Package ‘flowGraph’

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Type Package

Title Identifying differential cell populations in flow cytometry data accounting for marker frequency

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Description Identifies maximal differential cell populations in flow cytometry data taking into account dependencies between cell populations; flowGraph calculates and plots SpecEnr abundance scores given cell population cell counts.

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**cell_type_layers**

Determines the layer on which a phenotype resides.

**Description**

Determines the layer on which the given phenotypes reside.

**Usage**

`cell_type_layers(phen)`

**Arguments**

- **phen**  
  A string vector of phenotype or cell population name labels.

**Details**

Given a vector of phenotypes, returns an equal length vector of the number of markers in each phenotype.
extract_markers

Value

A numeric vector with the same length as phen indicating which layer each phenotype resides on.

See Also

get_phen_list get_phen_meta

Examples

```r
phen <- c('A+B+C-D++', 'A+B-', '', 'B++D-E+')
cell_type_layers(phen)
```

Description

Extracts all unique markers from cell population phenotypes

Usage

```
extract_markers(phen)
```

Arguments

phen A vector of cell population phenotypes.

Value

A vector of unique markers

See Also

str_split
fg_add_feature

Description

Adds a feature created using feat_fun from fg OR m into a given flowGraph object. Only use this function if you cannot generate the desired features using the existing flowGraph functions starting with fg_feat_<feature name>.

Usage

fg_add_feature(
  fg,
  type = "node",
  feature,
  m = NULL,
  feat_fun = NULL,
  overwrite = FALSE,
  ...
)

Arguments

fg
  flowGraph object.

type
  A string specifying the type of the feature being added i.e. 'node' or 'edge'.

feature
  A string indicating the unique name of the feature added.

m
  A numeric matrix with feature values; it should contain the same sample id's on row names as in fg_get_meta(fg)$id and node or edge names as column names (i.e. if m is a node feature, it would have the same column names as those in fg_get_graph(fg)$v$phenotype; if it is an edge feature, its column names should be the same as paste0(fg_get_graph(fg)$e$from, '_', fg_get_graph(fg)$e$to)).

feat_fun
  A function that ouputs a feature matrix as in m given fg and other optional parameters.

overwrite
  A logical variable indicating whether or not the function should replace the existing feature with the same name if one is already in fg.

...
  Other parameters that would be used as input into feat_fun.

Details

fg_add_feature adds the given new feature matrix to the given flowGraph object fg updating slots feat and feat_desc. See flowGraph-class slot feat and feat_desc for what should be in these slots. We do not recommend users to directly use this method unless there is a clear understanding on how the row and column names should be specified. Instead, we recommend users to use the functions listed in the "See also" sections prefixed with "fg_feat_".
Value
flowGraph object.

See Also
flowGraph-class fg_feat_node_prop fg_feat_node_specenr fg_get_feature fg_rm_feature fg_get_feature_desc

Examples
no_cores <- 1
data(fg_data_pos30)
fg <- flowGraph(fg_data_pos30$count, class=fg_data_pos30$meta$class,
       prop=FALSE, specenr=FALSE,
       no_cores=no_cores)
fg_get_feature_desc(fg)

fg <- fg_add_feature(fg, type="node", feature="count_copy",
       m=fg_data_pos30$count)
fg_get_feature_desc(fg)

fg_add_summary

Adds a feature summary.

Description
Adds a feature summary into a given flowGraph object. Only use this function if your summary statistic cannot be calculated using the fg_summary function.

Usage
fg_add_summary(
   fg, 
   type = "node", 
   summary_meta = NULL, 
   p = NULL, 
   summ_fun = NULL, 
   overwrite = FALSE, 
   ...
)

Arguments
fg flowGraph object.
type A string indicating feature type the summary was created for; 'node' or 'edge'.
The user must provide type and summary_meta.

summary_meta is a list containing feature (feature name), test_name (summary statistic name), class (class), label1, and label2 (class labels compared). See \texttt{fg\_get\_summary\_desc} for details.

\textit{p} A list containing summary values; this list contains elements: values (a vector containing summary statistics e.g. p-values; this vector should be named by their associated phenotype or edge name), test_custom (a function of the statistical test used), and adjust_custom (a function of the p-value correction method used). This list must contain the values element.

\textit{summ\_fun} A function that outputs a feature summary matrix as in \textit{p} given \textit{fg} and other optional parameters.

\textit{overwrite} A logical variable indicating whether or not the function should replace the existing feature summary with the same name if one is already in \textit{fg}.

... Other parameters that would be used as input into \textit{summ\_fun}.

\textbf{Details}

\texttt{fg\_add\_summary} adds the given feature summary list \textit{p} or the output of the given function \textit{summ\_fun} to the given flowGraph object \textit{fg} updating slots \textit{summary} and \textit{summary\_desc}. See \texttt{flowGraph\_class} slot \textit{summary} and \textit{summary\_desc} for what should be in these slots. We do not recommend users directly use this function unless what is required is duly in the above slots is well understood — note these slots are used in plotting functions e.g. \texttt{fg\_plot}. We instead recommend users to use the \texttt{fg\_summary} function.

\textbf{Value}

flowGraph object.

\textbf{See Also}

\texttt{flowGraph\_class fg\_summary fg\_get\_summary fg\_rm\_summary fg\_get\_summary\_desc fg\_add\_feature}

\textbf{Examples}

```r
no\_cores <- 1
data(fg\_data\_pos30)
fg <- flowGraph(fg\_data\_pos30\$count, class=fg\_data\_pos30\$meta\$class,
               no\_cores=no\_cores)

# get samples that we are going to compare
m <- fg\_get\_feature(fg, type="node", feature="prop")
m1\_ <- m[fg\_data\_pos30\$meta\$class=="control",,drop=FALSE]
m2\_ <- m[fg\_data\_pos30\$meta\$class=="exp",,drop=FALSE]

# define test or summary function to conduct comparison
test\_custom <- function(x,y)
  tryCatch(stats::t.test(x,y)$p\_value, error=function(e) 1)
values\_p <- sapply(seq\_len(ncol(m)), function(j)
  test\_custom(m1\_[,j], m2\_[,j])
```
values_p <- p.adjust(values_p, method="BY")

# the user can choose to fill either parameter "p" or "summ_fun",
# the latter of which must output a list with the same elements as "p".
# see documentation for ?flowGraph-class, slot "summary" for
# details on what should be in "p".
p <- list(values=values_p, test_fun=test_custom, adjust_fun="BY")
fg <- fg_add_summary(fg, type="node", summary_meta=list(
  feature="prop", test_name="wilcox_BY", 
  class="class", label1="control", label2="exp"), p=p)

fg_get_summary_desc(fg)

---

### fg_clean_phen

Reformats phenotype

**Description**

Reformats cell population phenotypes into flowGraph format

**Usage**

```
fg_clean_phen(phen, markers = NULL)
```

**Arguments**

- `phen`: Vector of cell population phenotype names as character strings.
- `markers`: markers extracted from `phen`.

**Value**

Vector with the same length as `phen` containing reformatted and not necessarily changed cell population phenotype names.

**See Also**

`str_extract`, `str_split`

**Examples**

```
# fg_clean_phen(c("A+_B+","B+_notC","A-_C"))
```
fg_clear_features  Clears all features in a flowGraph object.

Description
Returns a flowGraph object with only the count feature.

Usage
fg_clear_features(fg)

Arguments
fg  flowGraph object.

Value
flowGraph object with only the count node feature.

See Also
flowGraph-class

Examples
no_cores <- 1
data(fg_data_pos30)
fg <- flowGraph(fg_data_pos30$count, class=fg_data_pos30$meta$class,
               no_cores=no_cores)
fg <- fg_clear_features(fg)
fg_get_summary_desc(fg)

fg_clear_summary  Removes all summary statistics.

Description
Removes all summary statistics in a flowGraph object; we recommend doing this to save space.

Usage
fg_clear_summary(fg)
Arguments

fg

flowGraph object.

Value

flowGraph object with an empty summary slot.

See Also

flowGraph-class fg_summary

Examples

no_cores <- 1
data(fg_data_pos30)
fg <- flowGraph(fg_data_pos30$count, class=fg_data_pos30$meta$class,
    prop=FALSE, specenr=FALSE,
    no_cores=no_cores, node_features="count")
fg_get_summary_desc(fg)

fg <- fg_clear_summary(fg)
fg_get_summary_desc(fg)

fg_data_fca

Description

fg_data_fca

Usage

fg_data_fca

Format

A list containing the following elements derived from the flowCAP-II AML data set for cell populations up to layer 3.

- count: A numeric sample x cell population node matrix with cell count values.
- meta: A data frame containing meta information on samples in count; it contains columns:
  - class: a string indicating whether a sample is from a "control" or "aml" subject.
  - id: a string containing sample id’s.
  - train: a logical variable indicating whether a sample is from the train or test set.
  - subject: a numeric variable containing the id of the subject from whom the sample came from.
  - tube: the tube or panel number; all samples in this data set is analyzed under the 6th panel.
Source


---

**fg_data_pos2**

**Description**

**fg_data_pos2**

**Usage**

**fg_data_pos2**

**Format**

A list containing the following elements for a positive control data set with markers A, B, C, D. This is a positive control data set where node A+B+C+ increased by 50

- count: A numeric sample x cell population node matrix with cell count values
- meta: A data frame containing meta information on samples in count; it contains columns:
  - id: a string containing sample id’s.
  - class: a string indicating whether a sample is from a "control" or "exp" (experiment) subject.

---

**fg_data_pos30**

**Description**

**fg_data_pos30**

**Usage**

**fg_data_pos30**
fg_extract_phenotypes

Format

A list containing the following elements for a positive control data set with markers A, B, C, D; note it was made with two and three thresholds for markers A and B to test functions with multiple thresholds (this is a positive control data set where nodes A+.B+.C+ increased by 50

- count: A numeric sample x cell population node matrix with cell count values
- meta: A data frame containing meta information on samples in count; it contains columns:
  - id: a string containing sample id’s.
  - class: a string indicating whether a sample is from a "control" or "exp" (experiment) subject.

fg_extract_phenotypes  Extracts a set of phenotypes from a flowGraph object.

Description

Extracts or removes a specified set of phenotypes from a flowGraph object.

Usage

fg_extract_phenotypes(fg, phenotypes)

Arguments

fg  flowGraph object.
phenotypes  A string vector of phenotype or cell population name labels.

Details

The summary in fg will not be modified; we recommend users recalculate them.

Value

flowGraph object.

See Also

flowGraph-class fg_get_feature_desc fg_merge fg_extract_samples fg_merge_samples
Examples

```r
no_cores <- 1
data(fg_data_pos30)
fg0 <- flowGraph(fg_data_pos30$count, class=fg_data_pos30$meta$class,
    prop=FALSE, specenr=FALSE,
    no_cores=no_cores)
fg_get_feature_desc(fg0)

fg <- fg_extract_phenotypes(fg0, fg_get_graph(fg0)$v$phenotype[1:10])
fg_get_feature_desc(fg)
```

Clears all features and feature summaries in a flowGraph object.

Description

Returns a flowGraph object with only the count feature and meta data. This function clears all other features and feature summaries to save space.

Usage

```r
fg_extract_raw(fg)
```

Arguments

- `fg` flowGraph object.

Value

flowGraph object with all summary statistics and feature values removed except for the node count feature.

See Also

`flowGraph-class`

Examples

```r
no_cores <- 1
data(fg_data_pos30)
fg <- flowGraph(fg_data_pos30$count, class=fg_data_pos30$meta$class,
    no_cores=no_cores)

fg <- fg_extract_raw(fg)
show(fg)
```
fg_extract_samples  Extracts a set of samples from a flowGraph object.

Description

Extracts or removes a specified set of samples from a flowGraph object.

Usage

fg_extract_samples(fg, sample_ids, rm_summary = TRUE)

Arguments

fg  flowGraph object.

sample_ids  A string vector of sample id's that the user wants to keep in fg.

rm_summary  A logical indicating whether or not to clear summary.

Details

The summaries in fg will not be modified; we recommend the user recalculates them.

Value

flowGraph object.

See Also

flowGraph-class  fg_get_feature_desc  fg_merge  fg_extract_phenotypes

Examples

no_cores <- 1
data(fg_data_pos30)
fg0 <- flowGraph(fg_data_pos30$count, class=fg_data_pos30$meta$class,
prop=FALSE, specenr=FALSE,
no_cores=no_cores)
fg_get_feature_desc(fg0)

fg <- fg_extract_samples(fg0, fg_get_meta(fg0)$id[1:5])
fg_get_feature_desc(fg)
fg_feat_cumsum

Converts cell counts into cumulated cell counts.

