Package 'COTAN'

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Type Package

Title COexpression Tables ANalysis

Version 2.9.4

Description Statistical and computational method to analyze the co-expression of gene pairs at single cell level. It provides the foundation for single-cell gene interactome analysis. The basic idea is studying the zero UMI counts' distribution instead of focusing on positive counts; this is done with a generalized contingency tables framework. COTAN can effectively assess the correlated or anti-correlated expression of gene pairs. It provides a numerical index related to the correlation and an approximate p-value for the associated independence test. COTAN can also evaluate whether single genes are differentially expressed, scoring them with a newly defined global differentiation index. Moreover, this approach provides ways to plot and cluster genes according to their co-expression pattern with other genes, effectively helping the study of gene interactions and becoming a new tool to identify cell-identity marker genes.

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ClustersList Clusters *utilities*

Description

Handle *clusterization* <-> *clusters* `list` conversions, *clusters* grouping and merge

Usage

```
asClusterization(clusters, allCells = NULL)

toClustersList(clusters)

fromClustersList(
  clustersList,
  elemNames = vector(mode = "character"),
  throwOnOverlappingClusters = TRUE
)

groupByClustersList(elemNames, clustersList, throwOnOverlappingClusters = TRUE)

groupByClusters(clusters)

mergeClusters(clusters, names, mergedName = "")

multiMergeClusters(clusters, namesList, mergedNames = NULL)
```

Arguments

`clusters`	A named `vector` or `factor` that defines the *clusters*
`allCells`	A `vector` of cells' names that should list the same names in the `clusters` in any order
`clustersList`	A named `list` whose elements define the various clusters
`elemNames`	A `list` of names to which associate a cluster
`throwOnOverlappingClusters`	When `TRUE`, in case of overlapping clusters, the function `fromClustersList` and `groupByClustersList` will throw. This is the default. When FALSE, instead, in case of overlapping clusters, `fromClustersList` will return the last cluster to which each element belongs, while `groupByClustersList` will return a vector of positions that is longer than the given `elemNames`
`names`	A list of *clusters* names to be merged
`mergedName`	The name of the new merged clusters
`namesList`	A `list` of `list`s of *clusters* names to be respectively merged
`mergedNames`	The names of the new merged *clusters*

Details

`asClusterization()` given a *clusterization* in the form of a `data.frame` or a `vector` or a `factor`, returns a named `factor`

`toClustersList()` given a *clusterization*, creates a `list` of *clusters* (i.e. for each *cluster*, which elements compose the *cluster*)

`fromClustersList()` given a `list` of *clusters* returns a *clusterization* (i.e. a named `vector` that for each element indicates to which cluster it belongs)

`groupByClusters()` given a *clusterization* returns a permutation, such that using the permutation on the input the *clusters* are grouped together

`groupByClustersList()` given the elements' names and a `list` of *clusters* returns a permutation, such that using the permutation on the given names the *clusters* are grouped together.

`mergeClusters()` given a *clusterization*, creates a new one where the given *clusters* are merged.

`multiMergeClusters()` given a *clusterization*, creates a new one where the given sets of *clusters* are merged.

Value

`asClusterization()` returns the *clusterization* as a named `factor`

`toClustersList()` returns a `list` of clusters

`fromClustersList()` returns a clusterization. If the given `elemNames` contain values not present in the `clustersList`, those will be marked as `"-1"`

`groupByClusters()` and `groupByClustersList()` return a permutation that groups the clusters together. For each cluster the positions are guaranteed to be in increasing order. In case, all elements not corresponding to any cluster are grouped together as the last group

`mergeClusters()` returns a new *clusterization* with the wanted *clusters* being merged. If less than 2 *cluster* names were passed the function will emit a warning and return the initial *clusterization*

`multiMergeClusters()` returns a new *clusterization* with the wanted *clusters* being merged by consecutive iterations of `mergeClusters()` on the given `namesList`

Examples

```
## create a clusterization
clusters <- paste0("",sample(7, 100, replace = TRUE))
names(clusters) <- paste0("E_",formatC(1:100,  width = 3, flag = "0"))

## create a clusters list from a clusterization
clustersList <- toClustersList(clusters)
head(clustersList, 1)

## recreate the clusterization from the cluster list
clusters2 <- fromClustersList(clustersList, names(clusters))
all.equal(factor(clusters), clusters2)

cl1Size <- length(clustersList[["1"]])

## establish the permutation that groups clusters together
perm <- groupByClusters(clusters)
!is.unsorted(head(names(clusters)[perm],cl1Size))
head(clusters[perm], cl1Size)
```

```
## it is possible to have the list of the element names different
## from the names in the clusters list
selectedNames <- paste0("E_",formatC(11:110,  width = 3, flag = "0"))
perm2 <- groupByClustersList(selectedNames, toClustersList(clusters))
all.equal(perm2[91:100], c(91:100))

## is is possible to merge a few clusters together
clustersMerged <- mergeClusters(clusters, names = c("7", "2"),
                                mergedName = "7__2")
sum(table(clusters)[c(2, 7)]) == table(clustersMerged)[["7__2"]]

## it is also possible to do multiple merges at once!
## Note the default new clusters' names
clustersMerged2 <-
  multiMergeClusters(clusters2, namesList = list(c("2", "7"),
                                                 c("1", "3", "5")))
table(clustersMerged2)
```

`Conversions` *Data class conversions*

Description

All functions to convert a COTAN object to/from other data classes used by the `BioConductor` analysis packages

Usage

```
convertToSingleCellExperiment(objCOTAN)

convertFromSingleCellExperiment(objSCE, clNamesPattern = "")
```

Arguments

`objCOTAN`	a `COTAN` object
`objSCE`	A SingleCellExperiment::SingleCellExperiment object to be converted
`clNamesPattern`	A regular expression pattern used to identify the clustering columns in `colData`. Default supports `Seurat` conventions: `"^(COTAN_clusters_|seurat_clusters$|.*_snn_res\\..`

Details

`convertToSingleCellExperiment()` converts a COTAN object into a SingleCellExperiment::SingleCellExperiment object. Stores the raw counts in the `"counts"` SummarizedExperiment::Assays, the metadata for genes and cells as `rowData` and `colData` slots respectively and finally the genes' and cells' `COEX` along the dataset metadata into the `metadata` slot.

The function performs the following steps:

- Extracts the raw counts matrix, gene metadata, cell metadata, gene and cell *co-expression* matrix from the `COTAN` object; the `clustersCoex` slot is not converted
- Identifies *clusterizations* and *conditions* in the cell metadata by the prefixes `"CL_"` and `"COND_"`

- Renames *clusterization* columns with the prefix `"COTAN_clusters_"` and *condition* columns with the prefix `"COTAN_conditions_"`
- Constructs a `SingleCellExperiment` object with the counts matrix, gene metadata, updated cell metadata, and stores the *co-expression* matrices in the `metadata` slot.

The resulting `SingleCellExperiment` object is compatible with downstream analysis packages and workflows within the Bioconductor ecosystem

`convertFromSingleCellExperiment()` converts a SingleCellExperiment::SingleCellExperiment object back into a COTAN object. It supports `SCE` objects that were originally created from either a `COTAN` object or a `Seurat` object. The function extracts the `"counts"` matrix, genes' metadata, cells' metadata, *co-expression* matrices (if available), and reconstructs the `COTAN` object accordingly. The function performs the following steps:

- Extracts the raw matrix from the `"counts"` SummarizedExperiment::Assays
- Extracts gene metadata from `rowData`
- Extracts cell metadata from `colData`, excluding any *clusterizations* or *conditions* present
- Attempts to retrieve *co-expression* matrices from the `metadata` slot if they exist
- Constructs a `COTAN` object using the extracted data
- Adds back the *clusterizations* and *conditions* using `COTAN` methods If the COEX is not present (e.g., in `SCE` objects created from `Seurat`), the `genesCoex` and `cellsCoex` slots in the resulting `COTAN` object will be empty matrices

Value

A SingleCellExperiment::SingleCellExperiment object containing the data from the input COTAN object, with *clusterizations* and *conditions* appropriately prefixed and stored in the cell metadata.

A COTAN object containing the data extracted from the input SingleCellExperiment::SingleCellExperiment object

See Also

COTAN, SingleCellExperiment::SingleCellExperiment

COTAN, SingleCellExperiment::SingleCellExperiment

Examples

```
data("test.dataset")
obj <- COTAN(raw = test.dataset)
obj <- proceedToCoex(obj, calcCoex = FALSE, saveObj = FALSE)

sce <- convertToSingleCellExperiment(objCOTAN = obj)

newObj <- convertFromSingleCellExperiment(sce)

stopifnot(identical(getDims(newObj), getDims(obj)))
```

COTAN-class *Definition of the* COTAN *class*

Description

Definition of the COTAN class

Slots

raw dgCMatrix - the raw UMI count matrix $n \times m$ (gene number × cell number)

genesCoex dspMatrix - the correlation of COTAN between genes, $n \times n$

cellsCoex dspMatrix - the correlation of COTAN between cells, $m \times m$

metaDataset data.frame

metaCells data.frame

clustersCoex a list of COEX data.frames for each clustering in the metaCells

COTAN_Legacy *Handle legacy* scCOTAN*-class and related symmetric matrix* <-> *vector conversions*

Description

A class and some functions related to the V1 version of the COTAN package

Usage

```
clustersDeltaExpression(objCOTAN, clName = "", clusters = NULL)

vec2mat_rfast(x, genes = "all")

mat2vec_rfast(mat)
```

Arguments

objCOTAN	a COTAN object
clName	The name of the *clusterization*. If not given the last available *clusterization* will be used, as it is probably the most significant!
clusters	A *clusterization* to use. If given it will take precedence on the one indicated by clName
x	a list formed by two arrays: genes with the unique gene names and values with all the values.
genes	an array with all wanted genes or the string "all". When equal to "all" (the default), it recreates the entire matrix.
mat	a square (possibly symmetric) matrix with all genes as row and column names.

Details

Define the legacy scCOTAN-class

Automatically converts an object from class scCOTAN into COTAN

Explicitly converts an object from class COTAN into scCOTAN

clustersDeltaExpression() is a legacy function now superseded by DEAOnClusters(). It estimates the change in genes' expression inside the *cluster* compared to the average situation in the data set.

This is a deprecated function related to old scCOTAN objects. Use the more appropriate Matrix::dspMatrix type for similar functionality.

mat2vec_rfast converts a compacted symmetric matrix (that is an array) into a symmetric matrix.

This is a deprecated function related to old scCOTAN objects. Use the more appropriate Matrix::dspMatrix type for similar functionality.

vec2mat_rfast converts a symmetric matrix into a compacted symmetric matrix. It will forcibly make its argument symmetric.

Value

a scCOTAN object

clustersDeltaExpression() returns a data.frame with the ν weighted discrepancy of the expression of each gene within the *cluster* against the corresponding model expectations

mat2vec_rfast returns a list formed by two arrays:

- "genes" with the unique gene names,
- "values" with all the values.

vec2mat_rfast returns the reconstructed symmetric matrix

Slots

raw ANY. To store the raw data matrix

raw.norm ANY. To store the raw data matrix divided for the cell efficiency estimated (nu)

coex ANY. The COEX matrix

nu vector.

lambda vector.

a vector.

hk vector.

n_cells numeric.

meta data.frame.

yes_yes ANY. Unused and deprecated. Kept for backward compatibility only

clusters vector.

cluster_data data.frame.

Examples

```
v <- list("genes" = paste0("gene_", c(1:9)), "values" = c(1:45))

M <- vec2mat_rfast(v)
all.equal(rownames(M), v[["genes"]])
all.equal(colnames(M), v[["genes"]])

genes <- paste0("gene_", sample.int(ncol(M), 3))

m <- vec2mat_rfast(v, genes)
all.equal(rownames(m), v[["genes"]])
all.equal(colnames(m), genes)

v2 <- mat2vec_rfast(M)
all.equal(v, v2)
```

COTAN_ObjectCreation COTAN *shortcuts*

Description

These functions create a COTAN object and/or also run all the necessary steps until the genes' COEX matrix is calculated.

Usage

```
COTAN(raw = "ANY")

## S4 method for signature 'COTAN'
proceedToCoex(
  objCOTAN,
  calcCoex = TRUE,
  optimizeForSpeed = TRUE,
  deviceStr = "cuda",
  cores = 1L,
  cellsCutoff = 0.003,
  genesCutoff = 0.002,
  cellsThreshold = 0.99,
  genesThreshold = 0.99,
  saveObj = TRUE,
  outDir = "."
)

automaticCOTANObjectCreation(
  raw,
  GEO,
  sequencingMethod,
  sampleCondition,
  calcCoex = TRUE,
  optimizeForSpeed = TRUE,
  deviceStr = "cuda",
```

```
  cores = 1L,
  cellsCutoff = 0.003,
  genesCutoff = 0.002,
  cellsThreshold = 0.99,
  genesThreshold = 0.99,
  saveObj = TRUE,
  outDir = "."
)
```

Arguments

raw	a matrix or dataframe with the raw counts
objCOTAN	a newly created COTAN object
calcCoex	a Boolean to determine whether to calculate the genes' COEX or stop just after the estimateDispersionBisection() step
optimizeForSpeed	Boolean; when TRUE COTAN tries to use the torch library to run the matrix calculations. Otherwise, or when the library is not available will run the slower legacy code
deviceStr	On the torch library enforces which device to use to run the calculations. Possible values are "cpu" to us the system *CPU*, "cuda" to use the system *GPUs* or something like "cuda:0" to restrict to a specific device
cores	number of cores to use. Default is 1.
cellsCutoff	clean() will delete from the raw data any gene that is expressed in less cells than threshold times the total number of cells. Default cutoff is 0.003 (0.3%)
genesCutoff	clean() will delete from the raw data any cell that is expressing less genes than threshold times the total number of genes. Default cutoff is 0.002 (0.2%)
cellsThreshold	any gene that is expressed in more cells than threshold times the total number of cells will be marked as **fully-expressed**. Default threshold is 0.99 (99.0%)
genesThreshold	any cell that is expressing more genes than threshold times the total number of genes will be marked as **fully-expressing**. Default threshold is 0.99 (99.0%)
saveObj	Boolean flag; when TRUE saves intermediate analyses and plots to file
outDir	an existing directory for the analysis output.
GEO	a code reporting the GEO identification or other specific dataset code
sequencingMethod	a string reporting the method used for the sequencing
sampleCondition	a string reporting the specific sample condition or time point.

