof\_cluster\_kmeans

## **Arguments**

tof_tibble	A 'tof_tbl' or 'tibble'.
group_cols	An unquoted column name indicating which columns should be used to group cells before clustering. Clustering is then performed on each group independently.
	Additional arguments to pass to the 'tof_cluster_*' function family member corresponding to the chosen method.
augment	A boolean value indicating if the output should column-bind the cluster ids of each cell as a new column in 'tof_tibble' (TRUE, the default) or if a single-column tibble including only the cluster ids should be returned (FALSE).
method	A string indicating which clustering methods should be used. Valid values include "flowsom", "phenograph", "kmeans", "ddpr", and "xshift".

# Value

A 'tof\_tbl' or 'tibble' If augment = FALSE, it will have a single column encoding the cluster ids for each cell in 'tof\_tibble'. If augment = TRUE, it will have ncol(tof\_tibble) + 1 columns: each of the (unaltered) columns in 'tof\_tibble' plus an additional column encoding the cluster ids.

tof\_cluster\_kmeans

Perform k-means clustering on high-dimensional cytometry data.

## **Description**

This function performs k-means clustering on high-dimensional cytometry data using a user-specified selection of input variables/high-dimensional cytometry measurements. It is mostly a convenient wrapper around kmeans.

# Usage

```
tof_cluster_kmeans(
  tof_tibble,
  cluster_cols = where(tof_is_numeric),
  num_clusters = 20,
  ...
)
```

## **Arguments**

tof_tibble	A 'tof_tibble'.
cluster_cols	Unquoted column names indicating which columns in 'tof_tibble' to use in computing the k-means clusters. Defaults to all numeric columns in 'tof_tibble'. Supports tidyselect helpers.
num_clusters	An integer indicating the maximum number of clusters that should be returned. Defaults to 20.
	Optional additional arguments that can be passed to kmeans.

#### Value

A tibble with one column named '.kmeans\_cluster'. This column will contain an integer vector of length 'nrow(tof\_tibble)' indicating the id of the k-means cluster to which each cell (i.e. each row) in 'tof\_tibble' was assigned.

#### See Also

Other clustering functions: tof\_cluster(), tof\_cluster\_ddpr(), tof\_cluster\_flowsom(), tof\_cluster\_phenograph()

### **Examples**

```
sim_data <-
    dplyr::tibble(
        cd45 = rnorm(n = 1000),
        cd38 = rnorm(n = 1000),
        cd34 = rnorm(n = 1000),
        cd19 = rnorm(n = 1000)
    )

tof_cluster_kmeans(tof_tibble = sim_data)
tof_cluster_kmeans(tof_tibble = sim_data, cluster_cols = c(cd45, cd19))</pre>
```

tof\_cluster\_phenograph

Perform PhenoGraph clustering on high-dimensional cytometry data.

### **Description**

This function performs PhenoGraph clustering on high-dimensional cytometry data using a user-specified selection of input variables/high-dimensional cytometry measurements.

## Usage

```
tof_cluster_phenograph(
  tof_tibble,
  cluster_cols = where(tof_is_numeric),
  num_neighbors = 30,
  distance_function = c("euclidean", "cosine"),
  ...
)
```

## **Arguments**

tof\_tibble A 'tof\_tbl' or 'tibble'.

cluster\_cols Unquoted column names indicating which columns in 'tof\_tibble' to use in computing the PhenoGraph clusters. Defaults to all numeric columns in 'tof\_tibble'. Supports tidyselect helpers.

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num\_neighbors

An integer indicating the number of neighbors to use when constructing Pheno-Graph's k-nearest-neighbor graph. Smaller values emphasize local graph structure; larger values emphasize global graph structure (and will add time to the computation). Defaults to 30.

distance\_function

A string indicating which distance function to use for the nearest-neighbor calculation. Options include "euclidean" (the default) and "cosine" distances.

... Optional additional parameters that can be passed to tof\_find\_knn.

#### **Details**

For additional details about the Phenograph algorithm, see this paper.

#### Value

A tibble with one column named '.phenograph\_cluster'. This column will contain an integer vector of length 'nrow(tof\_tibble)' indicating the id of the PhenoGraph cluster to which each cell (i.e. each row) in 'tof\_tibble' was assigned.

#### See Also

Other clustering functions: tof\_cluster(), tof\_cluster\_ddpr(), tof\_cluster\_flowsom(), tof\_cluster\_kmeans()

## **Examples**

```
sim_data <-
    dplyr::tibble(
        cd45 = rnorm(n = 1000),
        cd38 = rnorm(n = 1000),
        cd34 = rnorm(n = 1000),
        cd19 = rnorm(n = 1000)
    )

tof_cluster_phenograph(tof_tibble = sim_data)
tof_cluster_phenograph(tof_tibble = sim_data, cluster_cols = c(cd45, cd19))</pre>
```

tof\_cluster\_tibble

Cluster (ungrouped) high-dimensional cytometry data.

# Description

This function is a wrapper around tidytof's tof\_cluster\_\* function family and provides a low-level API for clustering ungrouped data frames. It is a subroutine of tof\_cluster and shouldn't be called directly by users.

## Usage

```
tof_cluster_tibble(tof_tibble, ..., augment = TRUE, method)
```

tof\_compute\_km\_curve

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## **Arguments**

tof\_tibble A 'tof\_tbl' or 'tibble'.

.. Additional arguments to pass to the 'tof\_cluster\_\*' function family member cor-

responding to the chosen method.

augment A boolean value indicating if the output should column-bind the cluster ids of

each cell as a new column in 'tof\_tibble' (TRUE, the default) or if a single-

column tibble including only the cluster ids should be returned (FALSE).

method A string indicating which clustering methods should be used. Valid values in-

clude "flowsom", "phenograph", "kmeans", "ddpr", and "xshift".

#### Value

A 'tof\_tbl' or 'tibble' If augment = FALSE, it will have a single column encoding the cluster ids for each cell in 'tof\_tibble'. If augment = TRUE, it will have ncol(tof\_tibble) + 1 columns: each of the (unaltered) columns in 'tof\_tibble' plus an additional column encoding the cluster ids.

### **Description**

Compute a Kaplan-Meier curve from sample-level survival data

## Usage

```
tof_compute_km_curve(survival_curves)
```

### **Arguments**

survival\_curves

A tibble from which the Kaplan-Meier curve will be computed. Each row must represent an observation and must have two columns named "time\_to\_event" and "event".

### Value

A tibble with 3 columns: time\_to\_event, survival\_probability, and is\_censored (whether or not an event was censored at that timepoint).

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tof_cosine_dist A function for finding the cosine distance between each of the rows of a numeric matrix and a numeric vector.	tof_cosine_dist	A function for finding the cosine distance between each of the rows of a numeric matrix and a numeric vector.
---	-----------------	---

## **Description**

A function for finding the cosine distance between each of the rows of a numeric matrix and a numeric vector.

## Usage

```
tof_cosine_dist(matrix, vector)
```

## Arguments

matrix A numeric matrix.

vector A numeric vector.

#### Value

A numeric vector of distances of length 'nrow(matrix)' in which the ith entry represents the cosine distance between the ith row of 'matrix' and 'vector'.

## **Examples**

NULL

tof\_create\_grid

Create an elastic net hyperparameter search grid of a specified size

# Description

This function creates a regular hyperparameter search grid (in the form of a tibble) specifying the search space for the two hyperparameters of a generalized linear model using the glmnet package: the regularization penalty term and the lasso/ridge regression mixture term.

## Usage

```
tof_create_grid(
  penalty_values,
  mixture_values,
  num_penalty_values = 5,
  num_mixture_values = 5
)
```

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## Arguments

penalty\_values A numeric vector of the unique elastic net penalty values ("lambda") to include

in the hyperparameter grid. If unspecified, a regular grid with 'num\_penalty\_values'

between  $10^{-10}$  and  $10^{0}$  will be used.

mixture\_values A numeric vector of all elastic net mixture values ("alpha") to include in the hyperparameter grid. If unspecified, a regular grid with 'num\_mixture\_values'

between 0 and 1 will be used.

num\_penalty\_values

Optional. If 'penalty\_values' is not supplied, 'num\_penalty\_values' (an integer) can be given to specify how many equally-spaced penalty values between 10^(-10) and 1 should be included in the hyperparameter grid. If this method is used, the regular grid will always be returned. Defaults to 5.

num\_mixture\_values

Optional. If 'mixture\_values' is not supplied, 'num\_mixture\_values' (an integer) can be given to specify how many equally-spaced penalty values between 0 (ridge regression) and 1 (lasso) should be included in the hyperparameter grid. If this method is used, the regular grid will always be returned. Defaults to 5.

#### Value

A tibble with two numeric columns: 'penalty' and 'mixture'.

#### See Also

Other modeling functions: tof\_assess\_model(), tof\_predict(), tof\_split\_data(), tof\_train\_model()

## **Examples**

```
tof_create_grid()
tof_create_grid(num_penalty_values = 10, num_mixture_values = 5)
tof_create_grid(penalty_values = c(0.01, 0.1, 0.5))
```

tof\_create\_recipe

Create a recipe for preprocessing sample-level cytometry data for an elastic net model

### **Description**

Create a recipe for preprocessing sample-level cytometry data for an elastic net model

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### Usage

```
tof_create_recipe(
  feature_tibble,
  predictor_cols,
  outcome_cols,
  standardize_predictors = TRUE,
  remove_zv_predictors = FALSE,
  impute_missing_predictors = FALSE)
```

## **Arguments**

feature\_tibble A tibble in which each row represents a sample- or patient- level observation, such as those produced by tof\_extract\_features.

predictor\_cols Unquoted column names indicating which columns in the data contained in 'feature\_tibble' should be used as predictors in the elastic net model. Supports tidyselect helpers.

outcome\_cols Unquoted column names indicating which columns in 'feature\_tibble' should be used as outcome variables in the elastic net model. Supports tidyselect helpers.

standardize\_predictors

A logical value indicating if numeric predictor columns should be standardized (centered and scaled) before model fitting. Defaults to TRUE.

remove\_zv\_predictors

A logical value indicating if predictor columns with near-zero variance should be removed before model fitting using step\_nzv. Defaults to FALSE.

impute\_missing\_predictors

A logical value indicating if predictor columns should have missing values imputed using k-nearest neighbors before model fitting (see step\_impute\_knn). Imputation is performed using an observation's 5 nearest-neighbors. Defaults to FALSE.

#### Value

A recipe object.

tof\_downsample

Downsample high-dimensional cytometry data.

## **Description**

This function downsamples the number of cells in a 'tof\_tbl' using the one of three methods (randomly sampling a constant number of cells, randomly sampling a proportion of cells, or performing density-dependent downsampling per the algorithm in Qiu et al., (2011)).

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### Usage

```
tof_downsample(
  tof_tibble,
  group_cols = NULL,
  ...,
  method = c("constant", "prop", "density")
)
```

## Arguments

tof\_tibble A 'tof\_tbl' or a 'tibble'.

group\_cols Unquoted names of the columns in 'tof\_tibble' that should be used to define groups within which the downsampling will be performed. Supports tidyselect helpers. Defaults to 'NULL' (no grouping).

... Additional arguments to pass to the 'tof\_downsample\_\*' function family member corresponding to the chosen method.

Method A string indicating which downsampling method to use: "constant" (the default), "prop", or "density".

### Value

A downsampled 'tof\_tbl' with the same number of columns as the input 'tof\_tibble', but fewer rows. The number of rows in the result will depend on the chosen downsampling method.

### See Also

Other downsampling functions: tof\_downsample\_constant(), tof\_downsample\_density(), tof\_downsample\_prop()

```
sim_data <-
    dplyr::tibble(
        cd45 = rnorm(n = 1000),
        cd38 = rnorm(n = 1000),
        cd34 = rnorm(n = 1000),
        cd19 = rnorm(n = 1000),
        cluster_id = sample(letters, size = 1000, replace = TRUE)
   )
# sample 200 cells from the input data
tof_downsample(
   tof_tibble = sim_data,
   num_cells = 200L,
   method = "constant"
# sample 10% of all cells from the input data
tof_downsample(
   tof_tibble = sim_data,
   prop_cells = 0.1,
```

```
method = "prop"
)

# sample ~10% of cells from the input data using density dependence
tof_downsample(
    tof_tibble = sim_data,
    target_prop_cells = 0.1,
    method = "density"
)
```

tof\_downsample\_constant

Downsample high-dimensional cytometry data by randomly selecting a constant number of cells per group.

# Description

This function downsamples the number of cells in a 'tof\_tbl' by randomly selecting 'num\_cells' cells from each unique combination of values in 'group\_cols'.

### Usage

```
tof_downsample_constant(tof_tibble, group_cols = NULL, num_cells)
```

# **Arguments**

tof\_tibble A 'tof\_tbl' or a 'tibble'.

group\_cols

Unquoted names of the columns in 'tof\_tibble' that should be used to define groups from which 'num\_cells' will be downsampled. Supports tidyselect helpers. Defaults to 'NULL' (no grouping).

An integer number of cells that should be sampled from each group defined by 'group\_cols'.

#### Value

A 'tof\_tbl' with the same number of columns as the input 'tof\_tibble', but fewer rows. Specifically, the number of rows will be 'num\_cells' multiplied by the number of unique combinations of the values in 'group\_cols'. If any group has fewer than 'num\_cells' number of cells, all cells from that group will be kept.

### See Also

Other downsampling functions: tof\_downsample(), tof\_downsample\_density(), tof\_downsample\_prop()

## **Examples**

```
sim_data <-
   dplyr::tibble(
       cd45 = rnorm(n = 1000),
        cd38 = rnorm(n = 1000),
        cd34 = rnorm(n = 1000),
        cd19 = rnorm(n = 1000),
        cluster_id = sample(letters, size = 1000, replace = TRUE)
    )
# sample 500 cells from the input data
tof_downsample_constant(
    tof_tibble = sim_data,
   num_cells = 500L
)
# sample 20 cells per cluster from the input data
tof_downsample_constant(
    tof_tibble = sim_data,
   group_cols = cluster_id,
   num_cells = 20L
)
```

tof\_downsample\_density

Downsample high-dimensional cytometry data by randomly selecting a proportion of the cells in each group.

# Description

This function downsamples the number of cells in a 'tof\_tbl' using the density-dependent downsampling algorithm described in Qiu et al., (2011).

### Usage

```
tof_downsample_density(
  tof_tibble,
  group_cols = NULL,
  density_cols = where(tof_is_numeric),
  target_num_cells,
  target_prop_cells,
  target_percentile = 0.03,
  outlier_percentile = 0.01,
  distance_function = c("euclidean", "cosine", "12", "ip"),
  density_estimation_method = c("mean_distance", "sum_distance", "spade"),
  ...
)
```

### **Arguments**

tof\_tibble A 'tof\_tbl' or a 'tibble'.

group\_cols Unquoted names of the columns in 'tof\_tibble' that should be used to define

groups within which the downsampling will be performed. Supports tidyselect

helpers. Defaults to 'NULL' (no grouping).

density\_cols Unquoted names of the columns in 'tof\_tibble' to use in the density estimation

for each cell. Defaults to all numeric columns in 'tof\_tibble'.

target\_num\_cells

An approximate constant number of cells (between 0 and 1) that should be sampled from each group defined by 'group\_cols'. Slightly more or fewer cells may be returned due to how the density calculation is performed.

target\_prop\_cells

An approximate proportion of cells (between 0 and 1) that should be sampled from each group defined by 'group\_cols'. Slightly more or fewer cells may be returned due to how the density calculation is performed. Ignored if 'target\_num\_cells' is specified.

target\_percentile

The local density percentile (i.e. a value between 0 and 1) to which the down-sampling procedure should adjust all cells. In short, the algorithm will continue to remove cells from the input 'tof\_tibble' until the local densities of all remaining cells is equal to 'target\_percentile'. Lower values will result in more cells being removed. See Qiu et al., (2011) for details. Defaults to 0.1 (the 10th percentile of local densities). Ignored if either 'target\_num\_cells' or 'target\_prop\_cells' are specified.

outlier\_percentile

The local density percentile (i.e. a value between 0 and 1) below which cells should be considered outliers (and discarded). Cells with a local density below 'outlier\_percentile' will never be selected during the downsampling procedure. Defaults to 0.01 (cells below the 1st local density percentile will be removed).

distance\_function

A string indicating which distance function to use for the cell-to-cell distance calculations. Options include "euclidean" (the default) and "cosine" distances.

density\_estimation\_method

A string indicating which algorithm should be used to calculate the local density estimate for each cell. Options include k-nearest neighbor density estimation using the mean distance to a cell's k-nearest neighbors ("mean\_distance"; the default), k-nearest neighbor density estimation using the summed distance to a cell's k nearest neighbors ("sum\_distance") and counting the number of neighboring cells within a spherical radius around each cell as described in Qiu et al., 2011 ("spade"). While "spade" often produces the best results, it is slower than knn-density estimation methods.

Optional additional arguments to pass to tof\_knn\_density or tof\_spade\_density.

## Value

A 'tof\_tbl' with the same number of columns as the input 'tof\_tibble', but fewer rows. The number of rows will depend on the chosen value of 'target\_percentile', with fewer cells selected with lower

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values of 'target\_percentile'.

### See Also

Other downsampling functions: tof\_downsample(), tof\_downsample\_constant(), tof\_downsample\_prop()

## **Examples**

```
sim_data <-
    dplyr::tibble(
       cd45 = rnorm(n = 1000),
        cd38 = rnorm(n = 1000),
       cd34 = rnorm(n = 1000),
        cd19 = rnorm(n = 1000)
   )
tof_downsample_density(
    tof_tibble = sim_data,
   density\_cols = c(cd45, cd34, cd38),
   target_prop_cells = 0.5,
   density_estimation_method = "spade"
)
tof_downsample_density(
    tof_tibble = sim_data,
   density\_cols = c(cd45, cd34, cd38),
   target_num_cells = 200L,
   density_estimation_method = "spade"
)
tof_downsample_density(
    tof_tibble = sim_data,
   density\_cols = c(cd45, cd34, cd38),
   target_num_cells = 200L,
   density_estimation_method = "mean_distance"
)
```

tof\_downsample\_prop

Downsample high-dimensional cytometry data by randomly selecting a proportion of the cells in each group.

### **Description**

This function downsamples the number of cells in a 'tof\_tbl' by randomly selecting a 'prop\_cells' proportion of the total number of cells with each unique combination of values in 'group\_cols'.

## Usage

```
tof_downsample_prop(tof_tibble, group_cols = NULL, prop_cells)
```

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# Arguments

```
tof_tibble A 'tof_tbl' or a 'tibble'.

group_cols

Unquoted names of the columns in 'tof_tibble' that should be used to define groups from which 'prop_cells' will be downsampled. Supports tidyselect helpers. Defaults to 'NULL' (no grouping).

A proportion of cells (between 0 and 1) that should be sampled from each group defined by 'group_cols'.
```

### Value

A 'tof\_tbl' with the same number of columns as the input 'tof\_tibble', but fewer rows. Specifically, the number of rows should be 'prop\_cells' times the number of rows in the input 'tof\_tibble'.

# See Also

Other downsampling functions: tof\_downsample(), tof\_downsample\_constant(), tof\_downsample\_density()

```
sim_data <-
    dplyr::tibble(
       cd45 = rnorm(n = 1000),
        cd38 = rnorm(n = 1000),
        cd34 = rnorm(n = 1000),
        cd19 = rnorm(n = 1000),
        cluster_id = sample(letters, size = 1000, replace = TRUE)
    )
# sample 10% of all cells from the input data
tof_downsample_prop(
   tof_tibble = sim_data,
   prop_cells = 0.1
)
# sample 10% of all cells from each cluster in the input data
tof_downsample_prop(
    tof_tibble = sim_data,
   group_cols = cluster_id,
   prop_cells = 0.1
)
```

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## **Description**

This function is a wrapper around tidytof's tof\_\*\_density() function family. It performs local density estimation on high-dimensional cytometry data using a user-specified method (of 3 choices) and each method's corresponding input parameters.

### Usage

```
tof_estimate_density(
  tof_tibble,
  distance_cols = where(tof_is_numeric),
  distance_function = c("euclidean", "cosine", "12", "ip"),
  normalize = TRUE,
  ...,
  augment = TRUE,
  method = c("mean_distance", "sum_distance", "spade")
)
```

# **Arguments**

tof\_tibble A 'tof\_tbl' or a 'tibble'.

cell distances during the local density estimation for each cell. Defaults to all

numeric columns in 'tof tibble'.

distance\_function

A string indicating which distance function to use for calculating cell-to-cell dis-

tances during local density estimation. Options include "euclidean" (the default)

and "cosine".

normalize A boolean value indicating if the vector of local density estimates should be

normalized to values between 0 and 1. Defaults to TRUE.

... Additional arguments to pass to the 'tof\_\*\_density()' function family member

corresponding to the chosen 'method'.

augment A boolean value indicating if the output should column-bind the local density

estimates of each cell as a new column in 'tof\_tibble' (TRUE; the default) or if a single-column tibble including only the local density estimates should be

returned (FALSE).

method A string indicating which local density estimation method should be used. Valid

values include "mean distance", "sum distance", and "spade".

## Value

A 'tof\_tbl' or 'tibble' If augment = FALSE, it will have a single column encoding the local density estimates for each cell in 'tof\_tibble'. If augment = TRUE, it will have ncol(tof\_tibble) + 1 columns: each of the (unaltered) columns in 'tof\_tibble' plus an additional column encoding the local density estimates.