Description

Converts the cell counts in a flowGraph object into cumulated cell counts; this is optional and can be done only for there is more than one threshold for one or more markers. This should also only be ran when initializing a flowGraph object as converting back and forth is computationally expensive. If the user is interested in seeing non- and cumulated counts, we recommend keeping two flowGraph objects, one for each version. This function simply converts e.g. the count of A+ or A++ into the sum of count of A+, A++, and A+++ or A++, and A++.

Usage

fg_feat_cumsum(fg, no_cores)

Arguments

fg flowGraph object.
no_cores An integer indicating how many cores to parallelize on.

Details

fg_feat_cumsum returns the given flowGraph object with an adjusted count feature. As in our example,

Value

flowGraph object with cumulated counts.

See Also

flowGraph-class Matrix

Examples

no_cores <- 1
data(fg_data_pos30)
fg <- flowGraph(fg_data_pos30$count, class=fg_data_pos30$meta$class,
    prop=FALSE, specenr=FALSE,
    no_cores=no_cores)

fg <- flowGraph:::fg_feat_cumsum(fg, no_cores=no_cores)
fg_feat_edge_prop

Generates the proportion edge feature.

Description
Generates the proportion edge feature and returns it inside the flowGraph object.

Usage
fg_feat_edge_prop(fg, no_cores = 1, overwrite = FALSE)

Arguments
- **fg**: flowGraph object.
- **no_cores**: An integer indicating how many cores to parallelize on.
- **overwrite**: A logical variable indicating whether to overwrite the existing proportion edge feature if it exists.

Details
Given a flowGraph object, `fg_feat_edge_prop` returns the same flowGraph object with an additional proportions prop edge feature and its meta data. The proportions feature is made using the node count feature and is the cell count of each cell population (e.g. A+B+) over the cell count of its parent (e.g. A+); each edge then corresponds with such a relationship. The edge feature matrix has column names `<from>_<to>` e.g. A+_A+B+.

Value
flowGraph object containing the proportion edge feature.

See Also
- `flowGraph-class`
- `fg_feat_node_prop`
- `fg_feat_node_specenr`
- `fg_add_feature`
- `fg_get_feature`
- `fg_rm_feature`
- `fg_get_feature_desc`

Examples
```r
no_cores <- 1
data(fg_data_pos30)
fg <- flowGraph(fg_data_pos30$count, class=fg_data_pos30$meta$class,
prop=FALSE, specenr=FALSE,
no_cores=no_cores)

fg <- fg_feat_edge_prop(fg)
```
fg_feat_edge_specenr

Generates the SpecEnr edge feature.

Description

Generates the SpecEnr edge feature and returns it inside the flowGraph object.

Usage

fg_feat_edge_specenr(fg, no_cores = 1, overwrite = FALSE)

Arguments

- **fg**: flowGraph object.
- **no_cores**: An integer indicating how many cores to parallelize on.
- **overwrite**: A logical variable indicating whether to overwrite the existing proportion edge feature if it exists.

Details

Given a flowGraph object, `fg_feat_edge_specenr` returns the same flowGraph object with an additional SpecEnr and expected proportions expect_prop edge feature and its meta data. The expected proportions edge feature is calculated by taking the ratio of the child nodes’ (e.g. A+B+) expected proportion value over its parent nodes’ (e.g. A+) actual proportion value. The SpecEnr feature is the actual over expected proportion ratio, logged. The edge feature matrix has column names `<from>_<to>` e.g. A+_A+B+.

Value

flowGraph object containing the proportion edge feature.

See Also

- `flowGraph-class`
- `fg_feat_node_prop`
- `fg_feat_node_specenr`
- `fg_add_feature`
- `fg_get_feature`
- `fg_rm_feature`
- `fg_get_feature_desc`

Examples

```r
no_cores <- 1
data(fg_data_pos30)
fg <- flowGraph(fg_data_pos30$count, class=fg_data_pos30$meta$class, 
               prop=FALSE, specenr=FALSE, 
               no_cores=no_cores)

fg <- fg_feat_edge_specenr(fg)
```
**Description**

For each class label in column `class` of `meta`, `fg_feat_mean_class` takes the column mean of the rows in the given feature matrices (as specified in `node_features` and `edge_features`) associated with that class; it then takes the difference point by point between these means and the original rows for that class.

**Usage**

```r
fg_feat_mean_class(
  fg,
  class,
  no_cores = 1,
  node_features = NULL,
  edge_features = NULL
)
```

**Arguments**

- **fg**
  a column name in `fg_get_meta(fg)` indicating the meta data that should be used as the class label of each sample while conducting normalization.

- **class**
  An integer indicating how many cores to parallelize on.

- **node_features**
  A string vector indicating the node features to perform normalization on; set as `NULL` to normalize all.

- **edge_features**
  A string vector indicating the edge features to perform normalization on; set as `NULL` to normalize all.

**Details**

For all features in the given `flowGraph` object and for each class label in column `class` of `meta`, `fg_feat_mean_class`. It takes the column mean of the rows in the given feature matrices (as specified in `node_features` and `edge_features`) associated with that class; it then takes the difference point by point between these means and the original rows for that class. `fg_feat_mean_class`

**Value**

A numeric matrix whose dimensions equate to that of the input and whose values are normalized per class.

flowGraph object with normalized features.
Generates the proportion node feature.

Description
Generates the proportion node feature and returns it inside the returned flowGraph object.

Usage
fg_feat_node_prop(fg, overwrite = FALSE)

Arguments
fg          flowGraph object.
overwrite   A logical variable indicating whether to overwrite the existing proportion node
            feature if it exists.

Details
Given a flowGraph object, fg_feat_node_prop returns the same flowGraph object, inside of which
is an additional proportions prop node feature and its meta data. The proportions feature is made
using the node count feature and is the cell count of each cell population over the total cell count.

Value
flowGraph object containing the proportion node feature.

See Also
flowGraph-class fg_feat_node_specenr fg_add_feature fg_get_feature fg_rm_feature
fg_get_feature_desc
fg_feat_node_specenr

Examples

```r
no_cores <- 1
data(fg_data_pos30)
fg <- flowGraph(fg_data_pos30$count, class=fg_data_pos30$meta$class,
    prop=FALSE, specenr=FALSE,
    no_cores=no_cores)

fg <- fg_feat_node_prop(fg)
```

fg_feat_node_specenr  Generates the SpecEnr node feature.

Description

Generates the SpecEnr node feature and returns it inside the returned flowGraph object.

Usage

```r
fg_feat_node_specenr(fg, no_cores = 1, feature = "prop", overwrite = FALSE)
```

Arguments

- **fg**: flowGraph object
- **no_cores**: An integer indicating how many cores to parallelize on.
- **feature**: A string indicating feature name; this is the feature SpecEnr will be calculated on.
- **overwrite**: A logical variable indicating whether to overwrite the existing SpecEnr node feature if it exists.

Details

Given a flowGraph object, `fg_feat_node_specenr` returns the same flowGraph object with an additional SpecEnr and expect_prop node feature and its meta data. The expected proportions feature is made using the prop node and edge features; therefore, the returned flowGraph will also contain these two features. For details on how these feature is calculated.

Value

flowGraph object containing the SpecEnr node feature.

References

fg_get_feature

Retrieves a feature matrix.

Description

Retrieves a feature matrix from a given flowGraph object, the feature type, and feature name.

Usage

fg_get_feature(fg, type = "node", feature = "count")

Arguments

fg  
flowGraph object.

type  
A string indicating feature type ‘node’ or ‘edge’.

feature  
A string indicating feature name;

Details

Returns NULL if the requested feature does not exist.

Value

A numeric matrix of the specified feature values.

See Also

flowGraph-class fg_get_feature_desc fg_add_feature fg_rm_feature fg_get_summary
fg_get_feature_desc

Retrieves and/or recalculates a feature description table.

Description

Retrieves and/or recalculates a feature description table for a given flowGraph object.

Usage

fg_get_feature_desc(fg, re_calc = FALSE)

Arguments

fg  
flowGraph object.

re_calc  
A logical variable specifying whether or not a feature summary should be recalculated or directly retrieved from fg.

Value

A data frame where each row contains information on a feature from the given flowGraph object; its columns is as in the feat_desc slot of flowGraph-class.

See Also

flowGraph-class fg_get_feature fg_add_feature fg_rm_feature fg_get_summary_desc

Examples

```r
no_cores <- 1
data(fg_data_pos30)
fg <- flowGraph(fg_data_pos30$count, class=fg_data_pos30$meta$class,
    prop=FALSE, specenr=FALSE, 
    no_cores=no_cores)

feature_matrix <- fg_get_feature(fg, type='node', feature='count')

fg_get_feature_desc(fg, re_calc=TRUE)
```
fg_get_feature_means

Retrieves feature summaries.

Description

Retrieves a feature summary (e.g. colMeans) for samples specified by sample id’s id OR class label label for class class given a feature specified by type and feat.

Usage

fg_get_feature_means(
  fg,
  type = c("node", "edge"),
  feature = "count",
  class = NULL,
  label = NULL,
  id = NULL,
  summary_fun = colMeans
)

Arguments

fg      flowGraph object.
type    A string indicating feature type the summary was created for 'node' or 'edge'.
feature A string indicating feature name the summary was created for;
class   A string corresponding to a column name of the meta slot of fg whose values represent the class label of each sample on which the summary was created to compare or analyze;
label   A string indicating a class label.
id      A string vector containing the sample id’s corresponding to the id column of the meta slot of fg.
summary_fun A function that takes in a matrix and outputs a vector the same length as the number of columns this matrix has.

Value

A list containing two numeric vectors calculated using the summary_fun function on the subset of samples specified by sample id’s id OR class label label for class class from a feature matrix specified by type and feat.

See Also

flowGraph-class fg_get_summary_desc fg_add_summary fg_rm_summary fg_get_summary
Examples

```r
no_cores <- 1
data(fg_data_pos30)
fg <- flowGraph(fg_data_pos30$count, class=fg_data_pos30$meta$class, no_cores=no_cores)
fg <- fg_summary(fg, no_cores=no_cores, class="class", label1="control", overwrite=FALSE, test_name="t", diminish=FALSE)
show(fg)
feat_mean <- fg_get_feature_means(fg, type="node", feature="count", class="class", label="control")
```

---

fg_get_graph

*Retrieves a graph list from a given flowGraph object.*

**Description**

Retrieves a graph list from a given flowGraph object.

**Usage**

`fg_get_graph(fg)`

**Arguments**

fg

- flowGraph object.

**Value**

A list containing two data frames (`v` and `e`) from the `graph` slot of the given flowGraph object containing information on the cell populations phenotype nodes and edges representing relation between cell populations.

**See Also**

`flowGraph-class`  `fg_plot`  `ggdf`  `plot_gr`

**Examples**

```r
no_cores <- 1
data(fg_data_pos30)
fg <- flowGraph(fg_data_pos30$count, class=fg_data_pos30$meta$class, prop=FALSE, specenr=FALSE, no_cores=no_cores)
gr <- fg_get_graph(fg)
head(gr$v)
head(gr$e)
```
fg_get_markers

Retrieves the markers from a given flowGraph object.

Description

Retrieves the markers from a given flowGraph object.

Usage

fg_get_markers(fg)

Arguments

fg          flowGraph object.

Value

A character vector containing the markers used in a flowGraph object.

See Also

flowGraph-class

Examples

no_cores <- 1
data(fg_data_pos30)
fg <- flowGraph(fg_data_pos30$count, class=fg_data_pos30$meta$class,
                    prop=FALSE, specenr=FALSE,
                    no_cores=no_cores)
fg_get_markers(fg)

fg_get_meta

Retrieves sample meta.

Description

Retrieves sample meta from a given flowGraph object.

Usage

fg_get_meta(fg)

Arguments

fg          flowGraph object.
fg_get_summary

Retrieves a summary statistic.

Description

Retrieves a summary statistic from a given flowGraph object; while fg is required, the user can choose to input parameters summary_meta, index, or all of type, feat, test_name, class, label1, and label2. See fg_get_summary_desc for details.