Details

Constructor of the class COTAN

proceedToCoex() takes a newly created COTAN object (or the result of a call to dropGenesCells()) and runs calculateCoex()

automaticCOTANObjectCreation() takes a raw dataset, creates and initializes a COTAN object and runs proceedToCoex()

Value

a COTAN object

proceedToCoex() returns the updated COTAN object with genes' COEX calculated. If asked to, it will also store the object, along all relevant clean-plots, in the output directory.

automaticCOTANObjectCreation() returns the new COTAN object with genes' COEX calculated. When asked, it will also store the object, along all relevant clean-plots, in the output directory.

Examples

```
data("test.dataset")
obj <- COTAN(raw = test.dataset)

#
# In case one needs to run more steps to clean the datatset
# the following might apply
if (FALSE) {
  objCOTAN <- initializeMetaDataset(objCOTAN,
                                    GEO = "test",
                                    sequencingMethod = "artificial",
                                    sampleCondition = "test dataset")
#
# doing all the cleaning...
#
# in case the genes' `COEX` is not needed it can be skipped
# (e.g. when calling [cellsUniformClustering()])
  objCOTAN <- proceedToCoex(objCOTAN, calcCoex = FALSE,
                            cores = 6L, optimizeForSpeed = TRUE,
                            deviceStr = "cuda", saveObj = FALSE)
}


## Otherwise it is possible to run all at once.
objCOTAN <- automaticCOTANObjectCreation(
  raw = test.dataset,
  GEO = "code",
  sequencingMethod = "10X",
  sampleCondition = "mouse_dataset",
  calcCoex = TRUE,
  saveObj = FALSE,
  outDir = tempdir(),
  cores = 6L)
```

Datasets *Data-sets*

Description

Simple data-sets included in the package

Usage

```
data(raw.dataset)

data(ERCCraw)

data(test.dataset)

data(test.dataset.clusters1)

data(test.dataset.clusters2)

data(vignette.split.clusters)

data(vignette.merge.clusters)

data(vignette.merge2.clusters)
```

Format

`raw.dataset` is a data frame with 2000 genes and 815 cells

`ERCCRaw` is a `data.frame`

`test.dataset` is a `data.frame` with 600 genes and 1200 cells

`test.dataset.clusters1` is a `character array`

`test.dataset.clusters2` is a `character array`

`vignette.split.clusters` is a `factor`

`vignette.merge.clusters` is a `factor`

`vignette.merge2.clusters` is a `factor`

Details

`raw.dataset` is a sub-sample of a real *scRNA-seq* data-set

`ERCCRaw` dataset

`test.dataset` is an artificial data set obtained by sampling target negative binomial distributions on a set of 600 genes on 2 two cells *clusters* of 600 cells each. Each *clusters* has its own set of parameters for the distributions even, but a fraction of the genes has the same expression in both *clusters*.

`test.dataset.clusters1` is the *clusterization* obtained running `cellsUniformClustering()` on the `test.dataset`

`test.dataset.clusters2` is the *clusterization* obtained running `mergeUniformCellsClusters()` on the `test.dataset` using the previous *clusterization*

`vignette.split.clusters` is the clusterization obtained running `cellsUniformClustering()` on the vignette dataset (mouse cortex E17.5, `GEO: GSM2861514`)

`vignette.merge.clusters` is the clusterization obtained running `mergeUniformCellsClusters()` on the vignette dataset (mouse cortex E17.5, `GEO: GSM2861514`) using the previous *clusterization*

`vignette.merge2.clusters` is the clusterization obtained re-running `mergeUniformCellsClusters()` on the vignette dataset (mouse cortex E17.5, `GEO: GSM2861514`) using the `vignette.split.clusters` *clusterization*, but with a sequence of progressively relaxed checks

Source

GEO GSM2861514

ERCC

`getColorsVector` `getColorsVector`

Description

This function returns a list of colors based on the `RColorBrewer::brewer.pal()` function

Usage

```
getColorsVector(numNeededColors = 0L)
```

Arguments

`numNeededColors`
: The number of returned colors. If omitted it returns all available colors

Details

The colors are taken from the `RColorBrewer::brewer.pal.info()` sets with `Set1`, `Set2`, `Set3` placed first.

Value

an array of `RGB` colors of the wanted size

Examples

```
colorsVector <- getColorsVector(17)
```

`getGDI,COTAN-method` *Calculations of genes statistics*

Description

A collection of functions returning various statistics associated to the genes. In particular the *discrepancy* between the expected probabilities of zero and their actual occurrences, both at single gene level or looking at genes' pairs

To make the `GDI` more specific, it may be desirable to restrict the set of genes against which `GDI` is computed to a selected subset, with the recommendation to include a consistent fraction of cell-identity genes, and possibly focusing on markers specific for the biological question of interest (for instance neural cortex layering markers). In this case we denote it as *Local Differentiation Index* (`LDI`) relative to the selected subset.

Usage

```
## S4 method for signature 'COTAN'
getGDI(objCOTAN)

## S4 method for signature 'COTAN'
storeGDI(objCOTAN, genesGDI)

genesCoexSpace(objCOTAN, primaryMarkers, numGenesPerMarker = 25L)

establishGenesClusters(
  objCOTAN,
  groupMarkers,
  numGenesPerMarker = 25L,
  kCuts = 6L,
  distance = "cosine",
  hclustMethod = "ward.D2"
)

calculateGenesCE(objCOTAN)

calculateGDIGivenCorr(corr, numDegreesOfFreedom, rowsFraction = 0.05)

calculateGDI(objCOTAN, statType = "S", rowsFraction = 0.05)

calculatePValue(
  objCOTAN,
  statType = "S",
  geneSubsetCol = vector(mode = "character"),
  geneSubsetRow = vector(mode = "character")
)

calculatePDI(
  objCOTAN,
  statType = "S",
  geneSubsetCol = vector(mode = "character"),
  geneSubsetRow = vector(mode = "character")
)
```

Arguments

objCOTAN	a COTAN object
genesGDI	the named genes' GDI array to store or the output data.frame of the function calculateGDI()
primaryMarkers	A vector of primary marker names.
numGenesPerMarker	the number of correlated genes to keep as other markers (default 25)
groupMarkers	a named list with an element for each group comprised of one or more marker genes
kCuts	the number of estimated *cluster* (this defines the height for the tree cut)
distance	type of distance to use. Default is "cosine". Can be chosen among those supported by parallelDist::parDist()

hclustMethod	default is "ward.D2" but can be any method defined by stats::hclust() function
corr	a matrix object, possibly a subset of the columns of the full symmetric matrix
numDegreesOfFreedom	a int that determines the number of degree of freedom to use in the χ^2 test
rowsFraction	The fraction of rows that will be averaged to calculate the GDI. Defaults to 5%
statType	Which statistics to use to compute the p-values. By default it will use the "S" (Pearson's χ^2 test) otherwise the "G" (G-test)
geneSubsetCol	an array of genes. It will be put in columns. If left empty the function will do it genome-wide.
geneSubsetRow	an array of genes. It will be put in rows. If left empty the function will do it genome-wide.

Details

getGDI() extracts the genes' GDI array as it was stored by the method storeGDI()

storeGDI() stored and already calculated genes' GDI array in a COTAN object. It can be retrieved using the method getGDI()

genesCoexSpace() calculates genes groups based on the primary markers and uses them to prepare the genes' COEX space data.frame.

establishGenesClusters() perform the genes' clustering based on a pool of gene markers, using the genes' COEX space

calculateGenesCE() is used to calculate the discrepancy between the expected probability of zero and the observed zeros across all cells for each gene as *cross-entropy*: $-\sum_c \mathbb{1}_{X_c==0} \log(p_c) - \mathbb{1}_{X_c!=0} \log(1-p_c)$ where X_c is the observed count and p_c the probability of zero

calculateGDIGivenCorr() produces a vector with the GDI for each column based on the given correlation matrix, using the *Pearson's χ^2 test*

calculateGDI() produces a data.frame with the GDI for each gene based on the COEX matrix

calculatePValue() computes the p-values for genes in the COTAN object. It can be used genome-wide or by setting some specific genes of interest. By default it computes the *p-values* using the S statistics (χ^2)

calculatePDI() computes the p-values for genes in the COTAN object using calculatePValue() and takes their $\log(-\log(\cdot))$ to calculate the genes' *Pair Differential Index*

Value

getGDI() returns the genes' GDI`` array if available or NULL‘ otherwise

storeGDI() returns the given COTAN object with updated GDI genes' information

genesCoexSpace() returns a list with:

- "SecondaryMarkers" a named list that for each secondary marker, gives the list of primary markers that selected for it
- "GCS" the relevant subset of COEX matrix
- "rankGenes" a data.frame with the rank of each gene according to its *p-value*

establishGenesClusters() a list of:

- "g.space" the genes' COEX space data.frame

- "plot.eig" the eigenvalues plot
- "pca_clusters" the PCA components data.frame
- "tree_plot" the tree plot for the genes' COEX space

calculateGenesCE() returns a named array with the *cross-entropy* of each gene

calculateGDIGivenCorr() returns a vector with the GDI data for each column of the input

calculateGDI() returns a data.frame with:

- "sum.raw.norm" the sum of the normalized data rows
- "GDI" the GDI data
- "exp.cells" the percentage of cells expressing the gene

calculatePValue() returns a *p-value* matrix as dspMatrix

calculatePDI() returns a *Pair Differential Index* matrix as dspMatrix

Examples

```
data("test.dataset")
objCOTAN <- COTAN(raw = test.dataset)
objCOTAN <- proceedToCoex(objCOTAN, cores = 6L, saveObj = FALSE)

markers <- getGenes(objCOTAN)[sample(getNumGenes(objCOTAN), 10)]
GCS <- genesCoexSpace(objCOTAN, primaryMarkers = markers,
                      numGenesPerMarker = 15)

groupMarkers <- list(G1 = c("g-000010", "g-000020", "g-000030"),
                     G2 = c("g-000300"),
                     G3 = c("g-000510", "g-000530", "g-000550",
                            "g-000570", "g-000590"))

resList <-  establishGenesClusters(objCOTAN, groupMarkers = groupMarkers,
                                   numGenesPerMarker = 11)
```

---

getMu *Calculating the COEX matrix for genes and cells*

Description

These are the functions and methods used to calculate the COEX matrices according to the COTAN model. From there it is possible to calculate the associated p-value and the GDI (*Global Differential Expression*)

The COEX matrix is defined by following formula:

$$\frac{\sum_{i,j\in\{\mathrm{Y,N}\}}(-1)^{\#\{i,j\}}\frac{O_{ij}-E_{ij}}{1\vee E_{ij}}}{\sqrt{n\sum_{i,j\in\{\mathrm{Y,N}\}}\frac{1}{1\vee E_{ij}}}}$$

where O and E are the observed and expected contingency tables and n is the relevant number of genes/cells (depending on given actOnCells flag).

The formula can be more effectively implemented as:

$$\sqrt{\frac{1}{n}\sum_{i,j\in\{\mathrm{Y,\,N}\}}\frac{1}{1\vee E_{ij}}\left(O_{\mathrm{YY}}-E_{\mathrm{YY}}\right)}$$

once one notices that $O_{ij}-E_{ij}=(-1)^{\#\{i,j\}}\,r$ for some constant r for all $i,j\in\{\mathrm{Y,\,N}\}$.

The latter follows from the fact that the relevant marginal sums of the expected contingency tables were enforced to match the marginal sums of the observed ones.

The new implementation of the function relies on the `torch` package. This implies that it is potentially able to use the system `GPU` to run the heavy duty calculations required by this method. However installing the `torch` package on a system can be *finicky*, so we tentatively provide a short help page Installing_torch hoping that it will help...

Usage

```
getMu(objCOTAN)

## S4 method for signature 'COTAN'
getGenesCoex(
  objCOTAN,
  genes = vector(mode = "character"),
  zeroDiagonal = TRUE,
  ignoreSync = FALSE
)

## S4 method for signature 'COTAN'
getCellsCoex(
  objCOTAN,
  cells = vector(mode = "character"),
  zeroDiagonal = TRUE,
  ignoreSync = FALSE
)

## S4 method for signature 'COTAN'
isCoexAvailable(objCOTAN, actOnCells = FALSE, ignoreSync = FALSE)

## S4 method for signature 'COTAN'
dropGenesCoex(objCOTAN)

## S4 method for signature 'COTAN'
dropCellsCoex(objCOTAN)

calculateLikelihoodOfObserved(objCOTAN, formula = "raw")

getDataMatrix(objCOTAN, dataMethod = "")

observedContingencyTablesYY(
  objCOTAN,
  actOnCells = FALSE,
  asDspMatrices = FALSE
)
```

```
observedPartialContingencyTablesYY(
  objCOTAN,
  columnsSubset,
  zeroOne = NULL,
  actOnCells = FALSE
)

observedContingencyTables(objCOTAN, actOnCells = FALSE, asDspMatrices = FALSE)

observedPartialContingencyTables(
  objCOTAN,
  columnsSubset,
  zeroOne = NULL,
  actOnCells = FALSE
)

expectedContingencyTablesNN(
  objCOTAN,
  actOnCells = FALSE,
  asDspMatrices = FALSE,
  optimizeForSpeed = TRUE
)

expectedPartialContingencyTablesNN(
  objCOTAN,
  columnsSubset,
  probZero = NULL,
  actOnCells = FALSE,
  optimizeForSpeed = TRUE
)

expectedContingencyTables(
  objCOTAN,
  actOnCells = FALSE,
  asDspMatrices = FALSE,
  optimizeForSpeed = TRUE
)

expectedPartialContingencyTables(
  objCOTAN,
  columnsSubset,
  probZero = NULL,
  actOnCells = FALSE,
  optimizeForSpeed = TRUE
)

contingencyTables(objCOTAN, g1, g2)

## S4 method for signature 'COTAN'
calculateCoex(
  objCOTAN,
  actOnCells = FALSE,
```

```
  returnPPFract = FALSE,
  optimizeForSpeed = TRUE,
  deviceStr = "cuda"
)

calculatePartialCoex(
  objCOTAN,
  columnsSubset,
  probZero = NULL,
  zeroOne = NULL,
  actOnCells = FALSE,
  optimizeForSpeed = TRUE
)

calculateS(
  objCOTAN,
  geneSubsetCol = vector(mode = "character"),
  geneSubsetRow = vector(mode = "character")
)

calculateG(
  objCOTAN,
  geneSubsetCol = vector(mode = "character"),
  geneSubsetRow = vector(mode = "character")
)

getSelectedGenes(objCOTAN, genesSel = "", numGenes = 2000L)

calculateReducedDataMatrix(
  objCOTAN,
  useCoexEigen = FALSE,
  dataMethod = "",
  numComp = 25L,
  genesSel = "",
  numGenes = 2000L
)
```

