

# Package ‘COTAN’

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**Type** Package

**Title** COexpression Tables ANalysis

**Version** 2.11.4

**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.

**URL** <https://github.com/seriph78/COTAN>

**BugReports** <https://github.com/seriph78/COTAN/issues>

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ClustersList	Clusters <i>utilities</i>
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## 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

<code>clusters</code>	A named vector or factor that defines the <i>clusters</i>
<code>allCells</code>	A vector of cells' names that should list the same names in the clusters in any order
<code>clustersList</code>	A named list whose elements define the various clusters
<code>elemNames</code>	A list of names to which associate a cluster
<code>throwOnOverlappingClusters</code>	When TRUE, in case of overlapping clusters, the function <code>fromClustersList</code> and <code>groupByClustersList</code> will throw. This is the default. When FALSE, instead, in case of overlapping clusters, <code>fromClustersList</code> will return the last

	cluster to which each element belongs, while <code>groupByClustersList</code> will return a vector of positions that is longer than the given <code>elemNames</code>
<code>names</code>	A list of <i>clusters</i> names to be merged
<code>mergedName</code>	The name of the new merged clusters
<code>namesList</code>	A list of lists of <i>clusters</i> names to be respectively merged
<code>mergedNames</code>	The names of the new merged <i>clusters</i>

### 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)

c11Size <- length(clustersList[["1"]])

## establish the permutation that groups clusters together
perm <- groupByClusters(clusters)
!is.unsorted(head(names(clusters)[perm],c11Size))
head(clusters[perm], c11Size)

## 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,
  assayName = "counts",
  clNamesPattern = "",
  condNamesPattern = "",

```

```

genesNamesPattern = "",
cellsIDsPattern = ""
)

```

### Arguments

<code>objCOTAN</code>	a COTAN object
<code>objSCE</code>	A <code>SingleCellExperiment::SingleCellExperiment</code> object to be converted
<code>assayName</code>	Name of the assay in <code>objSCE</code> to be used as raw counts. Default is "counts". If not found, the function falls back to "X" and then to the first available assay, emitting a warning in both cases.
<code>clNamesPattern</code>	A regular expression pattern used to identify the clustering columns in <code>colData</code> . Default supports Seurat conventions: <code>"^(COTAN_clusters_ seurat_clusters\$ *_snn_res\\.\\.\\.*) w"</code>
<code>condNamesPattern</code>	A regular expression pattern used to identify the condition columns in <code>colData</code> . Default supports Seurat conventions: <code>"^(COTAN_conditions_ condition\$ orig.ident\$)"</code>
<code>genesNamesPattern</code>	A regular expression pattern (case insensitive) used to identify the genes' names column in <code>rowData</code> . It used only if no names are available from the data matrix or the genes' data-set. Default supports is: <code>"^((Gene Feature)s?[ _-]?)(Name ID)s? IDs? Symbols?"</code>
<code>cellsIDsPattern</code>	A regular expression pattern (case insensitive) used to identify the cells' names column in <code>colData</code> . It used only if no names are available from the data matrix or the cells' data-set. Default supports is: <code>"^(Cells?[ _-]?IDs? Barcodes? Cells?)\$"</code>

### 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)[-5:-6], getDims(obj)[-5:-6]),
          identical(unlist(getDims(newObj)[5:6]),
                    unlist(getDims(obj)[5:6]) + 1L))
```

---

COTAN-class

*The COTAN class*

---

### Description

Definition of the COTAN class

### Value

A COTAN object.