Usage

```
fg_get_summary(
  fg,
  type = "node",
  index = NULL,
  summary_meta = NULL,
  adjust_custom = "byLayer",
  SpecEnr_filt = TRUE,
  summary_fun = colMeans,
  adjust0_lim = c(-0.1, 0.1),
  filter_adjust0 = 1,
  filter_es = 0,
  filter_btwn_tpthres = 0.05,
  filter_btwn_es = 0.5,
  default_p_thres = 1
)
```
Arguments

fg  
flowGraph object.

type  
A string indicating feature type the summary was created for 'node' or 'edge'.

index  
The user must provide type and additionally, one of summary_meta or index.  
index is an integer indicating the row in \texttt{fg\_get\_summary\_desc(<flowGraph>)}  
of the corresponding type and summary the user would like to retrieve.

summary_meta  
The user must provide type and additionally, one of summary_meta or index.  
summary_meta is a list containing feat (feature name), test_name (summary  
statistic name), class (class), label1, and label2 (class labels compared). See  
\texttt{fg\_get\_summary\_desc} for details.

adjust_custom  
A function or a string indicating the test adjustment method to use. If a string is  
provided, it should be one of \texttt{c("holm", "hochberg", "hommel", "bonferroni",  
"BH", "BY", "fdr", "none")} (see \texttt{p.adjust.methods}). If a function is pro-  
vided, it should take as input a numeric vector and output the same vector ad-  
justed.

SpecEnr_filt  
A logical indicating whether or not to filter p-values for SpecEnr.

summary_fun  
A function that takes in a matrix and outputs a vector the same length as the  
number of columns this matrix has. Set to \texttt{NULL} to not calculate this summary  
(i.e. returned list will not contain m1 and m2). See \texttt{fg\_get\_feature\_means}.

adjust0_lim  
A vector of two numeric values indicating a range around 0, default set to \(-0.1\)  
and \(0.1\).

filter_adjust0  
A numeric variable indicating what percentage of SpecEnr values compared  
(minimum) should be not close to 0. Set to 1 to not conduct filtering. Original  
p-values stored in \texttt{values\_original}.

filter_es  
A numeric variable between 0 and 1 indicating what the Cohen’s D value of the  
nodes/edges in question must be greater or equal to, to be significant.

filter_btwn_tpthres  
A numeric variable between 0 and 1 indicating the unadjusted T-test p-value  
threshold used to test whether the actual and expected feature values used to  
calculate the specified SpecEnr feature are significantly different for each sam-  
ple class. Note this only needs to be specified for SpecEnr features. Combined  
with \texttt{filter\_btwn\_es}, we conduct three tests to understand if there is an actual  
large difference between actual and expected features: (1,2) T-test of signifi-  
cance between the actual and expected raw feature value (e.g. proportion) for  
samples in each of the compared classes, (3) and the T-test of significance be-  
tween the differences of actual and expected feature values of the two classes.  
If any two of the three tests come out as insignificant, we set the p-value for the  
associated node/edge to 1.

filter_btwn_es  
A numeric variable between 0 and 1 indicating what the Cohen’s D value of  
the nodes/edges in question must be greater or equal to, to be significant – see  
\texttt{filter\_btwn\_tpthres}.

default_p_thres  
A numeric variable indicating the p-value threshold user is using. Currently,  
all nodes/edges not passing the filter criterion will be defaulted to 1; if this  
parameter is set, then all of these nodes/edges will be set to a minimum of  
\texttt{default\_p\_thres}.  


Value

A list containing elements on feature summary retrieved by the user as in the summary slot of
flowGraph-class. If summary_fun is not NULL, this list also includes:

- **m1**: a numeric vector the same length as values; this is a summary of the samples compared
e.g. mean.
- **m2**: a numeric vector the same length as values; this is a summary of the samples compared
e.g. mean.
- **cohensd**: a numeric vector indicating cohen’s d values considering effect size.
- **cohensd_size**: a factor vector interpreting cohen’s d values.
- **adjust0**: a numeric vector indicating the percentage of samples that have a SpecEnr value in
  the range of adjust0_lim around 0; if there are two classes of samples being compared, we
  output the smaller percentage between the two classes.
- **btwn**: a data frame containing columns:
  - **tpv1**: unadjusted p-value calculated between the actual and expected raw feature values
    of class 1.
  - **tpv2**: unadjusted p-value calculated between the actual and expected raw feature values
    of class 2.
  - **cd1**: Cohen’s D between the actual and expected raw feature values of class 1.
  - **cd2**: Cohen’s D between the actual and expected raw feature values of class 2.
  - **btp**: unadjusted p-value calculated between the difference between actual and expected
    raw feature of the two classes.
  - **bcd**: Cohen’s D calculated between the difference between actual and expected raw feature
    of the two classes.
  - **btp_**: unadjusted p-value calculated between the log ratio between actual and expected
    raw feature of the two classes.
  - **bcd_**: Cohen’s D calculated between the log ratio between actual and expected raw feature
    of the two classes.

See Also

flowGraph-class fg_get_feature_means fg_get_summary_desc fg_add_summary fg_rm_summary
fg_get_feature

Examples

```r
no_cores <- 1
data(fg_data_pos30)
fg <- flowGraph(fg_data_pos30$count, class=fg_data_pos30$meta$class,
    no_cores=no_cores)

# set features to NULL to apply summary statistic to all features.
fg <- fg_summary(fg, no_cores=no_cores, class="class", label="control",
    overwrite=FALSE, test_name="t", diminish=FALSE,
    node_features=NULL, edge_features=NULL)
show(fg)
```

fg_get_summary_desc

feat_summ <- fg_get_summary(fg, type="node", summary_meta=list(  feature="SpecEnr", test_name="t", class="class",  label1="control", label2="exp"))

fg_get_summary_desc
Retrieves a feature summary description table.

Description
Retrieves a feature summary description table for a given flowGraph object.

Usage
fg_get_summary_desc(fg)

Arguments
fg  flowGraph object.

Value
A data frame where each row contains information on a feature summary from fg:

- type: feature type (i.e. 'node' or 'edge').
- feat: feature name.
- test_name: summary name.
- class: class or the column name of fg_get_meta(fg) whose values represent the class label of each sample on which the summary was created for.
- label1: A string from the class column of the meta slot indicating the label of samples compared.
- label2: A string from the class column of the meta slot indicating the label of samples compared.

See Also
flowGraph-class fg_get_summary fg_add_summary fg_rm_summary fg_get_feature_desc

Examples
no_cores <- 1
data(fg_data_pos30)
f <- flowGraph(fg_data_pos30$count, class=fg_data_pos30$meta$class,
  no_cores=no_cores)

fg_get_summary_desc(fg)
**fg_get_summary_index**  
Retrieves the index of the requested summary.

---

**Description**

Retrieves the index of the requested summary from a given flowGraph object.

**Usage**

```r
fg_get_summary_index(fg, type = "node", index = NULL, summary_meta = NULL)
```

**Arguments**

- `fg`: flowGraph object.
- `type`: A string indicating feature type the summary was created for 'node' or 'edge'.
- `index`: The user must provide type and additionally, one of summary_meta or index. index is an integer indicating the row in `fg_get_summary_desc(<flowGraph>)` of the corresponding type and summary the user would like to retrieve.
- `summary_meta`: The user must provide type and additionally, one of summary_meta or index. summary_meta is a list containing type (feature type: node or edge), feature (feature name), test_name (summary statistic name), class (class), label1, and label2 (class labels compared). See `fg_get_summary_desc` for details.

**Value**

An integer analogous to index. If both index and summary_meta are NULL, returns 1.

**See Also**

`flowGraph-class` `fg_get_summary_desc` `fg_add_summary` `fg_rm_summary` `fg_plot`

**Examples**

```r
do_cores <- 1
data(fg_data_pos30)
fg <- flowGraph(fg_data_pos30$count, class=fg_data_pos30$meta$class,
do_cores=do_cores)

# set features to NULL to apply summary statistic to all features.
fg <- fg_summary(fg, no_cores=do_cores, class="class", label1="control",
overwrite=FALSE, test_name="t", diminish=FALSE,
node_features=NULL, edge_features=NULL)
show(fg)

index <- flowGraph:::fg_get_summary_index(
f, type="node", summary_meta=list(
  feature="SpecEnr", test_name="t", class="class",
  label1="control", label2="exp"))
```
fg_get_summary_tables  
Retrieves a table containing all node or edge summary statistics.

Description
Retrieves a table containing all node or edge summary statistics given a flowGraph object.

Usage
fg_get_summary_tables(fg, type = "node")

Arguments
fg  
flowGraph object.

type  
A string indicating feature type the summaries the user wants to retrieve were created for, 'node' or 'edge'.

Value
A list; this output is the same as that of function fg_get_graph with additional columns. These columns contain summary statistics from the summary slot of the flowGraph object. These columns are named: <feature type: node/edge>.<feature>.<summary name>.<class>.<class labels>.

See Also
flowGraph-class fg_get_feature_means fg_get_summary_desc fg_add_summary fg_rm_summary fg_get_summary fg_get_feature

Examples
no_cores <- 1
data(fg_data_pos30)
fg <- flowGraph(fg_data_pos30$count, class=fg_data_pos30$meta$class,
no_cores=no_cores)

fg <- fg_summary(fg, no_cores=no_cores, class="class", label1="control",
overwrite=FALSE, test_name="t", diminish=FALSE)
show(fg)

feat_summ_table_node <- fg_get_summary_tables(fg, type="node")
head(feat_summ_table_node)
fg_gsub_ids

Replace sample id's.

Description

Replace sample id's in a flowGraph object.

Usage

fg_gsub_ids(fg, ids_new, ids_old = NULL)

Arguments

fg
flowGraph object.

ids_new
A string vector of new sample id's; if ids_old is set to NULL, each id in ids_new should correspond to each id in fg_get_meta(fg)$id.

ids_old
A string vector of old sample id's the user wants to replace; these marker names corresponding to those in fg_get_meta(fg)$id with the same length as ids_new. If ids_old=NULL, ids_new should be the same length as fg_get_meta(fg)$id.

Value

flowGraph object with sample id's replaced.

See Also

flowGraph-class fg_get_feature_desc fg_gsub_markers

Examples
	no_cores <- 1
data(fg_data_pos30)
fg <- flowGraph(fg_data_pos30$count, class=fg_data_pos30$meta$class, prop=FALSE, specenr=FALSE, no_cores=no_cores)

fg <- fg_gsub_ids(fg, ids_new=paste0(fg_get_meta(fg)$id, "_new"))
fg_gsub_markers

Replace marker names.

Description

Replace marker names in a flowGraph object.

Usage

fg_gsub_markers(fg, markers_new, markers_old = NULL)

Arguments

- **fg**: flowGraph object.
- **markers_new**: A string vector of new marker names; if **markers_old** is set to NULL, each marker in **markers_new** should correspond to each marker in the markers slot of the flowGraph object.
- **markers_old**: A string vector of old marker names user wants to replace; these marker names corresponding to those in **fg_get_markers(fg)** with the same length as **markers_new**.
  If **markers_old=NULL**, **markers_new** should be the same length as **fg_get_markers(fg)**.

Value

flowGraph object with marker names replaced.

See Also

- `flowGraph-class`
- `fg_gsub_ids`

Examples

```r
no_cores <- 1
data(fg_data_pos30)
fg <- flowGraph(fg_data_pos30$count, class=fg_data_pos30$meta$class,
                prop=FALSE, specenr=FALSE,
                no_cores=no_cores)

fg <- fg_gsub_markers(fg, c("Anew", "Bnew", "Cnew", "Dnew"))
fg_get_feature_desc(fg)
```
fg_load

Load a flowGraph object from a specified folder path.

Description

Load a flowGraph object from a specified folder path.

Usage

fg_load(folder_path)

Arguments

folder_path A string indicating the folder path to where a flowGraph object was saved using the fg_save function.

Details

see function fg_save

Value

flowGraph object

See Also

fg_save

Examples

no_cores <- 1
data(fg_data_pos2)
fg <- flowGraph(fg_data_pos2$count, class=fg_data_pos2$meta$class, no_cores=no_cores)

fg_save(fg, "tmp")
fg <- fg_load("tmp")
**fg_merge**

Merges two flowGraph objects together.

### Description

Merges two flowGraph objects together.