Arguments

objCOTAN	a COTAN object
genes	The given genes' names to select the wanted COEX columns. If missing all columns will be returned. When not empty a proper result is provided by calculating the partial COEX matrix on the fly
zeroDiagonal	When TRUE sets the diagonal to zero.
ignoreSync	When TRUE ignores whether the lambda/nu/dispersion have been updated since the COEX matrix was calculated.
cells	The given cells' names to select the wanted COEX columns. If missing all columns will be returned. When not empty a proper result is provided by calculating the partial COEX matrix on the fly
actOnCells	Boolean; when TRUE the function works for the cells, otherwise for the genes
formula	a string indicating which function of the likelihood is actually returned. Supported formulas are:

- `"raw"` just the likelihood (default): $p^{(1-z)} \times (1-p)^z = (1.0-z)p+z(1.0-p)$
- `"log"` the log of the likelihood: $(1.0-z)\log(p) + z\log(1.0-p)$
- `"der"` the derivative of the log of the likelihood: $(1.0-z)/p - z/(1.0-p)$
- `"sLog"` the *signed* log of the likelihood: $(1.0-z)\log(p) - z\log(1.0-p)$

where z is the *binarized projection* and p is the *probability of zero*

`dataMethod` selects the method to use to create the `data.frame` to pass to the `UMAPPlot()`. To calculate, for each cell, a statistic for each gene based on available data/model, the following methods are supported:

- `"RW", "Raw", "RawData"` uses the *raw* counts
- `"NN", "NuNorm", "Normalized"` uses the *ν-normalized* counts
- `"LN", "LogNorm", "LogNormalized"` uses the *log-normalized* counts (default)
- `"BI", "Bin", "Binarized"` uses the *binarized* data matrix
- `"BD", "BinDiscr", "BinarizedDiscrepancy"` uses the *difference* between the *binarized* data matrix and the estimated *probability of one*
- `"AB", "AdjBin", "AdjBinarized"` uses the absolute value of the *binarized discrepancy* above
- `"LH", "Like", "Likelihood"` uses the *likelihood* of *binarized* data matrix
- `"LL", "LogLike", "LogLikelihood"` uses the *log-likelihood* of *binarized* data matrix
- `"DL", "DerLogL", "DerivativeLogLikelihood"` uses the *derivative* of the *log-likelihood* of *binarized* data matrix
- `"SL", "SignLogL", "SignedLogLikelihood"` uses the *signed log-likelihood* of *binarized* data matrix

For the last four options see `calculateLikelihoodOfObserved()` for more details

`asDspMatrices` Boolean; when `TRUE` the function will return only packed dense symmetric matrices

`columnsSubset` a sub-set of the columns of the matrices that will be returned

`zeroOne` the raw count matrix projected to `0` or `1`. If not given the appropriate one will be calculated on the fly

`optimizeForSpeed` Boolean; deprecated: always TRUE

`probZero` is the expected **probability of zero** for each gene/cell pair. If not given the appropriate one will be calculated on the fly

`g1` a gene

`g2` another gene

`returnPPFract` Boolean; when `TRUE` the function returns the fraction of genes/cells pairs for which the *expected contingency table* is smaller than 0.5. Default is FALSE

`deviceStr` On the `torch` library enforces which device to use to run the calculations. Possible values are `"cpu"` to us the system *CPU*, `"cuda"` to use the system *GPUs* or something like `"cuda:0"` to restrict to a specific device

`geneSubsetCol` an array of genes. It will be put in columns. If left empty the function will do it genome-wide.

`geneSubsetRow` an array of genes. It will be put in rows. If left empty the function will do it genome-wide.

genesSel — Decides whether and how to perform the gene-selection. used for the clustering and the UMAP. It is a string indicating one of the following selection methods:

- "HGDI" Will pick-up the genes with highest GDI (default)
- "HVG_Seurat" Will pick-up the genes with the highest variability via the **Seurat** package
- "HVG_Scanpy" Will pick-up the genes with the highest variability according to the Scanpy package (using the **Seurat** implementation)

numGenes — the number of genes to select using the above method. Will be ignored when an explicit list of genes has been passed in

useCoexEigen — Boolean to determine whether to project the data matrix onto the first eigenvectors of the **COEX** matrix or instead restrict the data matrix to the selected genes before applying the PCA reduction

numComp — Number of components of the reduced matrix, it defaults to 25L.

Details

getMu() calculates the vector $\mu = \lambda \times \nu^T$

getGenesCoex() extracts a complete (or a partial after genes dropping) genes' COEX matrix from the COTAN object.

getCellsCoex() extracts a complete (or a partial after cells dropping) cells' COEX matrix from the COTAN object.

isCoexAvailable() allows to query whether the relevant COEX matrix from the COTAN object is available to use

dropGenesCoex() drops the genesCoex member from the given COTAN object

dropCellsCoex() drops the cellsCoex member from the given COTAN object

calculateLikelihoodOfObserved() gives for each cell and each gene the likelihood of the observed zero/one data

getDataMatrix() gives for each cell and each gene the result of the selected formula as function of the observed counts and their expected value

observedContingencyTablesYY() calculates observed *Yes/Yes* field of the contingency table

observedPartialContingencyTablesYY() calculates observed *Yes/Yes* field of the contingency table

observedContingencyTables() calculates the observed contingency tables. When the parameter asDspMatrices == TRUE, the method will effectively throw away the lower half from the returned observedYN and observedNY matrices, but, since they are transpose one of another, the full information is still available.

observedPartialContingencyTables() calculates the observed contingency tables.

expectedContingencyTablesNN() calculates the expected *No/No* field of the contingency table

expectedPartialContingencyTablesNN() calculates the expected *No/No* field of the contingency table

expectedContingencyTables() calculates the expected values of contingency tables. When the parameter asDspMatrices == TRUE, the method will effectively throw away the lower half from the returned expectedYN and expectedNY matrices, but, since they are transpose one of another, the full information is still available.

expectedPartialContingencyTables() calculates the expected values of contingency tables, restricted to the specified column sub-set

contingencyTables() returns the observed and expected contingency tables for a given pair of genes. The implementation runs the same algorithms used to calculate the full observed/expected contingency tables, but restricted to only the relevant genes and thus much faster and less memory intensive

calculateCoex() estimates and stores the COEX matrix in the cellCoex or genesCoex field depending on given actOnCells flag. It also calculates the percentage of *problematic* genes/cells pairs. A pair is *problematic* when one or more of the expected counts were significantly smaller than 1 (< 0.5). These small expected values signal that scant information is present for such a pair.

calculatePartialCoex() estimates a sub-section of the COEX matrix in the cellCoex or genesCoex field depending on given actOnCells flag. It also calculates the percentage of *problematic* genes/cells pairs. A pair is *problematic* when one or more of the expected counts were significantly smaller than 1 (< 0.5). These small expected values signal that scant information is present for such a pair.

calculateS() calculates the statistics **S** for genes contingency tables. It always has the diagonal set to zero.

calculateG() calculates the statistics *G-test* for genes contingency tables. It always has the diagonal set to zero. It is proportional to the genes' presence mutual information.

getSelectedGenes() selects the *most representative* genes of the data.set

calculateReducedDataMatrix() calculates the reduced data-matrix to be used for *clusterizations* or UMAP plots.

It uses the given dataMethod to determine with which data to start, then, depending on the value of useCoexEigen, either uses the genesSel to restrict evaluation to the relevant genes' before the PCA is run, or it calculates the first **COEX** eigenvectors and projects the data matrix to their sub-space.

Value

getMu() returns the mu matrix

getGenesCoex() returns the genes' COEX values

getCellsCoex() returns the cells' COEX values

isCoexAvailable() returns whether relevant COEX matrix has been calculated and, in case, if it is still aligned to the estimators.

dropGenesCoex() returns the updated COTAN object

dropCellsCoex() returns the updated COTAN object

calculateLikelihoodOfObserved() returns a matrix with the selected *formula* of the likelihood of the observed zero/one

getDataMatrix() returns a matrix with the same shape as the *raw* data

observedContingencyTablesYY() returns a list with:

- observedYY the *Yes/Yes* observed contingency table as matrix
- observedY the full *Yes* observed vector

observedPartialContingencyTablesYY() returns a list with:

- observedYY the *Yes/Yes* observed contingency table as matrix, restricted to the selected columns as named list with elements
- observedY the full *Yes* observed vector

observedContingencyTables() returns the observed contingency tables as named list with elements:

- "observedNN"
- "observedNY"
- "observedYN"
- "observedYY"

observedPartialContingencyTables() returns the observed contingency tables, restricted to the selected columns, as named list with elements:

- "observedNN"
- "observedNY"
- "observedYN"
- "observedYY"

expectedContingencyTablesNN() returns a list with:

- expectedNN the *No/No* expected contingency table as matrix
- expectedN the *No* expected vector

expectedPartialContingencyTablesNN() returns a list with:

- expectedNN the *No/No* expected contingency table as matrix, restricted to the selected columns, as named list with elements
- expectedN the full *No* expected vector

expectedContingencyTables() returns the expected contingency tables as named list with elements:

- "expectedNN"
- "expectedNY"
- "expectedYN"
- "expectedYY"

expectedPartialContingencyTables() returns the expected contingency tables, restricted to the selected columns, as named list with elements:

- "expectedNN"
- "expectedNY"
- "expectedYN"
- "expectedYY"

contingencyTables() returns a list containing the observed and expected contingency tables

calculateCoex() returns the updated COTAN object

calculatePartialCoex() returns the asked section of the COEX matrix

calculateS() returns the S matrix

calculateG() returns the G matrix

getSelectedGenes() returns an array with the genes' names

calculateReducedDataMatrix() returns the reduced matrix. The returned matrix has dimensions: (number of cells, number of components)

Note

The sum of the matrices returned by the function `observedContingencyTables()` and `expectedContingencyTables()` will have the same value on all elements. This value is the number of genes/cells depending on the parameter `actOnCells` being `TRUE/FALSE`.

See Also

ParametersEstimations for more details.

Installing_torch about the `torch` package

Examples

```
data("test.dataset")
objCOTAN <- COTAN(raw = test.dataset)
objCOTAN <- initializeMetaDataset(objCOTAN, GEO = "test_GEO",
                                  sequencingMethod = "distribution_sampling",
                                  sampleCondition = "reconstructed_dataset")
objCOTAN <- clean(objCOTAN)

objCOTAN <- estimateLambdaLinear(objCOTAN)
objCOTAN <- estimateDispersionBisection(objCOTAN, cores = 6L)

## Now the `COTAN` object is ready to calculate the genes' `COEX`

## mu <- getMu(objCOTAN)
## observedY <- observedContingencyTablesYY(objCOTAN, asDspMatrices = TRUE)
obs <- observedContingencyTables(objCOTAN, asDspMatrices = TRUE)

## expectedN <- expectedContingencyTablesNN(objCOTAN, asDspMatrices = TRUE)
exp <- expectedContingencyTables(objCOTAN, asDspMatrices = TRUE)

objCOTAN <- calculateCoex(objCOTAN, actOnCells = FALSE)

stopifnot(isCoexAvailable(objCOTAN))
genesCoex <- getGenesCoex(objCOTAN)
genesSample <- sample(getNumGenes(objCOTAN), 10)
partialGenesCoex <- calculatePartialCoex(objCOTAN, genesSample,
                                         actOnCells = FALSE)

stopifnot(all(1e-6 >
                abs(partialGenesCoex -
                      getGenesCoex(objCOTAN,
                                   getGenes(objCOTAN)[sort(genesSample)],
                                   zeroDiagonal = FALSE))))

## S <- calculateS(objCOTAN)
## G <- calculateG(objCOTAN)
## pValue <- calculatePValue(objCOTAN)
gdiDF <- calculateGDI(objCOTAN)
objCOTAN <- storeGDI(objCOTAN, genesGDI = gdiDF)

## Touching any of the `lambda`/`nu`/`dispersion` parameters invalidates the
## `COEX` matrix and derivatives, so it can be dropped it from the `COTAN`
## object
objCOTAN <- dropGenesCoex(objCOTAN)
stopifnot(!isCoexAvailable(objCOTAN))
```

```
objCOTAN <- estimateDispersionNuBisection(objCOTAN, cores = 6L)

## Now the `COTAN` object is ready to calculate the cells' `COEX`
## In case one needs to calculate both, it is more sensible to run the above
## before any `COEX` evaluation

g1 <- getGenes(objCOTAN)[sample(getNumGenes(objCOTAN), 1)]
g2 <- getGenes(objCOTAN)[sample(getNumGenes(objCOTAN), 1)]
tables <- contingencyTables(objCOTAN, g1 = g1, g2 = g2)
tables

objCOTAN <- calculateCoex(objCOTAN, actOnCells = TRUE)
stopifnot(isCoexAvailable(objCOTAN, actOnCells = TRUE, ignoreSync = TRUE))
cellsCoex <- getCellsCoex(objCOTAN, zeroDiagonal = FALSE)

cellsSample <- sample(getNumCells(objCOTAN), 10)
partialCellsCoex <- calculatePartialCoex(objCOTAN, cellsSample,
                                         actOnCells = TRUE)

stopifnot(all(1e-6 >
                abs(partialCellsCoex - cellsCoex[, sort(cellsSample)])))

objCOTAN <- dropCellsCoex(objCOTAN)
stopifnot(!isCoexAvailable(objCOTAN, actOnCells = TRUE))

signedLikelhood <- calculateLikelihoodOfObserved(objCOTAN, formula = "sLog")
```

HandleMetaData *Handling* meta-data *in* COTAN *objects*

Description

Much of the information stored in the COTAN object is compacted into three data.frames:

- "metaDataset" - contains all general information about the data-set
- "metaGenes" - contains genes' related information along the lambda and dispersion vectors and the fully-expressed flag
- "metaCells" - contains cells' related information along the nu vector, the fully-expressing flag, the *clusterizations* and the *conditions*