### See Also

Other local density estimation functions: tof\_knn\_density(), tof\_spade\_density()

## **Examples**

```
sim_data <-
   dplyr::tibble(
        cd45 = rnorm(n = 1000),
        cd38 = rnorm(n = 1000),
        cd34 = rnorm(n = 1000),
        cd19 = rnorm(n = 1000)
   )
# perform the density estimation
tof_estimate_density(tof_tibble = sim_data, method = "spade")
# perform the density estimation with a smaller search radius around
# each cell
tof_estimate_density(
    tof_tibble = sim_data,
   alpha_multiplier = 2,
   method = "spade"
)
```

tof\_extract\_central\_tendency

Extract the central tendencies of CyTOF markers in each cluster in a 'tof\_tibble'.

## **Description**

This feature extraction function calculates a user-specified measurement of central tendency (i.e. median or mode) of the cells in each cluster in a 'tof\_tibble' across a user-specified selection of CyTOF markers. These calculations can be done either overall (across all cells in the dataset) or after breaking down the cells into subgroups using 'group\_cols'.

# Usage

```
tof_extract_central_tendency(
  tof_tibble,
  cluster_col,
  group_cols = NULL,
  marker_cols = where(tof_is_numeric),
  stimulation_col = NULL,
  central_tendency_function = stats::median,
  format = c("wide", "long")
)
```

### **Arguments**

tof\_tibble A 'tof\_tibble' or a 'tibble' in which each row represents a single cell and each

column represents a CyTOF measurement or a piece of metadata (i.e. cluster id,

patient id, etc.) about each cell.

cluster\_col An unquoted column name indicating which column in 'tof\_tibble' stores the

cluster ids of the cluster to which each cell belongs. Cluster labels can be produced via any method the user chooses - including manual gating, any of the

functions in the 'tof\_cluster\_\*' function family, or any other method.

group\_cols Unquoted column names representing which columns in 'tof\_tibble' should be

used to break the rows of 'tof\_tibble' into subgroups for the feature extraction calculation. Defaults to NULL (i.e. performing the extraction without sub-

groups).

marker\_cols Unquoted column names representing which columns in 'tof\_tibble' (i.e. which

CyTOF protein measurements) should be included in the feature extraction calculation. Defaults to all numeric (integer or double) columns. Supports tidyse-

lection.

stimulation\_col

Optional. An unquoted column name that indicates which column in 'tof\_tibble' contains information about which stimulation condition each cell was exposed to during data acquisition. If provided, the feature extraction will be further broken down into subgroups by stimulation condition (and features from each stimulation condition will be included as their own features in wide format).

central\_tendency\_function

The function that will be used to calculate the measurement of central tendency for each cluster (to be used as the dependent variable in the linear model). De-

faults to median.

format A string indicating if the data should be returned in "wide" format (the default;

each cluster feature is given its own column) or in "long" format (each cluster

feature is provided as its own row).

#### Value

A tibble.

If format == "wide", the tibble will have 1 row for each combination of the grouping variables provided in 'group\_cols' and one column for each grouping variable, one column for each extracted feature (the central tendency of a given marker in a given cluster). The names of each column containing cluster features is obtained using the following pattern: "{marker\_id}@{cluster\_id}\_ct".

If format == "long", the tibble will have 1 row for each combination of the grouping variables in 'group\_cols', each cluster id (i.e. level) in 'cluster\_col', and each marker in 'marker\_cols'. It will have one column for each grouping variable, one column for the cluster ids, one column for the CyTOF channel names, and one column ('value') containing the features.

#### See Also

Other feature extraction functions: tof\_extract\_emd(), tof\_extract\_features(), tof\_extract\_jsd(), tof\_extract\_proportion(), tof\_extract\_threshold()

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## **Examples**

```
sim_data <-
    dplyr::tibble(
        cd45 = rnorm(n = 1000),
        cd38 = rnorm(n = 1000),
        cd34 = rnorm(n = 1000),
        cd19 = rnorm(n = 1000),
        cluster_id = sample(letters, size = 1000, replace = TRUE),
        patient = sample(c("kirby", "mario"), size = 1000, replace = TRUE),
        stim = sample(c("basal", "stim"), size = 1000, replace = TRUE)
   )
# extract proportion of each cluster in each patient in wide format
tof_extract_central_tendency(
    tof_tibble = sim_data,
   cluster_col = cluster_id,
   group_cols = patient
)
# extract proportion of each cluster in each patient in long format
tof_extract_central_tendency(
    tof_tibble = sim_data,
   cluster_col = cluster_id,
   group_cols = patient,
    format = "long"
)
```

tof\_extract\_emd

Extract aggregated features from CyTOF data using earth-mover's distance (EMD)

## Description

This feature extraction function calculates the earth-mover's distance (EMD) between the stimulated and unstimulated ("basal") experimental conditions of samples in a CyTOF experiment. This calculation is performed across a user-specified selection of CyTOF antigens and can be performed either overall (across all cells in the dataset) or after breaking down the cells into subgroups using 'group\_cols'.

# Usage

```
tof_extract_emd(
  tof_tibble,
  cluster_col,
  group_cols = NULL,
  marker_cols = where(tof_is_numeric),
  emd_col,
  reference_level,
```

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```
format = c("wide", "long"),
num_bins = 100
)
```

### **Arguments**

tof\_tibble

A 'tof\_tbl' or a 'tibble'.

cluster\_col

An unquoted column name indicating which column in 'tof\_tibble' stores the cluster ids of the cluster to which each cell belongs. Cluster labels can be produced via any method the user chooses - including manual gating, any of the functions in the 'tof\_cluster\_\*' function family, or any other method.

group\_cols

Unquoted column names representing which columns in 'tof\_tibble' should be used to break the rows of 'tof\_tibble' into subgroups for the feature extraction calculation. Defaults to NULL (i.e. performing the extraction without subgroups).

marker\_cols

Unquoted column names representing which columns in 'tof\_tibble' (i.e. which CyTOF protein measurements) should be included in the earth-mover's distance calculation. Defaults to all numeric (integer or double) columns. Supports tidyselect helpers.

emd\_col

An unquoted column name that indicates which column in 'tof\_tibble' should be used to group cells into different distributions to be compared with one another during the EMD calculation. For example, if you want to compare marker expression distributions across stimulation conditions, 'emd\_col' should be the column in 'tof\_tibble' containing information about which stimulation condition each cell was exposed to during data acquisition.

If provided, the feature extraction will be further broken down into subgroups by stimulation condition (and features from each stimulation condition will be included as their own features in wide format).

reference\_level

A string indicating what the value in 'emd\_col' corresponds to the "reference" value to which all other values in 'emd\_col' should be compared. For example, if 'emd\_col' represents the stimulation condition for a cell, reference\_level might take the value of "basal" or "unstimulated" if you want to compare each stimulation to the basal state.

format

A string indicating if the data should be returned in "wide" format (the default; each cluster feature is given its own column) or in "long" format (each cluster feature is provided as its own row).

num\_bins

Optional. The number of bins to use in dividing one-dimensional marker distributions into discrete segments for the EMD calculation. Defaults to 100.

## Value

A tibble.

If format == "wide", the tibble will have 1 row for each combination of the grouping variables provided in 'group\_cols' and one column for each grouping variable, one column for each extracted feature (the EMD between the distribution of a given marker in a given cluster in the basal

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condition and the distribution of that marker in a given cluster in a stimulated condition). The names of each column containing cluster features is obtained using the following pattern: "{stimulation\_id}\_{marker\_id}@{cluster\_id}\_emd".

If format == "long", the tibble will have 1 row for each combination of the grouping variables in 'group\_cols', each cluster id (i.e. level) in 'cluster\_col', and each marker in 'marker\_cols'. It will have one column for each grouping variable, one column for the cluster ids, one column for the CyTOF channel names, and one column ('value') containing the features.

### See Also

```
Other feature extraction functions: tof_extract_central_tendency(), tof_extract_features(), tof_extract_jsd(), tof_extract_proportion(), tof_extract_threshold()
```

```
sim_data <-
    dplyr::tibble(
        cd45 = rnorm(n = 1000),
        cd38 = rnorm(n = 1000),
        cd34 = rnorm(n = 1000),
        cd19 = rnorm(n = 1000),
        cluster_id = sample(letters, size = 1000, replace = TRUE),
        patient = sample(c("kirby", "mario"), size = 1000, replace = TRUE),
        stim = sample(c("basal", "stim"), size = 1000, replace = TRUE)
    )
# extract emd of each cluster in each patient (using the "basal" stim
# condition as a reference) in wide format
tof_extract_emd(
    tof_tibble = sim_data,
    cluster_col = cluster_id,
   group_cols = patient,
    emd_col = stim,
    reference_level = "basal"
)
# extract emd of each cluster (using the "basal" stim
# condition as a reference) in long format
tof_extract_emd(
    tof_tibble = sim_data,
    cluster_col = cluster_id,
    emd_col = stim,
    reference_level = "basal",
    format = "long"
)
```

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### **Description**

This function wraps other members of the 'tof\_extract\_\*' function family to extract sample-level features from both lineage (i.e. cell surface antigen) CyTOF channels assumed to be stable across stimulation conditions and signaling CyTOF channels assumed to change across stimulation conditions. Features are extracted for each cluster within each independent sample (as defined with the 'group\_cols' argument).

# Usage

```
tof_extract_features(
  tof_tibble,
  cluster_col,
  group_cols = NULL,
  stimulation_col = NULL,
  lineage_cols,
  signaling_cols,
  central_tendency_function = stats::median,
  signaling_method = c("threshold", "emd", "jsd", "central tendency"),
  basal_level = NULL,
  ...
)
```

# Arguments

tof\_tibble A 'tof\_tbl' or a 'tibble'.

cluster\_col An unquoted column name indicating which column in 'tof\_tibble' stores the cluster ids of the cluster to which each cell belongs. Cluster labels can be pro-

duced via any method the user chooses - including manual gating, any of the

functions in the 'tof\_cluster\_\*' function family, or any other method.

Unquoted column names representing which columns in 'tof\_tibble' should be used to break the rows of 'tof\_tibble' into subgroups for the feature extraction calculation. Defaults to NULL (i.e. performing the extraction without sub-

groups).

stimulation\_col

Optional. An unquoted column name that indicates which column in 'tof\_tibble' contains information about which stimulation condition each cell was exposed to during data acquisition. If provided, the feature extraction will be further broken down into subgroups by stimulation condition (and features from each stimulation condition will be included as their own features in wide format).

lineage\_cols Unquoted column names representing which columns in 'tof\_tibble' (i.e. which CyTOF protein measurements) should be considered lineage markers in the feature extraction calculation. Supports tidyselect helpers.

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signaling\_cols Unquoted column names representing which columns in 'tof\_tibble' (i.e. which CyTOF protein measurements) should be considered signaling markers in the feature extraction calculation. Supports tidyselect helpers.

central\_tendency\_function

The function that will be used to calculate the measurement of central tendency for each cluster (to be used as the dependent variable in the linear model). Defaults to median.

signaling\_method

A string indicating which feature extraction method to use for signaling markers (as identified by the 'signaling\_cols' argument). Options are "threshold" (the default), "emd", "jsd", and "central tendency".

basal\_level A string indicating what the value in 'stimulation\_col' corresponds to the basal stimulation condition (i.e. "basal" or "unstimulated").

... Optional additional arguments to be passed to tof\_extract\_threshold, tof\_extract\_emd, or tof\_extract\_jsd.

### **Details**

Lineage channels are specified using the 'lineage\_cols' argument, and their extracted features will be measurements of central tendency (as computed by the user-supplied 'central\_tendency\_function').

Signaling channels are specified using the 'signaling\_cols' argument, and their extracted features will depend on the user's chosen 'signaling\_method'. If 'signaling method' == "threshold" (the default), tof\_extract\_threshold will be used to calculate the proportion of cells in each cluster with signaling marker expression over 'threshold' in each stimulation condition. If 'signaling\_method' == "emd" or 'signaling\_method' == "jsd", tof\_extract\_emd or tof\_extract\_jsd will be used to calculate the earth-mover's distance (EMD) or Jensen-Shannon Distance (JSD), respectively, between the basal condition and each of the stimulated conditions in each cluster for each sample. Finally, if none of these options are chosen, tof\_extract\_central\_tendency will be used to calculate measurements of central tendency.

In addition, tof\_extract\_proportion will be used to extract the proportion of cells in each cluster will be computed for each sample.

These calculations can be performed either overall (across all cells in the dataset) or after breaking down the cells into subgroups using 'group\_cols'.

### Value

A tibble.

The output tibble will have 1 row for each combination of the grouping variables provided in 'group\_cols' (thus, each row will represent what is considered a single "sample" based on the grouping provided). It will have one column for each grouping variable and one column for each extracted feature ("wide" format).

### See Also

Other feature extraction functions: tof\_extract\_central\_tendency(), tof\_extract\_emd(), tof\_extract\_jsd(), tof\_extract\_proportion(), tof\_extract\_threshold()

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### **Examples**

```
sim_data <-
    dplyr::tibble(
       cd45 = rnorm(n = 1000),
        cd38 = rnorm(n = 1000),
        cd34 = rnorm(n = 1000),
        cd19 = rnorm(n = 1000),
        cluster_id = sample(letters, size = 1000, replace = TRUE),
        patient = sample(c("kirby", "mario"), size = 1000, replace = TRUE),
        stim = sample(c("basal", "stim"), size = 1000, replace = TRUE)
   )
# extract the following features from each cluster in each
# patient/stimulation:
    - proportion of each cluster
    - central tendency (median) of cd45 and cd38 in each cluster
    - the proportion of cells in each cluster with cd34 expression over
      the default threshold (asinh(10 / 5))
tof_extract_features(
    tof_tibble = sim_data,
   cluster_col = cluster_id,
    group_cols = patient,
    lineage_cols = c(cd45, cd38),
    signaling_cols = cd34,
    stimulation_col = stim
)
# extract the following features from each cluster in each
# patient/stimulation:
    - proportion of each cluster
     - central tendency (mean) of cd45 and cd38 in each cluster
     - the earth mover's distance between each cluster's cd34 histogram in
      the "basal" and "stim" conditions
tof_extract_features(
    tof_tibble = sim_data,
   cluster_col = cluster_id,
    group_cols = patient,
    lineage_cols = c(cd45, cd38),
    signaling_cols = cd34,
   central_tendency_function = mean,
   stimulation_col = stim,
    signaling_method = "emd",
   basal_level = "basal"
)
```

Extract aggregated features from CyTOF data using the Jensen-Shannon Distance (JSD)

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## **Description**

This feature extraction function calculates the Jensen-Shannon Distance (JSD) between the stimulated and unstimulated ("basal") experimental conditions of samples in a CyTOF experiment. This calculation is performed across a user-specified selection of CyTOF antigens and can be performed either overall (across all cells in the dataset) or after breaking down the cells into subgroups using 'group\_cols'.

### Usage

```
tof_extract_jsd(
  tof_tibble,
  cluster_col,
  group_cols = NULL,
  marker_cols = where(tof_is_numeric),
  jsd_col,
  reference_level,
  format = c("wide", "long"),
  num_bins = 100
)
```

## Arguments

tof\_tibble A 'tof\_tbl' or a 'tibble'.

cluster\_col An unquoted column name indicating which column in 'tof\_tibble' stores the

cluster ids of the cluster to which each cell belongs. Cluster labels can be produced via any method the user chooses - including manual gating, any of the

functions in the 'tof\_cluster\_\*' function family, or any other method.

group\_cols Unquoted column names representing which columns in 'tof\_tibble' should be used to break the rows of 'tof\_tibble' into subgroups for the feature extraction

calculation. Defaults to NULL (i.e. performing the extraction without sub-

groups).

marker\_cols Unquoted column names representing which columns in 'tof\_tibble' (i.e. which

CyTOF protein measurements) should be included in the feature extraction calculation. Defaults to all numeric (integer or double) columns. Supports tidyse-

lect helpers.

jsd\_col An unquoted column name that indicates which column in 'tof\_tibble' contains

information about which stimulation condition each cell was exposed to during

data acquisition.

If provided, the feature extraction will be further broken down into subgroups by stimulation condition (and features from each stimulation condition will be

included as their own features in wide format).

reference\_level

A string indicating what the value in 'jsd\_col' corresponds to the basal stimula-

tion condition (i.e. "basal" or "unstimulated").

format A string indicating if the data should be returned in "wide" format (the default; each cluster feature is given its own column) or in "long" format (each cluster

feature is provided as its own row).

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num\_bins

Optional. The number of bins to use in dividing one-dimensional marker distributions into discrete segments for the JSD calculation. Defaults to 100.

### Value

A tibble.

If format == "wide", the tibble will have 1 row for each combination of the grouping variables provided in 'group\_cols' and one column for each grouping variable, one column for each extracted feature (the JSD between the distribution of a given marker in a given cluster in the basal condition and the distribution of that marker in the same cluster in a stimulated condition). The names of each column containing cluster features is obtained using the following pattern: "{stimulation\_id}\_{marker\_id}@{cluster\_id}\_jsd".

If format == "long", the tibble will have 1 row for each combination of the grouping variables in 'group\_cols', each cluster id (i.e. level) in 'cluster\_col', and each marker in 'marker\_cols'. It will have one column for each grouping variable, one column for the cluster ids, one column for the CyTOF channel names, and one column ('value') containing the features.

#### See Also

```
Other feature extraction functions: tof_extract_central_tendency(), tof_extract_emd(), tof_extract_features(), tof_extract_proportion(), tof_extract_threshold()
```

```
sim_data <-
   dplyr::tibble(
        cd45 = rnorm(n = 1000),
        cd38 = rnorm(n = 1000),
        cd34 = rnorm(n = 1000),
        cd19 = rnorm(n = 1000),
        cluster_id = sample(letters, size = 1000, replace = TRUE),
        patient = sample(c("kirby", "mario"), size = 1000, replace = TRUE),
        stim = sample(c("basal", "stim"), size = 1000, replace = TRUE)
   )
# extract jsd of each cluster in each patient (using the "basal" stim
# condition as a reference) in wide format
tof_extract_jsd(
    tof_tibble = sim_data,
   cluster_col = cluster_id,
   group_cols = patient,
   jsd_col = stim,
    reference_level = "basal"
)
# extract jsd of each cluster (using the "basal" stim
# condition as a reference) in long format
tof_extract_jsd(
    tof_tibble = sim_data,
   cluster_col = cluster_id,
    jsd_col = stim,
```

```
reference_level = "basal",
format = "long"
)
```

tof\_extract\_proportion

Extract the proportion of cells in each cluster in a 'tof\_tibble'.

# Description

This feature extraction function allows you to calculate the proportion of cells in each cluster in a 'tof\_tibble' - either overall or when broken down into subgroups using 'group\_cols'.

### Usage

```
tof_extract_proportion(
  tof_tibble,
  cluster_col,
  group_cols = NULL,
  format = c("wide", "long")
)
```

# **Arguments**

tof\_tibble A 'tof\_tbl' or a 'tibble'.

cluster\_col An unquoted column name indicating which column in 'tof\_tibble' stores the

cluster ids of the cluster to which each cell belongs. Cluster labels can be produced via any method the user chooses - including manual gating, any of the

functions in the 'tof\_cluster\_\*' function family, or any other method.

group\_cols Unquoted column names representing which columns in 'tof\_tibble' should be

used to break the rows of 'tof\_tibble' into subgroups for the feature extraction calculation. Defaults to NULL (i.e. performing the extraction without sub-

groups).

format A string indicating if the data should be returned in "wide" format (the default;

each cluster proportion is given its own column) or in "long" format (each cluster

proportion is provided as its own row).

## Value

A tibble.

If format == "wide", the tibble will have 1 row for each combination of the grouping variables provided in 'group\_cols' and one column for each grouping variable as well as one column for the proportion of cells in each cluster. The names of each column containing cluster proportions is obtained using the following pattern: "prop@{cluster\_id}".

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If format == "long", the tibble will have 1 row for each combination of the grouping variables in 'group\_cols' and each cluster id (i.e. level) in 'cluster\_col'. It will have one column for each grouping variable, one column for the cluster ids, and one column ('prop') containing the cluster proportions.

### See Also

```
Other feature extraction functions: tof_extract_central_tendency(), tof_extract_emd(), tof_extract_features(), tof_extract_jsd(), tof_extract_threshold()
```

## **Examples**

```
sim_data <-
   dplyr::tibble(
        cd45 = rnorm(n = 1000),
        cd38 = rnorm(n = 1000),
        cd34 = rnorm(n = 1000),
        cd19 = rnorm(n = 1000),
        cluster_id = sample(letters, size = 1000, replace = TRUE),
        patient = sample(c("kirby", "mario"), size = 1000, replace = TRUE),
        stim = sample(c("basal", "stim"), size = 1000, replace = TRUE)
    )
# extract proportion of each cluster in each patient in wide format
tof_extract_proportion(
    tof_tibble = sim_data,
   cluster_col = cluster_id,
   group_cols = patient
)
# extract proportion of each cluster in each patient in long format
tof_extract_proportion(
    tof_tibble = sim_data,
   cluster_col = cluster_id,
   group_cols = patient,
    format = "long"
)
```

### **Description**

This feature extraction function calculates the proportion of cells in a given cluster that have a CyTOF antigen expression over a user-specified threshold across a user-specified selection of CyTOF markers. These calculations can be done either overall (across all cells in the dataset) or after breaking down the cells into subgroups using 'group\_cols'.

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## Usage