### Usage

```r
fg_merge(
  fg1,
  fg2,
  method_sample = c("union", "intersect", "setdiff", "none"),
  method_phenotype = c("intersect", "setdiff", "none")
)
```

### Arguments

- `fg1`: flowGraph object.
- `fg2`: flowGraph object.
- `method_sample`: A string indicating how samples from flowGraph objects should be merged:
  - `union`: keep all samples from both flowGraph objects; in this case `method_phenotype` must be `intersect`.
  - `intersect`: keep only samples that exist in both `fg1` and `fg2`.
  - `setdiff`: keep only samples that exist in `fg1` and not in `fg2`.
  - `none`: keep all samples in `fg1`.
- `method_phenotype`: A string indicating how phenotypes from flowGraph objects should be merged:
  - `intersect`: keep only phenotypes that exist in both `fg1` and `fg2`.
  - `setdiff`: keep only phenotypes that exist in `fg1` and not in `fg2`.

### Details

`fg_merge` is a generic function that merges the samples and phenotypes of two flowGraph objects. Note that if `method_sample="union"` then `method_phenotype` must be set to "intersect".

### Value

flowGraph object.

### See Also

`flowGraph-class`, `fg_extract_samples`, `fg_extract_phenotypes`, `fg_merge_samples`
Examples

```r
no_cores <- 1
data(fg_data_pos30)
fg0 <- flowGraph(fg_data_pos30$count, class=fg_data_pos30$meta$class,
                prop=FALSE, specenr=FALSE,
                no_cores=no_cores)

fg1 <- fg_extract_samples(fg0, fg_get_meta(fg0)$id[1:5])
fg2 <- fg_extract_samples(fg0, fg_get_meta(fg0)$id[4:7])
fg <- fg_merge(fg1, fg2, method_sample="intersect",
               method_phenotype="intersect")
fg_get_feature_desc(fg)
```

---

fg_merge_samples

*Merges the samples from two flowGraph objects.*

Description

Merges the samples from two flowGraph objects together; we recommend removing all summary statistics from the new flowGraph object as those won’t be adjusted: `fg_clear_summary`.

Usage

`fg_merge_samples(fg1, fg2)`

Arguments

- `fg1` : flowGraph object.
- `fg2` : flowGraph object.

Details

Appends the samples from `fg2` onto those in `fg1`. This function requires that the two flowGraph objects must have the same phenotypes. Therefore, we recommend users to use, instead, `fg_merge`.

Value

flowGraph object.

See Also

`flowGraph-class fg_get_feature_desc fg_merge fg_extract_samples`
Examples

```r
no_cores <- 1
data(fg_data_pos30)
fg0 <- flowGraph(fg_data_pos30$count, class=fg_data_pos30$meta$class,
                 prop=FALSE, specenr=FALSE,
                 no_cores=no_cores)

fg1 <- fg_extract_samples(fg0, fg_get_meta(fg0)$id[1:5])
fg2 <- fg_extract_samples(fg0, fg_get_meta(fg0)$id[4:7])
fg <- fg_merge_samples(fg1, fg2)
fg_get_feature_desc(fg)
```

fg_plot

*Creates a cell hierarchy plot.*

Description

Creates a cell hierarchy plot given a flowGraph object. If a path is not provided for `fg_plot` to save the plot, please use `plot_gr` to view plot given the output of `fg_plot`.

Usage

```r
fg_plot(
  fg,
  type = "node",
  index = 1,
  summary_meta = NULL,
  adjust_custom = "byLayer",
  show_nodes_edges = NULL,
  label_max = 30,
  p_thres = 0.05,
  filter_adjust0 = 1,
  filter_es = 0,
  filter_btwn_tpthres = 1,
  filter_btwn_es = 0,
  node_labels = c("prop", "expect_prop"),
  summary_fun = colMeans,
  layout_fun = NULL,
  show_bgedges = TRUE,
  main = NULL,
  interactive = FALSE,
  visNet_plot = TRUE,
  path = NULL,
  width = 9,
  height = 9
)
```
Arguments

**fg**

flowGraph object.

**type**

A string indicating feature type the summary was created for 'node' or 'edge'.

**index**

The user must provide type and additionally, one of summary_meta or index. index is an integer indicating the row in `fg_get_summary_desc(<flowGraph>)` of the corresponding type and summary the user would like to retrieve.

**summary_meta**

The user must provide type and additionally, one of summary_meta or index. summary_meta is a list containing feature (feature name), test_name (summary statistic name), class (class), label1, and label2 (class labels compared). See `fg_get_summary_desc` for details.

**adjust_custom**

A function or a string indicating the test adjustment method to use. If a string is provided, it should be one of c("holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none") (see `p.adjust.methods`). If a function is provided, it should take as input a numeric vector and output the same vector adjusted.

**show_nodes_edges**

A logical vector indicating which nodes/edges (type) to show in the plot; if this is not specified, only nodes/edges with significant summary statistics will be shown.

**label_max**

An integer specifying the maximum number of nodes to label.

**p_thres**

A double indicating a summary statistic threshold e.g. if we are plotting a T test summary statistic, we can set the threshold to .05; nodes with a p-value greater than .05 will not be plotted.

**filter_adjust0**

A numeric variable indicating what percentage of SpecEnr values compared (minimum) should be not close to 0. Set to 1 to not conduct filtering.

**filter_es**

A numeric variable between 0 and 1 indicating what the Cohen's D value of the nodes/edges in question must be greater or equal to, to be significant.

**filter_btwn_tpthres**

A numeric variable between 0 and 1 indicating the unadjusted T-test p-value threshold used to test whether the actual and expected feature values used to calculate the specified SpecEnr feature are significantly different for each sample class. Note this only needs to be specified for SpecEnr features. Combined with `filter_btwn_es`, we conduct three tests to understand if there is an actual large difference between actual and expected features: (1,2) T-test of significance between the actual and expected raw feature value (e.g. proportion) for samples in each of the compared classes, (3) and the T-test of significance between the differences of actual and expected feature values of the two classes. If any two of the three tests come out as insignificant, we set the p-value for the associated node/edge to 1.

**filter_btwn_es**

A numeric variable between 0 and 1 indicating what the Cohen's D value of the nodes/edges in question must be greater or equal to, to be significant – see `filter_btwn_tpthres`.

**node_labels**

A string vector indicating which node feature(s) should be used to label a node. We recommend keeping the length of this vector to below 2. Set to "NONE" if no p-value labels are needed.
summary_fun  A function that takes in a matrix and outputs a vector the same length as the number of columns this matrix has; see `fg_summary`.

layout_fun  A string representing a function from the igraph package that indicates what layout should be used if a cell hierarchy is to be plotted; all such functions have prefix `layout_`. Only specify if different from the default one already calculated in the fg flowGraph object given.

show_bgedges  A logical variable indicating whether or not edges not specified for plotting should be plotted as light grey in the background.

main  A string or the title of the plot; if left as NULL, a default title will be applied.

interactive  A logical variable indicating whether the plot should be an interactive plot; see package ggiraph.

visNet_plot  A logical variable indicating if an interactive plot is chosen, if function should output a visNetwork plot; if set to FALSE, ggplot’s girafe will be used instead.

path  A string indicating the path to where the function should save the plot; leave as NULL to not save the plot. Static plots are saved as PNG, interactive plots are saved as HTML.

width  A numeric variable specifying, in inches, what the plot width should be.

height  A numeric variable specifying, in inches, what the plot height should be.

Details

fg_plot takes a flowGraph object as input and returns the graph slot of the given object with additional columns to serve as input into `plot_gr` for plotting using functions in the ggplot2 package. Users can choose to save a PNG version of the plot by filling out the path parameter with a full path to the PNG plot. In addition to specifying columns added from ggdf, fg_plot also adds label column(s) whose values serve as labels in the interactive version of the plot.

Value

A list of nodes and edges for plotting with the `plot_gr` function. Other elements in this list include show_bgedges, which has the same value as parameter show_bgedges, and main, the title of the plot.

See Also

flowGraph-class get_phen_meta ggdf plot_gr fg_get_feature fg_get_summary

Examples

```r
no_cores <- 1
data(fg_data_pos2)
fg <- flowGraph(fg_data_pos2$count, class=fg_data_pos2$meta$class,
               no_cores=no_cores)

gr <- fg_plot(fg, type="node", index=1, label_max=30,
               show_nodes_edges=NULL, p_thres=.01, node_labels=c("prop", "expect_prop"),
               path=NULL) # set path to a full path to save plot as a PNG
# plot_gr(gr)
```
fg_plot_box

Description

Creates a boxplot of the values of one node/edge

Usage

fg_plot_box(
  fg,
  type = "node",
  index = 1,
  summary_meta = NULL,
  node_edge = 1,
  adjust_custom = "byLayer",
  p_thres = 0.05,
  filter_adjust0 = 0.5,
  filter_es = 0.5,
  filter_btwn_tpthres = 0.05,
  filter_btwn_es = 0.5,
  paired = FALSE,
  dotplot = TRUE,
  outlier = TRUE,
  all_labels = FALSE,
  show_mean = TRUE,
  main = NULL,
  path = NULL
)

Arguments

fg     flowGraph object.
type    A string indicating feature type the summary was created for 'node' or 'edge'.
index   The user must provide type and additionally, one of summary_meta or index. index is an integer indicating the row in fg_get_summary_desc(<flowGraph>) of the corresponding type and summary the user would like to retrieve.
summary_meta    The user must provide type and additionally, one of summary_meta or index. summary_meta is a list containing feature (feature name), test_name (summary statistic name), class (class), label1, and label2 (class labels compared). See fg_get_summary_desc for details.
node_edge    An integer/index of or a string of the cell population (node) / edge name (edge) the user wants to plot.
adjust_custom  A function or a string indicating the test adjustment method to use. If a string is provided, it should be one of c("holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none") (see p.adjust.methods). If a function is provided, it should take as input a numeric vector and output the same vector adjusted.

p_thres  A numeric variable indicating a p-value threshold

filter_adjust0  A numeric variable indicating what percentage of SpecEnr values compared (minimum) should be not close to 0. Set to 1 to not conduct filtering.

filter_es  A numeric variable between 0 and 1 indicating what the Cohen’s D value of the nodes/edges in question must be greater or equal to, to be significant.

filter_btwn_tpthres  A numeric variable between 0 and 1 indicating the unadjusted T-test p-value threshold used to test whether the actual and expected feature values used to calculate the specified SpecEnr feature are significantly different for each sample class. Note this only needs to be specified for SpecEnr features. Combined with filter_btwn_es, we conduct three tests to understand if there is an actual large difference between actual and expected features: (1,2) T-test of significance between the actual and expected raw feature value (e.g. proportion) for samples in each of the compared classes, (3) and the T-test of significance between the differences of actual and expected feature values of the two classes. If any two of the three tests come out as insignificant, we set the p-value for the associated node/edge to 1.

filter_btwn_es  A numeric variable between 0 and 1 indicating what the Cohen’s D value of the nodes/edges in question must be greater or equal to, to be significant – see filter_btwn_tpthres.

paired  A logical indicating whether the summary is paired.

dotplot  A logical indicating whether or not to plot sample points.

outlier  A logical indicating whether or not outliers should be plotted.

all_labels  A logical indicating whether or not to plot samples of all classes outside of just those used in the summary statistic test.

show_mean  A logical indicating whether or not to label the mean.

main  A string or the title of the plot; if left as NULL, a default title will be applied.

path  A string indicating the path to where the function should save the plot; leave as NULL to not save the plot. Static plots are saved as PNG.

Details

The plot is made using the ggplot2 package. The interactive version is the same as the static version, it is only here to support the shiny app.

Value

A static boxplot.

See Also

flowGraph-class fg_plot plot_gr fg_get_feature fg_get_summary fg_plot_qq
Examples

```r
no_cores <- 1
data(fg_data_pos2)
fg <- flowGraph(fg_data_pos2$count, class=fg_data_pos2$meta$class,
               no_cores=no_cores)

fg_plot_box(fg, type="node", summary_meta=NULL, adjust_custom="byLayer", index=1, node_edge=10)
```

---

`fg_plot_pVSdiff`  
Creates a p value vs feature difference plot

Description

Creates a p value vs feature difference plot where the difference is that of the features of samples belonging to different classes corresponding to an existing summary statistic.