**Slots**

raw dgCMatrix - the raw UMI count matrix  $n \times m$  (gene number  $\times$  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	<i>Handle legacy sCCOTAN-class and related symmetric matrix &lt;-&gt; vector conversions</i>
--------------	--

---

**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 <i>clusterization</i> . If not given the last available <i>clusterization</i> will be used, as it is probably the most significant!
clusters	A <i>clusterization</i> 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  $\nu$  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 = FALSE,
  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 = FALSE,
    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 <code>estimateDispersionViaSolver()</code> 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 use 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	<code>clean()</code> 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	<code>clean()</code> 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 <b>fully-expressed</b> . 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 <b>fully-expressing</b> . 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)

options(parallelly.fork.enable = TRUE)

#
# In case one needs to run more steps to clean the dataset
# 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.cells.origin)
data(vignette.split.clusters)
data(vignette.merge.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.cells.origin` is a factor  
`vignette.split.clusters` is a factor  
`vignette.merge.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.cells.origin` is the factor discriminating between the *cortical* cells and the *other* cells. This was constructed from the following dataset: Mouse Cortex E17.5, GEO: GSM2861514 <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM2861514>

`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) starting from the `vignette.split.clusters` *clusterization*, with a sequence of progressively relaxed *UT* checks

### Value

Objects loaded by `data()`:

- `raw.dataset, ERCCraw, test.dataset`: `data.frames`
- `test.dataset.clusters1, test.dataset.clusters2`: character vectors of cluster labels
- `vignette.split.clusters, vignette.merge.clusters, vignette.cells.origin`: factors of cluster labels

### Source

GEO GSM2861514

ERCC

---

<code>getColorsVector</code>	<code>getColorsVector</code>
------------------------------	------------------------------

---

### 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)
```

```

calculateGDIgivenS(S, rowsFraction = 0.05, cores = 1L, chunkSize = 1024L)

calculateGDIgivenCorr(corr, numDegreesOfFreedom, rowsFraction = 0.05)

calculateGDI(
  objCOTAN,
  statType = "S",
  rowsFraction = 0.05,
  cores = 1L,
  chunkSize = 1024L
)

calculatePValue(
  objCOTAN,
  statType = "S",
  geneSubsetCol = vector(mode = "character"),
  geneSubsetRow = vector(mode = "character"),
  cores = 1L,
  chunkSize = 1024L
)

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 <a href="#">calculateGDI()</a>
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 <i>cluster</i> (this defines the height for the tree cut)
distance	type of distance to use. Default is "cosine". Can be chosen among those supported by <a href="#">parallelDist::parDist()</a>
hclustMethod	default is "ward.D2" but can be any method defined by <a href="#">stats::hclust()</a> function
S	a matrix object
rowsFraction	The fraction of rows that will be averaged to calculate the GDI. Defaults to 5%

cores	number of cores to use. Default is 1.
chunkSize	number of elements to solve in batch in a single core. Default is 1024.
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 $\chi^2$ test
statType	Which statistics to use to compute the p-values. By default it will use the "S" (Pearson's $\chi^2$ 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*:  $-\sum_c \mathbb{1}_{X_c=0} \log(p_c) - \mathbb{1}_{X_c \neq 0} \log(1 - p_c)$  where  $X_c$  is the observed count and  $p_c$  the probability of zero

calculateGDIGivenS() produces a vector with the GDI for each column based on the S matrix (*Pearson's  $\chi^2$  test*)

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

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



## 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:

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

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:

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

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

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: <ul style="list-style-type: none"> <li>• "raw" just the likelihood (default): <math>p^{(1-z)} \times (1-p)^z = (1.0-z)p + z(1.0-p)</math></li> <li>• "log" the log of the likelihood: <math>(1.0-z) \log(p) + z \log(1.0-p)</math></li> <li>• "der" the derivative of the log of the likelihood: <math>(1.0-z)/p - z/(1.0-p)</math></li> <li>• "sLog" the <i>signed</i> log of the likelihood: <math>(1.0-z) \log(p) - z \log(1.0-p)</math></li> </ul> where $z$ is the <i>binarized projection</i> and $p$ is the <i>probability of zero</i>
dataMethod	selects the method to use to create the data.frame to pass to the <code>UMAPPlot()</code> . To calculate, for each cell, a statistic for each gene based on available data/model, the following methods are supported: <ul style="list-style-type: none"> <li>• "RW", "Raw", "RawData" uses the <i>raw</i> counts</li> <li>• "NN", "NuNorm", "Normalized" uses the <i><math>\nu</math>-normalized</i> counts</li> <li>• "LN", "LogNorm", "LogNormalized" uses the <i>log-normalized</i> counts (default)</li> </ul>