```
tof_extract_threshold(
  tof_tibble,
  cluster_col,
  group_cols = NULL,
  marker_cols = where(tof_is_numeric),
  stimulation_col = NULL,
  threshold = asinh(10/5),
  format = c("wide", "long")
)
```

#### Arguments

tof\_tibble A 'tof\_tbl' or a 'tibble'.

cluster\_col An unquoted column name indicating which column in 'tof\_tibble' stores the

cluster ids of the cluster to which each cell belongs. Cluster labels can be produced via any method the user chooses - including manual gating, any of the

functions in the 'tof\_cluster\_\*' function family, or any other method.

group\_cols Unquoted column names representing which columns in 'tof\_tibble' should be

used to break the rows of 'tof\_tibble' into subgroups for the feature extraction calculation. Defaults to NULL (i.e. performing the extraction without sub-

groups).

marker\_cols Unquoted column names representing which columns in 'tof tibble' (i.e. which

CyTOF protein measurements) should be included in the feature extraction calculation. Defaults to all numeric (integer or double) columns. Supports tidyse-

lect helpers.

stimulation col

Optional. An unquoted column name that indicates which column in 'tof\_tibble' contains information about which stimulation condition each cell was exposed to during data acquisition. If provided, the feature extraction will be further broken down into subgroups by stimulation condition (and features from each

stimulation condition will be included as their own features in wide format).

threshold A double or integer of length 1 indicating what threshold should be used.

A string indicating if the data should be returned in "wide" format (the default;

each cluster feature is given its own column) or in "long" format (each cluster

feature is provided as its own row).

#### Value

A tibble.

format

If format == "wide", the tibble will have 1 row for each combination of the grouping variables provided in 'group\_cols' and one column for each grouping variable, one column for each extracted feature (the proportion of cells in a given cluster over with marker expression values over 'threshold'). The names of each column containing cluster features is obtained using the following pattern: "{marker\_id}@{cluster\_id}\_threshold".

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If format == "long", the tibble will have 1 row for each combination of the grouping variables in 'group\_cols', each cluster id (i.e. level) in 'cluster\_col', and each marker in 'marker\_cols'. It will have one column for each grouping variable, one column for the cluster ids, one column for the CyTOF channel names, and one column ('value') containing the features.

### See Also

```
Other feature extraction functions: tof_extract_central_tendency(), tof_extract_emd(), tof_extract_features(), tof_extract_jsd(), tof_extract_proportion()
```

## **Examples**

```
sim_data <-
    dplyr::tibble(
        cd45 = rnorm(n = 1000),
        cd38 = rnorm(n = 1000),
        cd34 = rnorm(n = 1000),
        cd19 = rnorm(n = 1000),
        cluster_id = sample(letters, size = 1000, replace = TRUE),
        patient = sample(c("kirby", "mario"), size = 1000, replace = TRUE),
        stim = sample(c("basal", "stim"), size = 1000, replace = TRUE)
   )
# extract proportion of each cluster in each patient in wide format
tof_extract_threshold(
    tof_tibble = sim_data,
   cluster_col = cluster_id,
   group_cols = patient
)
# extract proportion of each cluster in each patient in long format
tof_extract_threshold(
    tof_tibble = sim_data,
   cluster_col = cluster_id,
   group_cols = patient,
   format = "long"
)
```

tof\_find\_best

Find the optimal hyperparameters for an elastic net model from candidate performance metrics

### **Description**

Find the optimal hyperparameters for an elastic net model from candidate performance metrics

## Usage

```
tof_find_best(performance_metrics, model_type, optimization_metric)
```

### **Arguments**

```
performance_metrics
```

A tibble of performance metrics for an elastic net model (in wide format)

model\_type A string indicating which type of glmnet model was trained.

optimization\_metric

A string indicating which performance metric should be used to select the optimal model.

### Value

A tibble with 3 columns: "mixture", "penalty", and a column containing the chosen optimization metric. If the returned tibble has more than 1 column, it means that more than 1 mixture/penalty combination yielded the optimal result (i.e. the tuning procedure resulted in a tie).

```
tof_find_cv_predictions
```

Calculate and store the predicted outcomes for each validation set observation during model tuning

# Description

Calculate and store the predicted outcomes for each validation set observation during model tuning

## Usage

```
tof_find_cv_predictions(
   split_data,
   prepped_recipe,
   lambda,
   alpha,
   model_type,
   outcome_colnames
)
```

# **Arguments**

split\_data An 'rsplit' object from the rsample package. Alternatively, an unsplit tbl\_df

can be provided, though this is not recommended.

prepped\_recipe A trained recipe

lambda A single numeric value indicating which penalty (lambda) value should be used

to make the predictions

alpha A single numeric value indicating which mixture (alpha) value should be used

to make the predictions

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model\_type

A string indicating which kind of elastic net model to build. If a continuous response is being predicted, use "linear" for linear regression; if a categorical response with only 2 classes is being predicted, use "two-class" for logistic regression; if a categorical response with more than 2 levels is being predicted, use "multiclass" for multinomial regression; and if a time-to-event outcome is being predicted, use "survival" for Cox regression.

outcome\_colnames

Quoted column names indicating which columns in the data being fit represent the outcome variables (with all others assumed to be predictors).

### Value

A tibble containing the predicted and true values for the outcome for each of the validation observations in 'split\_data'.

tof\_find\_emd

Find the earth-mover's distance between two numeric vectors

# Description

Find the earth-mover's distance between two numeric vectors

# Usage

```
tof_find_emd(vec_1, vec_2, num_bins = 100)
```

## **Arguments**

vec\_1 A numeric vector.
vec\_2 A numeric vector.

num\_bins An integer number of bins to use when performing kernel density estimation on

the two vectors. Defaults to 100.

### Value

A double (of length 1) representing the EMD between the two vectors.

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tof_find_jsd	Find the Jensen-Shannon Divergence (JSD) between two numeric vectors

# Description

Find the Jensen-Shannon Divergence (JSD) between two numeric vectors

# Usage

```
tof_find_jsd(vec_1, vec_2, num_bins = 100)
```

# Arguments

vec\_1 A numeric vector.

vec\_2 A numeric vector.

num\_bins An integer number of bins to use when binning across the two vectors' combined

range. Defaults to 100.

# Value

A double (of length 1) representing the JSD between the two vectors.

tof\_find\_knn Find the k-nearest neighbors of each cell in a high-dimensional cytometry dataset.

# Description

Find the k-nearest neighbors of each cell in a high-dimensional cytometry dataset.

# Usage

```
tof_find_knn(
   .data,
   k = min(10, nrow(.data)),
   distance_function = c("euclidean", "cosine", "12", "ip"),
   .query,
   ...
)
```

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# **Arguments**

k

. data A 'tof\_tibble' or 'tibble' in which each row represents a cell and each column

represents a high-dimensional cytometry measurement.

distance\_function

A string indicating which distance function to use for the nearest-neighbor calculation. Options include "euclidean" (the default) and "cosine" distances.

An integer indicating the number of nearest neighbors to return for each cell.

. query A set of cells to be queried against .data (i.e. a set of cells for which to find

nearest neighbors within .data). Defaults to .data itself, i.e. finding nearest

neighbors for all cells in .data.

... Optional additional arguments to pass to hnsw\_knn

### Value

A list with two elements: "neighbor\_ids" and "neighbor\_distances," both of which are n by k matrices (in which n is the number of cells in the input '.data'. The [i,j]-th entry of "neighbor\_ids" represents the row index for the j-th nearest neighbor of the cell in the i-th row of '.data'. The [i,j]-th entry of "neighbor\_distances" represents the distance between those two cells according to 'distance\_function'.

```
sim_data <-
   dplyr::tibble(
        cd45 = rnorm(n = 1000),
        cd38 = rnorm(n = 1000),
        cd34 = rnorm(n = 1000),
        cd19 = rnorm(n = 1000)
   )
# Find the 10 nearest neighbors of each cell in the dataset
tof_find_knn(
    .data = sim_data,
   k = 10.
   distance_function = "euclidean"
)
# Find the 10 approximate nearest neighbors
tof_find_knn(
    .data = sim_data,
   k = 10,
   distance_function = "euclidean",
)
```

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tof\_find\_log\_rank\_threshold

Compute the log-rank test p-value for the difference between the two survival curves obtained by splitting a dataset into a "low" and "high" risk group using all possible relative-risk thresholds.

## **Description**

Compute the log-rank test p-value for the difference between the two survival curves obtained by splitting a dataset into a "low" and "high" risk group using all possible relative-risk thresholds.

### Usage

```
tof_find_log_rank_threshold(input_data, relative_risk_col, time_col, event_col)
```

# **Arguments**

input\_data A tbl\_df or data.frame in which each observation is a row. relative\_risk\_col

An unquote column name indicating which column contains the relative-risk estimates for each observation.

time\_col An unquoted column name indicating which column contains the true time-to-

event information for each observation.

event\_col An unquoted column name indicating which column contains the outcome (event

or censorship). Must be a binary column - all values should be either 0 or 1 (with 1 indicating the adverse event and 0 indicating censorship) or FALSE and TRUE (with TRUE indicating the adverse event and FALSE indicating censorship).

### Value

A tibble with 3 columns: "candidate\_thresholds" (the relative-risk threshold used for the log-rank test), "log\_rank\_p\_val" (the p-values of the log-rank tests) and "is\_best" (a logical value indicating which candidate threshold gave the optimal, i.e. smallest, p-value).

## Description

Using the character vectors obtained from the 'name' and 'desc' columns of the parameters of the data of a flowFrame, figure out the high-dimensional cytometry panel used to collect the data and return it as a tidy tibble.

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### Usage

```
tof_find_panel_info(input_flowFrame)
```

### **Arguments**

```
input_flowFrame
```

a raw flowFrame (just read from an .fcs file) from which a high-dimensional cytometry panel should be extracted

## Value

A tibble with 2 columns ('metals' and 'antigens') that correspond to the metals and antigens of the high-dimensional cytometry panel used during data acquisition.

tof\_fit\_split

Fit a glmnet model and calculate performance metrics using a single rsplit object

## **Description**

This function trains a glmnet model on the training set of an rsplit object, then calculates performance metrics of that model on the validation/holdout set at all combinations of the mixture and penalty hyperparameters provided in a hyperparameter grid.

# Usage

```
tof_fit_split(
   split_data,
   prepped_recipe,
   hyperparameter_grid,
   model_type,
   outcome_colnames
)
```

# **Arguments**

split\_data An 'rsplit' object from the rsample package. Alternatively, an unsplit tbl\_df can be provided, though this is not recommended.

prepped\_recipe A trained recipe
hyperparameter\_grid

A tibble containing the hyperparameter values to tune. Can be created using tof\_create\_grid

model\_type A string representing the type of glmnet model being fit. outcome\_colnames

Quoted column names indicating which columns in the data being fit represent the outcome variables (with all others assumed to be predictors). 100 tof\_generate\_palette

### Value

A tibble with the same number of rows as the input hyperparameter grid. Each row represents a combination of mixture and penalty, and each column contains a performance metric for the fitted glmnet model on 'split\_data's holdout set. The specific performance metrics depend on the type of model being fit:

- "linear" mean-squared error ('mse') and mean absolute error ('mae')
- "two-class" binomial deviance ('binomial\_deviance'); misclassification error rate 'misclassification\_error'; the area under the receiver-operating curve ('roc\_auc'); and 'mse' and 'mse' as above
- "multiclass" multinomial deviance ('multinomial\_deviance'); misclassification error rate 'misclassification\_error'; the area under the receiver-operating curve ('roc\_auc') computed using the Hand-Till method in roc\_auc; and 'mse' and 'mse' as above
- "survival" the negative log2-transformed partial likelihood ('neg\_log\_partial\_likelihood') and Harrel's concordance index (often simply called "C"; 'concordance\_index')

### References

Harrel Jr, F. E. and Lee, K. L. and Mark, D. B. (1996) Tutorial in biostatistics: multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing error, Statistics in Medicine, 15, pages 361–387.

### **Description**

This function generates a color palette based on the color palette of the author's favorite pokemon.

# Usage

```
tof_generate_palette(num_colors)
```

### **Arguments**

num\_colors An integer specifying the number of colors you'd like to generate.

### Value

A character vector of hex codes specifying the colors in the palette.

```
tof_generate_palette(num_colors = 5L)
```

# **Description**

Get a 'tof\_model''s optimal mixture (alpha) value

### Usage

```
tof_get_model_mixture(tof_model)
```

# **Arguments**

tof\_model A tof\_model

### Value

A numeric value

```
feature_tibble <-
   dplyr::tibble(
        sample = as.character(1:100),
        cd45 = runif(n = 100),
        pstat5 = runif(n = 100),
       cd34 = runif(n = 100),
       outcome = (3 * cd45) + (4 * pstat5) + rnorm(100),
        class =
            as.factor(
                dplyr::if_else(outcome > median(outcome), "class1", "class2")
        multiclass =
            as.factor(
                c(rep("class1", 30), rep("class2", 30), rep("class3", 40))
        event = c(rep(0, times = 30), rep(1, times = 70)),
        time_{to}=to_{event}=rnorm(n=100, mean=10, sd=2)
split_data <- tof_split_data(feature_tibble, split_method = "simple")</pre>
# train a regression model
regression_model <-
    tof_train_model(
        split_data = split_data,
        predictor_cols = c(cd45, pstat5, cd34),
       response_col = outcome,
       model_type = "linear"
   )
```

```
tof_get_model_mixture(regression_model)
```

```
tof_get_model_outcomes
```

Get a 'tof\_model''s outcome variable name(s)

# **Description**

```
Get a 'tof_model''s outcome variable name(s)
```

### Usage

```
tof_get_model_outcomes(tof_model)
```

### **Arguments**

```
tof_model A tof_model
```

### Value

A character vector

```
feature_tibble <-</pre>
    dplyr::tibble(
        sample = as.character(1:100),
        cd45 = runif(n = 100),
        pstat5 = runif(n = 100),
        cd34 = runif(n = 100),
        outcome = (3 * cd45) + (4 * pstat5) + rnorm(100),
        class =
            as.factor(
                dplyr::if_else(outcome > median(outcome), "class1", "class2")
            ),
        multiclass =
            as.factor(
                c(rep("class1", 30), rep("class2", 30), rep("class3", 40))
        event = c(rep(0, times = 30), rep(1, times = 70)),
        time_{to}=rnorm(n = 100, mean = 10, sd = 2)
    )
split_data <- tof_split_data(feature_tibble, split_method = "simple")</pre>
# train a regression model
regression_model <-</pre>
   tof_train_model(
```

tof\_get\_model\_penalty

```
split_data = split_data,
    predictor_cols = c(cd45, pstat5, cd34),
    response_col = outcome,
    model_type = "linear"
)

tof_get_model_outcomes(regression_model)
```

tof\_get\_model\_penalty Get a 'tof\_model''s optimal penalty (lambda) value

# **Description**

Get a 'tof\_model''s optimal penalty (lambda) value

## Usage

```
tof_get_model_penalty(tof_model)
```

### **Arguments**

tof\_model A tof\_model

### Value

A numeric value

```
feature_tibble <-
    dplyr::tibble(
        sample = as.character(1:100),
        cd45 = runif(n = 100),
       pstat5 = runif(n = 100),
        cd34 = runif(n = 100),
        outcome = (3 * cd45) + (4 * pstat5) + rnorm(100),
        class =
            as.factor(
                dplyr::if_else(outcome > median(outcome), "class1", "class2")
            ),
        multiclass =
            as.factor(
                c(rep("class1", 30), rep("class2", 30), rep("class3", 40))
            ),
        event = c(rep(0, times = 30), rep(1, times = 70)),
        time_{to}=rnorm(n = 100, mean = 10, sd = 2)
   )
split_data <- tof_split_data(feature_tibble, split_method = "simple")</pre>
```

```
# train a regression model
regression_model <-
    tof_train_model(
        split_data = split_data,
        predictor_cols = c(cd45, pstat5, cd34),
        response_col = outcome,
        model_type = "linear"
    )

tof_get_model_penalty(regression_model)</pre>
```

# **Description**

Get a 'tof\_model''s training data

# Usage

```
tof_get_model_training_data(tof_model)
```

### **Arguments**

```
tof_model A tof_model
```

## Value

A tibble of (non-preprocessed) training data used to fit the model

tof\_get\_model\_type 105

```
event = c(rep(0, times = 30), rep(1, times = 70)),
    time_to_event = rnorm(n = 100, mean = 10, sd = 2)
)

split_data <- tof_split_data(feature_tibble, split_method = "simple")

# train a regression model
regression_model <-
    tof_train_model(
        split_data = split_data,
        predictor_cols = c(cd45, pstat5, cd34),
        response_col = outcome,
        model_type = "linear"
    )

tof_get_model_training_data(regression_model)</pre>
```

tof\_get\_model\_type

Get a 'tof\_model''s model type

# Description

Get a 'tof\_model''s model type

## Usage

```
tof_get_model_type(tof_model)
```

# **Arguments**

tof\_model A tof\_model

### Value

A string

tof\_get\_model\_x

```
multiclass =
            as.factor(
                c(rep("class1", 30), rep("class2", 30), rep("class3", 40))
            ),
        event = c(rep(0, times = 30), rep(1, times = 70)),
        time_{to}=rnorm(n = 100, mean = 10, sd = 2)
   )
split_data <- tof_split_data(feature_tibble, split_method = "simple")</pre>
# train a regression model
regression_model <-</pre>
    tof_train_model(
        split_data = split_data,
        predictor_cols = c(cd45, pstat5, cd34),
        response_col = outcome,
       model_type = "linear"
   )
tof_get_model_type(regression_model)
```

tof\_get\_model\_x

Get a 'tof\_model''s processed predictor matrix (for glmnet)

# Description

Get a 'tof\_model''s processed predictor matrix (for glmnet)

### Usage

```
tof_get_model_x(tof_model)
```

### **Arguments**

tof\_model A tof\_model

### Value

An x value formatted for glmnet

```
feature_tibble <-
    dplyr::tibble(
        sample = as.character(1:100),
        cd45 = runif(n = 100),
        pstat5 = runif(n = 100),
        cd34 = runif(n = 100),
        outcome = (3 * cd45) + (4 * pstat5) + rnorm(100),</pre>
```

tof\_get\_model\_y

```
class =
            as.factor(
                dplyr::if_else(outcome > median(outcome), "class1", "class2")
            ),
        multiclass =
            as.factor(
                c(rep("class1", 30), rep("class2", 30), rep("class3", 40))
            ),
        event = c(rep(0, times = 30), rep(1, times = 70)),
        time_{to}=rnorm(n = 100, mean = 10, sd = 2)
   )
split_data <- tof_split_data(feature_tibble, split_method = "simple")</pre>
# train a regression model
regression_model <-
    tof_train_model(
        split_data = split_data,
        predictor_cols = c(cd45, pstat5, cd34),
       response_col = outcome,
       model_type = "linear"
   )
tof_get_model_x(regression_model)
```

tof\_get\_model\_y

Get a 'tof\_model''s processed outcome variable matrix (for glmnet)

# Description

Get a 'tof\_model''s processed outcome variable matrix (for glmnet)

### Usage

```
tof_get_model_y(tof_model)
```

### **Arguments**

tof\_model A tof\_model

### Value

A y value formatted for glmnet

tof\_get\_panel

### **Examples**

```
feature_tibble <-
    dplyr::tibble(
       sample = as.character(1:100),
        cd45 = runif(n = 100),
        pstat5 = runif(n = 100),
        cd34 = runif(n = 100),
        outcome = (3 * cd45) + (4 * pstat5) + rnorm(100),
        class =
            as.factor(
                dplyr::if_else(outcome > median(outcome), "class1", "class2")
            ),
        multiclass =
            as.factor(
                c(rep("class1", 30), rep("class2", 30), rep("class3", 40))
            ),
        event = c(rep(0, times = 30), rep(1, times = 70)),
        time_{to}=rnorm(n = 100, mean = 10, sd = 2)
   )
split_data <- tof_split_data(feature_tibble, split_method = "simple")</pre>
# train a regression model
regression_model <-
    tof_train_model(
       split_data = split_data,
        predictor_cols = c(cd45, pstat5, cd34),
       response_col = outcome,
       model_type = "linear"
   )
tof_get_model_y(regression_model)
```

tof\_get\_panel

Get panel information from a tof\_tibble

# **Description**

Get panel information from a tof\_tibble

### **Usage**

```
tof_get_panel(tof_tibble)
```

# **Arguments**

```
tof_tibble A 'tof_tbl'.
```

tof\_is\_numeric 109

# Value

A tibble containing information about the CyTOF panel that was used during data acquisition for the data contained in 'tof\_tibble'.

# See Also

```
Other tof_tbl utilities: new_tof_tibble(), tof_set_panel()
```

# **Examples**

```
input_file <- dir(tidytof_example_data("aml"), full.names = TRUE)[[1]]
tof_tibble <- tof_read_data(input_file)
tof_get_panel(tof_tibble)</pre>
```

tof\_is\_numeric

Find if a vector is numeric

# Description

This function takes an input vector '.vec' and checks if it is either an integer or a double (i.e. is the type of vector that might encode high-dimensional cytometry measurements).

# Usage

```
tof_is_numeric(.vec)
```

# Arguments

.vec

A vector.

### Value

A boolean value indicating if .vec is of type integer or double.

110 tof\_knn\_density

tof_knn_density	Estimate cells' local densities using K-nearest-neighbor density esti- mation
-----------------	--

## **Description**

This function uses the distances between a cell and each of its K nearest neighbors to estimate local density of each cell in a 'tof\_tbl' or 'tibble' containing high-dimensional cytometry data.

## **Usage**