Usage

```r
fg_plot_pVSdiff(
  fg,  
  type = "node",  
  index = 1,  
  summary_meta = NULL,  
  adjust_custom = "byLayer",  
  logged = TRUE,  
  label_max = 5,  
  p_thres = 0.05,  
  filter_adjust0 = 1,  
  filter_es = 0,  
  filter_btwn_tpthres = 1,  
  filter_btwn_es = 0,  
  shiny_plot = FALSE,  
  nodes_max = 30,  
  main = NULL,  
  interactive = FALSE,  
  path = NULL
)
```

Arguments

- **fg**: flowGraph object.
- **type**: A string indicating feature type the summary was created for 'node' or 'edge'.
- **index**: The user must provide type and additionally, one of summary_meta or index. Index is an integer indicating the row in `fg_get_summary_desc(<flowGraph>)` of the corresponding type and summary the user would like to retrieve.
The user must provide type and additionally, one of summary_meta or index. summary_meta is a list containing feature (feature name), test_name (summary statistic name), class (class), label1, and label2 (class labels compared). See fg_get_summary_desc for details.

A function or a string indicating the test adjustment method to use. If a string is provided, it should be one of c("holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none") (see p.adjust.methods). If a function is provided, it should take as input a numeric vector and output the same vector adjusted.

A logical indicating whether or not to log the summary statistic p value.

An integer indicating the maximum number of max difference and/or min p value nodes/edges that should be labelled.

A numeric variable indicating a p-value threshold; a line will be plotted at this threshold.

A numeric variable indicating what percentage of SpecEnr values compared (minimum) should be not close to 0. Set to 1 to not conduct filtering.

A numeric variable between 0 and 1 indicating what the Cohen’s D value of the nodes/edges in question must be greater or equal to, to be significant.

A numeric variable between 0 and 1 indicating the unadjusted T-test p-value threshold used to test whether the actual and expected feature values used to calculate the specified SpecEnr feature are significantly different for each sample class. Note this only needs to be specified for SpecEnr features. Combined with filter_tptthres, we conduct three tests to understand if there is an actual large difference between actual and expected features: (1,2) T-test of significance between the actual and expected raw feature value (e.g. proportion) for samples in each of the compared classes, (3) and the T-test of significance between the differences of actual and expected feature values of the two classes. If any two of the three tests come out as insignificant, we set the p-value for the associated node/edge to 1.

A numeric variable between 0 and 1 indicating what the Cohen’s D value of the nodes/edges in question must be greater or equal to, to be significant – see filter_tptthres.

A logical indicating whether this plot is made for shiny; users don’t need to change this.

An integer indicating maximum number of nodes to plot; this limit is set for interactive plots only.

A string or the title of the plot; if left as NULL, a default title will be applied.

A logical variable indicating whether the plot should be an interactive plot; see package ggiraph.

A string indicating the path to where the function should save the plot; leave as NULL to not save the plot. Static plots are saved as PNG.

The interactive plot is made using the ggiraph package.
Value
A static or interactive p value vs difference plot.

See Also
flowGraph-class fg_plot plot_gr fg_get_feature fg_get_summary fg_plot_qq

Examples

```r
no_cores <- 1
data(fg_data_pos2)
fg <- flowGraph(fg_data_pos2$count, class=fg_data_pos2$meta$class,
    no_cores=no_cores)

gp <- fg_plot_pVSdiff(fg, type="node", summary_meta=NULL,
    adjust_custom="byLayer", index=1, label_max=10)
```

---

fg_plot_qq

*Creates a QQ plot of a summary statistic.*

Description

Creates a QQ plot of a summary statistic.

Usage

```r
fg_plot_qq(
    fg, 
    type = "node", 
    index = 1, 
    summary_meta = NULL, 
    adjust_custom = "byLayer", 
    logged = TRUE, 
    p_thres = 0.05, 
    filter_adjust0 = 1, 
    filter_es = 0, 
    filter_btwn_tpthres = 1, 
    filter_btwn_es = 0, 
    shiny_plot = FALSE, 
    main = NULL, 
    interactive = FALSE, 
    path = NULL
)```
Arguments

fg flowGraph object.

**type** A string indicating feature type the summary was created for 'node' or 'edge'.

**index** The user must provide type and additionally, one of summary_meta or index. index is an integer indicating the row in \( \text{fg\_get\_summary\_desc}(<\text{flowGraph}>) \) of the corresponding type and summary the user would like to retrieve.

**summary_meta** The user must provide type and additionally, one of summary_meta or index. summary_meta is a list containing feature (feature name), test_name (summary statistic name), class (class), label1, and label2 (class labels compared). See \( \text{fg\_get\_summary\_desc} \) for details.

**adjjust_custom** A function or a string indicating the test adjustment method to use. If a string is provided, it should be one of \( \text{c("holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none")} \) (see \( \text{p.adjust.methods} \)). If a function is provided, it should take as input a numeric vector and output the same vector adjusted.

**logged** A logical indicating whether or not to log the summary statistic p value.

**p_thres** A double indicating a summary statistic threshold e.g. if we are plotting a T-test summary statistic, we can set the threshold to .05; nodes with a p-value greater than .05 will not be plotted.

**filter_adjust0** A numeric variable indicating what percentage of SpecEnr values compared (minimum) should be not close to 0. Set to 1 to not conduct filtering.

**filter_es** A numeric variable between 0 and 1 indicating what the Cohen’s D value of the nodes/edges in question must be greater or equal to, to be significant.

**filter_btwn_tpthres** A numeric variable between 0 and 1 indicating the unadjusted T-test p-value threshold used to test whether the actual and expected feature values used to calculate the specified SpecEnr feature are significantly different for each sample class. Note this only needs to be specified for SpecEnr features. Combined with filter_es, we conduct three tests to understand if there is an actual large difference between actual and expected features: (1,2) T-test of significance between the actual and expected raw feature value (e.g. proportion) for samples in each of the compared classes, (3) and the T-test of significance between the differences of actual and expected feature values of the two classes. If any two of the three tests come out as insignificant, we set the p-value for the associated node/edge to 1.

**filter_btwn_es** A numeric variable between 0 and 1 indicating what the Cohen’s D value of the nodes/edges in question must be greater or equal to, to be significant – see filter_btwn_tpthres.

**shiny_plot** A logical indicating whether this plot is made for shiny; users don’t need to change this.

**main** A string or the title of the plot; if left as NULL, a default title will be applied.

**interactive** A logical indicating whether or not plot should be an interactive ggiraph plot as opposed to a static plot.
path

A string indicating the path to where the function should save the plot; leave as NULL to not save the plot. Static plots are saved as PNG, interactive plots are saved as HTML.

Details

The interactive plot is made using the ggiraph package.

Value

A static or interactive qq plot.

See Also

flowGraph-class fg_plot plot_gr fg_get_feature fg_get_summary

Examples

no_cores <- 1
data(fg_data_pos2)
fg <- flowGraph(fg_data_pos2$count, class=fg_data_pos2$meta$class,
    no_cores=no_cores)

fg_plot_qq(fg, type="node", summary_meta=NULL, adjust_custom="byLayer", index=1,
    interactive=TRUE, logged=FALSE)

fg_plot_qq(fg, type="node", summary_meta=NULL, adjust_custom="byLayer", index=1,
    interactive=FALSE, logged=FALSE)

fg_replace_meta

Replaces sample meta.

Description

Replaces sample meta in a given flowGraph object.

Usage

fg_replace_meta(fg, meta)

Arguments

fg flowGraph object.
meta A data frame containing meta data; see details in flowGraph-class.

Value

A flowGraph object with an updated sample meta.
\textbf{fg\_rm\_feature}\hfill {}47

\textbf{See Also}

\texttt{flowGraph-class \texttt{fg\_get\_meta}}

\textbf{Examples}

\begin{verbatim}
no_cores <- 1
data(fg_data_pos30)
fg <- flowGraph(fg_data_pos30$\texttt{count}, \texttt{class}=fg_data_pos30$\texttt{meta}$\texttt{class},
              \texttt{prop}=\texttt{FALSE}, \texttt{specenr}=\texttt{FALSE},
              \texttt{no\_cores}=\texttt{no\_cores})
head(fg\_get\_meta(fg))

new_df <- fg_data_pos30$\texttt{meta}
new_df$id[1] <- "newID"

fg <- fg\_replace\_meta(fg, new_df)
head(fg\_get\_meta(fg))
\end{verbatim}

\textbf{fg\_rm\_feature} \hspace{2cm} \textit{Removes a feature.}

\textbf{Description}

Removes a feature from a given flowGraph object.

\textbf{Usage}

\texttt{fg\_rm\_feature(fg, type = \"node\", feature = NULL)}

\textbf{Arguments}

\begin{itemize}
  \item \texttt{fg} \hspace{1cm} flowGraph object.
  \item \texttt{type} \hspace{1cm} A string specifying the type of the feature being removed i.e. \texttt{\textquote{node}} or \texttt{\textquote{edge}}.
  \item \texttt{feature} \hspace{1cm} A string indicating the unique name of the feature removed; note we cannot remove the \texttt{\textquote{node}} \texttt{\textquote{count}} feature type.
\end{itemize}

\textbf{Details}

\texttt{fg\_rm\_feature} removes a specified feature matrix from the given flowGraph object \texttt{fg} updating slots \texttt{feat} and \texttt{feat\_desc}. See \texttt{flowGraph-class} slot \texttt{feat} and \texttt{feat\_desc} for what should be in these slots.

\textbf{Value}

flowGraph object with specified feature removed.
fg_rm_summary

Removes a feature summary.

Description

Removes a feature summary from a given flowGraph object; while fg is required, the user can choose to input parameters summary_meta, index, or all of type, feat, test_name, class, label1, and label2. See `fg_get_summary_desc` for details.

Usage

```
fg_rm_summary(fg, type = "node", index = NULL, summary_meta = NULL)
```

Arguments

- `fg`  
  flowGraph object.

- `type`  
  A string indicating feature type the summary was created for; 'node' or 'edge'.

- `index`  
  The user must provide type and additionally, one of summary_meta or index. index is an integer indicating the row in `fg_get_summary_desc(<flowGraph>)` of the corresponding type and summary the user would like to retrieve.

- `summary_meta`  
  The user must provide type and additionally, one of summary_meta or index. summary_meta is a list containing feat (feature name), test_name (summary statistic name), class (class), label1, and label2 (class labels compared). See `fg_get_summary_desc` for details.

Value

flowGraph object.

See Also

- `flowGraph-class`
- `fg_add_feature`
- `fg_get_feature`
- `fg_get_feature_desc`
- `fg_rm_summary`
Examples

```r
no_cores <- 1
data(fg_data_pos30)
fg <- flowGraph(fg_data_pos30$count, class=fg_data_pos30$meta$class,
               prop=FALSE, specenr=FALSE,
               no_cores=no_cores)

fg <- fg_summary(fg, no_cores=no_cores, class="class", label1="control",
                 overwrite=FALSE, test_name="wilcox_byLayer", diminish=FALSE,
                 node_features=NULL, edge_features=NULL)
fg_get_summary_desc(fg)

fg <- fg_rm_summary(fg, summary_meta=c(
  feature="count", test_name="wilcox_byLayer",
  class="class", label1="control", label2="exp"))
fg_get_summary_desc(fg)
```

fg_save

Saves flowGraph object to a specified path.

Description

Saves flowGraph object to a specified path.

Usage

```r
fg_save(fg, folder_path = NULL, save_plots = TRUE, paired = FALSE, ...)
```

Arguments

- **fg**: flowGraph object to save.
- **folder_path**: A string indicating the folder path to where the flowGraph object should save its elements; if this is the first time the object is being saved, this folder should be empty or if it is not yet created, the function will create it. If the object has previously been saved before and this parameter is set to NULL, the function will save the object into the save folder it was previously saved in.
- **save_plots**: A logical indicating whether or not to save plots.
- **paired**: A logical indicating whether the summary is paired; used in function `fg_plot_box`.
- **...**: Other parameters for the `fg_save_plots` function.

Details

See generated README.md file.

Value

TRUE if flowGraph object successfully saved.
See Also
length.c("nrow", "nrow").NULL

Examples

```r
no_cores <- 1
data(fg_data_pos2)
fg <- flowGraph(fg_data_pos2$count, class=fg_data_pos2$meta$class,
                no_cores=no_cores)

fg_save(fg, "tmp")
```

Description

Saves numerous plots for all summary statistics in a given flowGraph object to a user specified folder.

Usage