- "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 <b>probability of zero</b> 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 <i>expected contingency table</i> 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 use the system <i>CPU</i> , "cuda" to use the system <i>GPUs</i> 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: <ul style="list-style-type: none"> <li>• "HGDI" Will pick-up the genes with highest GDI (default)</li> <li>• "HVG_Seurat" Will pick-up the genes with the highest variability via the <b>Seurat</b> package</li> <li>• "HVG_Scanpy" Will pick-up the genes with the highest variability according to the Scanpy package (using the <b>Seurat</b> implementation)</li> </ul>
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 <b>COEX</b> 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**

```
options(parallelly.fork.enable = TRUE)

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 <- estimateDispersionViaSolver(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(5.0e-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)[138L]
tables <- contingencyTables(objCOTAN, g1 = g1, g2 = g2)
tables

objCOTAN <- calculateCoex(objCOTAN, actOnCells = TRUE,
                          optimizeForSpeed = FALSE)
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))

signedLikelihood <- 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 <code>data.frame</code> has no real row names, the new row names of the resulting <code>data.frame</code>
meta	The information <code>data.frame</code> 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**

```
options(parallelly.fork.enable = TRUE)

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

<code>clName</code>	The name of the <i>clusterization</i> . If not given the last available <i>clusterization</i> will be used, as it is probably the most significant!
<code>clusters</code>	A <i>clusterization</i> to use. If given it will take precedence on the one indicated by <code>clName</code>
<code>dropNoCoex</code>	When TRUE drops the names from the <i>clusterizations</i> with empty associated COEX data. <code>frame</code>
<code>keepPrefix</code>	When TRUE returns the internal name of the <i>clusterization</i> : the one with the <code>CL_</code> prefix.
<code>coexDF</code>	a <code>data.frame</code> where each column indicates the COEX for each of the <i>clusters</i> of the <i>clusterization</i>
<code>override</code>	When TRUE silently allows overriding data for an existing <i>clusterization</i> name. Otherwise the default behavior will avoid potential data losses
<code>gene</code>	a gene
<code>cells</code>	a sub-set of the cells
<code>numCells</code>	the number of overall cells in all <i>clusters</i>
<code>adjustmentMethod</code>	<i>p-value</i> multi-test adjustment method, see <code>stats::p.adjust.methods()</code> . Defaults to "bonferroni"; use "none" for no adjustment
<code>floorLambdaFraction</code>	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%
<code>useDEA</code>	Boolean indicating whether to use the <i>DEA</i> to define the distance; alternatively it will use the average <i>Zero-One</i> counts, that is faster but less precise.
<code>distance</code>	type of distance to use. Default is "cosine" for <i>DEA</i> and "euclidean" for <i>Zero-One</i> . Can be chosen among those supported by <code>parallelDist::parDist()</code>
<code>dataIn</code>	The matrix to plot. It must have a row names containing the given elements (the columns are features)
<code>elements</code>	a named list of elements to label. Each array in the list will be shown with a different color
<code>title</code>	a string giving the plot title. Will default to UMAP Plot if not specified
<code>colors</code>	an array of colors to use in the plot. If not sufficient colors are given it will complete the list using colors from <code>getColorVector()</code>
<code>numNeighbors</code>	Overrides the default <code>n_neighbors</code> value
<code>minPointsDist</code>	Overrides the default <code>min_dist</code> value
<code>useCoexEigen</code>	Boolean to determine whether to project the data matrix onto the first eigenvectors of the <b>COEX</b> matrix or instead restrict the data matrix to the selected genes before applying the PCA reduction
<code>dataMethod</code>	selects the method to use to create the <code>data.frame</code> to pass to the <code>UMAPPlot()</code> . See <code>getDataMatrix()</code> for more details.
<code>numComp</code>	Number of components of the reduced matrix, it defaults to 25L.
<code>genesSel</code>	Decides whether and how to perform gene-selection. See <code>getSelectedGenes()</code> 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 <i>cluster</i> (this defines the height for the tree cut)
condNameList	a list of <i>conditions</i> ' 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 <code>clustersSummaryData()</code>
conditionsList	a list of <i>conditions</i> to use. If given they will take precedence on the ones indicated by <code>condNameList</code>
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 <i>conditions</i> to use. If given it will take precedence on the one indicated by <code>condName</code> that will only indicate the relevant column name in the returned <code>data.frame</code>
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 <code>stats::hclust()</code> function.
n	the number of extreme COEX values to return
markers	a list of marker genes
clustersCoex	the COEX <code>data.frame</code>
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* dendrogram 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 dendrogram 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:

- "c1Name" 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



```

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)

if (FALSE) {
  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 = "LogLikelihood",
                                   useCoexEigen = TRUE, numComp = 25L,
                                   clName = "first_clusterization")
plot(cellsUmapPlotAndDF[["plot"]])

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

clHeatmapPlotAndData <- clustersMarkersHeatmapPlot(objCOTAN, groupMarkers)
clHeatmapPlotAndData[["heatmapPlot"]]

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

objCOTAN <- addCondition(objCOTAN, condName = "High/Low",
                       conditions = conditions)

clHeatmapPlotAndData2 <-

```

```

clustersMarkersHeatmapPlot(objCOTAN, groupMarkers, kCuts = 2,
                           condNameList = list("High/Low"))
clHeatmapPlotAndData2[["heatmapPlot"]]

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 <i>condition</i> : the one with the COND_ prefix.
condName	the name of an existing <i>condition</i> .
name	the name of the <i>clusterization/condition</i> . If not given the last available <i>clusterization</i> will be used, or no <i>conditions</i>
labels	a <i>clusterization/condition</i> 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 <i>clusterizations</i> FALSE or <i>conditions</i> TRUE
conditions	a (factors) array of <i>condition labels</i>
override	When TRUE silently allows overriding data for an existing <i>condition</i> 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, cores = 1L)

heatmapPlot(
  objCOTAN = NULL,
  genesLists,
  sets = NULL,
  pValueThreshold = 0.01,
  cores = 1L,
  conditions = NULL,
  dir = "."
)

genesHeatmapPlot(
  objCOTAN,
  primaryMarkers,

```

```

    secondaryMarkers = vector(mode = "character"),
    pValueThreshold = 0.01,
    symmetric = TRUE,
    cores = 1L
)

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
cores	number of cores to use. Default is 1.
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,
                          optimizeForSpeed = FALSE)

## some genes
primaryMarkers <- c("g-000010", "g-000020", "g-000138")

## 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, cores = 3L,
                    genesLists = groupMarkers, sets = 2L:3L)
plot(hPlot)

ghPlot <- genesHeatmapPlot(objCOTAN, primaryMarkers = primaryMarkers,
                          secondaryMarkers = groupMarkers, cores = 3L,
                          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")
```

## 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!

These are just *suggestions*, no guarantees are given: **try at your own peril!**

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.

At the time of writing only cuda 12.8 is 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.

Below a link to install cuda 12.8 for WSL2 given: use a local installer to be sure the wanted cuda version is being installed, and not the latest one: [cuda 12.8 for WSL2](#)

It can happen that after the installation of the new torch version, including the dependencies, torch actually fails to run claiming that lantern dependency was not installed. This can happen due to presence of old cache files, so one can simply delete the relevant cache folder as in the example below...

## Value

No return value. This topic is documentation-only.