```
tof_knn_density(
  tof_tibble,
  distance_cols = where(tof_is_numeric),
  num_neighbors = min(15L, nrow(tof_tibble)),
 distance_function = c("euclidean", "cosine", "l2", "ip"),
  estimation_method = c("mean_distance", "sum_distance"),
  normalize = TRUE,
)
```

## **Arguments**

tof\_tibble A 'tof\_tbl' or a 'tibble'.

distance\_cols Unquoted names of the columns in 'tof tibble' to use in calculating cell-to-

cell distances during the local density estimation for each cell. Defaults to all

numeric columns in 'tof tibble'.

num\_neighbors An integer indicating the number of nearest neighbors to use in estimating the local density of each cell. Defaults to the minimum of 15 and the number of

rows in 'tof\_tibble'.

distance\_function

A string indicating which distance function to use for calculating cell-to-cell distances during local density estimation. Options include "euclidean" (the default) and "cosine".

estimation\_method

A string indicating how the relative density for each cell should be calculated from the distances between it and each of its k nearest neighbors. Options are "mean distance" (the default; estimates the relative density for a cell's neighborhood by taking the negative average of the distances to its nearest neighbors) and "sum\_distance" (estimates the relative density for a cell's neighborhood by taking the negative sum of the distances to its nearest neighbors).

normalize A boolean value indicating if the vector of local density estimates should be normalized to values between 0 and 1. Defaults to TRUE.

Additional optional arguments to pass to tof\_find\_knn.

tof\_log\_rank\_test 111

#### Value

A tibble with a single column named ".knn\_density" containing the local density estimates for each input cell in 'tof\_tibble'.

### See Also

Other local density estimation functions: tof\_estimate\_density(), tof\_spade\_density()

tof\_log\_rank\_test

Compute the log-rank test p-value for the difference between the two survival curves obtained by splitting a dataset into a "low" and "high" risk group using a given relative-risk threshold.

# **Description**

Compute the log-rank test p-value for the difference between the two survival curves obtained by splitting a dataset into a "low" and "high" risk group using a given relative-risk threshold.

## Usage

```
tof_log_rank_test(
   input_data,
   relative_risk_col,
   time_col,
   event_col,
   threshold
)
```

### **Arguments**

input\_data A tbl\_df or data.frame in which each observation is a row. relative\_risk\_col

An unquote column name indicating which column contains the relative-risk

estimates for each observation.

time\_col An unquoted column name indicating which column contains the true time-to-

event information for each observation.

event\_col An unquoted column name indicating which column contains the outcome (event

or censorship). Must be a binary column - all values should be either 0 or 1 (with 1 indicating the adverse event and 0 indicating censorship) or FALSE and TRUE (with TRUE indicating the adverse event and FALSE indicating censorship).

threshold A numeric value indicating the relative-risk threshold that should be used to split

observations into low- and high-risk groups.

## Value

A numeric value <1, the p-value of the log-rank test.

112 tof\_make\_knn\_graph

### **Examples**

NULL

```
tof_make_knn_graph
                         Title
```

### **Description**

Title

# Usage

```
tof_make_knn_graph(
  tof_tibble,
 knn_cols,
 num_neighbors,
 distance_function = c("euclidean", "cosine"),
 graph_type = c("weighted", "unweighted"),
)
```

# **Arguments**

tof\_tibble A tibble or tof\_tbl.

Unquoted column names indicating which columns in tof\_tibble should be used knn\_cols

for the KNN calculation.

num\_neighbors An integer number of neighbors to find for each cell (not including itself).

distance\_function

A string indicating which distance function to use for the nearest-neighbor cal-

culation. Options include "euclidean" (the default) and "cosine" distances.

graph\_type

A string indicating if the graph's edges should have weights ("weighted"; the

default) or not ("unweighted").

Optional additional arguments to pass to tof\_find\_knn

## Value

A tbl\_graph.

# **Examples**

NULL

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tof_make_roc_curve	Compute a receiver-operating curve (ROC) for a two-class or multi-
	class dataset

# **Description**

Compute a receiver-operating curve (ROC) for a two-class or multiclass dataset

# Usage

```
tof_make_roc_curve(input_data, truth_col, prob_cols)
```

## **Arguments**

input_data	A tof_tbl, tbl_df, or data.frame in which each row is an observation.
truth_col	An unquoted column name indicating which column in 'input_data' contains the true class labels for each observation. Must be a factor.
prob_cols	Unquoted column names indicating which columns in 'input_data' contain the probability estimates for each class in 'truth_col'. These columns must be specified in the same order as the factor levels in 'truth_col'.

### Value

A tibble that can be used to plot the ROC for a classification task. For each candidate probability threshold, the following are reported: specificity, sensitivity, true-positive rate (tpr), and false-positive rate (fpr).

```
feature_tibble <-</pre>
    dplyr::tibble(
        sample = as.character(1:100),
        cd45 = runif(n = 100),
        pstat5 = runif(n = 100),
        cd34 = runif(n = 100),
        outcome = (3 * cd45) + (4 * pstat5) + rnorm(100),
        class =
            as.factor(
                dplyr::if_else(outcome > median(outcome), "class1", "class2")
            )
    )
split_data <- tof_split_data(feature_tibble, split_method = "simple")</pre>
# train a logistic regression classifier
log_model <-
    tof_train_model(
        split_data = split_data,
```

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```
predictor_cols = c(cd45, pstat5, cd34),
        response_col = class,
        model_type = "two-class"
   )
# make predictions
predictions <-
    tof_predict(
       log_model,
        new_data = feature_tibble,
        prediction_type = "response"
prediction_tibble <-</pre>
   dplyr::tibble(
        truth = feature_tibble$class,
        prediction = predictions$.pred
   )
# make ROC curve
tof_make_roc_curve(
    input_data = prediction_tibble,
    truth_col = truth,
   prob_cols = prediction
)
```

tof\_metacluster

Metacluster clustered CyTOF data.

# Description

This function is a wrapper around tidytof's tof\_metacluster\_\* function family. It performs metaclustering on CyTOF data using a user-specified method (of 5 choices) and each method's corresponding input parameters.

# Usage

```
tof_metacluster(
  tof_tibble,
  cluster_col,
  metacluster_cols = where(tof_is_numeric),
  central_tendency_function = stats::median,
  ...,
  augment = TRUE,
  method = c("consensus", "hierarchical", "kmeans", "phenograph", "flowsom")
)
```

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### **Arguments**

tof\_tibble A 'tof tbl' or 'tibble'.

cluster\_col

An unquoted column name indicating which column in 'tof\_tibble' stores the cluster ids for the cluster to which each cell belongs. Cluster labels can be produced via any method the user chooses - including manual gating, any of the functions in the 'tof\_cluster\_\*' function family, or any other method.

metacluster\_cols

Unquoted column names indicating which columns in 'tof\_tibble' to use in computing the metaclusters. Defaults to all numeric columns in 'tof\_tibble'. Supports tidyselect helpers.

central\_tendency\_function

The function that should be used to calculate the measurement of central tendency for each cluster before metaclustering. This function will be used to compute a summary statistic for each input cluster in 'cluster\_col' across all columns specified by 'metacluster\_cols', and the resulting vector (one for each cluster) will be used as the input for metaclustering. Defaults to median.

... Additional arguments to pass to the 'tof\_metacluster\_\*' function family member

corresponding to the chosen 'method'.

augment A boolean value indicating if the output should column-bind the metacluster ids

of each cell as a new column in 'tof\_tibble' (TRUE; the default) or if a single-column tibble including only the metacluster ids should be returned (FALSE).

method A string indicating which clustering method should be used. Valid values in-

clude "consensus", "hierarchical", "kmeans", "phenograph", and "flowsom".

#### Value

A 'tof\_tbl' or 'tibble' If augment = FALSE, it will have a single column encoding the metacluster ids for each cell in 'tof\_tibble'. If augment = TRUE, it will have ncol(tof\_tibble) + 1 columns: each of the (unaltered) columns in 'tof\_tibble' plus an additional column encoding the metacluster ids.

#### See Also

Other metaclustering functions: tof\_metacluster\_consensus(), tof\_metacluster\_flowsom(), tof\_metacluster\_hierarchical(), tof\_metacluster\_kmeans(), tof\_metacluster\_phenograph()

```
sim_data <-
    dplyr::tibble(
        cd45 = rnorm(n = 1000),
        cd38 = rnorm(n = 1000),
        cd34 = rnorm(n = 1000),
        cd19 = rnorm(n = 1000),
        cluster_id = sample(letters, size = 1000, replace = TRUE)
    )

tof_metacluster(
    tof_tibble = sim_data,</pre>
```

```
cluster_col = cluster_id,
  clustering_algorithm = "consensus",
  method = "flowsom"
)

tof_metacluster(
  tof_tibble = sim_data,
  cluster_col = cluster_id,
  method = "phenograph"
)
```

tof\_metacluster\_consensus

Metacluster clustered CyTOF data using consensus clustering

# **Description**

This function performs consensus metaclustering on a 'tof\_tbl' containing CyTOF data using a user-specified selection of input variables/CyTOF measurements and the number of desired metaclusters. See ConsensusClusterPlus for additional details.

## Usage

# **Arguments**

tof\_tibble A 'tof\_tbl' or 'tibble'.

cluster\_col

An unquoted column name indicating which column in 'tof\_tibble' stores the cluster ids for the cluster to which each cell belongs. Cluster labels can be produced via any method the user chooses - including manual gating, any of the functions in the 'tof\_cluster\_\*' function family, or any other method.

#### metacluster\_cols

Unquoted column names indicating which columns in 'tof\_tibble' to use in computing the metaclusters. Defaults to all numeric columns in 'tof\_tibble'. Supports tidyselect helpers.

#### central\_tendency\_function

The function that should be used to calculate the measurement of central tendency for each cluster before metaclustering. This function will be used to compute a summary statistic for each input cluster in 'cluster\_col' across all columns specified by 'metacluster\_cols', and the resulting vector (one for each cluster) will be used as the input for metaclustering. Defaults to median.

#### num\_metaclusters

An integer indicating the number of clusters that should be returned. Defaults to 10.

#### proportion\_clusters

A numeric value between 0 and 1 indicating the proportion of clusters to subsample (from the total number of clusters in 'cluster\_col') during each iteration of the consensus clustering. Defaults to 0.9

#### proportion\_features

A numeric value between 0 and 1 indicating the proportion of features (i.e. the proportion of columns specified by 'metacluster\_cols') to subsample during each iteration of the consensus clustering. Defaults to 1 (all features are included).

num\_reps

An integer indicating how many subsampled replicates to run during consensus clustering. Defaults to 20.

## clustering\_algorithm

A string indicating which clustering algorithm ConsensusClusterPlus should use to metacluster the subsampled clusters during each resampling. Options are "hierarchical" (the default), "pam" (partitioning around medoids), and "kmeans".

## distance\_function

A string indicating which distance function should be used to compute the distances between clusters during consensus clustering. Options are "euclidean" (the default), "manhattan", "minkowski", "pearson", "spearman", "maximum", "binary", and "canberra". See ConsensusClusterPlus.

. Optional additional arguments to pass to ConsensusClusterPlus.

#### Value

A tibble with a single column ('.consensus\_metacluster') and the same number of rows as the input 'tof\_tibble'. Each entry in the column indicates the metacluster label assigned to the same row in 'tof tibble'.

#### See Also

Other metaclustering functions: tof\_metacluster(), tof\_metacluster\_flowsom(), tof\_metacluster\_hierarchical() tof\_metacluster\_kmeans(), tof\_metacluster\_phenograph()

### **Examples**

```
sim_data <-
    dplyr::tibble(
        cd45 = rnorm(n = 1000),
        cd38 = rnorm(n = 1000),
        cd34 = rnorm(n = 1000),
        cd19 = rnorm(n = 1000),
        cluster_id = sample(letters, size = 1000, replace = TRUE)
    )

tof_metacluster_consensus(tof_tibble = sim_data, cluster_col = cluster_id)</pre>
```

tof\_metacluster\_flowsom

Metacluster clustered CyTOF data using FlowSOM's built-in metaclustering algorithm

# Description

This function performs metaclustering on a 'tof\_tbl' containing CyTOF data using a user-specified selection of input variables/CyTOF measurements and the number of desired metaclusters. It takes advantage of the FlowSOM package's built-in functionality for automatically detecting the number of metaclusters and can use several strategies as adapted by the FlowSOM team: consensus metaclustering, hierarchical metaclustering, k-means metaclustering, or metaclustering using the FlowSOM algorithm itself. See MetaClustering for additional details.

### Usage

```
tof_metacluster_flowsom(
  tof_tibble,
  cluster_col,
  metacluster_cols = where(tof_is_numeric),
  central_tendency_function = stats::median,
  num_metaclusters = 10L,
  clustering_algorithm = c("consensus", "hierarchical", "kmeans", "som"),
  ...
)
```

# **Arguments**

tof\_tibble A 'tof\_tbl' or 'tibble'.

cluster\_col

An unquoted column name indicating which column in 'tof\_tibble' stores the cluster ids for the cluster to which each cell belongs. Cluster labels can be produced via any method the user chooses - including manual gating, any of the functions in the 'tof\_cluster\_\*' function family, or any other method.

#### metacluster\_cols

Unquoted column names indicating which columns in 'tof\_tibble' to use in computing the metaclusters. Defaults to all numeric columns in 'tof\_tibble'. Supports tidyselect helpers.

#### central\_tendency\_function

The function that should be used to calculate the measurement of central tendency for each cluster before metaclustering. This function will be used to compute a summary statistic for each input cluster in 'cluster\_col' across all columns specified by 'metacluster\_cols', and the resulting vector (one for each cluster) will be used as the input for metaclustering. Defaults to median.

#### num\_metaclusters

An integer indicating the maximum number of clusters that should be returned. Defaults to 10. Note that for this function, the output may provide a small number of metaclusters than requested. This is because MetaClustering uses the "Elbow method" to automatically detect the optimal number of metaclusters.

# clustering\_algorithm

A string indicating which clustering algorithm MetaClustering should use to perform the metaclustering. Options are "consensus" (the default), "hierarchical", "kmeans", and "som" (i.e. self-organizing map; the FlowSOM algorithm itself).

... Optional additional arguments to pass to MetaClustering.

#### Value

A tibble with a single column ('.flowsom\_metacluster') and the same number of rows as the input 'tof\_tibble'. Each entry in the column indicates the metacluster label assigned to the same row in 'tof\_tibble'.

#### See Also

Other metaclustering functions: tof\_metacluster(), tof\_metacluster\_consensus(), tof\_metacluster\_hierarchicaltof\_metacluster\_kmeans(), tof\_metacluster\_phenograph()

```
sim_data <-
    dplyr::tibble(
        cd45 = rnorm(n = 1000),
        cd38 = rnorm(n = 1000),
        cd34 = rnorm(n = 1000),
        cd19 = rnorm(n = 1000),
        cluster_id = sample(letters, size = 1000, replace = TRUE)
)

tof_metacluster_flowsom(
    tof_tibble = sim_data,
    cluster_col = cluster_id,
    clustering_algorithm = "consensus"
)</pre>
```

```
tof_metacluster_flowsom(
   tof_tibble = sim_data,
   cluster_col = cluster_id,
   clustering_algorithm = "som"
)
```

tof\_metacluster\_hierarchical

Metacluster clustered CyTOF data using hierarchical agglomerative clustering

#### **Description**

This function performs hierarchical metaclustering on a 'tof\_tbl' containing CyTOF data using a user-specified selection of input variables/CyTOF measurements and the number of desired metaclusters. See hclust.

# Usage

```
tof_metacluster_hierarchical(
  tof_tibble,
  cluster_col,
  metacluster_cols = where(tof_is_numeric),
  central_tendency_function = stats::median,
  num_metaclusters = 10L,
  distance_function = c("euclidean", "manhattan", "minkowski", "maximum", "canberra",
        "binary"),
  agglomeration_method = c("complete", "single", "average", "median", "centroid",
        "ward.D", "ward.D2", "mcquitty")
)
```

# **Arguments**

tof\_tibble A 'tof\_tbl' or 'tibble'.

cluster\_col

An unquoted column name indicating which column in 'tof\_tibble' stores the cluster ids for the cluster to which each cell belongs. Cluster labels can be produced via any method the user chooses - including manual gating, any of the functions in the 'tof\_cluster\_\*' function family, or any other method.

metacluster\_cols

Unquoted column names indicating which columns in 'tof\_tibble' to use in computing the metaclusters. Defaults to all numeric columns in 'tof\_tibble'. Supports tidyselect helpers.

central\_tendency\_function

The function that should be used to calculate the measurement of central tendency for each cluster before metaclustering. This function will be used to compute a summary statistic for each input cluster in 'cluster\_col' across all columns

specified by 'metacluster\_cols', and the resulting vector (one for each cluster) will be used as the input for metaclustering. Defaults to median.

num\_metaclusters

An integer indicating the number of clusters that should be returned. Defaults to

distance\_function

A string indicating which distance function should be used to compute the distances between clusters during the hierarchical metaclustering. Options are "euclidean" (the default), "manhattan", "minkowski", "maximum", "canberra", and "binary". See dist for additional details.

agglomeration\_method

A string indicating which agglomeration algorithm should be used during hierarchical cluster combination. Options are "complete" (the default), "single", "average", "median", "centroid", "ward.D", "ward.D2", and "mcquitty". See hclust for details.

#### Value

A tibble with a single column ('.hierarchical\_metacluster') and the same number of rows as the input 'tof\_tibble'. Each entry in the column indicates the metacluster label assigned to the same row in 'tof\_tibble'.

#### See Also

Other metaclustering functions: tof\_metacluster(), tof\_metacluster\_consensus(), tof\_metacluster\_flowsom(), tof\_metacluster\_kmeans(), tof\_metacluster\_phenograph()

### **Examples**

```
sim_data <-
    dplyr::tibble(
        cd45 = rnorm(n = 1000),
        cd38 = rnorm(n = 1000),
        cd34 = rnorm(n = 1000),
        cd19 = rnorm(n = 1000),
        cluster_id = sample(letters, size = 1000, replace = TRUE)
    )

tof_metacluster_hierarchical(tof_tibble = sim_data, cluster_col = cluster_id)</pre>
```

tof\_metacluster\_kmeans

Metacluster clustered CyTOF data using k-means clustering

### **Description**

This function performs k-means metaclustering on a 'tof\_tbl' containing CyTOF data using a user-specified selection of input variables/CyTOF measurements and the number of desired metaclusters. See hclust.

#### Usage

```
tof_metacluster_kmeans(
  tof_tibble,
  cluster_col,
  metacluster_cols = where(tof_is_numeric),
  central_tendency_function = stats::median,
  num_metaclusters = 10L,
  ...
)
```

# **Arguments**

tof\_tibble

A 'tof\_tbl' or 'tibble'.

cluster\_col

An unquoted column name indicating which column in 'tof\_tibble' stores the cluster ids for the cluster to which each cell belongs. Cluster labels can be produced via any method the user chooses - including manual gating, any of the functions in the 'tof\_cluster\_\*' function family, or any other method.

metacluster\_cols

Unquoted column names indicating which columns in 'tof\_tibble' to use in computing the metaclusters. Defaults to all numeric columns in 'tof\_tibble'. Supports tidyselect helpers.

central\_tendency\_function

The function that should be used to calculate the measurement of central tendency for each cluster before metaclustering. This function will be used to compute a summary statistic for each input cluster in 'cluster\_col' across all columns specified by 'metacluster\_cols', and the resulting vector (one for each cluster) will be used as the input for metaclustering. Defaults to median.

num\_metaclusters

An integer indicating the number of clusters that should be returned. Defaults to 10.

.. Optional additional method specifications to pass to tof\_cluster\_kmeans.

## Value

A tibble with a single column ('.kmeans\_metacluster') and the same number of rows as the input 'tof\_tibble'. Each entry in the column indicates the metacluster label assigned to the same row in 'tof tibble'.

#### See Also

```
Other metaclustering functions: tof_metacluster(), tof_metacluster_consensus(), tof_metacluster_flowsom(), tof_metacluster_hierarchical(), tof_metacluster_phenograph()
```

### **Examples**

```
sim_data <-
    dplyr::tibble(
        cd45 = rnorm(n = 1000),
        cd38 = rnorm(n = 1000),
        cd34 = rnorm(n = 1000),
        cd19 = rnorm(n = 1000),
        cluster_id = sample(letters, size = 1000, replace = TRUE)
    )

tof_metacluster_kmeans(tof_tibble = sim_data, cluster_col = cluster_id)</pre>
```

tof\_metacluster\_phenograph

Metacluster clustered CyTOF data using PhenoGraph clustering

## Description

This function performs PhenoGraph metaclustering on a 'tof\_tbl' containing CyTOF data using a user-specified selection of input variables/CyTOF measurements. The number of metaclusters is automatically detected by the PhenoGraph algorithm. See tof\_cluster\_phenograph.

#### Usage

```
tof_metacluster_phenograph(
  tof_tibble,
  cluster_col,
  metacluster_cols = where(tof_is_numeric),
  central_tendency_function = stats::median,
  num_neighbors = 5L,
  ...
)
```

## Arguments

tof\_tibble A 'tof\_tbl' or 'tibble'.

cluster\_col

An unquoted column name indicating which column in 'tof\_tibble' stores the cluster ids for the cluster to which each cell belongs. Cluster labels can be produced via any method the user chooses - including manual gating, any of the functions in the 'tof\_cluster\_\*' function family, or any other method.

metacluster\_cols

Unquoted column names indicating which columns in 'tof\_tibble' to use in computing the metaclusters. Defaults to all numeric columns in 'tof\_tibble'. Supports tidyselect helpers.

central\_tendency\_function

The function that should be used to calculate the measurement of central tendency for each cluster before metaclustering. This function will be used to compute a summary statistic for each input cluster in 'cluster\_col' across all columns specified by 'metacluster\_cols', and the resulting vector (one for each cluster) will be used as the input for metaclustering. Defaults to median.

num\_neighbors

An integer indicating the number of neighbors to use when constructing Pheno-Graph's k-nearest-neighbor graph. Smaller values emphasize local graph structure; larger values emphasize global graph structure (and will add time to the computation). Defaults to 5.