```r
fg_save_plots(
  fg,
  plot_path,
  plot_types = "node",
  interactive = FALSE,
  adjust_custom = "byLayer",
  label_max = 10,
  box_no = 20,
  paired = FALSE,
  logged = FALSE,
  filter_adjust0 = 1,
  filter_es = 0,
  filter_btwn_tpthres = 1,
  filter_btwn_es = 0,
  overwrite = TRUE,
  node_labels = "NONE",
  ...
)
```

Arguments

- `fg`: flowGraph object.
- `plot_path`: A string indicating the folder path to where the function should save the plots.
plot_types A string or a vector of strings indicating what feature types and their summaries the function should plot for: 'node' or 'edge'.

interactive A logical indicating whether the QQ plot, p-value vs difference plot, and the cell hierarchy plots should be interactive; see functions fg_plot and fg_plot_qq.

adjust_custom A function or a string indicating the test adjustment method to use. If a string is provided, it should be one of c("holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none") (see p.adjust.methods). If a function is provided, it should take as input a numeric vector and output the same vector adjusted.

label_max An integer indicating how many labels should be shown in the functions fg_plot_pVSdiff and fg_plot.

box_no An integer indicating the maximum number of boxplots to save; used in function fg_plot_box.

paired A logical indicating whether the summary is paired; used in function fg_plot_box.

logged A logical indicating whether or not to log the summary statistic p value in the qq plots.

filter_adjust0 A numeric variable indicating what percentage of SpecEnr values compared (minimum) should be not close to 0. Set to 1 to not conduct filtering. This parameter is used for the QQ and the pVSdifference plots.

filter_es A numeric variable between 0 and 1 indicating what the Cohen’s D value of the nodes/edges in question must be greater or equal to, to be significant.

filter_btwn_tpthres A numeric variable between 0 and 1 indicating the unadjusted T-test p-value threshold used to test whether the actual and expected feature values used to calculate the specified SpecEnr feature are significantly different for each sample class. Note this only needs to be specified for SpecEnr features. Combined with filter_btwn_es, we conduct three tests to understand if there is an actual large difference between actual and expected features: (1,2) T-test of significance between the actual and expected raw feature value (e.g., proportion) for samples in each of the compared classes, (3) and the T-test of significance between the differences of actual and expected feature values of the two classes. If any two of the three tests come out as insignificant, we set the p-value for the associated node/edge to 1.

filter_btwn_es A numeric variable between 0 and 1 indicating what the Cohen’s D value of the nodes/edges in question must be greater or equal to, to be significant – see filter_btwn_tpthres.

overwrite A logical variable indicating whether or not to replace old plots if they exist under the same folder name.

node_labels Parameter for the fg_plot function.

... Other parameters for the fg_plot function.

Details

The interactive plots are made using the ggiraph package.
**fg_set_layout**

Determines cell hierarchy layout.

**Description**

Determines cell hierarchy layout and returns the X, Y coordinate of each cell population. This function is a wrapper for `set_layout_graph`.

**Usage**

```r
fg_set_layout(fg, layout_fun = "layout.reingold.tilford")
```

**Arguments**

- `fg` flowGraph object.
- `layout_fun` A string version of a function name from the igraph package that indicates what layout should be used if a cell hierarchy is to be plotted; all such functions have prefix `layout_` e.g. `layout_fun="layout.reingold.tilford"`.

**Details**

Given a flowGraph object, modifies the graph slot such that it contains X, Y axes for each node in accordance to a user specified layout.

**Value**

flowGraph object with coordinate meta data on cell populations and edges for plotting use.
fg_summary

Examples

```r
no_cores <- 1
data(fg.data_pos30)
fg <- flowGraph(fg.data_pos30$count, class=fg.data_pos30$meta$class,
prop=FALSE, specenr=FALSE,
no_cores=no_cores)

fg <- fg_set_layout(fg)
head(fg.get_graph(fg)$v)
```

fg_summary  Calculates feature summary statistics.

Description

Calculates feature summary statistics for flowGraph features; users can choose from a list of statistical significance tests/adjustments or define custom summary functions. For special cases, see example in function fg_add_summary on how to manually calculate summary statistics without using this function.

Usage

```r
fg_summary(
  fg,
  no_cores = 1,
  class = "class",
  label1 = NULL,
  label2 = NULL,
  class_labels = NULL,
  node_features = "SpecEnr",
  edge_features = "NONE",
  test_name = "t_diminish",
  diminish = TRUE,
  p_thres = 0.05,
  p_rate = 2,
  test_custom = "t",
  effect_size = TRUE,
  adjust0 = TRUE,
  adjust0_lim = c(-0.1, 0.1),
  btwn = TRUE,
  btwn_test_custom = "t",
  save_functions = FALSE,
  overwrite = FALSE
)
```
Arguments

fg  
flowGraph object.

no_cores  
An integer indicating how many cores to parallelize on.

class  
A string corresponding to the column name or index of fg_get_meta(fg) whose values represent the class label of each sample.

label1  
A string from the class column of the meta slot indicating one of the labels compared to create the summary statistic. If you would like to compare all other class labels against one label, set label2 to NULL. If you would like to compare all labels against all labels, set label1 and label2 to NULL.

label2  
A string from the class column of the meta slot indicating one of the labels compared to create the summary statistic.

class_labels  
A list of vectors, each containing two strings representing labels to compare; this parameter is an alternative to parameters label1 and label2 that supports multiple label pairings.

node_features  
A string vector indicating which node feature(s) to perform summary statistics on; set to NULL or "NONE" and the function will perform summary statistics on all or no node features.

edge_features  
A string vector indicating which edge feature(s) to perform summary statistics on; set to NULL or "NONE" and the function will perform summary statistics on all or no edge features.

test_name  
A string with the name of the test you are performing.

diminish  
A logical variable indicating whether to use diminishing summary statistics; if TRUE, a summary statistic for a node or edge will only be done if at least one of its parent node or edge is significant. Otherwise, the test will be performed on all nodes or edges.

p_thres  
A double indicating the summary statistic threshold; if the result of a statistical test is greater than p_thres, then it is insignificant.

p_rate  
A double; if diminish=TRUE, then p_rate needs to be specified. To determine whether or not a node or edge’s parent is significant, we use p_thres. However, the higher the layer on which a node resides or to which an edge points to, the less stringent this p_thres should be. Therefore, we set p_thres as the threshold for the parent node or edge of the last layer and multiply p_thres by p_rate for each increasing layer e.g. given default values and 4 layers, the thresholds for layers 1 through 4 would be .4, .2, .1, and .05.

test_custom  
A function or a string indicating the statistical test to use. If a string is provided, it should be one of c("t","wilcox","ks","var","chisq"); these correspond to statistical tests stats::t.test, stats::wilcox.test, and so on. If a function is provided, it should take as input two numeric vectors and output a numeric variable.

effect_size  
A logical variable indicating whether or not to calculate effect size statistic (cohen’s d) for this set of class labels; later used for plotting.

adjust0  
A logical variable indicating whether or not to calculate the minimum percentage of values from samples of each class label that falls within the range of adjust0_lim. This is only done for SpecEnr values as p-values become unstable when comparing near 0 values.
adjust0_lim A vector of two numeric values indicating a range around 0, default set to -0.1 and 0.1.

btwn A logical variable indicating whether or not to calculate the btwn data frame given in the fg_get_summary function.

btwn_test_custom Same as test_custom but for btwn.

save_functions A logical variable indicating whether to save test and adjust functions.

overwrite A logical variable indicating whether to overwrite the existing summary statistics if it exists.

Details

fg_summary calculates a summary statistic as specified by the user in parameters test_name, diminish (p_thres, p_rate), and test_custom. The test is done for a node or edge feature of interest within a given flowGraph object as specified by parameters node_features, edge_features. It then returns information on the summary statistic inside the same flowGraph object and returns it to the user. See flowGraph-class slot summary for details on the contents.

Value

flowGraph object containing calculated summary statistics.

See Also

flowGraph-class fg_clear_summary

Examples

```r
no_cores <- 1
data(fg_data_pos30)
fg <- flowGraph(fg_data_pos30$count, class=fg_data_pos30$meta$class,
  prop=FALSE, specenr=FALSE,
  no_cores=no_cores)
fg_get_summary_desc(fg)
fg <- fg_summary(fg, no_cores=no_cores, class="class", label1="control",
  overwrite=FALSE, test_name="t", diminish=FALSE,
  node_features="count", edge_features="NONE")
fg_get_summary_desc(fg)
```

flowGraph

flowGraph object constructor.
**Description**

Initializes a `flowGraph` object given the cell counts for one or more flow cytometry sample(s). The `flowGraph` object returned holds meta data for each sample, each cell population node, edges representing how each cell population node relate to one another, and features for these nodes and edges.

**Usage**