Usage

```
## S4 method for signature 'COTAN'
getMetadataDataset(objCOTAN)

## S4 method for signature 'COTAN'
getMetadataElement(objCOTAN, tag)

## S4 method for signature 'COTAN'
getMetadataGenes(objCOTAN)
```

```
## S4 method for signature 'COTAN'
getMetadataCells(objCOTAN)

## S4 method for signature 'COTAN'
getDims(objCOTAN)

datasetTags()

## S4 method for signature 'COTAN'
initializeMetaDataset(objCOTAN, GEO, sequencingMethod, sampleCondition)

## S4 method for signature 'COTAN'
addElementToMetaDataset(objCOTAN, tag, value)

getColumnFromDF(df, colName)

setColumnInDF(df, colToSet, colName, rowNames = vector(mode = "character"))

getMetaInfoRow(meta, tag)

updateMetaInfo(meta, tag, value)
```

Arguments

objCOTAN	a COTAN object
tag	The tag associated to the wanted value
GEO	a code reporting the GEO identification or other specific data-set code
sequencingMethod	a string reporting the method used for the sequencing
sampleCondition	a string reporting the specific sample condition or time point
value	The value or the values to associate to the tag
df	the data.frame
colName	the name of the new or existing column in the data.frame
colToSet	the column to add
rowNames	when not empty, if the input data.frame has no real row names, the new row names of the resulting data.frame
meta	The information data.frame to update

Details

getMetadataDataset() extracts the meta-data stored for the current data-set.

getMetadataElement() extracts the value associated with the given tag if present or an empty string otherwise.

getMetadataGenes() extracts the meta-data stored for the genes

getMetadataCells() extracts the meta-data stored for the cells

getDims() extracts the sizes of all slots of the COTAN object

datasetTags() defines a list of short names associated to an enumeration. It also defines the relative long names as they appear in the meta-data

initializeMetaDataset() initializes meta-data data-set

addElementToMetaDataset() is used to add a line of information to the meta-data data.frame. If the tag was already used it will update the associated value(s) instead

getColumnFromDF() is a function to extract a column from a data.frame, while keeping the rowNames as vector names

setColumnInDF() is a function to append, if missing, or resets, if present, a column into a data.frame, whether the data.frame is empty or not. The given rowNames are used only in the case the data.frame has only the default row numbers, so this function cannot be used to override row names

getMetaInfoRow() is an internal function: it extracts the row\ associated with the given tag if present or zero otherwise.

updateMetaInfo() is an internal function: updates an information data.frame

Value

getMetadataDataset() returns the meta-data data.frame

getMetadataElement() returns a string with the relevant value

getMetadataGenes() returns the genes' meta-data data.frame

getMetadataCells() returns the cells' meta-data data.frame

getDims() returns a named list with the sizes of the slots

datasetTags() a named character array with the standard labels used in the metaDataset of the COTAN objects

initializeMetaDataset() returns the given COTAN object with the updated metaDataset

addElementToMetaDataset() returns the updated COTAN object

getColumnFromDF() returns the column in the data.frame as named array, NULL if the wanted column is not available

setColumnInDF() returns the updated, or the newly created, data.frame

getMetaInfoRow() returns the last relevant row position if any or zero otherwise.

updateMetaInfo() returns the updated data.frame

Examples

```
data("test.dataset")
objCOTAN <- COTAN(raw = test.dataset)

objCOTAN <- initializeMetaDataset(objCOTAN, GEO = "test_GEO",
                                  sequencingMethod = "distribution_sampling",
                                  sampleCondition = "reconstructed_dataset")

objCOTAN <- addElementToMetaDataset(objCOTAN, "Test",
                                    c("These are ", "some values"))

dataSetInfo <- getMetadataDataset(objCOTAN)

numInitialCells <- getMetadataElement(objCOTAN, datasetTags()[["cells"]])

metaGenes <- getMetadataGenes(objCOTAN)
```

```
metaCells <- getMetadataCells(objCOTAN)

allSizes <- getDims(objCOTAN)
```

HandleStrings *Handle names and factors' levels*

Description

Internal functions dedicated to solve strings or factors related simple tasks

Usage

```
handleNamesSubsets(names, subset = vector(mode = "character"))

conditionsFromNames(names, splitPattern = " ", fragmentNum = 2L)

isEmptyName(name)

niceFactorLevels(v)

factorToVector(f)
```

Arguments

names	The full list of the names to handle
subset	The names' subset. When empty all names are returned instead!
splitPattern	the pattern to use to split the names
fragmentNum	the string fragment to use as condition from the split names
name	the name to check
v	an array or factor object
f	a factor object

Details

handleNamesSubsets() returns the given subset or the full list of names if none were specified

conditionsFromNames() retrieves a condition from the given names by picking the asked fragment after having them split according to the given pattern

isEmptyName() returns whether the passed name is not null and has non-zero characters

niceFactorLevels() provides **nicer** factor labels that have all the same number of characters

factorToVector() converts a *named* factor to a *named* character vector

Value

`handleNamesSubsets()` returns the updated list of names' subset, reordered according to the given names' list

`conditionsFromNames()` returns the extracted conditions

`isEmptyName()` returns whether the passed name is equivalent to an empty string

`niceFactorLevels()` returns a `factor` that is preserving the *names* of the input with the new nicer levels

`factorToVector()` returns a `character vector` that preserves the *names* of the input factor

---

`HandlingClusterizations`
Handling cells' clusterization *and related functions*

Description

These functions manage the *clusterizations* and their associated *cluster* `COEX` `data.frames`.

A *clusterization* is any partition of the cells where to each cell it is assigned a **label**; a group of cells with the same label is called *cluster*.

For each *cluster* is also possible to define a `COEX` value for each gene, indicating its increased or decreased expression in the *cluster* compared to the whole background. A `data.frame` with these values listed in a column for each *cluster* is stored separately for each *clusterization* in the `clustersCoex` member.

The formulae for this *In/Out* `COEX` are similar to those used in the `calculateCoex()` method, with the **role** of the second gene taken by the *In/Out* status of the cells with respect to each *cluster*.

Usage

```
## S4 method for signature 'COTAN'
estimateNuLinearByCluster(objCOTAN, clName = "", clusters = NULL)

## S4 method for signature 'COTAN'
getClusterizations(objCOTAN, dropNoCoex = FALSE, keepPrefix = FALSE)

## S4 method for signature 'COTAN'
getClusterizationName(objCOTAN, clName = "", keepPrefix = FALSE)

## S4 method for signature 'COTAN'
getClusterizationData(objCOTAN, clName = "")

getClusters(objCOTAN, clName = "")

## S4 method for signature 'COTAN'
getClustersCoex(objCOTAN)

## S4 method for signature 'COTAN'
addClusterization(
  objCOTAN,
  clName,
```

```
  clusters,
  coexDF = data.frame(),
  override = FALSE
)

## S4 method for signature 'COTAN'
addClusterizationCoex(objCOTAN, clName, coexDF)

## S4 method for signature 'COTAN'
dropClusterization(objCOTAN, clName)

DEAOnClusters(objCOTAN, clName = "", clusters = NULL)

clusterGeneContingencyTables(objCOTAN, gene, cells)

pValueFromDEA(coexDF, numCells, adjustmentMethod)

logFoldChangeOnClusters(
  objCOTAN,
  clName = "",
  clusters = NULL,
  floorLambdaFraction = 0.05
)

distancesBetweenClusters(
  objCOTAN,
  clName = "",
  clusters = NULL,
  coexDF = NULL,
  useDEA = TRUE,
  distance = NULL
)

UMAPPlot(
  dataIn,
  clusters = NULL,
  elements = NULL,
  title = "",
  colors = NULL,
  numNeighbors = 0L,
  minPointsDist = NaN
)

cellsUMAPPlot(
  objCOTAN,
  clName = "",
  clusters = NULL,
  useCoexEigen = FALSE,
  dataMethod = "",
  numComp = 25L,
  genesSel = "",
  numGenes = 200L,
```

```
  colors = NULL,
  numNeighbors = 0L,
  minPointsDist = NA
)

clustersMarkersHeatmapPlot(
  objCOTAN,
  groupMarkers = list(),
  clName = "",
  clusters = NULL,
  coexDF = NULL,
  kCuts = 3L,
  adjustmentMethod = "bonferroni",
  condNameList = NULL,
  conditionsList = NULL
)

clustersSummaryData(
  objCOTAN,
  clName = "",
  clusters = NULL,
  condName = "",
  conditions = NULL
)

clustersSummaryPlot(
  objCOTAN,
  clName = "",
  clusters = NULL,
  condName = "",
  conditions = NULL,
  plotTitle = ""
)

clustersTreePlot(
  objCOTAN,
  kCuts,
  clName = "",
  clusters = NULL,
  useDEA = TRUE,
  distance = NULL,
  hclustMethod = "ward.D2"
)

findClustersMarkers(
  objCOTAN,
  n = 10L,
  markers = NULL,
  clName = "",
  clusters = NULL,
  coexDF = NULL,
  adjustmentMethod = "bonferroni"
```

```
)

geneSetEnrichment(clustersCoex, groupMarkers = list())

reorderClusterization(
  objCOTAN,
  clName = "",
  clusters = NULL,
  coexDF = NULL,
  reverse = FALSE,
  keepMinusOne = TRUE,
  useDEA = TRUE,
  distance = NULL,
  hclustMethod = "ward.D2"
)
```

Arguments

objCOTAN	a COTAN object
clName	The name of the *clusterization*. If not given the last available *clusterization* will be used, as it is probably the most significant!
clusters	A *clusterization* to use. If given it will take precedence on the one indicated by clName
dropNoCoex	When TRUE drops the names from the *clusterizations* with empty associated COEX data.frame
keepPrefix	When TRUE returns the internal name of the *clusterization*: the one with the CL_ prefix.
coexDF	a data.frame where each column indicates the COEX for each of the *clusters* of the *clusterization*
override	When TRUE silently allows overriding data for an existing *clusterization* name. Otherwise the default behavior will avoid potential data losses
gene	a gene
cells	a sub-set of the cells
numCells	the number of overall cells in all *clusters*
adjustmentMethod	*p-value* multi-test adjustment method, see stats::p.adjust.methods(). Defaults to "bonferroni"; use "none" for no adjustment
floorLambdaFraction	Indicates the lower bound to the average count sums inside or outside the cluster for each gene as fraction of the relevant lambda parameter. Default is 5%
useDEA	Boolean indicating whether to use the *DEA* to define the distance; alternatively it will use the average *Zero-One* counts, that is faster but less precise.
distance	type of distance to use. Default is "cosine" for *DEA* and "euclidean" for *Zero-One*. Can be chosen among those supported by parallelDist::parDist()
dataIn	The matrix to plot. It must have a row names containing the given elements (the columns are features)
elements	a named list of elements to label. Each array in the list will be shown with a different color

title	a string giving the plot title. Will default to UMAP Plot if not specified
colors	an array of colors to use in the plot. If not sufficient colors are given it will complete the list using colors from getColorsVector()
numNeighbors	Overrides the default n_neighbors value
minPointsDist	Overrides the default min_dist value
useCoexEigen	Boolean to determine whether to project the data matrix onto the first eigenvectors of the **COEX** matrix or instead restrict the data matrix to the selected genes before applying the PCA reduction
dataMethod	selects the method to use to create the data.frame to pass to the UMAPPlot(). See getDataMatrix() for more details.
numComp	Number of components of the reduced matrix, it defaults to 25L.
genesSel	Decides whether and how to perform gene-selection. See getSelectedGenes() for more details.
numGenes	the number of genes to select using the above method. Will be ignored when an explicit list of genes has been passed in
groupMarkers	an optional named list with an element for each group comprised of one or more marker genes
kCuts	the number of estimated *cluster* (this defines the height for the tree cut)
condNameList	a list of *conditions*' names to be used for additional columns in the final plot. When none are given no new columns will be added using data extracted via the function clustersSummaryData()
conditionsList	a list of *conditions* to use. If given they will take precedence on the ones indicated by condNameList
condName	The name of a condition in the COTAN object to further separate the cells in more sub-groups. When no condition is given it is assumed to be the same for all cells (no further sub-divisions)
conditions	The *conditions* to use. If given it will take precedence on the one indicated by condName that will only indicate the relevant column name in the returned data.frame
plotTitle	The title to use for the returned plot
hclustMethod	It defaults is "ward.D2" but can be any of the methods defined by the stats::hclust() function.
n	the number of extreme COEX values to return
markers	a list of marker genes
clustersCoex	the COEX data.frame
reverse	a flag to the output order
keepMinusOne	a flag to decide whether to keep the cluster "-1" (representing the non-clustered cells) untouched

Details

estimateNuLinearByCluster() does a linear estimation of nu: cells' counts averages normalized *cluster* by *cluster*

getClusterizations() extracts the list of the *clusterizations* defined in the COTAN object.

`getClusterizationName()` normalizes the given *clusterization* name or, if none were given, returns the name of last available *clusterization* in the `COTAN` object. It can return the *clusterization* **internal name** if needed

`getClusterizationData()` extracts the asked *clusterization* and its associated `COEX` `data.frame` from the `COTAN` object

`getClusters()` extracts the asked *clusterization* from the `COTAN` object

`getClustersCoex()` extracts the full `clusterCoex` member `list`

`addClusterization()` adds a *clusterization* to the current `COTAN` object, by adding a new column in the `metaCells` `data.frame` and adding a new element in the `clustersCoex` `list` using the passed in `COEX` `data.frame` or an empty `data.frame` if none were passed in.

`addClusterizationCoex()` adds a *clusterization* `COEX` `data.frame` to the current `COTAN` object. It requires the named *clusterization* to be already present.

`dropClusterization()` drops a *clusterization* from the current `COTAN` object, by removing the corresponding column in the `metaCells` `data.frame` and the corresponding `COEX` `data.frame` from the `clustersCoex` `list`.

`DEAOnClusters()` is used to run the Differential Expression analysis using the `COTAN` contingency tables on each *cluster* in the given *clusterization*

`clusterGeneContingencyTables()` returns the observed and expected contingency tables for a given gene and a given set of cells (a cluster). The implementation runs the same algorithms used to calculate the full observed/expected contingency tables used for DEA, but restricted to only the relevant gene and cluster, thus much faster and less memory intensive

`pValueFromDEA()` is used to convert to *p-value* the Differential Expression analysis using the `COTAN` contingency tables on each *cluster* in the given *clusterization*

`logFoldChangeOnClusters()` is used to get the log difference of the expression levels for each *cluster* in the given *clusterization* against the rest of the data-set

`distancesBetweenClusters()` is used to obtain a distance between the clusters. Depending on the value of the `useDEA` flag will base the distance on the *DEA* columns or the averages of the *Zero-One* matrix.