... Optional additional method specifications to pass to tof\_cluster\_phenograph.

### Value

A tibble with a single column ('.phenograph\_metacluster') and the same number of rows as the input 'tof\_tibble'. Each entry in the column indicates the metacluster label assigned to the same row in 'tof\_tibble'.

### See Also

Other metaclustering functions: tof\_metacluster(), tof\_metacluster\_consensus(), tof\_metacluster\_flowsom(), tof\_metacluster\_hierarchical(), tof\_metacluster\_kmeans()

### **Examples**

```
sim_data <-
    dplyr::tibble(
        cd45 = rnorm(n = 1000),
        cd38 = rnorm(n = 1000),
        cd34 = rnorm(n = 1000),
        cd19 = rnorm(n = 1000),
        cluster_id = sample(letters, size = 1000, replace = TRUE)
    )

tof_metacluster_phenograph(tof_tibble = sim_data, cluster_col = cluster_id)</pre>
```

```
tof_plot_cells_density
```

Plot marker expression density plots

## Description

This function plots marker expression density plots for a user-specified column in a tof\_tbl. Optionally, cells can be grouped to plot multiple vertically-arranged density plots

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# Usage

```
tof_plot_cells_density(
  tof_tibble,
  marker_col,
  group_col,
  num_points = 512,
  theme = ggplot2::theme_bw(),
  use_ggridges = FALSE,
  scale = 1,
  ...
)
```

# Arguments

tof_tibble	A 'tof_tbl' or a 'tibble'.
marker_col	An unquoted column name representing which column in 'tof_tibble' (i.e. which CyTOF protein measurement) should be included in the feature extraction calculation.
group_col	Unquoted column names representing which column in 'tof_tibble' should be used to break the rows of 'tof_tibble' into subgroups to be plotted as separate histograms. Defaults to plotting without subgroups.
num_points	The number of points along the full range of 'marker_col' at which the density should be calculated
theme	The ggplot2 theme for the plot. Defaults to theme_bw
use_ggridges	A boolean value indicting if geom_ridgeline should be used to plot overlain histograms. Defaults to FALSE. If TRUE, the ggridges package must be installed.
scale	Use to set the 'scale' argument in <code>geom_ridgeline</code> , which controls how far apart (vertically) density plots are arranged along the y-axis. Defaults to 1.
	Additional optional arguments to send to geom_ridgeline.

# Value

A ggplot object

```
sim_data <-
    dplyr::tibble(
        cd45 = rnorm(n = 1000),
        cd38 = rnorm(n = 1000),
        cd34 = rnorm(n = 1000),
        cd19 = rnorm(n = 1000),
        cluster_id = sample(c("a", "b"), size = 1000, replace = TRUE)
    )

density_plot <-</pre>
```

```
tof_plot_cells_density(
   tof_tibble = sim_data,
   marker_col = cd45,
   group_col = cluster_id
)
```

tof\_plot\_cells\_embedding

Plot scatterplots of single-cell data using low-dimensional feature embeddings

# **Description**

This function makes scatterplots using single-cell data embedded in a low-dimensional space (such as that generated by tof\_reduce\_dimensions, with each point colored using a user-specified variable.

### Usage

```
tof_plot_cells_embedding(
  tof_tibble,
  embedding_cols,
  color_col,
  facet_cols,
  compute_embedding_cols = where(tof_is_numeric),
  embedding_method = c("pca", "tsne", "umap"),
  embedding_args = list(),
  theme = ggplot2::theme_bw(),
   ...,
  method = c("ggplot2", "scattermore")
)
```

# **Arguments**

tof_tibble	A 'tof_tbl' or a 'tibble'.
embedding_cols	Unquoted column names indicating which columns in 'tof_tibble' should be used as the x and y axes of the scatterplot. Supports tidyselect helpers. Must select exactly 2 columns. If not provided, a feature embedding can be computed from scratch using the method provided using the 'embedding_method' argument and the tof_reduce_dimensions arguments passed to 'embedding_args'.
color_col	An unquoted column name specifying which column in 'tof_tibble' should be used to color each point in the scatterplot.
facet_cols	An unquoted column name specifying which column in 'tof_tibble' should be used to break the scatterplot into facets using facet_wrap.

```
compute_embedding_cols
```

Unquoted column names indicating which columns in 'tof\_tibble' to use for computing the embeddings with the method specified by 'embedding\_method'. Defaults to all numeric columns in 'tof tibble'. Supports tidyselect helpers.

#### embedding\_method

A string indicating which method should be used for the feature embedding (if 'embedding\_cols' are not provided). Options (which are passed to tof\_reduce\_dimensions) are "pca" (the default), "tsne", and "umap".

embedding\_args Optional additional arguments to pass to tof\_reduce\_dimensions. For example, for 'method = "tsne"', these might include 'num\_comp', 'perplexity', and 'theta'.

theme

A ggplot2 theme to apply to the scatterplot. Defaults to theme\_bw.

Optional additional arguments to pass to tof\_plot\_cells\_scatter.

method

A string indicating which plotting engine should be used. Valid values include "ggplot2" (the default) and "scattermore" (recommended if more than 100K cells are being plotted). Note that method = "scattermore" requires the scattermore package to be installed.

#### Value

A ggplot object.

#### See Also

Other visualization functions: tof\_plot\_cells\_layout(), tof\_plot\_cells\_scatter()

```
sim_data <-
   dplyr::tibble(
       cd45 = rnorm(n = 1000),
       cd38 = c(rnorm(n = 500), rnorm(n = 500, mean = 2)),
       cd34 = c(rnorm(n = 500), rnorm(n = 500, mean = 4)),
       cd19 = rnorm(n = 1000),
       cluster_id = c(rep("a", 500), rep("b", 500))
   )
# embed with pca
pca_plot <-
   tof_plot_cells_embedding(
       tof_tibble = sim_data,
       color_col = cd38,
       embedding_method = "pca",
        compute_embedding_cols = starts_with("cd")
   )
# embed with tsne
tsne_plot <-
   tof_plot_cells_embedding(
       tof_tibble = sim_data,
```

tof\_plot\_cells\_layout

```
color_col = cluster_id,
embedding_method = "tsne",
compute_embedding_cols = starts_with("cd")
)
```

tof\_plot\_cells\_layout Plot force-directed layouts of single-cell data

# **Description**

This function makes force-directed layouts using single-cell data embedded in a 2-dimensional space representing a k-nearest-neighbor graph constructed using cell-to-cell similarities. Each node in the force-directed layout represents a single cell colored using a user-specified variable.

## Usage

```
tof_plot_cells_layout(
  tof_tibble,
  knn_cols = where(tof_is_numeric),
  color_col,
  facet_cols,
  num_neighbors = 5,
  graph_type = c("weighted", "unweighted"),
  graph_layout = "fr",
  distance_function = c("euclidean", "cosine"),
  edge_alpha = 0.25,
  node_size = 2,
  theme = ggplot2::theme_void(),
  ...
)
```

# **Arguments**

tof_tibble	A 'tof_tbl' or a 'tibble'.
knn_cols	Unquoted column names indicating which columns in 'tof_tibble' should be used to compute the cell-to-cell distances used to construct the k-nearest-neighbor graph. Supports tidyselect helpers. Defaults to all numeric columns.
color_col	Unquoted column name indicating which column in 'tof_tibble' should be used to color the nodes in the force-directed layout.
facet_cols	Unquoted column names indicating which columns in 'tof_tibble' should be used to separate nodes into different force-directed layouts.
num_neighbors	An integer specifying how many neighbors should be used to construct the knearest neighbor graph.
graph_type	A string specifying if the k-nearest neighbor graph should be "weighted" (the default) or "unweighted".

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graph\_layout A string specifying which algorithm should be used to compute the force-directed

layout. Passed to ggraph. Defaults to "fr", the Fruchterman-Reingold algorithm. Other examples include "nicely", "gem", "kk", and many others. See

layout\_tbl\_graph\_igraph for other examples.

distance\_function

A string indicating which distance function to use in computing the cell-to-cell

distances. Valid options include "euclidean" (the default) and "cosine".

edge\_alpha A numeric value between 0 and 1 specifying the transparency of the edges drawn

in the force-directed layout. Defaults to 0.25.

node\_size A numeric value specifying the size of the nodes in the force-directed layout.

Defaults to 2.

theme A ggplot2 theme to apply to the force-directed layout. Defaults to theme\_void

... hnsw\_knn

#### Value

A ggraph/ggplot object.

#### See Also

Other visualization functions: tof\_plot\_cells\_embedding(), tof\_plot\_cells\_scatter()

```
sim_data <-
   dplyr::tibble(
        cd45 = rnorm(n = 1000),
        cd38 = c(rnorm(n = 500), rnorm(n = 500, mean = 2)),
        cd34 = c(rnorm(n = 500), rnorm(n = 500, mean = 4)),
        cd19 = rnorm(n = 1000),
        cluster_id = c(rep("a", 500), rep("b", 500))
   )
# make a layout colored by a marker
layout_cd38 <-
    tof_plot_cells_layout(
        tof_tibble = sim_data,
        color\_col = cd38
# make a layout colored by cluster id
layout_cluster <-
   tof_plot_cells_layout(
        tof_tibble = sim_data,
        color_col = cluster_id,
   )
```

tof\_plot\_cells\_scatter

```
tof_plot_cells_scatter
```

Plot scatterplots of single-cell data.

# Description

This function makes scatterplots of single-cell data using user-specified x- and y-axes. Additionally, each point in the scatterplot can be colored using a user-specified variable.

# Usage

```
tof_plot_cells_scatter(
  tof_tibble,
  x_col,
  y_col,
  color_col,
  facet_cols,
  theme = ggplot2::theme_bw(),
  ...,
  method = c("ggplot2", "scattermore")
)
```

# Arguments

tof_tibble	A 'tof_tbl' or a 'tibble'.
x_col	An unquoted column name specifying which column in 'tof_tibble' should be used as the x-axis.
y_col	An unquoted column name specifying which column in 'tof_tibble' should be used as the y-axis.
color_col	An unquoted column name specifying which column in 'tof_tibble' should be used to color each point in the scatterplot.
facet_cols	An unquoted column name specifying which column in 'tof_tibble' should be used to break the scatterplot into facets using facet_wrap.
theme	A ggplot2 theme to apply to the scatterplot. Defaults to theme_bw.
• • •	Optional additional arguments to pass to <pre>geom_point</pre> if method = "ggplot2" or <pre>geom_scattermore</pre> if method = "scattermore".
method	A string indicating which plotting engine should be used. Valid values include "ggplot2" (the default) and "scattermore" (recommended if more than 100K cells are being plotted). Note that method = "scattermore" requires the scattermore package to be installed.

# Value

A ggplot object.

### See Also

Other visualization functions: tof\_plot\_cells\_embedding(), tof\_plot\_cells\_layout()

## **Examples**

```
sim_data <-
    dplyr::tibble(
        cd45 = rnorm(n = 1000),
        cd38 = c(rnorm(n = 500), rnorm(n = 500, mean = 2)),
        cd34 = c(rnorm(n = 500), rnorm(n = 500, mean = 4)),
        cd19 = rnorm(n = 1000),
        cluster_id = c(rep("a", 500), rep("b", 500))
)</pre>
```

tof\_plot\_clusters\_heatmap

Make a heatmap summarizing cluster marker expression patterns in CyTOF data

# **Description**

This function makes a heatmap of cluster-to-cluster marker expression patterns in single-cell data. Markers are plotted along the horizontal (x-) axis of the heatmap and cluster IDs are plotted along the vertical (y-) axis of the heatmap.

### Usage

```
tof_plot_clusters_heatmap(
   tof_tibble,
   cluster_col,
   marker_cols = where(tof_is_numeric),
   central_tendency_function = stats::median,
   scale_markerwise = FALSE,
   scale_clusterwise = FALSE,
   cluster_markers = TRUE,
   cluster_clusters = TRUE,
   line_width = 0.25,
   theme = ggplot2::theme_minimal()
)
```

#### Arguments

tof\_tibble A 'tof\_tbl' or a 'tibble'.

cluster\_col

An unquoted column name indicating which column in 'tof\_tibble' stores the cluster ids for the cluster to which each cell belongs. Cluster labels can be produced via any method the user chooses - including manual gating, any of the functions in the 'tof\_cluster\_\*' function family, or any other method.

marker\_cols

Unquoted column names indicating which column in 'tof\_tibble' should be interpreted as markers to be plotted along the x-axis of the heatmap. Supports tidyselect helpers.

central\_tendency\_function

A function to use for computing the measure of central tendency that will be aggregated from each cluster in cluster\_col. Defaults to the median.

scale\_markerwise

A boolean value indicating if the heatmap should rescale the columns of the heatmap such that the maximum value for each marker is 1 and the minimum value is 0. Defaults to FALSE.

scale\_clusterwise

A boolean value indicating if the heatmap should rescale the rows of the heatmap such that the maximum value for each cluster is 1 and the minimum value is 0. Defaults to FALSE.

cluster\_markers

A boolean value indicating if the heatmap should order its columns (i.e. markers) using hierarchical clustering. Defaults to TRUE.

cluster\_clusters

A boolean value indicating if the heatmap should order its rows (i.e. clusters) using hierarchical clustering. Defaults to TRUE.

line\_width

A numeric value indicating how thick the lines separating the tiles of the heatmap should be. Defaults to 0.25.

theme

A ggplot2 theme to apply to the heatmap. Defaults to theme\_minimal

#### Value

A ggplot object.

```
sim_data <-
    dplyr::tibble(
        cd45 = rnorm(n = 1000),
        cd38 = rnorm(n = 1000),
        cd34 = rnorm(n = 1000),
        cd19 = rnorm(n = 1000),
        cluster_id = sample(letters, size = 1000, replace = TRUE)
)

heatmap <-
    tof_plot_clusters_heatmap(
        tof_tibble = sim_data,
        cluster_col = cluster_id
)</pre>
```

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tof\_plot\_clusters\_mst Visualize clusters in CyTOF data using a minimum spanning tree (MST).

### **Description**

This function plots a minimum-spanning tree using clustered single-cell data in order to summarize cluster-level characteristics. Each node in the MST represents a single cluster colored using a user-specified variable (either continuous or discrete).

# Usage

```
tof_plot_clusters_mst(
  tof_tibble,
  cluster_col,
  knn_cols = where(tof_is_numeric),
  color_col,
  num_neighbors = 5L,
  graph_type = c("unweighted", "weighted"),
  graph_layout = "nicely",
  central_tendency_function = stats::median,
  distance_function = c("euclidean", "cosine"),
  edge_alpha = 0.4,
  node_size = "cluster_size",
  theme = ggplot2::theme_void(),
  ...
)
```

# **Arguments**

tof_tibble	A 'tof_tbl' or a 'tibble'.
cluster_col	An unquoted column name indicating which column in 'tof_tibble' stores the cluster ids for the cluster to which each cell belongs. Cluster labels can be produced via any method the user chooses - including manual gating, any of the functions in the 'tof_cluster_*' function family, or any other method.
knn_cols	Unquoted column names indicating which columns in 'tof_tibble' should be used to compute the cluster-to-cluster distances used to construct the k-nearest-neighbor graph. Supports tidyselect helpers. Defaults to all numeric columns.
color_col	Unquoted column name indicating which column in 'tof_tibble' should be used to color the nodes in the MST.
num_neighbors	An integer specifying how many neighbors should be used to construct the knearest neighbor graph.
graph_type	A string specifying if the $k$ -nearest neighbor graph should be "weighted" (the default) or "unweighted".

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graph\_layout

This argument specifies a layout for the MST in one of two ways. Option 1: Provide a string specifying which algorithm should be used to compute the force-directed layout. Passed to ggraph. Defaults to "nicely", which tries to automatically select a visually-appealing layout. Other examples include "fr", "gem", "kk", and many others. See <a href="layout\_tbl\_graph\_igraph">layout\_tbl\_graph\_igraph</a> for other examples. Option 2: Provide a ggraph object previously generated with this function. The layout used to plot this ggraph object will then be used as a template for the new plot. Using this option, number of clusters (and their labels) must be identical to the template. This option is useful if you want to make multiple plots of the same tof\_tibble colored by different protein markers, for example.

central\_tendency\_function

A function to use for computing the measure of central tendency that will be aggregated from each cluster in cluster\_col. Defaults to the median.

distance\_function

A string indicating which distance function to use in computing the cluster-toclusters distances in constructing the MST. Valid options include "euclidean" (the default) and "cosine".

edge\_alpha

A numeric value between 0 and 1 specifying the transparency of the edges drawn in the force dispersed leavest Defaults to 0.25

in the force-directed layout. Defaults to 0.25.

node\_size

Either a numeric value specifying the size of the nodes in the MST or the string "cluster\_size", in which case the size of the node representing each cluster will be scaled according to the number of cells in that cluster (the default).

theme

A ggplot2 theme to apply to the force-directed layout. Defaults to  ${\tt theme\_void}$ 

... Optional additional arguments to hnsw\_knn

#### Value

A ggraph/ggplot object.

```
sim_data <-
   dplyr::tibble(
       cd45 = rnorm(n = 1000),
       cd38 = rnorm(n = 1000),
       cd34 = rnorm(n = 1000),
       cd19 = rnorm(n = 1000),
        cluster_id = sample(letters, size = 1000, replace = TRUE)
   )
# make a layout colored by a marker
layout_cd38 <-
   tof_plot_clusters_mst(
        tof_tibble = sim_data,
       cluster_col = cluster_id,
        color_col = cd38
   )
# use the same layout as the plot above to color the same
```

```
# tree using a different marker
layout_cd45 <-
    tof_plot_clusters_mst(
        tof_tibble = sim_data,
        cluster_col = cluster_id,
        color_col = cd45,
        graph_layout = layout_cd38
)</pre>
```

tof\_plot\_clusters\_volcano

Create a volcano plot from differential expression analysis results

# Description

This function makes a volcano plot using the results of a differential expression analysis (DEA) produced by one of the 'tof\_dea\_\*' verbs. Each point in the volcano plot represents a single cluster-marker pair, colored by significance level and the direction of the marker expression difference.

# Usage

```
tof_plot_clusters_volcano(
  dea_result,
  num_top_pairs = 10L,
  alpha = 0.05,
  point_size = 2,
  label_size = 3,
  nudge_x = 0,
  nudge_y = 0.25,
  increase_color = "#207394",
  decrease_color = "#cd5241",
  insignificant_color = "#cdcdcd",
  use_ggrepel = FALSE,
  theme = ggplot2::theme_bw()
)
```

# Arguments

dea_result	A tibble containing the differential expression analysis (DEA) results produced by one of the members of the 'tof_dea_*' function family.
num_top_pairs	An integer representing the number of most significant cluster-marker pairs that should be labeled in the volcano plot.
alpha	A numeric value between 0 and 1 representing the significance level below which a p-value should be considered statistically significant. Defaults to 0.05.
point_size	A numeric value specifying the size of the points in the volcano plot.
label_size	A numeric value specifying the size of the text labeling cluster-marker pairs.

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nudge\_x A numeric value specifying how far cluster-marker pair labels should be adjusted to the left (if 'nudge\_x' is negative) or to the right (if 'nudge\_x' is positive) to avoid overlap with the plotted points. Passed to geom\_text, and ignored

if 'use\_ggrepel' = TRUE. Defaults to 0.

nudge\_y A numeric value specifying how far cluster-marker pair labels should be ad-

justed downwards (if 'nudge\_y' is negative) or upwards (if 'nudge\_y' is positive) to avoid overlap with the plotted points. Passed to geom\_text, and ignored

if 'use\_ggrepel' = TRUE. Defaults to 0.25.

increase\_color A hex code specifying which fill color should be used for points corresponding

to cluster-marker pairs where significant increases were detected.

decrease\_color A hex code specifying which fill color should be used for points corresponding

to cluster-marker pairs where significant decreases were detected.

insignificant\_color

A hex code specifying which fill color should be used for points corresponding to cluster-marker pairs where no significant differences were detected.

use\_ggrepel A boolean value indicting if geom\_text\_repel should be used to plot labels for

cluster-marker pairs. Defaults to FALSE. If TRUE, the ggrepel package must be  $\,$ 

installed.

theme A ggplot2 theme to apply to the volcano plot. Defaults to theme\_bw

#### Value

A ggplot object.

#### **Examples**

```
# create a mock differential expression analysis result
sim_dea_result <-
    dplyr::tibble(
        cluster_id = rep(letters, 2),
        marker = rep(c("cd45", "cd34"), times = length(letters)),
        p_adj = runif(n = 2 * length(letters), min = 0, max = 0.5),
        mean_fc = runif(n = 2 * length(letters), min = 0.01, max = 10),
        significant = dplyr::if_else(p_adj < 0.05, "*", "")
    )

attr(sim_dea_result, which = "dea_method") <- "t_unpaired"

# create the volcano plot
volcano <- tof_plot_clusters_volcano(dea_result = sim_dea_result)</pre>
```

tof\_plot\_heatmap

Make a heatmap summarizing group marker expression patterns in high-dimensional cytometry data

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#### **Description**

This function makes a heatmap of group-to-group marker expression patterns in single-cell data. Markers are plotted along the horizontal (x-) axis of the heatmap and groups are plotted along the vertical (y-) axis of the heatmap.

# Usage

```
tof_plot_heatmap(
  tof_tibble,
 y_col,
 marker_cols = where(tof_is_numeric),
  central_tendency_function = stats::median,
  scale_markerwise = FALSE,
  scale_ywise = FALSE,
  cluster_markers = TRUE,
  cluster_groups = TRUE,
  line_width = 0.25,
  theme = ggplot2::theme_minimal()
)
```

### **Arguments**

tof\_tibble A 'tof tbl' or a 'tibble'.