## References

- torch installation guide: <https://torch.mlverse.org/docs/articles/installation.html>
- CRAN torch installation vignette: <https://cran.r-project.org/web/packages/torch/vignettes/installation.html>
- NVIDIA CUDA 12.8 for WSL2 download archive: [https://developer.nvidia.com/cuda-12-8-0-download-archi?target\\_os=Linux&target\\_arch=x86\\_64&Distribution=WSL-Ubuntu&target\\_version=2.0&target\\_type=deb\\_local](https://developer.nvidia.com/cuda-12-8-0-download-archi?target_os=Linux&target_arch=x86_64&Distribution=WSL-Ubuntu&target_version=2.0&target_type=deb_local)

### Examples

```
# find the relevant cache folder and delete it
folder <- tools::R_user_dir("torch","cache")
print(folder)
unlink(folder, recursive = TRUE, force = TRUE)

# reinstall explicitly for the toolchain you want
torch::install_torch(cuda_version = "12.8", reinstall = TRUE)

# run this only **AFTER** restarting `R`
COTAN::canUseTorch(TRUE, "cuda")
```

---

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

<code>newLevel</code>	the new default logging level. It defaults to 1
<code>logFileName</code>	the log file.
<code>msg</code>	the message to print
<code>logLevel</code>	the logging level of the current message. It defaults to 2
<code>appendLF</code>	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

`setLogLevel()` 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

```
setLogLevel(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))
```

### 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 [parallely::supportsMulticore\(\)](#) and [parallely::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 [parallely::supportsMulticore\(\)](#) about the flags influencing the multi-core support; e.g. the usage of R option `parallely.fork.enable`.

[torch::install\\_torch\(\)](#) and [torch::torch\\_is\\_installed\(\)](#) for installation. Note the [torch::torch\\_set\\_num\\_threads\(\)](#) 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
)

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

parallelDispersionNewton(
  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 <code>parallelDist::parDist()</code>
diag	logical value indicating whether the diagonal of the distance matrix should be printed by <code>print.dist.upper</code>
upper	logical value indicating whether the upper triangle of the distance matrix should be printed by <code>print.dist</code>

## 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  $\mu$  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  $\mu$ . 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 that was invoked by `estimateDispersionViaSolver()` for the estimation of the dispersion slot of a COTAN object via a parallel bisection solver. It is now deprecated

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

`dispersionNewton` is a private function for the estimation of dispersion slot of a COTAN object via a Newton-Raphson solver

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

`parallelDispersionNewton` is a private function invoked by `parallelDispersionNewton()` for the estimation of the dispersion slot of a COTAN object via a parallel Newton-Raphson 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 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'
estimateDispersionViaSolver(
  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

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

### Details

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

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

`estimateDispersionViaSolver()` 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 `estimateDispersionViaSolver()` 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  $\log_{10}$  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  
`estimateDispersionViaSolver()` 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  $\nu$ -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 <- estimateDispersionViaSolver(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	<i>Raw data cleaning</i>
-----------------	--------------------------

---

## 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'
setLambda(objCOTAN, lambda)

## S4 method for signature 'COTAN'
setDispersion(objCOTAN, dispersion)

## S4 method for signature 'COTAN'
setNu(objCOTAN, nu)

## 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)

genesPercentagePlot(
  objCOTAN,
  genes,
  title = "Genes' percentage of reads",
  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 <b>fully-expressed</b> . 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 <b>fully-expressing</b> . Default threshold is 0.99 (99.0%)
lambda	a named array that gives the values for lambda
dispersion	a named array that gives the values for the dispersion
nu	A named array that gives

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 <i>conditions</i> 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 <i>PCA</i> 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
title	The title of the plot; it defaults to "Genes' percentage of reads"
genePrefix	Prefix for the mitochondrial genes; use "^MT-" for Human (default) and "^mt-" for Mouse
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

setLambda() adds a column to the genes' metadata with the lambda (genes' counts averages) for the given batch

setDispersion() adds a column to the genes' metadata with the negative binomial dispersion factor for the given batch

setNu()

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

`genesPercentagePlot()` plots the percentage of expression of the passed-in genes against the overall cell's library size

`mitochondrialPercentagePlot()` plots the percentage of expression of the mitochondrial genes against the overall cell's library size

`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

`setLambda()` returns the updated COTAN object

`setDispersion()` returns the updated COTAN object

`setNu()` returns the updated COTAN object

`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 a half-violin-boxplot object

genesSizePlot() returns a half-violin-boxplot object

genesPercentagePlot() returns a list with:

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

mitochondrialPercentagePlot() returns a list with:

- "plot" a half-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
clPlots <- cleanPlots(objCOTAN)
plot(clPlots[["pcaCells"]])
plot(clPlots[["UDE"]])
plot(clPlots[["zoomedNu"]])
```

```

lsPlot <- cellSizePlot(objCOTAN)
plot(lsPlot)

gsPlot <- genesSizePlot(objCOTAN)
plot(gsPlot)

genes <- getGenes(objCOTAN)[1:30]
genesPercPlot <-
  genesPercentagePlot(objCOTAN, genes = genes)[["plot"]]
plot(genesPercPlot)

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,
  GDIIIn = 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,
minimumUTClusterSize = 50L,
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 <a href="#">SimpleGDIUniformityCheck</a> . 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 <i>cluster is uniform transcript</i> . See <a href="#">UniformTranscriptCheckers</a> for more details
initialResolution	a number indicating how refined are the clusters before checking for <b>uniformity</b> . It defaults to 0.8, the same as <a href="#">Seurat::FindClusters()</a>
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 use 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 <i>DEA</i> to define the distance; alternatively it will use the average <i>Zero-One</i> counts, that is faster but less precise.
distance	type of distance to use. Default is "cosine" for <i>DEA</i> and "euclidean" for <i>Zero-One</i> . Can be chosen among those supported by <a href="#">parallelDist::parDist()</a>
useCoexEigen	Boolean to determine whether to project the data matrix onto the first eigenvectors of the <b>COEX</b> 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 <a href="#">UMAPPlot()</a> . See <a href="#">getDataMatrix()</a> for more details.
genesSel	Decides whether and how to perform the gene-selection (defaults to "HVG_Seurat"). See <a href="#">getSelectedGenes()</a> 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 <b>RDM</b> components

hclustMethod	It defaults is "ward.D2" but can be any of the methods defined by the <code>stats::hclust()</code> function.
initialClusters	an existing <i>clusterization</i> to use as starting point: the <i>clusters</i> deemed <b>uniform</b> will be kept and the remaining cells will be processed as normal
minimumUTClusterSize	the minimum number of cells for a cluster to be deemed potentially <i>uniform transcript</i>
initialIteration	the number associated to the 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 placed in a sub-folder.
clusterName	the tag of the <i>cluster</i>
cells	the cells belonging to the <i>cluster</i>
clusters	The <i>clusterization</i> to merge. If not given the last available <i>clusterization</i> will be used, as it is probably the most significant!
checkers	a list of objects that defines the method and the <i>increasing</i> thresholds to discriminate whether to merge two <i>clusters</i> if deemed <i>uniform transcript</i> . See <a href="#">UniformTranscriptCheckers</a> 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 <i>merging</i> 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 ( $\leq 50$ ), 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 for 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)

objCOTAN <- storeGDI(objCOTAN, genesGDI = calculateGDI(objCOTAN, cores = 6L))

groupMarkers <- list(G1 = c("g-000010", "g-000020", "g-000138"),
                    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 |> methods::slot("firstCheck") |> methods::slot("GDIThreshold"),
  1.297
))

checker2 <- shiftCheckerThresholds(checker, 0.1)
```

```

stopifnot(identical(
  checker2 |> methods::slot("firstCheck") |> methods::slot("GDIThreshold"),
  1.397
))

splitList <- cellsUniformClustering(objCOTAN, cores = 6L,
  dataMethod = "LogLikelihood",
  useCoexEigen = TRUE,
  genesSel = "HGDI",
  numGenes = 2000L,
  numReducedComp = 50L,
  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),
  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 were 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 <i>cluster</i> is <i>uniform transcript</i> .
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 <i>cluster</i> is deemed <i>uniform transcript</i> .
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 <i>uniform transcript</i> . It is derived from <a href="#">BaseUniformityCheck</a>
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

`quantileAtRank` 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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