`UMAPPlot()` plots the given `data.frame` containing genes information related to clusters after applying the `umap` transformation via `Seurat::RunUMAP()`

`cellsUMAPPlot()` returns a `ggplot2` plot where the given *clusters* are placed on the base of their relative distance. Also if needed calculates and stores the `DEA` of the relevant *clusterization*.

`clustersMarkersHeatmapPlot()` returns the heatmap plot of a summary score for each *cluster* and each gene marker in the given *clusterization*. It also returns the size and percentage of each *cluster* on the right and a *clusterization* `dendogram` on the left, as returned by the function `clustersTreePlot()`. The heatmap cells' colors express the **DEA**, that is whether a gene is enriched or depleted in the cluster, while the stars are aligned to the corresponding adjusted $p-$value: *** for $p < 0.1\%$, ** for $p < 1\%$, * for $p < 5\%$, . for $p < 10\%$

`clustersSummaryData()` calculates various statistics about each cluster (with an optional further `condition` to separate the cells).

`clustersSummaryPlot()` calculates various statistics about each cluster via `clustersSummaryData()` and puts them together into a plot.

`clustersTreePlot()` returns the `dendogram` plot where the given *clusters* are placed on the base of their relative distance. Also if needed calculates and stores the `DEA` of the relevant *clusterization*.

`findClustersMarkers()` takes in a `COTAN` object and a *clusterization* and produces a `data.frame` with the `n` most positively enriched and the `n` most negatively enriched genes for each *cluster*. The

function also provides whether and the found genes are in the given `markers` list or not. It also returns the *adjusted p-value* for multi-tests using the `stats::p.adjust()`

`geneSetEnrichment()` returns a cumulative score of enrichment in a *cluster* over a gene set. In formulae it calculates $\frac{1}{n}\sum_i(1-e^{-\theta X_i})$, where the X_i are the positive values from `DEAOnClusters()` and $\theta = -\frac{1}{0.1}\ln(0.25)$

`reorderClusterization()` takes in a *clusterizations* and reorder its labels so that in the new order near labels indicate near clusters according to a *DEA* (or *Zero-One*) based distance

Value

`estimateNuLinearByCluster()` returns the updated `COTAN` object

`getClusterizations()` returns a vector of *clusterization* names, usually without the `CL_` prefix

`getClusterizationName()` returns the normalized *clusterization* name or `NULL` if no *clusterizations* are present

`getClusterizationData()` returns a `list` with 2 elements:

- `"clusters"` the named cluster labels array
- `"coex"` the associated `COEX` `data.frame`. This will be an **empty** `data.frame` when not specified for the relevant *clusterization*

`getClusters()` returns the named cluster labels array

`getClustersCoex()` returns the list with a `COEX` `data.frame` for each *clusterization*. When not empty, each `data.frame` contains a `COEX` column for each *cluster*.

`addClusterization()` returns the updated `COTAN` object

`addClusterizationCoex()` returns the updated `COTAN` object

`dropClusterization()` returns the updated `COTAN` object

`DEAOnClusters()` returns the co-expression `data.frame` for the genes in each *cluster*

`clusterGeneContingencyTables()` returns a list containing the observed and expected contingency tables

`pValueFromDEA()` returns a `data.frame` containing the *p-values* corresponding to the given `COEX` adjusted for *multi-test*

`logFoldChangeOnClusters()` returns the log-expression-change `data.frame` for the genes in each *cluster*

`distancesBetweenClusters()` returns a `dist` object

`UMAPPlot()` returns a `ggplot2` object

`cellsUMAPPlot()` returns a list with 2 objects:

- `"plot"` a `ggplot2` object representing the `umap` plot
- `"cellsRDM"` the *Reduced Data Matrix* used to create the plot

`clustersMarkersHeatmapPlot()` returns a list with:

- `"heatmapPlot"` the complete heatmap plot
- `"dataScore"` the `data.frame` with the score values
- `"pValueDF"` the `data.frame` with the corresponding adjusted $p-$values

`clustersSummaryData()` returns a `data.frame` with the following statistics: The calculated statistics are:

- "clName" the *cluster* **labels**
- "condName" the relevant condition (that sub-divides the *clusters*)
- "CellNumber" the number of cells in the group
- "MeanUDE" the average UDE in the group of cells
- "MedianUDE" the median UDE in the group of cells
- "ExpGenes25" the number of genes expressed in at the least 25% of the cells in the group
- "ExpGenes" the number of genes expressed at the least once in any of the cells in the group
- "CellPercentage" fraction of the cells with respect to the total cells

clustersSummaryPlot() returns a list with a data.frame and a ggplot objects

- "data" contains the data,
- "plot" is the returned plot

clustersTreePlot() returns a list with 2 objects:

- "dend" a ggplot2 object representing the dendrogram plot
- "objCOTAN" the updated COTAN object

findClustersMarkers() returns a data.frame containing n genes for each *cluster* scoring top/bottom COEX scores. The data.frame also contains:

- "CL" the cluster
- "Gene" the gene
- "Score" the COEX score of the gene
- "adjPVal" the *p-values* associated to the COEX adjusted for *multi-testing*
- "DEA" the differential expression of the gene
- "IsMarker" whether the gene is among the given markers
- "logFoldCh" the *log-fold-change* of the gene expression inside versus outside the cluster from logFoldChangeOnClusters()

geneSetEnrichment() returns a data.frame with the cumulative score

reorderClusterization() returns a list with 3 elements:

- "clusters" the newly reordered cluster labels array
- "coex" the associated COEX data.frame
- "permMap" the reordering mapping

Examples

```
data("test.dataset")
objCOTAN <- COTAN(raw = test.dataset)
objCOTAN <- proceedToCoex(objCOTAN, cores = 6L, calcCoex = FALSE,
                          optimizeForSpeed = TRUE, saveObj = FALSE)

data("test.dataset.clusters1")
clusters <- test.dataset.clusters1

coexDF <- DEAOnClusters(objCOTAN, clusters = clusters)

groupMarkers <- list(G1 = c("g-000010", "g-000020", "g-000030",
```

```
                          "g-000150", "g-000160", "g-000170"),
                    G2 = c("g-000300", "g-000330", "g-000450",
                          "g-000460", "g-000470"),
                    G3 = c("g-000510", "g-000530", "g-000550",
                          "g-000570", "g-000590"))

geneClusters <- rep(1:3, each = 240)[1:600]
names(geneClusters) <- getGenes(objCOTAN)

umapPlot <- UMAPPlot(coexDF, clusters = NULL, elements = groupMarkers)
plot(umapPlot)

objCOTAN <- addClusterization(objCOTAN, clName = "first_clusterization",
                              clusters = clusters, coexDF = coexDF)

lfcDF <- logFoldChangeOnClusters(objCOTAN, clusters = clusters)
umapPlot2 <- UMAPPlot(lfcDF, clusters = geneClusters)
plot(umapPlot2)

objCOTAN <- estimateNuLinearByCluster(objCOTAN, clusters = clusters)

clSummaryPlotAndData <-
  clustersSummaryPlot(objCOTAN, clName = "first_clusterization",
                      plotTitle = "first clusterization")
plot(clSummaryPlotAndData[["plot"]])

if (FALSE) {
  objCOTAN <- dropClusterization(objCOTAN, "first_clusterization")
}

clusterizations <- getClusterizations(objCOTAN, dropNoCoex = TRUE)
stopifnot(length(clusterizations) == 1)

cellsUmapPlotAndDF <- cellsUMAPPlot(objCOTAN, dataMethod = "LogNormalized",
                                    clName = "first_clusterization",
                                    genesSel = "HVG_Seurat")
plot(cellsUmapPlotAndDF[["plot"]])

enrichment <- geneSetEnrichment(clustersCoex = coexDF,
                                groupMarkers = groupMarkers)

clHeatmapPlotAndData <- clustersMarkersHeatmapPlot(objCOTAN, groupMarkers)

conditions <- as.integer(substring(getCells(objCOTAN), 3L))
conditions <- factor(ifelse(conditions <= 600, "L", "H"))
names(conditions) <- getCells(objCOTAN)

clHeatmapPlotAndData2 <-
  clustersMarkersHeatmapPlot(objCOTAN, groupMarkers, kCuts = 2,
                             condNameList = list("High/Low"),
                             conditionsList = list(conditions))

clName <- getClusterizationName(objCOTAN)

clusterDataList <- getClusterizationData(objCOTAN, clName = clName)

clusters <- getClusters(objCOTAN, clName = clName)
```

```
allClustersCoexDF <- getClustersCoex(objCOTAN)

summaryData <- clustersSummaryData(objCOTAN)

treePlotAndObj <- clustersTreePlot(objCOTAN, 2)
objCOTAN <- treePlotAndObj[["objCOTAN"]]
plot(treePlotAndObj[["dend"]])

clMarkers <- findClustersMarkers(objCOTAN, markers = list(),
                                 clusters = clusters)
```

HandlingConditions *Handling cells'* conditions *and related functions*

Description

These functions manage the *conditions*.

A *condition* is a set of **labels** that can be assigned to cells: one **label** per cell. This is especially useful in cases when the data-set is the result of merging multiple experiments' raw data

Usage

```
## S4 method for signature 'COTAN'
getAllConditions(objCOTAN, keepPrefix = FALSE)

## S4 method for signature 'COTAN'
getConditionName(objCOTAN, condName = "", keepPrefix = FALSE)

## S4 method for signature 'COTAN'
getCondition(objCOTAN, condName = "")

normalizeNameAndLabels(objCOTAN, name = "", labels = NULL, isCond = FALSE)

## S4 method for signature 'COTAN'
addCondition(objCOTAN, condName, conditions, override = FALSE)

## S4 method for signature 'COTAN'
dropCondition(objCOTAN, condName)
```

Arguments

objCOTAN	a COTAN object
keepPrefix	When TRUE returns the internal name of the *condition*: the one with the COND_ prefix.
condName	the name of an existing *condition*.
name	the name of the *clusterization*/*condition*. If not given the last available *clusterization* will be used, or no *conditions*
labels	a *clusterization*/*condition* to use. If given it will take precedence on the one indicated by name

isCond	a Boolean to indicate whether the function is dealing with *clusterizations* FALSE or *conditions* TRUE
conditions	a (factors) array of *condition* **labels**
override	When TRUE silently allows overriding data for an existing *condition* name. Otherwise the default behavior will avoid potential data losses

Details

getAllConditions() extracts the list of the *conditions* defined in the COTAN object.

getConditionName() normalizes the given *condition* name or, if none were given, returns the name of last available *condition* in the COTAN object. It can return the *condition* **internal name** if needed

getCondition() extracts the asked *condition* from the COTAN object

normalizeNameAndLabels() takes a pair of name/labels and normalize them based on the available information in the COTAN object

addCondition() adds a *condition* to the current COTAN object, by adding a new column in the metaCells data.frame

dropCondition() drops a *condition* from the current COTAN object, by removing the corresponding column in the metaCells data.frame

Value

getAllConditions() returns a vector of *conditions* names, usually without the COND_ prefix

getConditionName() returns the normalized *condition* name or NULL if no *conditions* are present

getCondition() returns a named factor with the *condition*

normalizeNameAndLabels() returns a list with:

- "name" the relevant name
- "labels" the relevant *clusterization*/*condition*

addCondition() returns the updated COTAN object

dropCondition() returns the updated COTAN object

Examples

```
data("test.dataset")
objCOTAN <- COTAN(raw = test.dataset)

cellLine <- rep(c("A", "B"), getNumCells(objCOTAN) / 2)
names(cellLine) <- getCells(objCOTAN)
objCOTAN <- addCondition(objCOTAN, condName = "Line", conditions = cellLine)

if (FALSE) {
  objCOTAN <- dropCondition(objCOTAN, "Genre")
}

conditionsNames <- getAllConditions(objCOTAN)

condName <- getConditionName(objCOTAN)

condition <- getCondition(objCOTAN, condName = condName)
isa(condition, "factor")
```

```
nameAndCond <- normalizeNameAndLabels(objCOTAN, name = condName,
                                      isCond = TRUE)
isa(nameAndCond[["labels"]], "factor")
```

HeatmapPlots *Heatmap Plots*

Description

These functions create heatmap COEX plots.