An unquoted column name indicating which column in 'tof tibble' stores the y\_col

ids for the group to which each cell belongs.

marker\_cols Unquoted column names indicating which column in 'tof\_tibble' should be in-

terpreted as markers to be plotted along the x-axis of the heatmap. Supports

tidyselect helpers.

central\_tendency\_function

A function to use for computing the measure of central tendency that will be

aggregated from each cluster in cluster col. Defaults to the median.

scale\_markerwise

A boolean value indicating if the heatmap should rescale the columns of the heatmap such that the maximum value for each marker is 1 and the minimum

value is 0. Defaults to FALSE.

scale\_ywise A boolean value indicating if the heatmap should rescale the rows of the heatmap

such that the maximum value for each group is 1 and the minimum value is 0.

Defaults to FALSE.

cluster\_markers

A boolean value indicating if the heatmap should order its columns (i.e. markers) using hierarchical clustering. Defaults to TRUE.

cluster\_groups A boolean value indicating if the heatmap should order its rows (i.e. groups)

using hierarchical clustering. Defaults to TRUE.

line\_width A numeric value indicating how thick the lines separating the tiles of the heatmap

should be. Defaults to 0.25.

theme A ggplot2 theme to apply to the heatmap. Defaults to theme\_minimal tof\_plot\_model

### Value

A ggplot object.

tof\_plot\_model

Plot the results of a glmnet model fit on sample-level data.

#### Description

Plot the results of a glmnet model fit on sample-level data.

### Usage

```
tof_plot_model(tof_model, new_data, theme = ggplot2::theme_bw())
```

# **Arguments**

tof\_model A 'tof\_model' trained using tof\_train\_model

new\_data A tibble of new observations for which a plot should be made. If new\_data isn't

provided, the plot will be made using the training data used to fit the model. Alternatively, the string "tuning\_data" can be provided, and the plot will be gen-

erated using the predictions generated during model tuning.

theme A ggplot2 theme to apply to the plot Defaults to theme\_bw

#### Value

A ggplot object. If the 'tof\_model' is a linear model, a scatterplot of the predicted outcome vs. the true outcome will be returned. If the 'tof\_model' is a two-class model, an ROC curve will be returned. If the 'tof\_model' is a multiclass model, a one-versus-all ROC curve will be returned for each class. If 'tof\_model' is a survival model, a Kaplan-Meier curve will be returned.

tof\_plot\_model\_linear

```
cd45 = runif(n = 20),
        pstat5 = runif(n = 20),
        cd34 = runif(n = 20),
        outcome = (3 * cd45) + (4 * pstat5) + rnorm(20),
        class =
            as.factor(
                dplyr::if_else(outcome > median(outcome), "class1", "class2")
   )
split_data <- tof_split_data(feature_tibble, split_method = "simple")</pre>
# train a regression model
regression_model <-
    tof_train_model(
        split_data = split_data,
        predictor_cols = c(cd45, pstat5, cd34),
        response_col = outcome,
        model_type = "linear"
    )
# make the plot
plot_1 <- tof_plot_model(tof_model = regression_model, new_data = new_tibble)</pre>
# train a logistic regression classifier
logistic_model <-</pre>
    tof_train_model(
        split_data = split_data,
        predictor_cols = c(cd45, pstat5, cd34),
        response_col = class,
        model_type = "two-class"
    )
# make the plot
plot_2 <- tof_plot_model(tof_model = logistic_model, new_data = new_tibble)</pre>
```

tof\_plot\_model\_linear Plot the results of a linear glmnet model fit on sample-level data.

#### **Description**

Plot the results of a linear glmnet model fit on sample-level data.

# Usage

```
tof_plot_model_linear(tof_model, new_data, theme = ggplot2::theme_bw())
```

#### **Arguments**

tof\_model A 'tof\_model' trained using tof\_train\_model

new\_data A tibble of new observations for which a plot should be made. If new\_data isn't

provided, the plot will be made using the training data used to fit the model. Alternatively, the string "tuning\_data" can be provided, and the plot will be gen-

erated using the predictions generated during model tuning.

theme A ggplot2 theme to apply to the plot Defaults to theme\_bw

### Value

A ggplot object. Specifically, a scatterplot of the predicted outcome vs. the true outcome will be returned.

tof\_plot\_model\_logistic

Plot the results of a two-class glmnet model fit on sample-level data.

### **Description**

Plot the results of a two-class glmnet model fit on sample-level data.

### Usage

```
tof_plot_model_logistic(tof_model, new_data, theme = ggplot2::theme_bw())
```

# Arguments

tof\_model A 'tof\_model' trained using tof\_train\_model

new\_data A tibble of new observations for which a plot should be made. If new\_data isn't

provided, the plot will be made using the training data used to fit the model. Alternatively, the string "tuning\_data" can be provided, and the plot will be gen-

erated using the predictions generated during model tuning.

theme A ggplot2 theme to apply to the plot. Defaults to theme\_bw

#### Value

A ggplot object. Specifically, an ROC curve...

```
tof_plot_model_multinomial
```

Plot the results of a multiclass glmnet model fit on sample-level data.

# **Description**

Plot the results of a multiclass glmnet model fit on sample-level data.

# Usage

```
tof_plot_model_multinomial(tof_model, new_data, theme = ggplot2::theme_bw())
```

# Arguments

tof\_model A 'tof\_model' trained using tof\_train\_model

new\_data A tibble of new observations for which a plot should be made. If new\_data isn't

provided, the plot will be made using the training data used to fit the model. Alternatively, the string "tuning\_data" can be provided, and the plot will be gen-

erated using the predictions generated during model tuning.

theme A ggplot2 theme to apply to the plot. Defaults to theme\_bw.

# Value

A ggplot object. Specifically, a one-versus-all ROC curve (one for each class).

```
tof_plot_model_survival
```

Plot the results of a survival glmnet model fit on sample-level data.

### **Description**

Plot the results of a survival glmnet model fit on sample-level data.

## Usage

```
tof_plot_model_survival(
  tof_model,
  new_data,
  censor_size = 2.5,
  theme = ggplot2::theme_bw()
)
```

## Arguments

tof\_model A 'tof\_model' trained using tof\_train\_model

new\_data A tibble of new observations for which a plot should be made. If new\_data isn't

provided, the plot will be made using the training data used to fit the model. Alternatively, the string "tuning\_data" can be provided, and the plot will be gen-

erated using the predictions generated during model tuning.

censor\_size A numeric value indicating how large to plot the tick marks representing cen-

sored values in the Kaplan-Meier curve.

theme A ggplot2 theme to apply to the plot. Defaults to theme\_bw

#### Value

A ggplot object. Specifically, a Kaplan-Meier curve.

```
tof_plot_sample_features
```

Make a heatmap summarizing sample marker expression patterns in CyTOF data

# **Description**

This function makes a heatmap of sample-to-sample marker expression patterns in single-cell data. Markers are plotted along the horizontal (x-) axis of the heatmap and sample IDs are plotted along the vertical (y-) axis of the heatmap.

### Usage

```
tof_plot_sample_features(
   feature_tibble,
   sample_col,
   feature_cols = where(tof_is_numeric),
   scale_featurewise = FALSE,
   scale_samplewise = FALSE,
   line_width = 0.25,
   theme = ggplot2::theme_minimal()
)
```

## Arguments

feature\_tibble A tbl\_df or data.frame of aggregated sample-level features, such as that gener-

ated by tof\_extract\_features.

sample\_col An unquoted column name indicating which column in 'tof\_tibble' stores the

IDs for each sample. If no sample IDs are present, a numeric ID will be assigned

to each row of 'feature\_tibble' based on its row index.

feature\_cols

Unquoted column names indicating which column in 'feature\_tibble' should be interpreted as features to be plotted along the x-axis of the heatmap. Supports tidyselect helpers.

scale\_featurewise

A boolean value indicating if the heatmap should rescale the columns of the heatmap such that the maximum value for each marker is 1 and the minimum value is 0. Defaults to FALSE.

scale\_samplewise

A boolean value indicating if the heatmap should rescale the rows of the heatmap such that the maximum value for each sample is 1 and the minimum value is 0. Defaults to FALSE.

line\_width

A numeric value indicating how thick the lines separating the tiles of the heatmap should be. Defaults to 0.25.

theme

A ggplot2 theme to apply to the heatmap. Defaults to theme\_minimal

### Value

A ggplot object.

# **Examples**

```
# simulate single-cell data
sim_data <-
    dplyr::tibble(
        cd45 = rnorm(n = 1000),
        cd38 = rnorm(n = 1000),
        cd34 = rnorm(n = 1000),
        cd19 = rnorm(n = 1000),
        cluster_id = sample(letters, size = 1000, replace = TRUE),
        sample_id = sample(paste0("sample", 1:5), size = 1000, replace = TRUE)
   )
# extract cluster proportions in each simulated patient
feature_data <-
    tof_extract_proportion(
        tof_tibble = sim_data,
        cluster_col = cluster_id,
        group_cols = sample_id
    )
# plot the heatmap
heatmap <- tof_plot_sample_features(feature_tibble = feature_data)</pre>
```

tof\_plot\_sample\_heatmap

Make a heatmap summarizing sample marker expression patterns in CyTOF data

# **Description**

This function makes a heatmap of sample-to-sample marker expression patterns in single-cell data. Markers are plotted along the horizontal (x-) axis of the heatmap and sample IDs are plotted along the vertical (y-) axis of the heatmap.

## Usage

```
tof_plot_sample_heatmap(
  tof_tibble,
  sample_col,
  marker_cols = where(tof_is_numeric),
  central_tendency_function = stats::median,
  scale_markerwise = FALSE,
  scale_samplewise = FALSE,
  line_width = 0.25,
  theme = ggplot2::theme_minimal()
)
```

# **Arguments**

tof\_tibble A 'tof\_tbl' or a 'tibble'.

sample\_col An unquoted column name indicating which column in 'tof\_tibble' stores the

ids for the sample to which each cell belongs.

marker\_cols Unquoted column names indicating which column in 'tof\_tibble' should be in-

terpreted as markers to be plotted along the x-axis of the heatmap. Supports

tidyselect helpers.

central\_tendency\_function

A function to use for computing the measure of central tendency that will be aggregated from each sample in cluster\_col. Defaults to the median.

scale\_markerwise

A boolean value indicating if the heatmap should rescale the columns of the heatmap such that the maximum value for each marker is 1 and the minimum value is 0. Defaults to FALSE.

scale\_samplewise

A boolean value indicating if the heatmap should rescale the rows of the heatmap such that the maximum value for each sample is 1 and the minimum value is 0.

Defaults to FALSE.

line\_width A numeric value indicating how thick the lines separating the tiles of the heatmap

should be. Defaults to 0.25.

theme A ggplot2 theme to apply to the heatmap. Defaults to theme\_minimal

#### Value

A ggplot object.

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#### **Examples**

```
sim_data <-
    dplyr::tibble(
        cd45 = rnorm(n = 1000),
        cd38 = rnorm(n = 1000),
        cd34 = rnorm(n = 1000),
        cd19 = rnorm(n = 1000),
        sample_id = sample(paste0("sample", 1:5), size = 1000, replace = TRUE)
)

heatmap <-
    tof_plot_sample_heatmap(
        tof_tibble = sim_data,
        sample_col = sample_id
)</pre>
```

tof\_postprocess

Post-process transformed CyTOF data.

# Description

This function transforms a 'tof\_tibble' of transformed ion counts from a mass cytometer back into something that looks more like an .fcs file that Fluidigm software generates.

## Usage

```
tof_postprocess(
  tof_tibble = NULL,
  channel_cols = where(tof_is_numeric),
  redo_noise = FALSE,
  transform_fun = function(x) rev_asinh(x, shift_factor = 0, scale_factor = 0.2)
)
```

#### **Arguments**

tof\_tibble A 'tof\_tibble' or a 'tibble'.

channel\_cols A vector of non-quoted column names indicating which columns in 'tof\_tibble' contain protein measurements. Supports tidyselect helpers. If nothing is specified, the default is to transform all numeric columns.

redo\_noise A boolean value indicating whether to add uniform noise that to each CyTOF measurement for aesthetic and visualization purposes. See this paper. Defaults to FALSE

transform\_fun A vectorized function to apply to each column specified by 'channel\_cols' for post-processing. Defaults to rev\_asinh transformation (with a cofactor of 5).

tof\_predict

#### Value

A 'tof\_tbl' with identical dimensions to the input 'tof\_tibble', with all columns specified in channel\_cols transformed using 'transform\_fun' (with noise added or not removed depending on 'redo\_noise').

#### See Also

```
[tof_preprocess()]
```

# **Examples**

```
# read in an example .fcs file from tidytof's internal datasets
input_file <- dir(tidytof_example_data("aml"), full.names = TRUE)[[1]]
tof_tibble <- tof_read_data(input_file)

# preprocess all numeric columns with default behavior
# arcsinh transformation with a cofactor of 5
preprocessed_tof_tibble <- tof_preprocess(tof_tibble)

# postprocess all numeric columns to reverse the preprocessing
tof_postprocess(tof_tibble)</pre>
```

tof\_predict

Use a trained elastic net model to predict fitted values from new data

#### **Description**

This function uses a trained 'tof\_model' to make predictions on new data.

# Usage

```
tof_predict(
  tof_model,
  new_data,
  prediction_type = c("response", "class", "link", "survival curve")
)
```

#### **Arguments**

tof\_model A 'tof\_model' trained using tof\_train\_model

new\_data A tibble of new observations for which predictions should be made. If new\_data

isn't provided, predictions will be made for the training data used to fit the

model.

prediction\_type

A string indicating which type of prediction should be provided by the model:

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"response" (the default) For "linear" models, the predicted response for each observation. For "two-class" and "multiclass" models, the fitted probabilities of each class for each observation. For "survival" models, the fitted relative-risk for each observation.

"class" Only applies to "two-class" and "multiclass" models. For both, the class label corresponding to the class with the maximum fitted probability.

"link" The linear predictions of the model (the output of the link function for each model family.)

"survival curve" Only applies to "survival" models. Returns a tibble indicating each patient's probability of survival (1 - probability(event)) at each timepoint in the dataset. Obtained using the survfit function.

#### Value

A tibble with a single column ('.pred') containing the predictions or, for multiclass models with 'prediction\_type' == "response", a tibble with one column for each class. Each row in the output corresponds to a row in 'new\_data' (or, if 'new\_data' is not provided, to a row in the 'tof\_model''s training data). In the latter case, be sure to check 'tof\_model\$training\_data' to confirm the order of observations, as the resampling procedure can change their ordering relative to the original input data.

#### See Also

Other modeling functions: tof\_assess\_model(), tof\_create\_grid(), tof\_split\_data(), tof\_train\_model()

```
feature_tibble <-
    dplyr::tibble(
        sample = as.character(1:100),
        cd45 = runif(n = 100),
        pstat5 = runif(n = 100),
        cd34 = runif(n = 100),
        outcome = (3 * cd45) + (4 * pstat5) + rnorm(100)
   )
new_tibble <-
   dplyr::tibble(
        sample = as.character(1:20),
        cd45 = runif(n = 20),
        pstat5 = runif(n = 20),
       cd34 = runif(n = 20),
        outcome = (3 * cd45) + (4 * pstat5) + rnorm(20)
    )
split_data <- tof_split_data(feature_tibble, split_method = "simple")</pre>
# train a regression model
regression_model <-
    tof_train_model(
        split_data = split_data,
```

tof\_preprocess

```
predictor_cols = c(cd45, pstat5, cd34),
    response_col = outcome,
    model_type = "linear"
)

# apply the model to new data
tof_predict(tof_model = regression_model, new_data = new_tibble)
```

tof\_preprocess

Preprocess raw high-dimensional cytometry data.

# **Description**

This function transforms a 'tof\_tbl' of raw ion counts, reads, or fluorescence intensity units directly measured on a cytometer using a user-provided function. It can be used to perform standard preprocessing steps (i.e. arcsinh transformation) before cytometry data analysis.

## Usage

```
tof_preprocess(
  tof_tibble = NULL,
  channel_cols = where(tof_is_numeric),
  undo_noise = FALSE,
  transform_fun = function(x) asinh(x/5)
)
```

## **Arguments**

tof\_tibble A 'tof\_tbl' or a 'tibble'. channel\_cols Unquoted column name

thannel\_cols Unquoted column names representing columns that contain single-cell protein measurements. Supports tidyselect helpers. If nothing is specified, the default is

to transform all numeric columns.

undo\_noise A boolean value indicating whether to remove the uniform noise that Fluidigm

software adds to CyTOF measurements for aesthetic and visualization purposes.

See this paper. Defaults to FALSE.

transform\_fun A vectorized function to apply to each protein value for variance stabilization.

Defaults to asinh transformation (with a co-factor of 5).

#### Value

A 'tof\_tbl' with identical dimensions to the input 'tof\_tibble', with all columns specified in channel\_cols transformed using 'transform\_fun' (with noise removed or not removed depending on 'undo\_noise').

## See Also

```
[tof_postprocess()]
```

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## **Examples**

```
# read in an example .fcs file from tidytof's internal datasets
input_file <- dir(tidytof_example_data("aml"), full.names = TRUE)[[1]]
tof_tibble <- tof_read_data(input_file)

# preprocess all numeric columns with default behavior
# arcsinh transformation with a cofactor of 5
tof_preprocess(tof_tibble)

# preprocess all numeric columns using the log base 10 tranformation
tof_preprocess(tof_tibble, transform_fun = log10)</pre>
```

tof\_prep\_recipe

Train a recipe or list of recipes for preprocessing sample-level cytometry data

# Description

Train a recipe or list of recipes for preprocessing sample-level cytometry data

### Usage

```
tof_prep_recipe(split_data, unprepped_recipe)
```

#### **Arguments**

split\_data

An 'rsplit' or 'rset' object from the rsample package containing the sample-level data to use for modeling. The easiest way to generate this is to use tof\_split\_data. Alternatively, an unsplit tbl\_df, though this is not recommended.

unprepped\_recipe

A recipe object (if 'split\_data' is an 'rsplit' object or a 'tbl\_df') or list of recipes (if 'split\_data' is an 'rset' object).

#### Value

If split\_data is an "rsplit" or "tbl\_df" object, will return a single prepped recipe. If split\_data is an "rset" object, will return a list of prepped recipes specific for each fold of the resampling procedure.

tof\_read\_data

tof_read_csv	Read high-dimensional cytometry data from a .csv file into a tidy tib- ble.

## **Description**

Read high-dimensional cytometry data from a .csv file into a tidy tibble.

## Usage

```
tof_read_csv(file_path = NULL, panel_info = dplyr::tibble())
```

# **Arguments**

file\_path A file path to a single .csv file.

panel\_info Optional. A tibble or data.frame containing information about the panel used

during high-dimensional cytometry data acquisition. Two columns are required:

"metals" and "antigens".

#### Value

A 'tof\_tbl' in which each row represents a single cell and each column represents a high-dimensional cytometry antigen channel.

A 'tof\_tbl' is an S3 class that extends the "tibble" class by storing one additional attribute: "panel" (a tibble storing information about the panel used during data acquisition). Because panel information isn't obvious from data read as a .csv file, this information must be provided manually from the user (unlike in 'tof\_read\_fcs').

tof\_read\_data

Read data from an .fcs/.csv file or a directory of .fcs/.csv files.

# **Description**

Read data from an .fcs/.csv file or a directory of .fcs/.csv files.

#### Usage

```
tof_read_data(path = NULL, sep = "|", panel_info = dplyr::tibble())
```

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#### **Arguments**

path A file path to a single file or to a directory of files. The only valid file types are

.fcs files or .csv files containing high-dimensional cytometry data.

sep Optional. A string to use to separate the antigen name and its associated metal

in the column names of the output tibble. Defaults to "I". Only used if the input

file is an .fcs file.

panel\_info Optional. A tibble or data.frame containing information about the panel used

during high-dimensional cytometry data acquisition. Two columns are required:

"metals" and "antigens". Only used if the input file is a .csv file.

#### Value

An [c by m+1] tibble in which each row represents a single cell (of c total in the dataset) and each column represents a high-dimensional cytometry measurement (of m total in the dataset). If more than one .fcs is read at once, the last column of the tibble ('file\_name') will represent the file name of the .fcs file from which each cell was read.

#### See Also

```
Other input/output functions: tof_write_csv(), tof_write_data(), tof_write_fcs()
```

# **Examples**

```
input_file <- dir(tidytof_example_data("aml"), full.names = TRUE)[[1]]
tof_read_data(input_file)</pre>
```

tof\_read\_fcs Read high-dimensional cytometry data from an .fcs file into a tidy tibble.

#### **Description**

This function reads high-dimensional cytometry data from a single .fcs file into a tidy data structure called a 'tof\_tbl' ("tof\_tibble"). tof\_tibbles are identical to normal tibbles except for an additional attribute ("panel") that stores information about the high-dimensional cytometry panel used during data acquisition.

#### Usage

```
tof_read_fcs(file_path = NULL, sep = "|")
```

#### **Arguments**

file\_path A file path to a single .fcs file.

sep A string to use to separate the antigen name and its associated metal in the

column names of the output tibble. Defaults to "I".

tof\_read\_file

# Value

a 'tof\_tbl' in which each row represents a single cell and each column represents a high-dimensional cytometry antigen channel.

A 'tof\_tbl' is an S3 class that extends the "tibble" class by storing one additional attribute: "panel" (a tibble storing information about the panel used during data acquisition).

tof_read_file	Read high-dimensional cytometry data from a single .fcs or .csv file into a tidy tibble.

# Description

Read high-dimensional cytometry data from a single .fcs or .csv file into a tidy tibble.