```r
define flowGraph(
    input_,
    meta = NULL,
    class = "class",
    no_cores = 1,
    markers = NULL,
    layout_fun = "layout.reingold.tilford",
    max_layer = NULL,
    cumsumpos = FALSE,
    prop = TRUE,
    specenr = TRUE,
    path = NULL,
    calculate_summary = TRUE,
    node_features = "SpecEnr",
    edge_features = "NONE",
    test_name = "t_diminish",
    test_custom = "t",
    diminish = TRUE,
    label1 = NULL,
    label2 = NULL,
    save_plots = FALSE
)
```

**Arguments**

- `input_` Any of the following:
  - a numeric matrix or vector of the cell counts; its column/names must be the phenotype names and its rownames must be sample ID's.

  All input samples should have the same markers and partitionsPerMarker.

- `meta` A data frame with meta data for each Phenotypes or sample; One of its column names should be "id" whose values correspond to the name of each Phenotypes object. We also recommend for it to have a column named "class" where one of its unique values is "control".

- `class` A string corresponding to the column name or index of `meta` whose values represent the class label of each sample; OR a vector the same length as the the number of samples in `input_` specifying the class of each given sample — this vector will be appended to `meta` under column name `class`.

- `no_cores` An integer indicating how many cores to parallelize on.

- `markers` A string vector of marker names used in `input_`. 
flowGraph

layout_fun  A string of a function from the igraph package that indicates what layout should be used if a cell hierarchy is to be plotted; all such functions have prefix layout_. This is defaulted to e.g. layout_fun="layout.reingold.tilford".

max_layer  An integer indicating the maximum layer in the cell hierarchy to analyze; set to ‘NULL’ to analyze all layers.

cumsumpos  A logical variable indicating whether or not to cumulate cell counts; this applies only when partitionsPerMarker > 3 and will convert e.g. the count of A+ or A++ into the sum of the counts of A+, A++, A++++, ..., or A++, A++, ..., .

prop  A logical variable indicating whether or not to calculate the proportion feature; this can be done later on with flowGraph_prop.

specenr  Logical variable: whether or not to calculate the SpecEnr feature, Default: T

path  A string indicating the folder path to where the flowGraph object should save its elements, Default = NULL (don’t save).

calculate_summary  A logical variable indicating whether or not to calculate the summary statistics for SpecEnr based on default parameters using the fg_summary summary function on class specified in parameter class.

node_features  A string vector indicating which node feature(s) to perform summary statistics on; set to NULL or “NONE” and the function will perform summary statistics on all or no node features.

edge_features  A string vector indicating which edge feature(s) to perform summary statistics on; set to NULL or “NONE” and the function will perform summary statistics on all or no edge features.

test_name  A string with the name of the test you are performing.

test_custom  See fg_summary.

diminish  A logical variable; applicable if calculate_summary is TRUE; see fg_summary.

label1  A string indicating a class label in fg_get_meta(fg)[,class]; set to NULL if you would like to compare all classes against all classes; applicable if calculate_summary is TRUE.

label2  A string indicating a class label in fg_get_meta(fg)[,class]; applicable if calculate_summary is TRUE.

save_plots  A logical indicating whether or not to save plots.

Details

flowGraph is the constructor for the flowGraph object. The user can choose to input as input_ a vector, a Phenotypes object (meaning there is only one sample), a matrix, or a Phenotypes object list. If the user is also inputting a sample meta data frame, it must contain a id column corresponding to sample names.

Value

flowGraph object
flowGraph-class

'flowGraph': A class for storing cell count feature values for the Phenotype class.

Description

'flowGraph': A class for storing cell count feature values for the Phenotype class.

Usage

## S4 method for signature 'flowGraph'
show(object)
Arguments

object A flowGraph object.

Value

a flowGraph object.

Methods (by generic)

• show: show method

Slots

feat A list containing elements node and edge, each containing a list with feature values; each element in this list is named by the feature name and contains a numeric matrix with the sample id's as row names and cell populations phenotype labels or edge labels as column names. Column names for edge features are labelled as <from>_<to> e.g. A+_A+B+.

feat_desc A list containing elements node and edge, each containing a data frame describing the features in the feat slot with columns:

• feat: feature name.
• nrow: number of samples.
• ncol: number of nodes or edges.
• inf: number of infinite values in the matrix.
• neginf: number of negative infinite values in the matrix.
• na: number of NA values in the matrix.
• nan: number of NaN values in the matrix.
• neg: number of negative values in the matrix.
• pos: number of positive values in the matrix.
• zero: number of 0's in the matrix.
• max: The maximum value in the matrix.
• min: The minimum value in the matrix.

summary A list containing elements node and edge, each containing a list with a feature summary list; each feature summary in this list contains elements:

• values: a numeric vector the same length as the number of nodes or edges.
• test_custom: a function or a string name of the summary test method used.

summary_desc A list containing elements node and edge, each containing a data frame describing the features in feat with columns:

• feat: A string indicating feature name the summary was created for.
• test_name: A string containing the name of the summary.
• class: A string corresponding to the column name of the meta slot whose values represent the class label of each sample on which the summary was created to compare or analyze.
• label1: A string from the class column of the meta slot indicating one of the labels compared to create the summary statistic.
• `label2`: A string from the `class` column of the `meta` slot indicating one of the labels compared to create the summary statistic.

`meta` A data frame containing the column(s) `id` (sample id’s corresponding to row names of features in the `feat` slot) and any other meta data pertaining to samples being analyzed.

`markers` A character vector containing markers used.

`edge_list` A list containing elements `child` and `parent`. These elements contain an edge list from child to parent and vice versa.

`graph` A list containing data frames `v` and `e` with information on cell population nodes and edges. `v` contains columns:

  • `phenotype`: The cell population node label names e.g. A+B+C+.
  • `phenocode`: A string of "0", "1", "2", ... indicating the whether each marker is expressed on a cell population.
  • `phenolayer`: The layer on which a cell population resides i.e. the number of markers in its phenotype label.
  • `phenogroup`: The markers used the make up the phenotype.

`plot_layout` A string indicating the name of the `igraph` layout function used to layout the cell population nodes for plotting.

`etc` A list containing other information (see `fg_get_summary` for other things stored in this slot):

  • `cumsumpos`: A logical indicating whether cell counts in `flowGraph` object contains cumulated cell counts; this is optional and can be done only for there is more than one threshold for one or more markers. This should also only be ran when initializing a `flowGraph` object as converting back and forth is computationally expensive. If the user is interested in seeing non- and cumulated counts, we recommend keeping two `flowGraph` objects, one for each version. This function simply converts e.g. the count of A+ or A++ into the sum of count of A+, A++, and A+++ or A++, and A+++.
  • `class_mean_normalized`: A logical indicating whether the features in the `flowGraph` object has been normalized according to some sample meta e.g. subject.
  • `save`: A list containing a string indicating the save ID of the object and a string indicating path where the object is saved – used in function `save_fg` to identify whether or not to save to the same folder.

Creating Objects

Objects can be created using `new("flowFrame")` or the constructor `flowGraph`, with mandatory argument `input_`. Creating objects using `new` is discouraged.

Methods

`'object'` represents a `flowGraph` object.

• `show(fg)`: Shows a description of the `flowGraph` object.
  • `fg_get_meta`: Retrieves the sample meta data from a given `flowGraph` object. See `fg_get_meta`.
  • `fg_get_graph`: Retrieves the cell population (v) and edge (e) meta data from a given `flowGraph` object. See `fg_get_graph`.
  • `fg_get_feature`: Retrieves the numeric feature matrix requested by the user from a given `flowGraph` object. See `fg_get_feature`. 
• `fg_get_summary`: Retrieves the feature summary list requested by the user from a given flowGraph object. See `fg_get_summary`.

• `fg_get_feature_desc`: Retrieves the data frame from the `feat_desc` slot of a given flowGraph object. See `fg_get_feature_desc`.

• `fg_get_summary_desc`: Retrieves the data frame from the `summary_desc` slot of a given flowGraph object. See `fg_get_summary_desc`.

• `fg_add_feature`: Adds a feature to a given flowGraph object; we do not recommend users directly use this method, instead please use wrapper functions e.g. `fg_feat_node_prop`, `fg_feat_node_specer`, See `fg_add_feature`.

• `fg_rm_feature`: Removes a user specified feature from a given flowGraph object. See `fg_rm_feature`.

• `fg_add_summary`: Adds a feature to a given flowGraph object; we do not recommend users directly use this method, instead please use wrapper function `fg_summary`.

• `fg_clear_summary`: Removes all feature summaries from a given flowGraph object. See `fg_clear_summary`.

• `fg_rm_summary`: Removes a user specified feature summaries from a given flowGraph object. See `fg_rm_summary`.

• `fg_gsub_markers`: Substitutes marker names in a given flowGraph object. See `fg_gsub_markers`.

• `fg_gsub_ids`: Substitutes sample id’s in a flowGraph object See `fg_gsub_ids`.

• `fg_merge_samples`: Merges the samples of two flowGraph objects; we recommend users use the wrapper function `fg_merge` instead. See `fg_merge_samples`.

• `fg_extract_samples`: Extract data for specific samples from a flowGraph object. See `fg_extract_samples`.

• `fg_extract_phenotypes`: Extract data for specific cell population nodes from a flowGraph object. See `fg_extract_phenotypes`.

• `fg_merge`: Merges two given flowGraph objects. See `fg_merge`.

• `fg_set_layout`: Sets layout for cell population nodes for the purpose of plotting. See `fg_set_layout`.

• `fg_plot`: Plots cell hierarchies in the flowGraph object. See `fg_plot`.

**Examples**

```r
showClass("flowGraph")
```

---

**Description**

Initializes a `flowGraph` object given the cell counts for one or more flow cytometry sample(s). The `flowGraph` object returned holds meta data for each sample, each cell population node, edges representing how each cell population node relate to one another, and features for these nodes and edges.
Usage

flowGraphSubset(
  input_,
  meta = NULL,
  class = "class",
  no_cores = 1,
  markers = NULL,
  layout_fun = "layout.reingold.tilford",
  max_layer = NULL,
  cumsumpos = FALSE,
  path = NULL,
  summary_pars = flowGraphSubset_summary_pars(),
  summary_adjust = flowGraphSubset_summary_adjust(),
  save_plots = TRUE
)

Arguments

input_  a numeric matrix of the cell counts; its column/names must be the phenotype names and its rownames must be sample ID’s.

meta  A data frame with meta data for each Phenotypes or sample; One of its column names should be "id" whose values correspond to the name of each Phenotypes object. We also recommend for it to have a column named "class" where one of its unique values is "control".

class  A string corresponding to the column name or index of meta whose values represent the class label of each sample, Default: 'class'

no_cores  An integer indicating how many cores to parallelize on, Default: 1

markers  A string vector of marker names used in input_, Default: NULL

layout_fun  A string of a function from the igraph package that indicates what layout should be used if a cell hierarchy is to be plotted; all such functions have prefix layout_. This is defaulted to e.g. layout_fun="layout.reingold.tilford".

max_layer  An integer indicating the maximum layer in the cell hierarchy to analyze; set to 'NULL' to analyze all layers.

cumsumpos  A logical variable indicating whether or not to cumulate cell counts; this applies only when partitionsPerMarker > 3 and will convert e.g. the count of A+ or A++ into the sum of the counts of A+, A++, A+++,... or A++, A+++, ... , Default: FALSE

path  A string indicating the folder path to where the flowGraph object should save its elements, Default = NULL (don’t save).

summary_pars  A list containing parameters for calculating the statistical significance summary significance that will determine whether to trim out phenotypes for this fast version of flowGraph. The lists’ elements are:

  • node_feature: "SpecEnr"; this is the feature we will be testing, don’t change this.
  • edge_feature: "NONE"; this unneeded for now.
• test_name: "t_diminish"; this unneeded for now.
• test_custom: "t"; a string or a function indicating the statistical test desires. These tests can be c("t", "wilcox", "ks", "var", "chisq") corresponding to functions t.test, wilcox.test, ks.test, var.test, chisq.test
• diminish: TRUE; whether or not to continue testing phenotypes whose parent phenotypes are all insignificant.
• class: "class"; the column name in meta that contains class labels you want to test.
• labels: c("aml", "control") for the flowcap data set; SET THIS!! to the class labels you want to test using test_custom.

summary_adjust A list of parameters on how to adjust the p-values; this also affects which phenotypes are tested. The elements in the list are:
• adjust_custom: "byLayer"; this is a string (corresponding to an option in p.adjust) or a function used to adjust p-values.
• btwn_test_custom: "t"; see test_custom in summary_pars; this statistical significance test is used in the filters.
• adjust0_lim: see fg_get_summary.
• filter_adjust0: see fg_get_summary.
• filter_es: see fg_get_summary.
• filter_btwn_tpthres: see fg_get_summary.
• filter_btwn_es: see fg_get_summary.

save_plots A logical indicating whether or not to save plots.

Details

All node and edge features are trimmed such that only the significant phenotypes are left; the original input is stored in the slot etc$original_count of the returned flowGraph object.

Value

flowGraph object

Examples

```r
## Not run:
if(interactive()){
  data(fg_data_pos2)
  fg <- flowGraph(fg_data_pos2$count, meta=fg_data_pos2$meta, no_cores=1)
}
```

## End(Not run)
**flowGraphSubset_summary_adjust**

*Default for flowGraphSubset's summary_adjust parameter.*

Description

Default input for flowGraphSubset's summary_adjust parameter. ONLY USE THIS OVER flowGraph IF: 1) your data set has more than 10,000 cell populations and you want to speed up your calculation time AND 2) you only have one set of classes you want to test on the SAME SET OF SAMPLES (e.g. control vs experiment). As flowGraphSubset does not calculate the SpecEnr for all cell populations, so if you want to test other sets of classes on the same samples, you will not be able to test all possible cell populations on the new set of classes.

Usage

```r
flowGraphSubset_summary_adjust()
```

Value

Default list parameter flowGraphSubset's summary_adjust parameter.

Examples

```r
flowGraphSubset_summary_adjust()
```

---

**flowGraphSubset_summary_pars**

*Default for flowGraphSubset's summary_pars parameter.*

Description

Default input for flowGraphSubset's summary_pars parameter.

Usage

```r
flowGraphSubset_summary_pars()
```

Value

Default list parameter flowGraphSubset's summary_pars parameter.

Examples

```r
flowGraphSubset_summary_pars()
```
fpurr_map

Description

= Wrapper for purrr::map and furrr::future_map to handle parallel-ization

Usage

fpurr_map(x, f, no_cores = 1, prll = TRUE, ...)

Arguments

x Variable to recurse over; must be indices!
f Function to recurse over.
no_cores Number of cores to use; future must have been ran already.
prll If set to FALSE, forces use of purrr::map instead of furrr::future_map, Default: TRUE
... Other parameters used by f.

Details

= Wrapper for purrr::map and furrr::future_map to handle parallel-ization easily; note that future
must have been ran already outside of the function and outputs will always be a list.

Value

= Unnested named list.

See Also

map future_map

get_child

Description

= Gets child populations of given cell populations

Usage

get_child(paren, pchild, pc_i, ac__, meta_cell__)

Arguments

- **parens**: Character vector of cell population phenotypes.
- **pchild**: Edge list where the name of the list is the cell population and the vector in each element contains the child cell populations of the named cell population.
- **pc_i**: A cell population x marker matrix where the values are 0/1/2/... corresponding to marker conditions /+/+... for possible PARENT populations.
- **ac__**: A list where the elements are marker index > "0"/"1"/"2"/... > a logical vector the same length as the number of cell population phenotypes indicating whether or not the marker condition exists in them; this is for the possible CHILD cell populations.
- **meta_cell__**: Data frame with meta data for cell population phenotypes from the flowGraph object; this is for the possible CHILD cell populations.

Value

A list containing child populations of parens; also globally updates pchild.

See Also

map, keep

---

**get_eprop**

*Gets edge proportions of a given edge matrix*

Description

Gets the edge proportions of the edges in edge matrix edf_, and updates ep edge proportion matrix if it didn’t contain the requested information.

Usage

`get_eprop(edf_, ep, mp_, no_cores = 1)`

Arguments

- **edf_**: Edge x from&to data frame containing edges and their from and to cell population phenotypes.
- **ep**: Sample x edge (parent_child) matrix with edge proportions.
- **mp_**: Sample x phenotype matrix with proportions.
- **no_cores**: Number of cores to use, Default: 1

Value

ep with only the specific columns (edges) requested; also updates ep globally.
**get_paren**

*Gets parent populations of given cell populations*

**Description**

Gets the parent populations of a vector of given cell childs and updates pparen the edge list if edge list doesn’t contain the requested information.

**Usage**