Usage

```
singleHeatmapDF(objCOTAN, genesLists, sets, pValueThreshold = 0.01)

heatmapPlot(
  objCOTAN = NULL,
  genesLists,
  sets = NULL,
  pValueThreshold = 0.01,
  conditions = NULL,
  dir = "."
)

genesHeatmapPlot(
  objCOTAN,
  primaryMarkers,
  secondaryMarkers = vector(mode = "character"),
  pValueThreshold = 0.01,
  symmetric = TRUE
)

cellsHeatmapPlot(objCOTAN, cells = NULL, clusters = NULL)

plotTheme(plotKind = "common", textSize = 14L)
```

Arguments

objCOTAN	a COTAN object
genesLists	A list of genes' arrays. The first array defines the genes in the columns
sets	A numeric array indicating which fields in the previous list should be used. Defaults to all fields
pValueThreshold	The p-value threshold. Default is 0.01
conditions	An array of prefixes indicating the different files
dir	The directory in which are all COTAN files (corresponding to the previous prefixes)
primaryMarkers	A set of genes plotted as rows

secondaryMarkers
: A set of genes plotted as columns

symmetric
: A Boolean: default TRUE. When TRUE the union of primaryMarkers and secondaryMarkers is used for both rows and column genes

cells
: Which cells to plot (all if no argument is given)

clusters
: Use this clusterization to select/reorder the cells to plot

plotKind
: a string indicating the plot kind

textSize
: axes and strip text size (default=14)

Details

singleHeatmapDF() creates the heatmap data.frame of one COTAN object

heatmapPlot() creates the heatmap of one or more COTAN objects

genesHeatmapPlot() is used to plot an *heatmap* made using only some genes, as markers, and collecting all other genes correlated with these markers with a p-value smaller than the set threshold. Than all relations are plotted. Primary markers will be plotted as groups of rows. Markers list will be plotted as columns.

cellsHeatmapPlot() creates the heatmap plot of the cells' COEX matrix

plotTheme() returns the appropriate theme for the selected plot kind. Supported kinds are: "common", "pca", "genes", "UDE", "heatmap", "GDI", "UMAP", "size-plot"

Value

singleHeatmapDF() returns a data.frame

heatmapPlot() returns a ggplot2 object

genesHeatmapPlot() returns a ggplot2 object

cellsHeatmapPlot() returns the cells' COEX *heatmap* plot

plotTheme() returns a ggplot2::theme object

See Also

ggplot2::theme() and ggplot2::ggplot()

Examples

```
data("test.dataset")
objCOTAN <- COTAN(raw = test.dataset)
objCOTAN <- clean(objCOTAN)
objCOTAN <- estimateLambdaLinear(objCOTAN)
objCOTAN <- estimateDispersionNuBisection(objCOTAN, cores = 6L)
objCOTAN <- calculateCoex(objCOTAN, actOnCells = FALSE)
objCOTAN <- calculateCoex(objCOTAN, actOnCells = TRUE)

## some genes
primaryMarkers <- c("g-000010", "g-000020", "g-000030")

## an example of named list of different gene set
groupMarkers <- list(G1 = primaryMarkers,
                     G2 = c("g-000300", "g-000330"),
                     G3 = c("g-000510", "g-000530", "g-000550",
                            "g-000570", "g-000590"))
```

```
hPlot <- heatmapPlot(objCOTAN, pValueThreshold = 0.05,
                     genesLists = groupMarkers, sets = 2L:3L)
plot(hPlot)

ghPlot <- genesHeatmapPlot(objCOTAN, primaryMarkers = primaryMarkers,
                           secondaryMarkers = groupMarkers,
                           pValueThreshold = 0.05, symmetric = FALSE)
plot(ghPlot)

clusters <- c(rep_len("1", getNumCells(objCOTAN)/2),
              rep_len("2", getNumCells(objCOTAN)/2))
names(clusters) <- getCells(objCOTAN)

chPlot <- cellsHeatmapPlot(objCOTAN, clusters = clusters)
## plot(chPlot)

theme <- plotTheme("pca")
```

Installing_torch *Installing torch R library (on Linux)*

Description

A brief explanation of how to install the torch package on WSL2 (Windows Subsystem for Linux), but it might work the same for other Linux systems. Naturally it makes a difference whether one wants to install support only for the CPU or also have the system GPU at the ready!

The main resources to install torch is https://torch.mlverse.org/docs/articles/installation.html or https://cran.r-project.org/web/packages/torch/vignettes/installation.html

Details

For the CPU-only support one need to ensure that also numeric libraries are installed, like BLAS and LAPACK and/or MKL if your CPU is from *Intel*. Otherwise torch will be stuck at using a single core for all computations.

For the GPU, currently only cuda devices are supported. Moreover only some specific versions of cuda (and corresponding cudnn) are effectively usable, so one needs to install them to actually use the GPU.

As of today only cuda 11.7 and 11.8 are supported, but check the torch documentation for more up-to-date information. Before downgrading your cuda version, please be aware that it is possible to maintain separate main versions of cuda at the same time on the system: that is one can have installed both 11.8 and a 12.4 cuda versions on the same system.

Below a link to install cuda 11.8 for WSL2 given: use a local installer to be sure the wanted cuda version is being installed, and not the latest one: cuda 11.8 for WSL2

LoggingFunctions *Logging in the* COTAN *package*

Description

Logging is currently supported for all COTAN functions. It is possible to see the output on the terminal and/or on a log file. The level of output on terminal is controlled by the COTAN.LogLevel option while the logging on file is always at its maximum verbosity

Usage

```
setLoggingLevel(newLevel = 1L)

setLoggingFile(logFileName)

logThis(msg, logLevel = 2L, appendLF = TRUE)
```

Arguments

newLevel	the new default logging level. It defaults to 1
logFileName	the log file.
msg	the message to print
logLevel	the logging level of the current message. It defaults to 2
appendLF	whether to add a new-line character at the end of the message

Details

setLoggingLevel() sets the COTAN logging level. It set the COTAN.LogLevel options to one of the following values:

- 0 - Always on log messages
- 1 - Major log messages
- 2 - Minor log messages
- 3 - All log messages

setLoggingFile() sets the log file for all COTAN output logs. By default no logging happens on a file (only on the console). Using this function COTAN will use the indicated file to dump the logs produced by all logThis() commands, independently from the log level. It stores the connection created by the call to bzfile() in the option: COTAN.LogFile

logThis() prints the given message string if the current log level is greater or equal to the given log level (it always prints its message on file if active). It uses message() to actually print the messages on the stderr() connection, so it is subject to suppressMessages()

Value

setLoggingLevel() returns the old logging level or default level if not set yet.

logThis() returns TRUE if the message has been printed on the terminal

Examples

```
setLoggingLevel(3) # for debugging purposes only

logFile <- file.path(".", "COTAN_Test1.log")
setLoggingFile(logFile)
logThis("Some log message")
setLoggingFile("") # closes the log file
file.remove(logFile)

logThis("LogLevel 0 messages will always show, ",
        logLevel = 0, appendLF = FALSE)
suppressMessages(logThis("unless all messages are suppressed",
                         logLevel = 0))
```

`MultiThreading` *Handling Multi-Core and GPU environments*

Description

Check whether session supports multi-core and/or GPU evaluation and utilities about their activation

Usage

```
handleMultiCore(cores)

canUseTorch(optimizeForSpeed, deviceStr)
```

Arguments

`cores`	the number of cores asked for
`optimizeForSpeed`	A Boolean to indicate whether to try to use the faster torch library
`deviceStr`	The name of the device to be used by torch

Details

`handleMultiCore()` uses `parallelly::supportsMulticore()` and `parallelly::availableCores()` to actually check whether the session supports multi-core evaluation. Provides an effective upper bound to the number of cores.

`canUseTorch()` is an internal function to handle the torch library: it returns whether **torch** is ready to be used. It obeys the opt-out flag set via the `COTAN.UseTorch` option

Value

`handleMultiCore()` returns the maximum sensible number of cores to use

`canUseTorch()` returns a list with 2 elements:

- `"useTorch"`: a Boolean indicating whether the torch library can be used
- `"deviceStr"`: the updated name of the device to be used: if no cuda GPU is available it will fallback to CPU calculations

See Also

the help page of `parallelly::supportsMulticore()` about the flags influencing the multi-core support; e.g. the usage of `R` option `parallelly.fork.enable`.

`torch::install_torch()` and `torch::torch_is_installed()` for installation. Note the `torch::torch_set_num_th` has effect also on the **Rfast** package methods

---

`NumericUtilities` *Numeric Utilities*

Description

A set of function helper related to the statistical model underlying the `COTAN` package

Usage

```
funProbZero(dispersion, mu)

dispersionBisection(
  sumZeros,
  lambda,
  nu,
  threshold = 0.001,
  maxIterations = 100L
)

parallelDispersionBisection(
  genes,
  sumZeros,
  lambda,
  nu,
  threshold = 0.001,
  maxIterations = 100L
)

nuBisection(
  sumZeros,
  lambda,
  dispersion,
  initialGuess,
  threshold = 0.001,
  maxIterations = 100L
)

parallelNuBisection(
  cells,
  sumZeros,
  lambda,
  dispersion,
  initialGuess,
  threshold = 0.001,
```

```
  maxIterations = 100L
)

calcDist(data, method, diag = FALSE, upper = FALSE)
```

Arguments

dispersion	the estimated dispersion (a n-sized vector)
mu	the lambda times nu values (a $n \times m$ matrix)
sumZeros	the number of genes not expressed in the relevant cell (a m-sized vector)
lambda	the estimated lambda (a n-sized vector)
nu	the estimated nu (a m-sized vector)
threshold	minimal solution precision
maxIterations	max number of iterations (avoids infinite loops)
genes	names of the relevant genes
initialGuess	the initial guess for nu (a m-sized vector)
cells	names of the relevant cells
data	a matrix or a data.frame of which we want to calculate the distance between columns
method	type of distance to use. Can be chosen among those supported by parallelDist::parDist()
diag	logical value indicating whether the diagonal of the distance matrix should be printed by print.dist.upper
upper	logical value indicating whether the upper triangle of the distance matrix should be printed by print.dist

Details

funProbZero is a private function that gives the probability that a sample gene's reads are zero, given the dispersion and mu parameters.

Using d for disp and μ for mu, it returns: $(1 + d\mu)^{-\frac{1}{d}}$ when $d > 0$ and $\exp((d-1)\mu)$ otherwise. The function is continuous in $d = 0$, increasing in d and decreasing in μ. It returns 0 when $d = -\infty$ or $\mu = \infty$. It returns 1 when $\mu = 0$.

dispersionBisection is a private function for the estimation of dispersion slot of a COTAN object via a bisection solver

The goal is to find a dispersion value that reduces to zero the difference between the number of estimated and counted zeros

parallelDispersionBisection is a private function invoked by estimateDispersionBisection() for the estimation of the dispersion slot of a COTAN object via a parallel bisection solver

The goal is to find a dispersion array that reduces to zero the difference between the number of estimated and counted zeros

nuBisection is a private function for the estimation of nu slot of a COTAN object via a bisection solver

The goal is to find a nu value that reduces to zero the difference between the number of estimated and counted zeros

parallelNuBisection is a private function invoked by estimateNuBisection() for the estimation of nu slot of a COTAN object via a parallel bisection solver

The goal is to find a nu array that reduces to zero the difference between the number of estimated and counted zeros

calcDist is a wrapper function that invokes parallelDist::parDist(): the main goal is to recover and finish the calculations via a fallback when there is a problem with the main algorithm

Value

the probability matrix that a *read count* is identically zero

the dispersion value

the dispersion values

the nu value

the dispersion values

a dist object with all distances

ParametersEstimations *Estimation of the* COTAN *model's parameters*

Description

These functions are used to estimate the COTAN model's parameters. That is the average count for each gene (lambda) the average count for each cell (nu) and the dispersion parameter for each gene to match the probability of zero.

The estimator methods are named Linear if they can be calculated as a linear statistic of the raw data or Bisection if they are found via a parallel bisection solver.

Usage

```
## S4 method for signature 'COTAN'
estimateLambdaLinear(objCOTAN)

## S4 method for signature 'COTAN'
estimateNuLinear(objCOTAN)

## S4 method for signature 'COTAN'
estimateDispersionBisection(
  objCOTAN,
  threshold = 0.001,
  cores = 1L,
  maxIterations = 100L,
  chunkSize = 1024L
)

## S4 method for signature 'COTAN'
estimateNuBisection(
  objCOTAN,
  threshold = 0.001,
  cores = 1L,
  maxIterations = 100L,
  chunkSize = 1024L
```

```
)

## S4 method for signature 'COTAN'
estimateDispersionNuBisection(
  objCOTAN,
  threshold = 0.001,
  cores = 1L,
  maxIterations = 100L,
  chunkSize = 1024L,
  enforceNuAverageToOne = TRUE
)

## S4 method for signature 'COTAN'
estimateDispersionNuNlminb(
  objCOTAN,
  threshold = 0.001,
  maxIterations = 50L,
  chunkSize = 1024L,
  enforceNuAverageToOne = TRUE
)

## S4 method for signature 'COTAN'
getNu(objCOTAN)

## S4 method for signature 'COTAN'
getLambda(objCOTAN)

## S4 method for signature 'COTAN'
getDispersion(objCOTAN)

estimatorsAreReady(objCOTAN)

getNuNormData(objCOTAN)

getLogNormData(objCOTAN)

getNormalizedData(objCOTAN, retLog = FALSE)

getProbabilityOfZero(objCOTAN)
```

Arguments

objCOTAN	a COTAN object
threshold	minimal solution precision
cores	number of cores to use. Default is 1.
maxIterations	max number of iterations (avoids infinite loops)
chunkSize	number of genes to solve in batch in a single core. Default is 1024.
enforceNuAverageToOne	a Boolean on whether to keep the average nu equal to 1
retLog	When TRUE calls getLogNormData(), calls getNuNormData()

Details

`estimateLambdaLinear()` does a linear estimation of `lambda` (genes' counts averages)

`estimateNuLinear()` does a linear estimation of `nu` (normalized cells' counts averages)

`estimateDispersionBisection()` estimates the negative binomial dispersion factor for each gene (`dispersion`). Determines the value such that, for each gene, the probability of zero count matches the number of observed zeros. It assumes `estimateNuLinear()` being already run.

`estimateNuBisection()` estimates the `nu` vector of a `COTAN` object by bisection. It determines the `nu` parameters such that, for each cell, the probability of zero counts matches the number of observed zeros. It assumes `estimateDispersionBisection()` being already run. Since this breaks the assumption that the average `nu` is one, it is recommended not to run this in isolation but use `estimateDispersionNuBisection()` instead.

`estimateDispersionNuBisection()` estimates the `dispersion` and `nu` field of a `COTAN` object by running sequentially a bisection for each parameter.

`estimateDispersionNuNlminb()` estimates the `nu` and `dispersion` parameters to minimize the discrepancy between the observed and expected probability of zero. It uses the `stats::nlminb()` solver, but since the joint parameters have too high dimensionality, it converges too slowly to be actually useful in real cases.

`getNu()` extracts the `nu` array (normalized cells' counts averages)

`getLambda()` extracts the `lambda` array (mean expression for each gene)

`getDispersion()` extracts the `dispersion` array (one value for each gene)

`estimatorsAreReady()` checks whether the estimators arrays `lambda`, `nu`, `dispersion` are available

`getNuNormData()` extracts the *ν-normalized* count table (i.e. where each column is divided by `nu`) and returns it

`getLogNormData()` extracts the *log-normalized* count table (i.e. where each column is divided by the `getCellsSize()`), takes its `log10` and returns it.