# Usage

```
tof_read_file(file_path = NULL, sep = "|", panel_info = dplyr::tibble())
```

## **Arguments**

file_path	A file path to a single .fcs or .csv file.
sep	A string to use to separate the antigen name and its associated metal in the column names of the output tibble. Defaults to "I". Only used if the input file is an .fcs file.
panel_info	Optional. A tibble or data.frame containing information about the panel used during high-dimensional cytometry data acquisition. Two columns are required: "metals" and "antigens". Only used if the input file is a .csv file.

#### Value

A 'tof\_tbl' in which each row represents a single cell and each column represents a high-dimensional cytometry antigen channel.

A 'tof\_tbl' is an S3 class that extends the "tibble" class by storing one additional attribute: "panel" (a tibble storing information about the panel used during data acquisition). Because panel information isn't obvious from data read as a .csv file, this information must be provided manually by the user.

tof\_reduce\_dimensions 153

tof\_reduce\_dimensions Apply dimensionality reduction to a single-cell dataset.

# **Description**

This function is a wrapper around tidytof's tof\_reduce\_\* function family. It performs dimensionality reduction on single-cell data using a user-specified method (of 3 choices) and each method's corresponding input parameters

# Usage

```
tof_reduce_dimensions(
  tof_tibble,
  ...,
  augment = TRUE,
  method = c("pca", "tsne", "umap")
)
```

#### **Arguments**

tof_tibble	A 'tof_tbl' or 'tibble'.
•••	Arguments to be passed to the tof_reduce_* function corresponding to the embedding method. See tof_reduce_pca, tof_reduce_tsne, and tof_reduce_umap.
augment	A boolean value indicating if the output should column-bind the dimensionality-reduced embedding vectors of each cell as a new column in 'tof_tibble' (TRUE, the default) or if a tibble including only the low-dimensionality embeddings should be returned (FALSE).
method	A method of dimensionality reduction. Currently, PCA, tSNE, and UMAP embedding are supported.

# Value

A tibble with the same number of rows as 'tof\_tibble', each representing a single cell. Each of the 'num\_comp' columns represents each cell's embedding in the calculated embedding space.

# See Also

Other dimensionality reduction functions: tof\_reduce\_pca(), tof\_reduce\_tsne(), tof\_reduce\_umap()

tof\_reduce\_pca

```
cd19 = rnorm(n = 100)
)

# calculate pca
tof_reduce_dimensions(tof_tibble = sim_data, method = "pca")

# calculate tsne
tof_reduce_dimensions(tof_tibble = sim_data, method = "tsne")

# calculate umap
tof_reduce_dimensions(tof_tibble = sim_data, method = "umap")
```

tof\_reduce\_pca

Perform principal component analysis on single-cell data

# Description

This function calculates principal components using single-cell data from a 'tof\_tibble'.

# Usage

```
tof_reduce_pca(
  tof_tibble,
  pca_cols = where(tof_is_numeric),
  num_comp = 5,
  threshold = NA,
  center = TRUE,
  scale = TRUE,
  return_recipe = FALSE
)
```

# Arguments

tof_tibble	A 'tof_tbl' or 'tibble'.
pca_cols	Unquoted column names indicating which columns in 'tof_tibble' to use for computing the principal components. Defaults to all numeric columns. Supports tidyselect helpers.
num_comp	The number of PCA components to calculate. Defaults to 5. See step_pca.
threshold	A double between 0 and 1 representing the fraction of total variance that should be covered by the components returned in the output. See step_pca.
center	A boolean value indicating if each column should be centered to mean $0$ before PCA analysis. Defaults to TRUE.
scale	A boolean value indicating if each column should be scaled to standard deviation = 1 before PCA analysis. Defaults to TRUE.

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return\_recipe

A boolean value indicating if instead of the UMAP result, a prepped recipe object containing the PCA embedding should be returned. Set this option to TRUE if you want to create the PCA embedding using one dataset but also want to project new observations onto the same embedding space later.

#### Value

A tibble with the same number of rows as 'tof\_tibble', each representing a single cell. Each of the 'num\_comp' columns represents each cell's embedding in the calculated principal component space.

#### See Also

Other dimensionality reduction functions: tof\_reduce\_dimensions(), tof\_reduce\_tsne(), tof\_reduce\_umap()

# Examples

```
# simulate single-cell data
sim_data <-
    dplyr::tibble(
        cd45 = rnorm(n = 200),
        cd38 = rnorm(n = 200),
        cd34 = rnorm(n = 200),
        cd19 = rnorm(n = 200)
   )
new_data <-
    dplyr::tibble(
       cd45 = rnorm(n = 50),
        cd38 = rnorm(n = 50),
        cd34 = rnorm(n = 50),
        cd19 = rnorm(n = 50)
   )
# calculate pca
tof_reduce_pca(tof_tibble = sim_data, num_comp = 2)
# return recipe instead of embeddings
pca_recipe <- tof_reduce_pca(tof_tibble = sim_data, return_recipe = TRUE)</pre>
# apply recipe to new data
recipes::bake(pca_recipe, new_data = new_data)
```

tof\_reduce\_tsne

Perform t-distributed stochastic neighborhood embedding on singlecell data

## Description

This function calculates a tSNE embedding using single-cell data from a 'tof\_tibble'.

tof\_reduce\_tsne

# Usage

```
tof_reduce_tsne(
  tof_tibble,
  tsne_cols = where(tof_is_numeric),
  num_comp = 2,
  perplexity = 30,
  theta = 0.5,
  max_iterations = 1000,
  verbose = FALSE,
  ...
)
```

# Arguments

tof_tibble	A 'tof_tbl' or 'tibble'.
tsne_cols	Unquoted column names indicating which columns in 'tof_tibble' to use in computing the tSNE embedding. Defaults to all numeric columns in 'tof_tibble'. Supports tidyselect helpers.
num_comp	The number of tSNE components to calculate for the embedding. Defaults to 2.
perplexity	A positive numeric value that represents represents the rough balance between the input data's local and global structure emphasized in the embedding. Smaller values emphasize local structure; larger values emphasize global structure. The recommended range is generally 5-50. Defaults to 30.
theta	A numeric value representing the speed/accuracy tradeoff for the embedding. Set to 0 for the exact tSNE; increase for a faster approximation. Defaults to 0.5
max_iterations	An integer number of iterations to use during embedding calculation. Defaults to 1000.
verbose	A boolean value indicating whether progress updates should be printed during embedding calculation. Default is FALSE.
	Additional arguments to pass to Rtsne.

### Value

A tibble with the same number of rows as 'tof\_tibble', each representing a single cell. Each of the 'num\_comp' columns represents each cell's embedding in the calculated tSNE space.

#### See Also

Other dimensionality reduction functions: tof\_reduce\_dimensions(), tof\_reduce\_pca(), tof\_reduce\_umap()

```
# simulate single-cell data
sim_data <-
    dplyr::tibble(
        cd45 = rnorm(n = 200),
        cd38 = rnorm(n = 200),</pre>
```

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```
cd34 = rnorm(n = 200),
    cd19 = rnorm(n = 200)
)

# calculate tsne
tof_reduce_tsne(tof_tibble = sim_data)

# calculate tsne with only 2 columns
tof_reduce_tsne(tof_tibble = sim_data, tsne_cols = c(cd34, cd38))
```

tof\_reduce\_umap

Apply uniform manifold approximation and projection (UMAP) to single-cell data

# Description

This function calculates a UMAP embedding from single-cell data in a 'tof\_tibble'.

# Usage

```
tof_reduce_umap(
  tof_tibble,
  umap_cols = where(tof_is_numeric),
  num_comp = 2,
  neighbors = 5,
  min_dist = 0.01,
  learn_rate = 1,
  epochs = NULL,
  verbose = FALSE,
  n_threads = 1,
  return_recipe = FALSE,
  ...
)
```

# **Arguments**

tof_tibble	A 'tof_tbl' or 'tibble'.
umap_cols	Unquoted column names indicating which columns in 'tof_tibble' to use in computing the UMAP embedding. Defaults to all numeric columns in 'tof_tibble'. Supports tidyselect helpers.
num_comp	An integer for the number of UMAP components.
neighbors	An integer for the number of nearest neighbors used to construct the target simplicial set.
min_dist	The effective minimum distance between embedded points.
learn_rate	Positive number of the learning rate for the optimization process.

tof\_reduce\_umap

epochs Number of iterations for the neighbor optimization. See umap for details.

verbose A boolean indicating if run details should be logged to the console. Defaults to

FALSE.

n\_threads Number of threads to use during UMAP calculation. Defaults to 1.

return\_recipe A boolean value indicating if instead of the UMAP result, a prepped recipe

object containing the UMAP embedding should be returned. Set this option to TRUE if you want to create the UMAP embedding using one dataset but also want to project new observations onto the same embedding space later.

want to project new observations onto the same embedding space

... Optional. Other options to be passed as arguments to umap.

#### Value

A tibble with the same number of rows as 'tof\_tibble', each representing a single cell. Each of the 'num\_comp' columns represents each cell's embedding in the calculated UMAP space.

#### See Also

Other dimensionality reduction functions: tof\_reduce\_dimensions(), tof\_reduce\_pca(), tof\_reduce\_tsne()

```
# simulate single-cell data
sim_data <-
    dplyr::tibble(
       cd45 = rnorm(n = 200),
        cd38 = rnorm(n = 200),
       cd34 = rnorm(n = 200),
        cd19 = rnorm(n = 200)
   )
new_data <-
   dplyr::tibble(
       cd45 = rnorm(n = 50),
        cd38 = rnorm(n = 50),
        cd34 = rnorm(n = 50),
        cd19 = rnorm(n = 50)
   )
# calculate umap
tof_reduce_umap(tof_tibble = sim_data)
# calculate umap with only 2 columns
tof_reduce_tsne(tof_tibble = sim_data, umap_cols = c(cd34, cd38))
# return recipe
umap_recipe <- tof_reduce_umap(tof_tibble = sim_data, return_recipe = TRUE)</pre>
# apply recipe to new data
recipes::bake(umap_recipe, new_data = new_data)
```

tof\_set\_panel

tof\_set\_panel

Set panel information from a tof\_tibble

#### **Description**

Set panel information from a tof\_tibble

## Usage

```
tof_set_panel(tof_tibble, panel)
```

# Arguments

```
tof_tibble A 'tof_tbl'.
```

panel A tibble containing two columns ('metals' and 'antigens') representing the in-

formation about a panel

## Value

A 'tof\_tibble' containing information about the CyTOF panel that was used during data acquisition for the data contained in the input 'tof\_tibble'. Two columns are required: "metals" and "antigens".

#### See Also

```
Other tof_tbl utilities: new_tof_tibble(), tof_get_panel()
```

```
# get current panel from an .fcs file
input_file <- dir(tidytof_example_data("aml"), full.names = TRUE)[[1]]
tof_tibble <- tof_read_data(input_file)
current_panel <- tof_get_panel(tof_tibble)

# create a new panel (remove empty channels)
new_panel <- dplyr::filter(current_panel, antigens != "empty")
tof_set_panel(tof_tibble = tof_tibble, panel = new_panel)</pre>
```

tof\_spade\_density

tof\_spade\_density

Estimate cells' local densities as done in Spanning-tree Progression Analysis of Density-normalized Events (SPADE)

#### **Description**

This function uses the algorithm described in Qiu et al., (2011) to estimate the local density of each cell in a 'tof\_tbl' or 'tibble' containing high-dimensional cytometry data. Briefly, this algorithm involves counting the number of neighboring cells within a sphere of radius alpha surrounding each cell. Here, we do so using the nn2 function.

## Usage

```
tof_spade_density(
  tof_tibble,
  distance_cols = where(tof_is_numeric),
  distance_function = c("euclidean", "cosine", "12", "ip"),
  num_alpha_cells = 2000L,
  alpha_multiplier = 5,
  max_neighbors = round(0.01 * nrow(tof_tibble)),
  normalize = TRUE,
  ...
)
```

#### **Arguments**

A 'tof\_tbl' or a 'tibble'.

distance\_cols

tof\_tibble

Unquoted names of the columns in 'tof\_tibble' to use in calculating cell-to-cell distances during the local density estimation for each cell. Defaults to all numeric columns in 'tof tibble'.

distance\_function

A string indicating which distance function to use for calculating cell-to-cell distances during local density estimation. Options include "euclidean" (the default) and "cosine".

num\_alpha\_cells

An integer indicating how many cells from 'tof\_tibble' should be randomly sampled from 'tof\_tibble' in order to estimate 'alpha', the radius of the sphere constructed around each cell during local density estimation. Alpha is calculated by taking the median nearest-neighbor distance from the 'num\_alpha\_cells' randomly-sampled cells and multiplying it by 'alpha\_multiplier'. Defaults to 2000.

alpha\_multiplier

An numeric value indicating the multiplier that should be used when calculating 'alpha', the radius of the sphere constructed around each cell during local density estimation. Alpha is calculated by taking the median nearest-neighbor distance from the 'num\_alpha\_cells' cells randomly-sampled from 'tof\_tibble' and multiplying it by 'alpha\_multiplier'. Defaults to 5.

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max\_neighbors An integer indicating the maximum number of neighbors that can be counted within the sphere surrounding any given cell. Implemented to reduce the density estimation procedure's speed and memory requirements. Defaults to 1% of the number of rows in 'tof\_tibble'.

A boolean value indicating if the vector of local density estimates should be normalized to values between 0 and 1. Defaults to TRUE.

Additional optional arguments to pass to tof\_find\_knn.

#### Value

A tibble with a single column named ".spade\_density" containing the local density estimates for each input cell in 'tof\_tibble'.

#### See Also

Other local density estimation functions: tof\_estimate\_density(), tof\_knn\_density()

```
sim_data <-
   dplyr::tibble(
       cd45 = rnorm(n = 1000),
       cd38 = rnorm(n = 1000),
       cd34 = rnorm(n = 1000),
        cd19 = rnorm(n = 1000)
    )
# perform the density estimation
tof_spade_density(tof_tibble = sim_data)
# perform the density estimation using cosine distance
tof_spade_density(
    tof_tibble = sim_data,
   distance_function = "cosine",
    alpha_multiplier = 2
)
# perform the density estimation with a smaller search radius around
# each cell
tof_spade_density(
    tof_tibble = sim_data,
   alpha_multiplier = 2
)
```

tof\_split\_data

tof\_split\_data

Split high-dimensional cytometry data into a training and test set

# Description

Split high-dimensional cytometry data into a training and test set

# Usage

```
tof_split_data(
  feature_tibble,
  split_method = c("k-fold", "bootstrap", "simple"),
  split_col,
  simple_prop = 3/4,
  num_cv_folds = 10,
  num_cv_repeats = 1L,
  num_bootstraps = 10,
  strata = NULL,
  ...
)
```

# Arguments

feature_tibble	A tibble in which each row represents a sample- or patient- level observation, such as those produced by tof_extract_features.
split_method	Either a string or a logical vector specifying how to perform the split. If a string, valid options include k-fold cross validation ("k-fold"; the default), bootstrapping ("bootstrap"), or a single binary split ("simple"). If a logical vector, it should contain one entry for each row in 'feature_tibble' indicating if that row should be included in the training set (TRUE) or excluded for the validation/test set (FALSE). Ignored entirely if 'split_col' is specified.
split_col	The unquoted column name of the logical column in 'feature_tibble' indicating if each row should be included in the training set (TRUE) or excluded for the validation/test set (FALSE).
simple_prop	A numeric value between 0 and 1 indicating what proportion of the data should be used for training. Defaults to 3/4. Ignored if split_method is not "simple".
num_cv_folds	An integer indicating how many cross-validation folds should be used. Defaults to 10. Ignored if split_method is not "k-fold".
num_cv_repeats	An integer indicating how many independent cross-validation replicates should be used (i.e. how many num_cv_fold splits should be performed). Defaults to 1. Ignored if split_method is not "k-fold".
num_bootstraps	An integer indicating how many independent bootstrap replicates should be used. Defaults to 25. Ignored if split_method is not "bootstrap".
strata	An unquoted column name representing the column in feature_tibble that should be used to stratify the data splitting. Defaults to NULL (no stratification).

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Optional additional arguments to pass to vfold\_cv for k-fold cross validation, bootstraps for bootstrapping, or initial\_split for simple splitting.

#### Value

If for k-fold cross validation and bootstrapping, an "rset" object; for simple splitting, an "rsplit" object. For details, see rsample.

#### See Also

Other modeling functions: tof\_assess\_model(), tof\_create\_grid(), tof\_predict(), tof\_train\_model()

```
feature_tibble <-</pre>
    dplyr::tibble(
       sample = as.character(1:100),
        cd45 = runif(n = 100),
        pstat5 = runif(n = 100),
        cd34 = runif(n = 100),
        outcome = (3 * cd45) + (4 * pstat5) + rnorm(100),
        class =
            as.factor(
                dplyr::if_else(outcome > median(outcome), "class1", "class2")
            ),
        multiclass =
            as.factor(
                c(rep("class1", 30), rep("class2", 30), rep("class3", 40))
            ),
        event = c(rep(0, times = 50), rep(1, times = 50)),
        time_{to}=rnorm(n = 100, mean = 10, sd = 2)
   )
# split the dataset into 10 CV folds
tof_split_data(
    feature_tibble = feature_tibble,
    split_method = "k-fold"
)
# split the dataset into 10 bootstrap resamplings
tof_split_data(
   feature_tibble = feature_tibble,
    split_method = "bootstrap"
)
# split the dataset into a single training/test set
# stratified by the "class" column
tof_split_data(
   feature_tibble = feature_tibble,
   split_method = "simple",
   strata = class
)
```

```
tof_split_tidytof_reduced_dimensions
```

Split the dimensionality reduction data that tidytof combines during SingleCellExperiment conversion

# Description

Split the dimensionality reduction data that tidytof combines during SingleCellExperiment conversion

# Usage

```
tof_split_tidytof_reduced_dimensions(sce)
```

#### **Arguments**

sce

A SingleCellExperiment with an entry named "tidytof\_reduced\_dimensions" in its reducedDims slot.

# Value

A SingleCellExperiment with separate entries named "tidytof\_pca", "tidytof\_umap", and "tidytof\_tsne" in its reducedDims slots (one for each of the dimensionality reduction methods for which tidytof has native support).

#### **Examples**

NULL

tof_train_model	Train an elastic net model to predict sample-level phenomena using
	high-dimensional cytometry data.

# **Description**

This function uses a training set/test set paradigm to tune and fit an elastic net model using a variety of user-specified details. Tuning can be performed using either a simple training vs. test set split, k-fold cross-validation, or bootstrapping, and multiple preprocessing options are available.

## Usage

```
tof_train_model(
  split_data,
  unsplit_data,
 predictor_cols,
  response_col = NULL,
  time_col = NULL,
  event_col = NULL,
 model_type = c("linear", "two-class", "multiclass", "survival"),
  hyperparameter_grid = tof_create_grid(),
  standardize_predictors = TRUE,
  remove_zv_predictors = FALSE,
  impute_missing_predictors = FALSE,
  optimization_metric = "tidytof_default",
 best_model_type = c("best", "best with sparsity"),
  num\_cores = 1
)
```

#### **Arguments**

An 'rsplit' or 'rset' object from the rsample package containing the sample-level data to use for modeling. The easiest way to generate this is to use tof\_split\_data.

unsplit\_data A tibble containing sample-level data to use for modeling without resampling.

While using a resampling method is advised, this argument provides an interface to fit a model without using cross-validation or bootstrap resampling. Ignored if

split\_data is provided.

'split\_data' should be used as predictors in the elastic net model. Supports tidys-

elect helpers.

response\_col Unquoted column name indicating which column in the data contained in 'split data'

should be used as the outcome in a "two-class", "multiclass", or "linear" elastic net model. Must be a factor for "two-class" and "multiclass" models and must

be a numeric for "linear" models. Ignored if 'model\_type' is "survival".

time\_col Unquoted column name indicating which column in the data contained in 'split\_data' represents the time-to-event outcome in a "survival" elastic net model. Must be

numeric. Ignored if 'model\_type' is "two-class", "multiclass", or "linear".

event\_col Unquoted column name indicating which column in the data contained in 'split\_data'

represents the time-to-event outcome in a "survival" elastic net model. Must be a binary column - all values should be either 0 or 1 (with 1 indicating the adverse event) or FALSE and TRUE (with TRUE indicating the adverse event). Ignored

if 'model\_type' is "two-class", "multiclass", or "linear".

model\_type A string indicating which kind of elastic net model to build. If a continuous

response is being predicted, use "linear" for linear regression; if a categorical response with only 2 classes is being predicted, use "two-class" for logistic regression; if a categorical response with more than 2 levels is being predicted, use "multiclass" for multinomial regression; and if a time-to-event outcome is

being predicted, use "survival" for Cox regression.

#### hyperparameter\_grid

A hyperparameter grid indicating which values of the elastic net penalty (lambda) and the elastic net mixture (alpha) hyperparametrs should be used during model tuning. Generate this grid using tof\_create\_grid.

#### standardize\_predictors

A logical value indicating if numeric predictor columns should be standardized (centered and scaled) before model fitting, as is standard practice during elastic net regularization. Defaults to TRUE.

#### remove\_zv\_predictors

A logical value indicating if predictor columns with near-zero variance should be removed before model fitting using step\_nzv. Defaults to FALSE.

#### impute\_missing\_predictors

A logical value indicating if predictor columns should have missing values imputed using k-nearest neighbors before model fitting (see <a href="step\_impute\_knn">step\_impute\_knn</a>). Imputation is performed using an observation's 5 nearest-neighbors. Defaults to FALSE.

#### optimization\_metric

A string indicating which optimization metric should be used for hyperparameter selection during model tuning. Valid values depend on the model\_type.

- For "linear" models, choices are "mse" (the mean squared error of the predictions; the default) and "mae" (the mean absolute error of the predictions).
- For "two-class" models, choices are "roc\_auc" (the area under the Receiver-Operating Curve for the classification; the default), "misclassification error" (the proportion of misclassified observations), "binomial\_deviance" (see deviance.glmnet), "mse" (the mean squared error of the logit function), and "mae" (the mean absolute error of the logit function).
- For "multiclass" models, choices are "roc\_auc" (the area under the Receiver-Operating Curve for the classification using the Hand-Till generalization of the ROC AUC for multiclass models in roc\_auc; the default), "misclassification error" (the proportion of misclassified observations), "multino-mial\_deviance" (see deviance.glmnet), and "mse" and "mae" as above.
- For "survival" models, choices are "concordance\_index" (Harrel's C index; see deviance.glmnet) and "partial\_likelihood\_deviance" (see deviance.glmnet).

## best\_model\_type

Currently unused.

num\_cores

Integer indicating how many cores should be used for parallel processing when fitting multiple models. Defaults to 1. Overhead to separate models across multiple cores can be high, so significant speedup is unlikely to be observed unless many large models are being fit.

#### Value

A 'tof\_model', an S3 class that includes the elastic net model with the best performance (assessed via cross-validation, bootstrapping, or simple splitting depending on 'split\_data') across all tested hyperparameter value combinations. 'tof\_models' store the following information:

**model** The final elastic net ("glmnet") model, which is chosen by selecting the elastic net hyperparameters with the best 'optimization\_metric' performance on the validation sets of each resample used to train the model (on average)

recipe The recipe used for data preprocessing

**mixture** The optimal mixture hyperparameter (alpha) for the glmnet model

penalty The optimal penalty hyperparameter (lambda) for the glmnet model

model\_type A string indicating which type of glmnet model was fit

**outcome\_colnames** A character vector representing the names of the columns in the training data modeled as outcome variables

**training\_data** A tibble containing the (not preprocessed) data used to train the model

**tuning\_metrics** A tibble containing the validation set performance metrics (and model predictions) during for each resample fold during model tuning.

**log\_rank\_thresholds** For survival models only, a tibble containing information about the relativerisk thresholds that can be used to split the training data into 2 risk groups (low- and high-risk) based on the final model's predictions. For each relative-risk threshold, the log-rank test p-value and an indicator of which threshold gives the most significant separation is provided.

**best\_log\_rank\_threshold** For survival models only, a numeric value representing the relative-risk threshold that yields the most significant log-rank test when separating the training data into low- and high-risk groups.

#### See Also

Other modeling functions: tof\_assess\_model(), tof\_create\_grid(), tof\_predict(), tof\_split\_data()