```r
get_paren(childs, pparen, pc__i, ac_, meta_cell_)
```

**Arguments**

- `childs` Character vector of cell population phenotypes.
- `pparen` Edge list where the name of the list is the cell population and the vector in each element contains the parent cell populations of the named cell population.
- `pc__i` A cell population x marker matrix where the values are 0/1/2/... corresponding to marker conditions /+-+/...; this is for the possible CHILD cell populations.
- `ac_` A list where the elements are marker index > "0"/"1"/"2"/... > a logical vector the same length as the number of cell population phenotypes indicating whether or not the marker condition exists in them; this is for the possible PARENT cell populations.
- `meta_cell_` data frame with meta data for cell population phenotypes from the flowGraph object; this is for the possible PARENT cell populations.

**Value**

A list containing parent populations of childs; also globally updates pparen.

---

**get_phen_list**

*Creates edge lists.*

**Description**

Creates edge lists indicating relationships between cell populations given meta data on these cell populations produced by the get_phen_meta function.

**Usage**

```r
get_phen_list(meta_cell = NULL, phen = NULL, no_cores = 1)
```
get_phen_meta

Arguments

  meta_cell  A data frame containing meta data on cell populations as produced by the get_phen_meta function.
  phen       A string vector of phenotype or cell population name labels. Cannot be set to NULL if meta_cell is set to NULL.
  no_cores   An integer indicating how many cores to parallelize on.

Value

A list containing 'pchild', an edge list indicating where edges point to, 'pparen', an edge list indicating where edges point from, and 'edf', a data frame where each row contains the nodes an edge points 'from' and 'to'.

See Also

  get_phen_meta cell_type_layers

Examples

  phen <- c('A+B-C+', 'A+B-', 'A+')
  get_phen_list(phen=phen)

get_phen_meta

Generates phenotype meta data.

Description

Generates phenotype meta data given a vector of phenotypes and optionally phenocodes.

Usage

  get_phen_meta(phen, phenocode = NULL)

Arguments

  phen       A string vector of phenotype or cell population name labels.
  phenocode  A string vector of phenocodes corresponding to the phenotypes in phen.

Value

A data frame with columns containing meta data on cell population nodes with columns:

  • phenotype: cell population node label e.g. "A+B+".
  • phenocode: a string penocode containing a numeric corresponding to the phenotype column e.g. "2200".
  • phenolayer: a numeric layer on which a cell population resides in e.g. 2.
ggdf

Prepares a given node and edge graph list for plotting.

Description

Prepares a given node and edge graph list for plotting by function plot_gr; do not use this function on its own.

Usage

```r
ggdf(gr0)
```

Arguments

- `gr0`: A list containing data frames `e` and `v`.

Details

codeggdf adds to the data frames `v` and `e` in slot `graph` from a `flowGraph` object specifying plotting options as required by `plot_gr`:

- `v`
  - `size`: a numeric indicating node size.
  - `colour`: a numeric or string indicating node colour.
  - `label`: a string indicating the label of a node.
  - `label_long`: a string indicating the long label of a node; used in interactive plots in `plot_gr`.
  - `label_ind`: a vector of logical variables indicating which nodes to add a label to in a static plot.
  - `v_ind`: a vector of logical variables indicating which nodes to plot.

- `e`
  - `colour`: a numeric or string indicating edge colour.
  - `e_ind`: a vector of logical variables indicating which edges to plot.

Value

A list containing data frames `e` and `v`, each with additional meta data column.

See Also

get_phen_list, cell_type_layers

Examples

```r
phen <- c('A+B+C-D++', 'A+B--', '', 'B++D-E+')
phenc <- c('22130', '21000', '00000', '03012')
get_phen_meta(phen, phenc)
```
See Also

- `flowGraph-class`
- `get_phen_meta`
- `plot_gr`

Examples

```r
no_cores <- 1
data(fg_data_pos30)
fg <- flowGraph(fg_data_pos30$count, class=fg_data_pos30$meta$class,
               prop=FALSE, specenr=FALSE,
               no_cores=no_cores)

gr_ <- ggdf(fg_get_graph(fg))
head(gr$_v)
head(gr$_e)
```

---

### loop_ind_f

*Prepares parallel loop indices.*

**Description**

`loop_ind_f` is a helper function that splits a vector of loop indices into a list of multiple loop indices for use in parallel processes within the `flowGraph` package.

**Usage**

```r
loop_ind_f(x, n)
```

**Arguments**

- `x`: A vector of loop indices.
- `n`: An integer, or the number of vectors to split `x` into.

**Value**

list of `n` vectors with elements from `x`.

**Examples**

```r
old_loop_inds <- 1:10
no_cores <- 5

new_loop_inds <- flowGraph:::loop_ind_f(old_loop_inds, no_cores)
# future::plan(future::multisession)
# example_indices <- furrr::future_map(new_loop_inds, function(ii) {
#   purrr::map(ii, function(i) i )
#})
```
mean_diff

Normalizes matrix values by class.

Description

Used only in the \texttt{fg_feat_mean_class} function; for each class in the \texttt{classes} vector, \texttt{meandiff} takes the column mean of the rows in the given matrix associated with that class; it then takes the difference point by point between these means and the original rows for that class.

Usage

\texttt{mean_diff(m0, classes)}

Arguments

- \texttt{m0} A numeric matrix.
- \texttt{classes} A vector whose length is equal to the number of rows in the given matrix.

Value

A numeric matrix whose dimensions equate to that of the input and whose values are normalized per class.

See Also

\texttt{fg_feat_mean_class}

Examples

\begin{verbatim}
classes <- append(rep('apples',4), rep('oranges',3))
m0 <- matrix(rnorm(35), nrow=7)
m <- flowGraph::mean_diff(m0, classes)
\end{verbatim}

ms_create

Calculate SpecEnr from proportion and expected proportion

Description

FUNCTION_DESCRIPTION

Usage

\texttt{ms_create(mp_, me_)}
Arguments

mp_ Numerical sample x cell population matrix w/ proportions.
me_ Numerical sample x cell population matrix w/ expected proportions.

Value

Numerical sample x cell population matrix w/ SpecEnr.

---

**ms_psig**

*Determines which phenotypes are statistically significant*

**Description**

Determines which phenotypes are statistically significant based on SpecEnr.

**Usage**

```r
ms_psig(
  ms_,
  summary_pars,
  summary_adjust,
  test_cust,
  test_custom,
  lyrno,
  mp_,
  me_
)
```

**Arguments**

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ms_</td>
<td>sample x phenotype SpecEnr matrix</td>
</tr>
<tr>
<td>summary_pars</td>
<td>See flowGraphSubset.</td>
</tr>
<tr>
<td>summary_adjust</td>
<td>See flowGraphSubset.</td>
</tr>
<tr>
<td>test_cust</td>
<td>Final significance test function.</td>
</tr>
<tr>
<td>test_custom</td>
<td>Raw significance test function.</td>
</tr>
<tr>
<td>lyrno</td>
<td>An integer indicating total number of layers in the cell hierarchy including layer 0.</td>
</tr>
<tr>
<td>mp_</td>
<td>sample x phenotype proportion matrix.</td>
</tr>
<tr>
<td>me_</td>
<td>sample x phenotype expected proportion matrix.</td>
</tr>
</tbody>
</table>

**Value**

A logical vector the same length as the number of columns in ms_ indicating whether or not each phenotype is significant; used only for the fast version of flowGraph to determine whether or not to keep testing the phenotypes' children.
plot_gr

Plots a cell hierarchy.

Description

Plots a cell hierarchy given the output from fg_plot, a list of nodes and edges.

Usage

```
plot_gr(
  gr,
  main = NULL,
  show_bgedges = TRUE,
  colour_palette = NULL,
  label_coloured = TRUE,
  shiny_plot = FALSE,
  interactive = FALSE,
  visNet_plot = TRUE,
  colour_edges = FALSE,
  ...
)
```

Arguments

- **gr**: A list containing data frames e and v.
- **main**: A string containing the plot title. If this is set to NULL, the function will look for a plot title in the main slot of gr; otherwise, this defaults to ""
- **show_bgedges**: A logical variable indicating whether or not edges not specified for plotting should be plotted as light grey in the background. If this is NULL, the function will look for a show_bgedges in the show_bgedges slot of gr; otherwise, this defaults to TRUE.
- **colour_palette**: A colour palette e.g. the default palette if the user sets this to NULL is c('blue', 'cyan', 'yellow', 'red').
- **label_coloured**: A logical indicating whether to colour the node labels using the same colours as the nodes in the non-interactive plot.
- **shiny_plot**: A logical indicating whether this plot is made for shiny; users don’t need to change this.
- **interactive**: A logical variable indicating whether the plot should be an interactive plot; see package ggiraph.
- **visNet_plot**: A logical variable indicating if an interactive plot is chosen, if function should output a visNetwork plot; if set to FALSE, ggplot’s girafe will be used instead.
- **colour_edges**: A logical variable indicating whether to colour edges if plotting a node feature summary.
- **...**: Other parameters for ggplot if interactive is set to FALSE; other parameters for plot_ly if interactive is set to TRUE.
set_layout_graph

Determines cell hierarchy layout.

Description

Determines cell hierarchy layout and returns the X, Y coordinate of each cell population.

Usage

set_layout_graph(gr, layout_fun = "layout.reingold.tilford")

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>gr</td>
<td>A list containing data frames e and v.</td>
</tr>
<tr>
<td>layout_fun</td>
<td>A string of a function from the igraph package that indicates what layout should be used if a cell hierarchy is to be plotted; all such functions have prefix layout_, e.g. layout_fun=&quot;layout.reingold.tilford&quot;.</td>
</tr>
</tbody>
</table>

Value

A list containing data frames e and v; each data frame contains an X, Y column or coordinate for each node and edge.

Examples

```r
no_cores <- 1
data(fg_data_pos2)
fg <- flowGraph(fg_data_pos2$count, class=fg_data_pos2$meta$class,
                no_cores=no_cores)

# fg <- fg_summary(fg, no_cores=no_cores, class="class", control="control",
# overwrite=FALSE, test_name="t_byLayer", diminish=FALSE)

gr_summary <- fg_plot(
    fg, type="node", p_thres=.05, show_bgedges=TRUE,
    path=NULL) # set path to a full path to save plot as a PNG

plot_gr(gr_summary, main=gr_summary$main, show_bgedges=TRUE)
plot_gr(gr_summary, main=gr_summary$main, show_bgedges=TRUE, interactive=TRUE)
```
Examples

```r
no_cores <- 1
data(fg_data_pos30)
fg <- flowGraph(fg_data_pos30$count, class=fg_data_pos30$meta$class,
    prop=FALSE, specenr=FALSE,
    no_cores=no_cores)

head(set_layout_graph(fg_get_graph(fg)))
```

summary_table

```
summarizes a numeric matrix.
```

Description

Summarizes a numeric matrix.

Usage

```r
summary_table(m, feat_type = "")
```

Arguments

- `m`: A numeric matrix.
- `feat_type`: Name of the matrix `m`.

Value

A data frame containing one row summarizing `m`; see `fg_get_feature_desc`.

Examples

```r
summary_table(matrix(rnorm(12), nrow=3), feat_type='random')
```

test_c

```
Converts input into a significance test function
```

Description

Converts input into a significance test function

Usage

```r
test_c(test_custom)
```
Arguments

test_custom a string c("t", "wilcox", "ks", "var", "chisq") or a function.

Value

a statistical significance test function.

See Also

t.test, wilcox.test, ks.test, var.test, chisq.test

time_output

 Outputs elapsed time.

Description

Given a time, prints the time elapsed from that time until now.

Usage

time_output(start, msg = "")

Arguments

start A time variable of class POSIXct, POSIXt.

msg A string with a message to print out after the elapsed time.

Value

Prints to console, the time from which process started start - ended, and > time elapsed from start until now.

Examples

start <- Sys.time()
flowGraph:::time_output(start,'start - now > time elapsed')
tstr

Formats time into string.

Description

Formats time into a string HH:MM:SS given time zone.

Usage

tstr(time)

Arguments

time A time variable of class POSIXct, POSIXt.

Value

Time formatted as a string; used in time_output function.

Examples

# NOT EXPORTED
flowGraph:::tstr(Sys.time())
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