`getNormalizedData()` is deprecated: please use `getNuNormData()` or `getLogNormData()` directly as appropriate

`getProbabilityOfZero()` gives for each cell and each gene the probability of observing zero reads

Value

`estimateLambdaLinear()` returns the updated `COTAN` object

`estimateNuLinear()` returns the updated `COTAN` object

`estimateDispersionBisection()` returns the updated `COTAN` object

`estimateNuBisection()` returns the updated `COTAN` object

`estimateDispersionNuBisection()` returns the updated `COTAN` object

`estimateDispersionNuNlminb()` returns the updated `COTAN` object

`getNu()` returns the `nu` array

`getLambda()` returns the `lambda` array

`getDispersion()` returns the `dispersion` array

`estimatorsAreReady()` returns a boolean specifying whether all three arrays are non-empty

`getNuNormData()` returns the *ν-normalized* count `data.frame`

`getLogNormData()` returns a `data.frame` after applying the formula $\log_{10}(10^4 * x + 1)$ to the raw counts normalized by *cells-size*

`getNormalizedData()` returns a `data.frame`

`getProbabilityOfZero()` returns a `data.frame` with the probabilities of zero

Examples

```
data("test.dataset")
objCOTAN <- COTAN(raw = test.dataset)

objCOTAN <- estimateLambdaLinear(objCOTAN)
lambda <- getLambda(objCOTAN)

objCOTAN <- estimateNuLinear(objCOTAN)
nu <- getNu(objCOTAN)

objCOTAN <- estimateDispersionBisection(objCOTAN, cores = 6L)
dispersion <- getDispersion(objCOTAN)

objCOTAN <- estimateDispersionNuBisection(objCOTAN, cores = 6L,
                                          enforceNuAverageToOne = TRUE)
nu <- getNu(objCOTAN)
dispersion <- getDispersion(objCOTAN)

nuNorm <- getNuNormData(objCOTAN)

logNorm <- getLogNormData(objCOTAN)

logNorm <- getNormalizedData(objCOTAN, retLog = TRUE)

probZero <- getProbabilityOfZero(objCOTAN)
```

`RawDataCleaning` *Raw data cleaning*

Description

These methods are to be used to clean the raw data. That is drop any number of genes/cells that are too sparse or too present to allow proper calibration of the `COTAN` model.

We call genes that are expressed in all cells *Fully-Expressed* while cells that express all genes in the data are called *Fully-Expressing*. In case it has been made quite easy to exclude the flagged genes/cells in the user calculations.

Usage

```
## S4 method for signature 'COTAN'
flagNotFullyExpressedGenes(objCOTAN)

## S4 method for signature 'COTAN'
flagNotFullyExpressingCells(objCOTAN)

## S4 method for signature 'COTAN'
getFullyExpressedGenes(objCOTAN)
```

```
## S4 method for signature 'COTAN'
getFullyExpressingCells(objCOTAN)

## S4 method for signature 'COTAN'
findFullyExpressedGenes(objCOTAN, cellsThreshold = 0.99)

## S4 method for signature 'COTAN'
findFullyExpressingCells(objCOTAN, genesThreshold = 0.99)

## S4 method for signature 'COTAN'
dropGenesCells(
  objCOTAN,
  genes = vector(mode = "character"),
  cells = vector(mode = "character")
)

ECDPlot(objCOTAN, yCut = NaN, condName = "", conditions = NULL)

## S4 method for signature 'COTAN'
clean(
  objCOTAN,
  cellsCutoff = 0.003,
  genesCutoff = 0.002,
  cellsThreshold = 0.99,
  genesThreshold = 0.99
)

cleanPlots(objCOTAN, includePCA = TRUE)

screePlot(pcaStdDev)

cellSizePlot(objCOTAN, condName = "", conditions = NULL)

genesSizePlot(objCOTAN, condName = "", conditions = NULL)

mitochondrialPercentagePlot(
  objCOTAN,
  genePrefix = "^MT-",
  condName = "",
  conditions = NULL
)

scatterPlot(objCOTAN, condName = "", conditions = NULL, splitSamples = TRUE)
```

Arguments

objCOTAN	a COTAN object
cellsThreshold	any gene that is expressed in more cells than threshold times the total number of cells will be marked as **fully-expressed**. Default threshold is 0.99 (99.0%)
genesThreshold	any cell that is expressing more genes than threshold times the total number of genes will be marked as **fully-expressing**. Default threshold is 0.99 (99.0%)
genes	an array of gene names

cells	an array of cell names
yCut	y threshold of library size to drop. Default is NaN
condName	The name of a condition in the COTAN object to further separate the cells in more sub-groups. When no condition is given it is assumed to be the same for all cells (no further sub-divisions)
conditions	The *conditions* to use. If given it will take precedence on the one indicated by condName that will only indicate the relevant column name in the returned data.frame
cellsCutoff	clean() will delete from the raw data any gene that is expressed in less cells than threshold times the total number of cells. Default cutoff is 0.003 (0.3%)
genesCutoff	clean() will delete from the raw data any cell that is expressing less genes than threshold times the total number of genes. Default cutoff is 0.002 (0.2%)
includePCA	a Boolean flag to determine whether to calculate the *PCA* associated with the normalized matrix. When TRUE the first four elements of the returned list will be NULL
pcaStdDev	a vector with the standard deviations of the various components
genePrefix	Prefix for the mitochondrial genes (default "^MT-" for Human, mouse "^mt-")
splitSamples	Boolean. Whether to plot each sample in a different panel (default FALSE)

Details

flagNotFullyExpressedGenes() returns a Boolean array with TRUE for those genes that are not fully-expressed.

flagNotFullyExpressingCells()returns a Boolean vector with TRUE for those cells that are not expressing all genes

getFullyExpressedGenes() returns the genes expressed in all cells of the dataset

getFullyExpressingCells() returns the cells that did express all genes of the dataset

findFullyExpressedGenes() determines the fully-expressed genes inside the raw data

findFullyExpressingCells() determines the cells that are expressing all genes in the dataset

dropGenesCells() removes an array of genes and/or cells from the current COTAN object.

ECDPlot() plots the *Empirical Cumulative Distribution* function of library sizes (UMI number). It helps to define where to drop "cells" that are simple background signal.

clean() is the main method that can be used to check and clean the dataset. It will discard any genes that has less than 3 non-zero counts per thousand cells and all cells expressing less than 2 per thousand genes. also produces and stores the estimators for nu

cleanPlots() creates the plots associated to the output of the clean() method.

screePlot() creates a plots showing the explained variance of the components of a PCA

cellSizePlot() plots the raw library size for each cell and sample.

genesSizePlot() plots the raw gene number (reads > 0) for each cell and sample

mitochondrialPercentagePlot() plots the raw library size for each cell and sample.

scatterPlot() creates a plot that check the relation between the library size and the number of genes detected.

Value

`flagNotFullyExpressedGenes()` returns a Booleans array with TRUE for genes that are not fully-expressed

`flagNotFullyExpressingCells()` returns an array of Booleans with TRUE for cells that are not expressing all genes

`getFullyExpressedGenes()` returns an array containing all genes that are expressed in all cells

`getFullyExpressingCells()` returns an array containing all cells that express all genes

`findFullyExpressedGenes()` returns the given `COTAN` object with updated **fully-expressed** genes' information

`findFullyExpressingCells()` returns the given `COTAN` object with updated **fully-expressing** cells' information

`dropGenesCells()` returns a completely new `COTAN` object with the new raw data obtained after the indicated genes/cells were expunged. All remaining data is dropped too as no more relevant with the restricted matrix. Exceptions are:

- the meta-data for the data-set that gets kept unchanged
- the meta-data of genes/cells that gets restricted to the remaining elements. The columns calculated via `estimate` and `find` methods are dropped too

`ECDPlot()` returns an `ECD` plot

`clean()` returns the updated `COTAN` object

`cleanPlots()` returns a `list` of `ggplot2` plots:

- `"pcaCells"` is for `PCA` cells
- `"pcaCellsData"` is the data of the `PCA` cells (can be plotted)
- `"genes"` is for `B` group cells' genes
- `"UDE"` is for cells' `UDE` against their `PCA`
- `"nu"` is for cell `nu`
- `"zoomedNu"` is the same but zoomed on the left and with an estimate for the low `nu` threshold that defines problematic cells

`screePlot()` returns a `ggplot2` plot for the explained variances

`cellSizePlot()` returns the `violin-boxplot` plot

`genesSizePlot()` returns the `violin-boxplot` plot

`mitochondrialPercentagePlot()` returns a `list` with:

- `"plot"` a `violin-boxplot` object
- `"sizes"` a sizes `data.frame`

`scatterPlot()` returns the scatter plot

Examples

```
library(zeallot)

data("test.dataset")
objCOTAN <- COTAN(raw = test.dataset)

genes.to.rem <- getGenes(objCOTAN)[grep('^MT', getGenes(objCOTAN))]
```

```
cells.to.rem <- getCells(objCOTAN)[which(getCellsSize(objCOTAN) == 0)]
objCOTAN <- dropGenesCells(objCOTAN, genes.to.rem, cells.to.rem)

objCOTAN <- clean(objCOTAN)

objCOTAN <- findFullyExpressedGenes(objCOTAN)
goodPos <- flagNotFullyExpressedGenes(objCOTAN)

objCOTAN <- findFullyExpressingCells(objCOTAN)
goodPos <- flagNotFullyExpressingCells(objCOTAN)

feGenes <- getFullyExpressedGenes(objCOTAN)

feCells <- getFullyExpressingCells(objCOTAN)

## These plots might help to identify genes/cells that need to be dropped
ecdPlot <- ECDPlot(objCOTAN, yCut = 100.0)
plot(ecdPlot)

# This creates many infomative plots useful to determine whether
# there is still something to drop...
# Here we use the tuple-like assignment feature of the `zeallot` package
c(pcaCellsPlot, ., genesPlot, UDEPlot, ., zNuPlot) %<-% cleanPlots(objCOTAN)
plot(pcaCellsPlot)
plot(UDEPlot)
plot(zNuPlot)

lsPlot <- cellSizePlot(objCOTAN)
plot(lsPlot)

gsPlot <- genesSizePlot(objCOTAN)
plot(gsPlot)

mitPercPlot <-
  mitochondrialPercentagePlot(objCOTAN, genePrefix = "g-0000")[["plot"]]
plot(mitPercPlot)

scPlot <- scatterPlot(objCOTAN)
plot(scPlot)
```

RawDataGetters *Raw data* COTAN *accessors*

Description

These methods extract information out of a just created COTAN object. The accessors have **read-only** access to the object.

Usage

```
## S4 method for signature 'COTAN'
getRawData(objCOTAN)
```

```
## S4 method for signature 'COTAN'
getNumCells(objCOTAN)

## S4 method for signature 'COTAN'
getNumGenes(objCOTAN)

## S4 method for signature 'COTAN'
getCells(objCOTAN)

## S4 method for signature 'COTAN'
getGenes(objCOTAN)

## S4 method for signature 'COTAN'
getZeroOneProj(objCOTAN)

## S4 method for signature 'COTAN'
getCellsSize(objCOTAN)

## S4 method for signature 'COTAN'
getNumExpressedGenes(objCOTAN)

## S4 method for signature 'COTAN'
getGenesSize(objCOTAN)

## S4 method for signature 'COTAN'
getNumOfExpressingCells(objCOTAN)
```

Arguments

objCOTAN	a COTAN object

Details

getRawData() extracts the raw count table.

getNumCells() extracts the number of cells in the sample (m)

getNumGenes() extracts the number of genes in the sample (n)

getCells() extract all cells in the dataset.

getGenes() extract all genes in the dataset.

getZeroOneProj() extracts the raw count table where any positive number has been replaced with 1

getCellsSize() extracts the cell raw library size.

getNumExpressedGenes() extracts the number of genes expressed for each cell. Exploits a feature of Matrix::CsparseMatrix

getGenesSize() extracts the genes raw library size.

getNumOfExpressingCells() extracts, for each gene, the number of cells that are expressing it. Exploits a feature of Matrix::CsparseMatrix

Value

getRawData() returns the raw count sparse matrix

`getNumCells()` returns the number of cells in the sample (m)

`getNumGenes()` returns the number of genes in the sample (n)

`getCells()` returns a character array with the cells' names

`getGenes()` returns a character array with the genes' names

`getZeroOneProj()` returns the raw count matrix projected to `0` or `1`

`getCellsSize()` returns an array with the library sizes

`getNumExpressedGenes()` returns an array with the library sizes

`getGenesSize()` returns an array with the library sizes

`getNumOfExpressingCells()` returns an array with the library sizes

Examples

```
data("test.dataset")
objCOTAN <- COTAN(raw = test.dataset)

rawData <- getRawData(objCOTAN)

numCells <- getNumCells(objCOTAN)

numGenes <- getNumGenes(objCOTAN)

cellsNames <- getCells(objCOTAN)

genesNames <- getGenes(objCOTAN)

zeroOne <- getZeroOneProj(objCOTAN)

cellsSize <- getCellsSize(objCOTAN)

numExpGenes <- getNumExpressedGenes(objCOTAN)

genesSize <- getGenesSize(objCOTAN)

numExpCells <- getNumOfExpressingCells(objCOTAN)
```

`UniformClusters` *Uniform Clusters*

Description

This group of functions takes in input a `COTAN` object and handle the task of dividing the dataset into **Uniform Clusters**, that is *clusters* that have an homogeneous genes' expression. This condition is checked by calculating the `GDI` of the *cluster* and verifying that no more than a small fraction of the genes have their `GDI` level above the given `GDIThreshold`

Usage

```
GDIPlot(
  objCOTAN,
  genes,
  condition = "",
  statType = "S",
  GDIThreshold = 1.43,
  GDIIn = NULL
)

cellsUniformClustering(
  objCOTAN,
  checker = NULL,
  GDIThreshold = NaN,
  initialResolution = 0.8,
  maxIterations = 25L,
  cores = 1L,
  optimizeForSpeed = TRUE,
  deviceStr = "cuda",
  useDEA = TRUE,
  distance = NULL,
  useCoexEigen = FALSE,
  dataMethod = "",
  genesSel = "HVG_Seurat",
  numGenes = 2000L,
  numReducedComp = 25L,
  hclustMethod = "ward.D2",
  initialClusters = NULL,
  initialIteration = 1L,
  saveObj = TRUE,
  outDir = "."
)

checkClusterUniformity(
  objCOTAN,
  clusterName,
  cells,
  checker,
  cores = 1L,
  optimizeForSpeed = TRUE,
  deviceStr = "cuda",
  saveObj = TRUE,
  outDir = "."
)

mergeUniformCellsClusters(
  objCOTAN,
  clusters = NULL,
  checkers = NULL,
  GDIThreshold = NaN,
  batchSize = 0L,
  cores = 1L,
```

```
  optimizeForSpeed = TRUE,
  deviceStr = "cuda",
  useDEA = TRUE,
  distance = NULL,
  hclustMethod = "ward.D2",
  allCheckResults = data.frame(),
  initialIteration = 1L,
  saveObj = TRUE,
  outDir = "."
)
```