```
feature_tibble <-
   dplyr::tibble(
       sample = as.character(1:100),
       cd45 = runif(n = 100),
       pstat5 = runif(n = 100),
       cd34 = runif(n = 100),
       outcome = (3 * cd45) + (4 * pstat5) + rnorm(100),
       class =
           as.factor(
                dplyr::if_else(outcome > median(outcome), "class1", "class2")
           ),
       multiclass =
           as.factor(
                c(rep("class1", 30), rep("class2", 30), rep("class3", 40))
       event = c(rep(0, times = 30), rep(1, times = 70)),
        time_{to}=rnorm(n = 100, mean = 10, sd = 2)
   )
split_data <- tof_split_data(feature_tibble, split_method = "simple")</pre>
# train a regression model
```

tof\_transform

```
tof_train_model(
    split_data = split_data,
   predictor_cols = c(cd45, pstat5, cd34),
   response_col = outcome,
   model_type = "linear"
)
# train a logistic regression classifier
tof_train_model(
    split_data = split_data,
   predictor_cols = c(cd45, pstat5, cd34),
   response_col = class,
   model_type = "two-class"
)
# train a cox regression survival model
tof_train_model(
    split_data = split_data,
   predictor_cols = c(cd45, pstat5, cd34),
   time_col = time_to_event,
   event_col = event,
   model_type = "survival"
)
```

tof\_transform

Transform raw high-dimensional cytometry data.

# **Description**

This function transforms a 'tof\_tbl' of raw ion counts, reads, or fluorescence intensity units directly measured on a cytometer using a user-provided function.

#### Usage

```
tof_transform(
  tof_tibble = NULL,
  channel_cols = where(tof_is_numeric),
  transform_fun
)
```

# Arguments

tof\_tibble A 'tof\_tbl' or a 'tibble'.

channel\_cols Unquoted column names representing columns that contain single-cell protein

measurements. Supports tidyselect helpers. If nothing is specified, the default is

to transform all numeric columns.

transform\_fun A vectorized function to apply to each protein value for variance stabilization.

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#### Value

A 'tof\_tbl' with identical dimensions to the input 'tof\_tibble', with all columns specified in channel\_cols transformed using 'transform\_fun'.

#### **Examples**

```
# read in an example .fcs file from tidytof's internal datasets
input_file <- dir(tidytof_example_data("aml"), full.names = TRUE)[[1]]</pre>
tof_tibble <- tof_read_data(input_file)</pre>
# preprocess all numeric columns with default behavior
# arcsinh transformation with a cofactor of 5
tof_preprocess(tof_tibble)
# preprocess all numeric columns using the log base 10 tranformation
tof_preprocess(tof_tibble, transform_fun = log10)
```

tof\_tune\_glmnet

Tune an elastic net model's hyperparameters over multiple resamples

## **Description**

Tune an elastic net model's hyperparameters over multiple resamples

# Usage

```
tof_tune_glmnet(
  split_data,
  prepped_recipe,
 hyperparameter_grid,
 model_type,
 outcome_cols,
 optimization_metric = "tidytof_default",
  num\_cores = 1
)
```

## **Arguments**

split\_data

An 'rsplit' or 'rset' object from the rsample package. The easiest way to generate this is to use tof\_split\_data. Alternatively, an unsplit tbl\_df can be provided, though this is not recommended.

prepped\_recipe Either a single recipe object (if 'split\_data' is an 'rsplit' object or a 'tbl\_df') or list of recipes (if 'split\_data' is an 'rset' object) such that each entry in the list corresponds to a resample in 'split\_data'.

tof\_upsample

hyperparameter\_grid

A hyperparameter grid indicating which values of the elastic net penalty (lambda) and the elastic net mixture (alpha) hyperparameters should be used during model tuning. Generate this grid using tof\_create\_grid.

model\_type

A string indicating which kind of elastic net model to build. If a continuous response is being predicted, use "linear" for linear regression; if a categorical response with only 2 classes is being predicted, use "two-class" for logistic regression; if a categorical response with more than 2 levels is being predicted, use "multiclass" for multinomial regression; and if a time-to-event outcome is being predicted, use "survival" for Cox regression.

outcome\_cols

Unquoted column name(s) indicating which column(s) in the data contained in 'split\_data' should be used as the outcome in the elastic net model. For survival models, two columns should be selected; for all others, only one column should be selected.

optimization\_metric

A string indicating which optimization metric should be used for hyperparameter selection during model tuning. Valid values depend on the model\_type.

num\_cores

Integer indicating how many cores should be used for parallel processing when fitting multiple models. Defaults to 1. Overhead to separate models across multiple cores can be high, so significant speedup is unlikely to be observed unless many large models are being fit.

#### Value

A tibble containing a summary of the model's performance in each resampling iteration across all hyperparameter combinations. Will contain 3 columns: "splits" (a list-col containing each resampling iteration's 'rsplit' object), "id" (the name of the resampling iteration), and "performance\_metrics" (a list-col containing the performance metrics for each resampling iteration. Each row of "performance\_metrics" is a tibble with the columns "mixture" and "penalty" and several additional columns containing the performance metrics of the model for each mixture/penalty combination). See tof\_fit\_split for additional details.

tof\_upsample

Upsample cells into the closest cluster in a reference dataset

#### **Description**

This function performs distance-based upsampling on CyTOF data by sorting single cells (passed into the function as 'tof\_tibble') into their most phenotypically similar cell subpopulation in a reference dataset (passed into the function as 'reference\_tibble'). It does so by calculating the distance (either mahalanobis, cosine, or pearson) between each cell in 'tof\_tibble' and the centroid of each cluster in 'reference\_tibble', then sorting cells into the cluster corresponding to their closest centroid.

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#### Usage

```
tof_upsample(
  tof_tibble,
  reference_tibble,
  reference_cluster_col,
  upsample_cols = where(tof_is_numeric),
  ...,
  augment = TRUE,
  method = c("distance", "neighbor")
)
```

#### **Arguments**

tof\_tibble

A 'tibble' or 'tof\_tbl' containing cells to be upsampled into their nearest reference subpopulation.

reference\_tibble

A 'tibble' or 'tof\_tibble' containing cells that have already been clustered or manually gated into subpopulations.

reference\_cluster\_col

An unquoted column name indicating which column in 'reference\_tibble' contains the subpopulation label (or cluster id) for each cell in 'reference\_tibble'.

upsample\_cols

Unquoted column names indicating which columns in 'tof\_tibble' to use in computing the distances used for upsampling. Defaults to all numeric columns in 'tof tibble'. Supports tidyselect helpers.

. . .

Additional arguments to pass to the 'tof\_upsample\_\*' function family member corresponding to the chosen method.

augment

A boolean value indicating if the output should column-bind the cluster ids of each cell as a new column in 'tof\_tibble' (TRUE, the default) or if a single-column tibble including only the cluster ids should be returned (FALSE).

method

A string indicating which clustering methods should be used. Valid values include "distance" (default) and "neighbor".

#### Value

A 'tof\_tbl' or 'tibble' If augment = FALSE, it will have a single column encoding the upsampled cluster ids for each cell in 'tof\_tibble'. If augment = TRUE, it will have ncol(tof\_tibble) + 1 columns: each of the (unaltered) columns in 'tof\_tibble' plus an additional column encoding the cluster ids.

```
# simulate single-cell data (and reference data with clusters to upsample
# into
sim_data <-
    dplyr::tibble(
        cd45 = rnorm(n = 1000),
        cd38 = rnorm(n = 1000),</pre>
```

```
cd34 = rnorm(n = 1000),
        cd19 = rnorm(n = 1000)
   )
reference_data <-
   dplyr::tibble(
       cd45 = rnorm(n = 200),
        cd38 = rnorm(n = 200),
        cd34 = rnorm(n = 200),
        cd19 = rnorm(n = 200),
        cluster_id = c(rep("a", times = 100), rep("b", times = 100))
   )
# upsample using distance to cluster centroids
tof_upsample(
    tof_tibble = sim_data,
    reference_tibble = reference_data,
    reference_cluster_col = cluster_id,
   method = "distance"
)
# upsample using distance to nearest neighbor
tof_upsample(
    tof_tibble = sim_data,
    reference_tibble = reference_data,
   reference_cluster_col = cluster_id,
   method = "neighbor"
)
```

tof\_upsample\_distance Upsample cells into the closest cluster in a reference dataset

# **Description**

This function performs distance-based upsampling on CyTOF data by sorting single cells (passed into the function as 'tof\_tibble') into their most phenotypically similar cell subpopulation in a reference dataset (passed into the function as 'reference\_tibble'). It does so by calculating the distance (either mahalanobis, cosine, or pearson) between each cell in 'tof\_tibble' and the centroid of each cluster in 'reference\_tibble', then sorting cells into the cluster corresponding to their closest centroid.

## Usage

```
tof_upsample_distance(
  tof_tibble,
  reference_tibble,
  reference_cluster_col,
  upsample_cols = where(tof_is_numeric),
  parallel_cols,
```

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```
distance_function = c("mahalanobis", "cosine", "pearson"),
num_cores = 1L,
return_distances = FALSE
)
```

#### **Arguments**

tof\_tibble

A 'tibble' or 'tof\_tbl' containing cells to be upsampled into their nearest reference subpopulation.

reference\_tibble

A 'tibble' or 'tof\_tibble' containing cells that have already been clustered or manually gated into subpopulations.

reference\_cluster\_col

An unquoted column name indicating which column in 'reference\_tibble' contains the subpopulation label (or cluster id) for each cell in 'reference\_tibble'.

upsample\_cols Unquoted column names indicating which columns in 'tof\_tibble' to use in computing the distances used for upsampling. Defaults to all numeric columns in 'tof\_tibble'. Supports tidyselect helpers.

parallel\_cols Optional. Unquoted column names indicating which columns in 'tof\_tibble' to use for breaking up the data in order to parallelize the upsampling using 'foreach' on a 'doParallel' backend. Supports tidyselect helpers.

distance\_function

A string indicating which distance function should be used to perform the upsampling. Options are "mahalanobis" (the default), "cosine", and "pearson".

num\_cores An integer indicating the number of CPU cores used to parallelize the classification. Defaults to 1 (a single core).

return\_distances

A boolean value indicating whether or not the returned result should include only one column, the cluster ids corresponding to each row of 'tof\_tibble' (return\_distances = FALSE, the default), or if the returned result should include additional columns representing the distance between each row of 'tof\_tibble' and each of the reference subpopulation centroids (return\_distances = TRUE).

## Value

If 'return\_distances = FALSE', a tibble with one column named '.upsample\_cluster', a character vector of length 'nrow(tof\_tibble)' indicating the id of the reference cluster to which each cell (i.e. each row) in 'tof\_tibble' was assigned.

If 'return\_distances = TRUE', a tibble with 'nrow(tof\_tibble)' rows and num\_clusters + 1 columns, where num\_clusters is the number of clusters in 'reference\_tibble'. Each row represents a cell from 'tof\_tibble', and num\_clusters of the columns represent the distance between the cell and each of the reference subpopulations' cluster centroids. The final column represents the cluster id of the reference subpopulation with the minimum distance to the cell represented by that row.

#### **Examples**

# simulate single-cell data (and reference data with clusters to upsample

```
# into
sim_data <-
   dplyr::tibble(
        cd45 = rnorm(n = 1000),
        cd38 = rnorm(n = 1000),
        cd34 = rnorm(n = 1000),
        cd19 = rnorm(n = 1000)
reference_data <-
    dplyr::tibble(
        cd45 = rnorm(n = 200),
        cd38 = rnorm(n = 200),
        cd34 = rnorm(n = 200),
        cd19 = rnorm(n = 200),
        cluster_id = c(rep("a", times = 100), rep("b", times = 100))
   )
# upsample using mahalanobis distance
tof_upsample_distance(
    tof_tibble = sim_data,
    reference_tibble = reference_data,
    reference_cluster_col = cluster_id
)
# upsample using cosine distance
tof_upsample_distance(
    tof_tibble = sim_data,
    reference_tibble = reference_data,
    reference_cluster_col = cluster_id,
   distance_function = "cosine"
)
```

tof\_upsample\_neighbor Upsample cells into the cluster of their nearest neighbor a reference dataset

### **Description**

This function performs upsampling on CyTOF data by sorting single cells (passed into the function as 'tof\_tibble') into their most phenotypically similar cell subpopulation in a reference dataset (passed into the function as 'reference\_tibble'). It does so by finding each cell in 'tof\_tibble''s nearest neighbor in 'reference\_tibble' and assigning it to the cluster to which its nearest neighbor belongs. The nearest neighbor calculation can be performed with either euclidean or cosine distance.

## Usage

```
tof_upsample_neighbor(
```

```
tof_tibble,
  reference_tibble,
  reference_cluster_col,
  upsample_cols = where(tof_is_numeric),
  num_neighbors = 1L,
  distance_function = c("euclidean", "cosine", "12", "ip")
)
```

#### Arguments

tof\_tibble

A 'tibble' or 'tof\_tbl' containing cells to be upsampled into their nearest reference subpopulation.

reference\_tibble

A 'tibble' or 'tof\_tibble' containing cells that have already been clustered or manually gated into subpopulations.

reference\_cluster\_col

An unquoted column name indicating which column in 'reference\_tibble' contains the subpopulation label (or cluster id) for each cell in 'reference\_tibble'.

upsample\_cols Unquoted column names indicating which columns in 'tof\_tibble' to use in computing the distances used for upsampling. Defaults to all numeric columns in 'tof\_tibble'. Supports tidyselect helpers.

num\_neighbors An integer indicating how many neighbors should be used in the nearest neighbor calculation. Clusters are assigned based on majority vote.

distance\_function

A string indicating which distance function should be used to perform the upsampling. Options are "euclidean" (the default) and "cosine".

#### Value

A tibble with one column named '.upsample\_cluster', a character vector of length 'nrow(tof\_tibble)' indicating the id of the reference cluster to which each cell (i.e. each row) in 'tof\_tibble' was assigned.

```
# simulate single-cell data (and reference data with clusters to upsample
# into
sim_data <-
    dplyr::tibble(
        cd45 = rnorm(n = 1000),
        cd38 = rnorm(n = 1000),
        cd34 = rnorm(n = 1000),
        cd19 = rnorm(n = 1000)
)

reference_data <-
    dplyr::tibble(
        cd45 = rnorm(n = 200),
        cd38 = rnorm(n = 200),</pre>
```

tof\_write\_csv

```
cd34 = rnorm(n = 200),
        cd19 = rnorm(n = 200),
        cluster_id = c(rep("a", times = 100), rep("b", times = 100))
   )
# upsample using euclidean distance
tof_upsample_neighbor(
    tof_tibble = sim_data,
    reference_tibble = reference_data,
    reference_cluster_col = cluster_id
)
# upsample using cosine distance
tof_upsample_neighbor(
    tof_tibble = sim_data,
    reference_tibble = reference_data,
    reference_cluster_col = cluster_id,
    distance_function = "cosine"
)
```

tof\_write\_csv

Write a series of .csv files from a tof\_tbl

# **Description**

This function takes a given 'tof\_tbl' and writes the single-cell data it contains into .csv files within the directory located at 'out\_path'. The 'group\_cols' argument specifies how the rows of the 'tof\_tbl' (each cell) should be broken into separate .csv files

# Usage

```
tof_write_csv(tof_tibble, group_cols, out_path, sep = "_", file_name)
```

# **Arguments**

tof_tibble	A 'tof_tbl' or a 'tibble'.
group_cols	Optional. Unquoted names of the columns in 'tof_tibble' that should be used to group cells into separate files. Supports tidyselect helpers. Defaults to NULL (all cells are written into a single file).
out_path	A system path indicating the directory where the output .csv files should be saved. If the directory doesn't exist, it will be created.
sep	Delimiter that should be used between each of the values of 'group_cols' to create the output .csv file names. Defaults to "_".
file_name	If 'group_cols' isn't specified, the name (without an extension) that should be used for the saved .csv file.

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# Value

This function does not return anything. Instead, it has the side-effect of saving .csv files to 'out\_path'.

#### See Also

```
Other input/output functions: tof_read_data(), tof_write_data(), tof_write_fcs()
```

tof\_write\_data Write high-dimensional cytometry data to a file or to a directory of files

# **Description**

Write data (in the form of a 'tof\_tbl') into either a .csv or an .fcs file for storage.

# Usage

```
tof_write_data(
  tof_tibble = NULL,
  group_cols,
  out_path = NULL,
  format = c("fcs", "csv"),
  sep = "_",
  file_name
)
```

# Arguments

tof_tibble	A 'tof_tbl' or a 'tibble'.
group_cols	Optional. Unquoted names of the columns in 'tof_tibble' that should be used to group cells into separate files. Supports tidyselect helpers. Defaults to no grouping (all cells are written into a single file).
out_path	Path to the directory where output files should be saved.
format	format for the files being written. Currently supports .csv and .fcs files
sep	Delimiter that should be used between each of the values of 'group_cols' to create the output .csv/.fcs file names. Defaults to "_".
file_name	If 'group_cols' isn't specified, the name (without an extension) that should be used for the saved file.

## Value

This function does not explicitly return any values. Instead, it writes .csv and/or .fcs files to the specified 'out\_path'.

tof\_write\_fcs

#### See Also

Other input/output functions: tof\_read\_data(), tof\_write\_csv(), tof\_write\_fcs()

# **Examples**

NULL

# Description

This function takes a given 'tof\_tbl' and writes the single-cell data it contains into .fcs files within the directory located at 'out\_path'. The 'group\_cols' argument specifies how the rows of the 'tof\_tbl' (each cell) should be broken into separate .fcs files

# Usage

```
tof_write_fcs(tof_tibble, group_cols, out_path, sep = "_", file_name)
```

# **Arguments**

tof_tibble	A 'tof_tbl' or a 'tibble'.
group_cols	Unquoted names of the columns in 'tof_tibble' that should be used to group cells into separate files. Supports tidyselect helpers. Defaults to NULL (all cells are written into a single file).
out_path	A system path indicating the directory where the output .csv files should be saved. If the directory doesn't exist, it will be created.
sep	Delimiter that should be used between each of the values of 'group_cols' to create the output .fcs file names. Defaults to "_".
file_name	If 'group_cols' isn't specified, the name (without an extension) that should be used for the saved .csv file.

#### Value

This function does not return anything. Instead, it has the side-effect of saving .fcs files to 'out\_path'.

## See Also

```
Other input/output functions: tof_read_data(), tof_write_csv(), tof_write_data()
```

# **Examples**

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where

Select variables with a function

# Description

This is a copy of where, a selection helper that selects the variables for which a predicate function returns TRUE. See language for more details about tidyselection.

# Usage

where(fn)

# **Arguments**

fn

A function that returns TRUE or FALSE (technically, a predicate function). Can also be a purrr-like formula.

# **Details**

This help file was replicated verbatim from tidyselect-package.

# Value

A predicate that can be used to select columns from a data.frame.

#### References

Lionel Henry and Hadley Wickham (2021). tidyselect: Select from a Set of Strings. R package version 1.1.1. https://CRAN.R-project.org/package=tidyselect

# **Examples**

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