Arguments

objCOTAN	a COTAN object
genes	a named list of genes to label. Each array will have different color.
condition	a string corresponding to the condition/sample (it is used only for the title).
statType	type of statistic to be used. Default is "S": Pearson's chi-squared test statistics. "G" is G-test statistics
GDIThreshold	legacy. The threshold level that is used in a SimpleGDIUniformityCheck. It defaults to 1.43
GDIIn	when the GDI data frame was already calculated, it can be put here to speed up the process (default is NULL)
checker	the object that defines the method and the threshold to discriminate whether a *cluster* is *uniform transcript*. See UniformTranscriptCheckers for more details
initialResolution	a number indicating how refined are the clusters before checking for **uniformity**. It defaults to 0.8, the same as Seurat::FindClusters()
maxIterations	max number of re-clustering iterations. It defaults to 25
cores	number of cores to use. Default is 1.
optimizeForSpeed	Boolean; when TRUE COTAN tries to use the torch library to run the matrix calculations. Otherwise, or when the library is not available will run the slower legacy code
deviceStr	On the torch library enforces which device to use to run the calculations. Possible values are "cpu" to us the system *CPU*, "cuda" to use the system *GPUs* or something like "cuda:0" to restrict to a specific device
useDEA	Boolean indicating whether to use the *DEA* to define the distance; alternatively it will use the average *Zero-One* counts, that is faster but less precise.
distance	type of distance to use. Default is "cosine" for *DEA* and "euclidean" for *Zero-One*. Can be chosen among those supported by parallelDist::parDist()
useCoexEigen	Boolean to determine whether to project the data matrix onto the first eigenvectors of the **COEX** matrix or instead restrict the data matrix to the selected genes before applying the PCA reduction
dataMethod	selects the method to use to create the data.frame to pass to the UMAPPlot(). See getDataMatrix() for more details.
genesSel	Decides whether and how to perform the gene-selection (defaults to "HVG_Seurat"). See getSelectedGenes() for more details.

numGenes — the number of genes to select using the above method. Will be ignored when an explicit list of genes has been passed in

numReducedComp — the number of calculated **RDM** components

hclustMethod — It defaults is "ward.D2" but can be any of the methods defined by the stats::hclust() function.

initialClusters — an existing *clusterization* to use as starting point: the *clusters* deemed **uniform** will be kept and the remaining cells will be processed as normal

initialIteration — the number associated tot he first iteration; it defaults to 1. Useful in case of restart of the procedure to avoid intermediate data override

saveObj — Boolean flag; when TRUE saves intermediate analyses and plots to file

outDir — an existing directory for the analysis output. The effective output will be paced in a sub-folder.

clusterName — the tag of the *cluster*

cells — the cells belonging to the *cluster*

clusters — The *clusterization* to merge. If not given the last available *clusterization* will be used, as it is probably the most significant!

checkers — a list of objects that defines the method and the *increasing* thresholds to discriminate whether to merge two *clusters* if deemed *uniform transcript*. See UniformTranscriptCheckers for more details

batchSize — Number pairs to test in a single round. If none of them succeeds the merge stops. Defaults to $2(\#cl)^{2/3}$

allCheckResults — An optional data.frame with the results of previous checks about the merging of clusters. Useful to restart the *merging* process after an interruption.

Details

GDIPlot() directly evaluates and plots the GDI for a sample.

cellsUniformClustering() finds a **Uniform** *clusterizations* by means of the GDI. Once a preliminary *clusterization* is obtained from the Seurat-package methods, each *cluster* is checked for **uniformity** via the function checkClusterUniformity(). Once all *clusters* are checked, all cells from the **non-uniform** clusters are pooled together for another iteration of the entire process, until all *clusters* are deemed **uniform**. In the case only a few cells are left out (≤ 50), those are flagged as "-1" and the process is stopped.

checkClusterUniformity() takes a COTAN object and a cells' *cluster* and checks whether the latter is **uniform** by looking at the genes' GDI distribution. The function runs checkObjIsUniform() on the given input checker

mergeUniformCellsClusters() takes in a **uniform** *clusterization* and progressively checks whether *merging* two *near clusters* would form a **uniform** *cluster* still. Multiple thresholds will be used from 1.37 up to the given one in order to prioritize merge of the best fitting pairs.

This function uses the *cosine distance* to establish the *nearest clusters pairs*. It will use the checkClusterUniformity() function to check whether the merged *clusters* are **uniform**. The function will stop once no *tested pairs* of clusters can be *merged* after testing all pairs in a single batch

Value

`GDIPlot()` returns a `ggplot2` object with a point got each gene, where on the ordinates are the `GDI` levels and on the abscissa are the average gene expression (log scaled). Also marked are the given *threshold* (in red) and the 50% and 75% quantiles (in blue).

`cellsUniformClustering()` returns a `list` with 2 elements:

- `"clusters"` the newly found cluster labels array
- `"coex"` the associated `COEX` `data.frame`

`checkClusterUniformity` returns a checker object of the same type as the input one, that contains both threshold and results of the check: see UniformTranscriptCheckers for more details

a `list` with:

- `"clusters"` the merged cluster labels array
- `"coex"` the associated `COEX` `data.frame`

Examples

```
data("test.dataset")

objCOTAN <- automaticCOTANObjectCreation(raw = test.dataset,
                                         GEO = "S",
                                         sequencingMethod = "10X",
                                         sampleCondition = "Test",
                                         cores = 6L,
                                         saveObj = FALSE)

groupMarkers <- list(G1 = c("g-000010", "g-000020", "g-000030"),
                     G2 = c("g-000300", "g-000330"),
                     G3 = c("g-000510", "g-000530", "g-000550",
                            "g-000570", "g-000590"))

gdiPlot <- GDIPlot(objCOTAN, genes = groupMarkers, cond = "test")
plot(gdiPlot)

## Here we override the default checker as a way to reduce the number of
## clusters as higher thresholds imply less stringent uniformity checks
##
## In real applications it might be appropriate to do so in the cases when
## the wanted resolution is lower such as in the early stages of the analysis
##

checker <- new("AdvancedGDIUniformityCheck")
stopifnot(identical(checker@firstCheck@GDIThreshold, 1.297))

checker2 <- shiftCheckerThresholds(checker, 0.1)
stopifnot(identical(checker2@firstCheck@GDIThreshold, 1.397))

splitList <- cellsUniformClustering(objCOTAN, cores = 6L,
                                    optimizeForSpeed = TRUE,
                                    deviceStr = "cuda",
                                    initialResolution = 0.8,
                                    checker = checker2,
                                    saveObj = FALSE)
```

```
clusters <- splitList[["clusters"]]

firstCluster <- getCells(objCOTAN)[clusters %in% clusters[[1L]]]

checkerRes <-
  checkClusterUniformity(objCOTAN, checker = checker2,
                         clusterName = clusters[[1L]], cells = firstCluster,
                         cores = 6L, optimizeForSpeed = TRUE,
                         deviceStr = "cuda", saveObj = FALSE)

objCOTAN <- addClusterization(objCOTAN,
                              clName = "split",
                              clusters = clusters,
                              coexDF = splitList[["coex"]],
                              override = FALSE)

stopifnot(identical(reorderClusterization(objCOTAN)[["clusters"]], clusters))

## It is possible to pass a list of checkers tot the merge function that will
## be applied each to the *resulting* merged *clusterization* obtained using
## the previous checker. This ensures that the most similar clusters are
## merged first improving the overall performance

mergedList <- mergeUniformCellsClusters(objCOTAN,
                                        checkers = c(checker, checker2),
                                        batchSize = 2L,
                                        clusters = clusters,
                                        cores = 6L,
                                        optimizeForSpeed = TRUE,
                                        deviceStr = "cpu",
                                        distance = "cosine",
                                        hclustMethod = "ward.D2",
                                        saveObj = FALSE)

objCOTAN <- addClusterization(objCOTAN,
                              clName = "merged",
                              clusters = mergedList[["clusters"]],
                              coexDF = mergedList[["coex"]],
                              override = TRUE)

stopifnot(identical(reorderClusterization(objCOTAN)[["clusters"]],
                    mergedList[["clusters"]]))
```

`UniformTranscriptCheckers`

Definition of the **Transcript Uniformity Checker** *classes*

Description

A hierarchy of classes to specify the method for checking whether a **cluster** has the *Uniform Transcript* property. It also doubles as result object.

`getCheckerThreshold()` extracts the main `GDI` threshold from the given checker object

`calculateThresholdShiftToUniformity()` calculates by how much the `GDI` thresholds in the given checker must be increased in order to have that the relevant cluster is deemed **uniform transcript**

`shiftCheckerThresholds()` returns a new checker object where the `GDI` thresholds where increased in order to *relax* the conditions to achieve **uniform transcript**

Usage

```
## S4 method for signature 'SimpleGDIUniformityCheck'
checkObjIsUniform(currentC, previousC = NULL, objCOTAN = NULL)

## S4 method for signature 'AdvancedGDIUniformityCheck'
checkObjIsUniform(currentC, previousC = NULL, objCOTAN = NULL)

checkersToDF(checkers)

dfToCheckers(df, checkerClass = "")

## S4 method for signature 'SimpleGDIUniformityCheck'
getCheckerThreshold(checker)

## S4 method for signature 'AdvancedGDIUniformityCheck'
getCheckerThreshold(checker)

## S4 method for signature 'SimpleGDIUniformityCheck'
calculateThresholdShiftToUniformity(checker)

## S4 method for signature 'AdvancedGDIUniformityCheck'
calculateThresholdShiftToUniformity(checker)

## S4 method for signature 'SimpleGDIUniformityCheck,numeric'
shiftCheckerThresholds(checker, shift)

## S4 method for signature 'AdvancedGDIUniformityCheck,numeric'
shiftCheckerThresholds(checker, shift)
```

Arguments

`currentC`	the object that defines the method and the threshold to discriminate whether a *cluster* is *uniform transcript*.
`previousC`	the optional result object of an already done check
`objCOTAN`	an optional `COTAN` object
`checkers`	a `list` of objects that defines the method, the thresholds and the results of the checks to discriminate whether a *cluster* is deemed *uniform transcript*.
`df`	a `data.frame` with col-names being the member names and row-names the names attached to each checker
`checkerClass`	the type of the checker to be reconstructed from the given `data.frame`
`checker`	An checker object that defines how to check for *uniform transcript*. It is derived from BaseUniformityCheck
`shift`	The amount by which to shift the `GDI` thresholds in the checker

Details

`BaseUniformityCheck` is the base class of the check methods

`GDICheck` represents a single unit check using `GDI` data. It defaults to an *above* check with threshold 1.4 and ratio 1%

`SimpleGDIUniformityCheck` represents the simplified (and legacy) mechanism to determine whether a cluster has the *Uniform Transcript* property

The method is based on checking whether the fraction of the genes' `GDI` below the given *threshold* is less than the given *ratio*

`AdvancedGDIUniformityCheck` represents the more precise and advanced mechanism to determine whether a cluster has the *Uniform Transcript* property

The method is based on checking the genes' `GDI` against three *thresholds*: if a cluster fails the first **below** check is deemed not *uniform*. Otherwise if it passes either of the other two checks (one above and one below) it is deemed *uniform*.

`checkObjIsUniform()` performs the check whether the given object is uniform according to the given checker

`checkersToDF()` converts a `list` of checkers (i.e. objects that derive from `BaseUniformityCheck`) into a `data.frame` with the values of the members

`dfToCheckers()` converts a `data.frame` of checkers values into an array of checkers ensuring given `data.frame` is compatible with member types

Value

a copy of `currentC` with the results of the check. Note that the slot `clusterSize` will be set to zero if it is not possible to get the result of the check

a `data.frame` with col-names being the member names and row-names the names attached to each checker

`dfToCheckers()` returns a `list` of checkers of the requested type, each created from one of `data.frame` rows

`getCheckerThreshold()` returns the appropriate member of the checker object representing the main `GDI` threshold

`calculateThresholdShiftToUniformity()` returns the positive shift that would make the `@isUniform` slot `TRUE` in the checker. It returns zero if the result is already `TRUE` and `NaN` in case no such shift can exist (e.g. the check have been not done yet)

`shiftCheckerThresholds()` returns a copy of the checker object where all `GDI` thresholds have been shifted by the same given `shift` amount

Slots

`isUniform` Logical. Output. The result of the check

`clusterSize` Integer. Output. The number of cells in the checked cluster. When zero implies no check has been run yet

`isCheckAbove` Logical. Determines how to compare quantiles against given thresholds. It is deemed passed if the relevant quantile is above/below the given threshold

`GDIThreshold` Numeric. The level of `GDI` beyond which the **cluster** is deemed not uniform. Defaults

`maxRatioBeyond` Numeric. The maximum fraction of the empirical `GDI` distribution that sits beyond the `GDI` threshold

`maxRankBeyond` Integer. The minimum rank in the empirical `GDI` distribution for the `GDI` threshold

`fractionBeyond` Numeric. Output. The fraction of genes whose `GDI` is above the threshold

`thresholdRank` Integer. Output. The rank that the `GDI` threshold would have in the genes' `GDI vector`

`quantileAtRatio` Numeric. Output. The quantile in the genes' `GDI` corresponding at the given ratio

`quantileAtRatio` Numeric. Output. The quantile in the genes' `GDI` corresponding at the given rank

`check` `GDICheck`. The single threshold check used to determine whether the **cluster** is deemed not *uniform*. Threshold defaults to 1.4, `maxRatioBeyond` to 1%

`firstCheck` `GDICheck`. Single threshold below check used to determine whether the **cluster** is deemed not *uniform*. Threshold defaults to 1.297, `maxRatioBeyond` to 5%

`secondCheck` `GDICheck`. Single threshold above check used to determine whether the **cluster** is deemed *uniform*. Threshold defaults to 1.307, `maxRatioBeyond` to 2%

`thirdCheck` `GDICheck`. Single threshold below check used to determine whether the **cluster** is deemed *uniform*. Threshold defaults to 1.4, `maxRatioBeyond` to 1%

`fourthCheck` `GDICheck`. Single threshold below check used to determine whether the **cluster** is deemed *uniform*. Threshold defaults to 1.4, `maxRankBeyond` to 2

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